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RESEARCH ARTICLE

Prognostic value of circulating tumor cells in metastatic breast cancer: a systemic review and meta-analysis

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Abstract
Objective Metastatic breast cancer (MBC) remains the main cause of cancer-related death, and the clinical significance and prognostic role of circulating tumor cells (CTCs) in metastatic breast cancer are still controversial. Here, we conducted a meta-analysis to clarify the correlation between CTCs and the clinicopathological features and prognosis of MBC.
Methods We performed a comprehensive search of Pubmed and the ISI Web of Science through December 2014. Only articles that focused on MBC patients and detected CTCs using the CellSearch system were included. The associations between CTCs and survival rate and clinicopathological parameters, including molecular pattern, metastatic region and treatment response, were evaluated.
Results This meta-analysis included 24 studies (3701 MBC patients), 13 prospective studies and 11 retrospective studies. We found that CTCs were more frequently detected with HER2 + primary tumors (pooled RR = 0.73, 95 % CI = 0.63–0.84). Additionally, higher CTC numbers indicated a worse treatment response (RR = 0.56, 95 % CI = 0.40–0.79), poorer PFS (RR = 0.64, 95 % CI = 0.56–0.73) and poorer OS (RR = 0.69, 95 % CI = 0.64–0.75) in MBC patients.
Conclusion Based on these results, we propose that HER2 positivity could be a significant risk factor for the presence of CTCs. Additionally, CTCs have a significant prognostic value for MBC patients. Therefore, CTCs should be continually monitored to guide the treatment of MBC patients, especially those with HER2 + primary tumors.

Keywords Breast cancer · Circulating tumor cells · Her2 · Prognosis · Meta-analysis

Introduction

Although great advancements have been made in the detection of and treatment strategies for treating breast cancer, metastatic breast cancer (MBC) remains an incurable disease and a main cause of cancer-related death [1]. Although imaging examination, sentinel lymph node biopsy and axillary lymph node dissection allow for the precise assessment of the status of lymph node metastases, minimal residual cancer cells that can spread to the blood or bone marrow are difficult to detect by conventional approaches or by examining tumor markers. In recent years, circulating tumor cells (CTCs) in the peripheral blood have been shown to play a crucial role in cancer

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metastasis and are considered important prognostic biomarkers for solid tumors, such as lung cancer, ovarian cancer, prostate cancer and breast cancer tumors [2].

Circulating tumor cells detach from primary tumors and then seed metastatic tumors in distant organs by traveling through the blood stream. The detection of CTCs is considered a type of "liquid biopsy" that can monitor cancer status and provide valuable information for risk stratification and subsequent treatment choices. In recent years, multiple methods have been reported to be capable of detecting CTCs, such as RT-PCR, MACS, the ChromaVision Medical System and the CellSearch system [3]. Among them, the CellSearch system has been the most commonly applied and is the only method approved by FDA [4]. Although most studies have shown that an increase in CTCs indicates poor outcome in MBC patients, some studies did not find a significant relationship between CTCs and a shorter survival time [5]. Two meta-analyses evaluated the clinical significance of CTCs in breast cancer [1, 6]; however, neither meta-analysis was focused on MBC patients, and the results of the meta-analyses may have been inaccurate because the included studies utilized different detection assays.

To address this issue, we performed a systemic review of published research on the clinical significance of CTCs with regard to MBC. In addition to overall survival (OS) and disease-free survival (DFS), we also evaluated the association between CTCs and metastatic region, treatment response and the expression of hormone receptors and HER-2 on primary tumors. Furthermore, to increase the accuracy of this meta-analysis, a subgroup analysis was also conducted for the prospective and retrospective studies included in this meta-analysis.

Materials and methods

Search strategy

We performed a comprehensive literature search of Medline and the ISI Web of Knowledge through December 2014. The search terms included "circulating tumor cell(s)", "metastatic", "breast cancer", "breast neoplasm", "breast carcinoma" and "prognosis". The titles and abstracts of publications identified by the search were examined manually to exclude reviews, letters and irrelevant studies. The references of the remaining articles were reviewed to supplement our initial search.

Eligibility criteria

The studies enrolled in this meta-analysis consisted of both prospective and retrospective studies. Publications were included if they met all of the following criteria: (a) focus on MBC and a study cohort of more than 20 patients; (b) CTC status assessed by the CellSearch system; (c) clear analysis of correlations between CTC status and clinicopathological features or survival outcomes (either disease-free survival or overall survival) and CTC status stratified by CTC numbers; (d) study participants were not included in other studies included in this meta-analysis; and (e) published in English.

To control the quality of this meta-analysis, all enrolled studies were examined using the critical review checklist provided by the Dutch Cochrane Centre [7], which includes seven key points: (a) clear definition of study population and country of origin, (b) clear definition of carcinoma type, (c) clear definition of study design, (d) clear definition of outcome assessment, (e) clear definition of cut-off value for CTC status, (f) clear definition of method of CTC assessment and (g) sufficient follow-up time.

Data extraction

All data were independently extracted by two reviewers using a standardized method. For each study, the following information was recorded: author's name, publication year, country, number of patients, detection markers, cut-off value for CTCs, metastatic region, hormonal receptor expression status, HER2 expression status, treatment response, PFS rate, OS rate and 95 % CI, if available. For metastatic region, the data were grouped as visceral or non-visceral metastasis, and subjects were also divided into different groups based on treatment response [stable disease (SD)/partial response (PR) or progressive disease (PD)] based on imaging studies.

In consideration of the high degree of malignancy of MBC and to avoid bias among studies, the observation times for PFS and OS were standardized to 1 and 2 years, respectively. For studies that did not provide OS or PFS directly, GetData Graph Digitizer 2.24 software (http://getdata-graph-digitizer.com/) was used to digitize and extract the data from Kaplan–Meier curves.

Quality assessment of primary studies

Two reviewers (Ting Zhang and Hongjun Yuan) independently evaluated the quality of the included studies using the Newcastle–Ottawa Quality Assessment Scale (NOS). Studies with NOS scores above 6 were identified as high-quality studies, and disagreements were resolved by joint discussion.

Statistical analysis

The statistical analysis was performed according to the guidelines proposed by the Meta-Analysis of Observational

Studies in Epidemiology group. Relative risks (RRs) with 95 % confidence intervals (95 % CIs) were determined using fixed and random models. Study heterogeneity was measured using the Q and I^2 tests, and when heterogeneity was observed ($p \leq 0.1$ and $I^2 \geq 50$ %), only the random model was applied for the statistical analysis. Potential causes of heterogeneity were explored by meta-regression analyses. Publication bias was assessed using the Begg rank correlation method and the Egger weighted regression method. p values <0.05 were considered statistically significant. All statistical analyses were conducted with STATA 11.0 software, and all p values were two-tailed.

Results

Search results

Initially, 976 publications were retrieved by the primary computerized search. However, based on their abstracts and titles, 913 studies were determined to be either laboratory studies, reviews, comments or written in a language other than English and were thus excluded. The full-texts of the remaining 63 studies were further reviewed in detail. Thirty-nine studies were excluded because they did not examine the relationships between CTCs and clinicopathological features or survival data or because CTCs were detected by a method other than the CellSearch system. Finally, 24 studies were identified as eligible for this meta-analysis, and 20 studies had NOS scores above 6 (Fig. 1).

Characteristics of eligible studies

The 24 studies analyzed here included a total of 3701 metastatic breast cancer patients (Table 1); 13 studies were prospective and included a total of 1831 subjects, and 11 studies were prospective and included a total of 1870 subjects. Eleven, 9 and 4 studies were conducted in the USA, Europe and Asia, respectively. All studies identified CTCs as CD45-cytokeratin + or CD45-Epcam + cells. In addition, the cut-off value for positive CTC status was five for all but one study, which considered one CTC as positive.

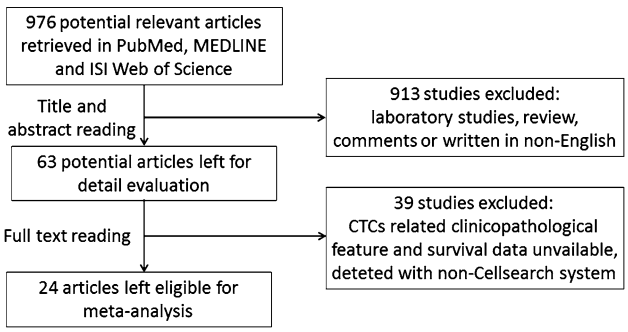

Fig. 1 Flow chart for selection of studies

Correlations between CTC number and clinicopathological features in MBC

The previous meta-analyses of CTCs in breast cancer did not evaluate the correlations between CTC number and HR (hormonal receptor) and HER2 expression in primary tumors. In the current meta-analysis, we found a significantly increased risk of CTC generation in patients with HER2-positive tumors (pooled RR = 0.73, 95 % CI = 0.63–0.84). When stratified by study type, both retrospective and prospective studies showed that HER2 expression was positively associated with CTC generation (prospective group RR = 0.73, 95 % CI = 0.58–0.86; retrospective group RR = 0.75, 95 % CI = 0.61–0.93; Fig. 2a). Moreover, the pooled analysis revealed that HR negativity was associated with higher CTC numbers (RR = 1.08, 95 % CI = 1.01–1.15); however, the stratified analysis based on study type did not find this correlation (prospective group RR = 1.06, 95 % CI = 0.96–1.17; retrospective group RR = 1.09, 95 % CI = 1.00–1.19; Fig. 2b). Because triple negative breast cancer (TNBC) is considered a distinct subpopulation of breast cancers, we also investigated if TNBCs tend to produce more CTCs; however, we did not observe an association between TNBC and CTC number (pooled RR = 0.87, 95 % CI = 0.73–1.03; prospective group RR = 0.89, 95 % CI = 0.66–1.20; retrospective group RR = 0.86, 95 % CI = 0.70–1.06; Fig. 2c). Furthermore, we examined whether CTC number was associated with metastatic region in MBC patients. The metastatic region was classified as visceral or non-visceral, and subjects with both regions affected were included in the non-visceral group. We did not find a significant correlation between CTC number and metastatic region in the pooled analysis or the stratified analysis based on study type (pooled RR = 1.05, 95 % CI = 0.98–1.12; prospective group RR = 0.95, 95 % CI = 0.83–1.08; retrospective group RR = 1.09, 95 % CI = 1.00–1.17; Fig. 2d).

CTC impact on survival and treatment response in MBC patients

One-year progression-free survival rates were available for 15 studies. Our pooled analysis showed that a CTC number of more than 5 cells per 7.5 ml is a significant risk factor for disease progression (RR = 0.64, 95 % CI = 0.56–0.73), and similar results were found in both the prospective and retrospective subgroups (prospective group

Table 1 Main characteristics of the included studies in this meta-analysis

References	Country	No. of patients	Marker	Cut off scores (pos/neg)	HR pos/neg	Her2 pos/neg	Triple neg/others	Metastasis (viseral/others)	Treatment response (SD&PR/PD)	PFS (1-year)	OS (2 years)	NOS score
Hayes [11]*	USA	177	CD45-CK+	CTC ≥ 5	L(60/29); H(61/25)	L(27/48); H(18/55)	NA	L(72/17); H(72/15)	L(50/40); H(19/68)	L(28/90); H(12/87)	L(39/90); H(26/87)	8
Budd [12]#	USA	138	CD45-CK+	CTC≥	NA	NA	NA	NA	L(83/20); H(13/22)	L(81/97); H(6/34)	L(53/103); H(5/35)	8
Cristofanilli, [13]#	USA	151	CD45-EpCam+	CTC ≥ 5	L(48/34); H(42/25)	L(24/58); H(11/52)	L(24/60); H(21/46)	L(52/31); H(42/25)	NA	NA	L(48/87); H(14/64)	7
Bidard [14]*	France	37	CK+	CTC ≥ 1	L(7/15); H(3/12)	NA	NA	NA	NA	NA	L(12/22); H(6/15)	7
Yagata [15]*	Japan	38	CD45-CK+ Epcam+	CTC ≥ 5	L(11/16); H(5/6)	L(13/14); H(5/6)	NA	NA	NA	L(24/27); H(5/11)	L(26/27); H(6/11)	6
De Giorgi [16]#	USA	102	CD45-CK+	CTC ≥ 5	L(23/27); H(15/35)	L(12/39); H(12/39)	NA	L(42/9); H(49/2)	NA	NA	L(30/51); H(19/51)	8
Liu [17]*	USA	72	CD45-CK+	CTC ≥ 5	NA	NA	NA	NA	NA	L(9/47); H(1/25)	NA	7
Mego [18]#	USA	149	CD45-CK+	CTC ≥ 5	L(40/51); H(37/21)	L(16/75); H(3/53)	L(33/58); H(18/40)	L(56/35); H(41/17)	NA	NA	L(52/91); H(24/58)	6
de Giorgi [19]#	USA	195	CD45-CK+	CTC ≥ 5	NA	NA	NA	NA	NA	NA	L(67/103); H(38/92)	7
Consoli [20]*	Italy	93	CD45-CK+	CTC ≥ 5	L(43/6); H(42/2)	L(13/36); H(5/39)	NA	L(40/9); H(37/7)	NA	L(11/49); H(3/44)	NA	5
Giuliano [21]#	USA	235	CD45-CK+	CTC ≥ 5	L(89/52); H(62/32)	L(28/113); H(15/79)	L(39/102); H(23/71)	L(80/61); H(60/34)	NA	L(75/141); H(23/94)	L(101/141; H(45/94)	6
Hartkopf [22]#	Germany	58	CD45-CK+	CTC ≥ 5	NA	L(6/18); H(11/23)	NA	L(20/4); H(28/6)	L(21/3); H(21/13)	NA	NA	5
Tokudome [23]*	Japan	28	CD45-CK+	CTC ≥ 5	L(12/7); H(4/5)	NA	NA	NA	L(17/2); H(6/3)	L(6/19); H(2/9)	L(12/19); H(2/9)	6
de Giorgi [24]#	USA	195	CD45-CK+	CTC ≥ 5	NA	NA	NA	NA	NA	L(44/103); H(26/92)	L(68/103); H(37/92)	7
Giordano [5]#	USA	517	CD45-CK+	CTC ≥ 5	L(196/115); H(146/60)	L(66/245); H(35/171)	L(82/229); H(42;164)	L(188/123); H(131/75)	NA	L(102/311; H(46/160)	L(208/311; H(85/206)	6
Hayashi [25]*	USA	52	CD45-CK+	CTC ≥ 5	NA	NA	NA	NA	NA	L(21/34); H(5/18)	L(20/34); H(5/18)	6
Müller [26]*	Germany	245	CD45-EpCam+	CTC ≥ 5	NA	NA	NA	NA	NA	L(49/123); H(36/122)	L(84/123); H(43/122)	7
Pierga [27]*	France	257	CD45-CK+	CTC ≥ 5	L(110/33); H(46/68)	L(7/136); H(34/80)	L(29/114); H(25/89)	NA	NA	L(59/143); H(24/114)	L(119/143; H(58/114)	7

Table 1 continued

References	Country	No. of patients	Marker	Cut off scores (pos/neg)	HR pos/neg	Her2 pos/neg	Triple neg/others	Metastasis (viseral/others)	Treatment response (SD&PR/PD)	PFS (1-year)	OS (2 years)	NOS score
Weissenstein [28]#	Switzerland	59	CK + Epcam+	CTC ≥ 5	L(23/9); H(18/4)	L(6/19); H(4/17)	NA	L(15/19); H(10/13)	NA	NA	L(26/35); H(4/24)	6
Jiang [4]*	China	294	CD45-CK+	CTC ≥ 5	L(112/63); H(79/33)	L(75/86); H(29/68)	NA	L(49/130); H(23/92)	NA	L(71/179); H(24/115)	L(108/179; H(38/115)	7
Liu [29]#	China	71	CD45-CK+ Epcam+	CTC ≥ 5	NA	L(48/4); H(12/7)	NA	L(31/21); H(15/4)	NA	NA	NA	5
Tarhan [30]*	Turkey	30	CD45-CK+	CTC ≥ 5	NA	NA	NA	NA	NA	NA	L(12/13); H(7/17)	6
Turker [31]*	Turkey	22	CD45-CK+	CTC ≥ 5	NA	NA	NA	NA	NA	L(9/16); H(0/6)	NA	5
Wallwiener [32]*	Germany	486	CD45-CK+	CTC ≥ 5	NA	L(74/207); H(43/162)	L(56/225); H(32/173)	L(123/158); H(52/153)	NA	L(73/256); H(30/189)	L(237/287); H(93/205)	8

HR hormonal receptor, *SD* stable disease, *PR* partial response, *PD* progression disease, *PFS* progression free survival, *OS* overall survival, *L* low CTC group, *H*, high CTC group, *NA* not available, *NOS* Newcastle–Ottawa quality assessment scale

* Prospective study; # retrospective study;

RR = 0.61, 95 % CI = 0.51–0.73; retrospective group RR = 69, 95 % CI = 0.56–0.84, Fig. 3a). The 2-year overall survival rate was extracted from 19 studies. Both the pooled and stratified analyses indicated that CTC number was significantly positively associated with increased risk of death (pooled RR = 0.69, 95 % CI = 0.64–0.75; prospective group RR = 0.69, 95 % CI = 0.61–0.77; retrospective group RR = 0.70, 95 % CI = 0.62–0.78, Fig. 3b).

Only four studies, which included a total of 303 MBC patients, provided treatment response data. The pooled analysis showed that CTC number was significantly correlated with a worse treatment response, identified as disease progression (RR = 0.56, 95 % CI = 0.40–0.79, I^2 = 0.64 random-effect); however, the sub-group analysis of the prospective studies did not find the same correlation (prospective group RR = 0.54, 95 % CI = 0.27–1.06, I^2 = 0.77 random-effect; retrospective group RR = 0.58, 95 % CI = 0.36–0.94, I^2 = 0.68 random-effect, Fig. 3c).

Publication bias

Begg's and Egger's tests were performed to determine publication bias, and the results of the funnel plots generated by those tests did not reveal any significant biases among the studies in our meta-analysis.

Discussion

Here, we present a meta-analysis based on a large pool of clinical studies (13 prospective and 11 retrospective studies) that assessed the prognostic value and clinical relevance of CTCs in MBC patients. Two meta-analyses regarding CTCs in breast cancer patients were published previously, one in 2011 and one in 2012 [1, 6]; however, the studies included in those meta-analyses included patients with different stages of breast cancer and used different CTC detection methods. Thus, the results of those meta-analyses may not have been accurate. Because laboratory research has shown that CTCs are closely associated with tumor metastasis, here, we identified 24 studies focused on CTCs in MBC patients. To ensure that the results of the meta-analysis were accurate, only studies that detected CTCs using the CellSearch System were included. We found that HER2 expression but not hormonal receptor expression or TNBC was a risk factor of higher CTC number. We also confirmed that the presence of CTCs was associated with increased risks of cancer progression and death.

Currently, the most commonly applied methods for detecting CTCs are RT-PCR and immunochemistry. Although approaches that rely on nucleic acid detection

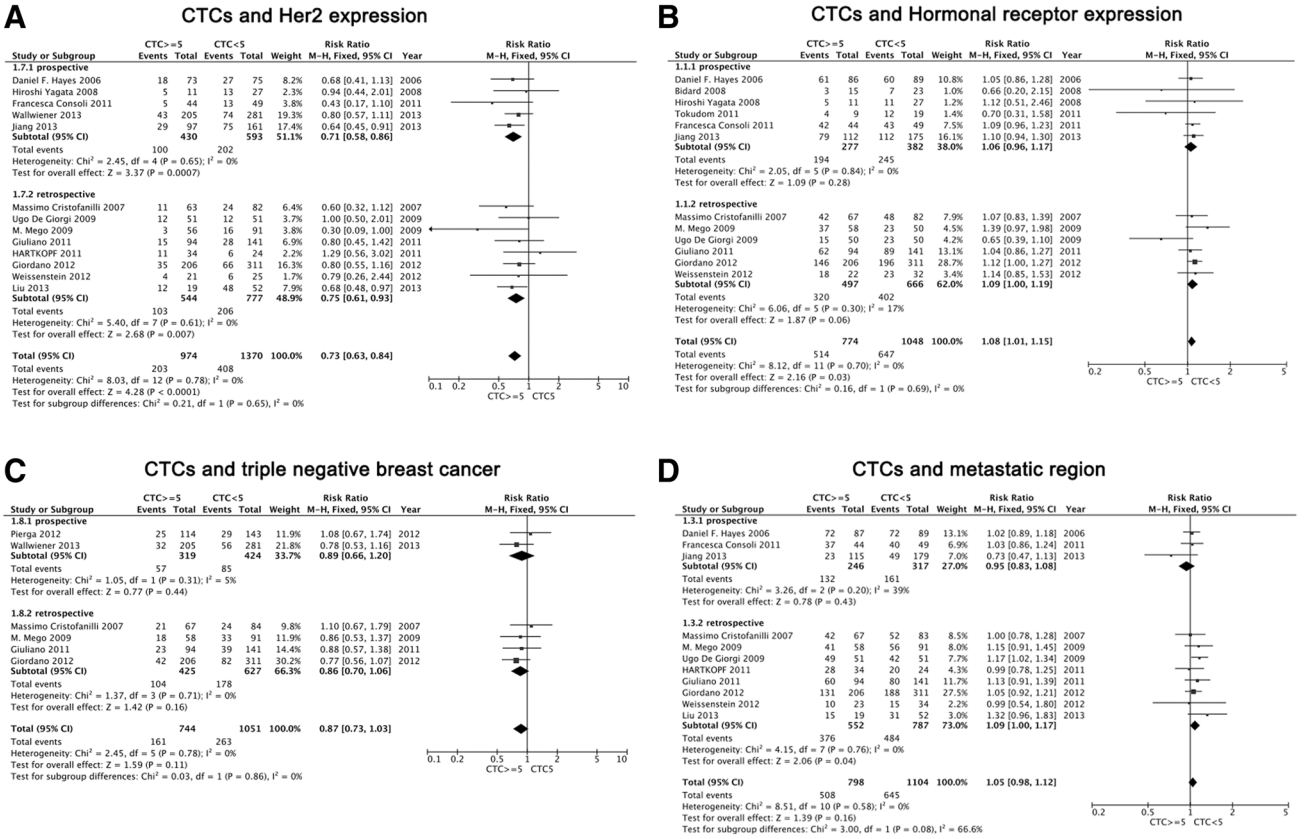

Fig. 2 The forest plot of RRs was assessed for association between CTCs and clinicopathological features, including Her2 expression (**a**), hormonal receptor expression (**b**), triple negative breast cancer (**c**) and metastatic region (visceral or non-visceral (**d**). Each result was shown by the RR with 95 % CIs (according to the fixed model)

possess the greatest sensitivity, they also possess relatively low specificity, which reduces their overall accuracy. For example, false signals can be generated by a small number of non-cancerous cells that enter into the circulation, such as immune cells, which have been reported to express CK, a widely used marker of CTCs [8]. Because of the drawbacks of other CTC detection methods, we only included studies that used the Cell Search System, which combines immunomagnetic sample enrichment and image cytometry technology and is the only FDA-approved approach for the detection and enumeration of CTCs in breast, prostate and colorectal cancer patients [9, 10].

Although the prognostic value of CTCs in breast cancer has been reported and most studies have shown that CTC number is an independent predictive factor for PFS and OS, some conflicting data exist. Giordano et al. [5] found that PFS and OS were similar between HER2 + MBC patients with ≥5 CTCs and those with <5 CTCs at baseline. Here, we performed the first meta-analysis focused on MBC patients, and we confirmed the prognostic value of CTCs and that a baseline CTC count of ≥5 was a significant risk factor for both disease progression and death.

Zhang et al. [1] found that CTC detection during or after cancer therapy cannot be used to monitor therapeutic effect and that different time points for CTC detection had the same predictive value. Thus, we examined whether CTC detection at baseline could predict therapeutic effect in MBC patients. The recently published SWOG S0500 study confirmed the prognostic value of CTC number in MBC patients receiving first-line chemotherapy; poor outcomes were observed in patients with persistently increased CTC numbers after first-line chemotherapy, and an early shift to an alternative therapeutic strategy did not effectively alter the outcomes of these patients [33]. Martin et al. [34] also reported that MBC patients with 0–4 CTCs after first-line chemotherapy had a significantly better PFS and OS than those with ≥5 CTCs after first-line chemotherapy, and patients with ≥5 CTCs at baseline and <5 CTCs after first-line chemotherapy had similar OS to those who had <5 CTC at baseline. In the present review, therapeutic effect data subgrouped by CTC number at baseline were limited. Our analysis showed that a CTC number of ≥5 could be a risk factor for worse therapeutic effect in MBC patients. A rational explanation for this finding is that most MBC patients included in this study had received first-line

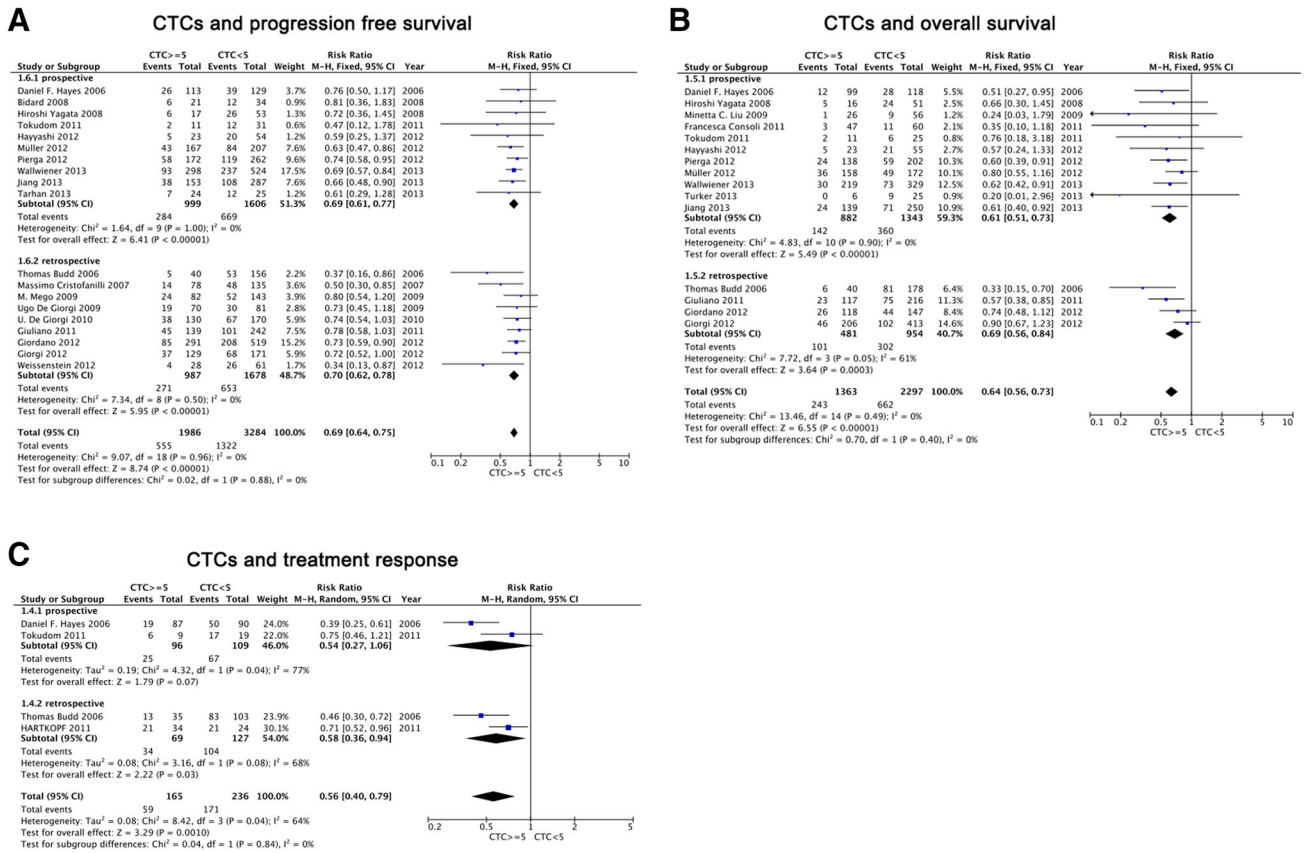

Fig. 3 The forest plot of RRs was assessed for association between CTCs and progression-free survival (**a**), overall survival (**b**) and treatment response (**c**). Each result was shown by the RR with 95 % CIs (according to the fixed model or random model)

chemotherapy or endocrinotherapy previously; thus, any remaining CTCs were expected to be chemoresistant generating the predictive value of CTC number for the effect of subsequent therapy. In summary, CTC number may help predict the efficacy of treatment, and CTC monitoring is valuable in guiding the therapeutic strategy used for breast cancer patients.

In addition to addressing the prognostic value of CTCs in MBC, several studies have investigated the relationship between CTCs and breast cancer of various molecular subtypes; however, the results of those studies are conflicting. HER2 positivity is a well-known risk factor of highly malignant breast cancer. Ignatiadis et al. [35] detected HER2-positive CTCs in breast cancer patients irrespective of their primary tumor HER2 status, but HER2-positive CTCs were more common in patients with HER2-positive cancer. Moreover, the existence of HER2-positive CTCs is also valuable for guiding anti-HER2 therapy; among patients with primary HER2-positive breast cancers undergoing anti-HER2 therapy, those with HER2-positive CTCs had significantly longer PFSs than these without HER2-positive CTCs [29]. In a retrospective study of 203 MBC patients, Munzone et al. [36] found that CTCs in MBC patients most commonly had the luminal-A/luminal-B HER2(-) phenotype. In contrast, Banys et al. [37] found that the most common CTC phenotype was triple negative and that the primary tumors of all CTC-positive patients were luminal [32]. Other studies did not indicate a significant correlation between the presence of CTCs and various breast cancer molecular subtypes [15]. Because of the inconsistent results of these past studies, which may be due to different grouping criteria and different detection methods, in this study, we analyzed the association between CTC number and the expression of hormonal receptors and HER2 in primary tumors. Our results showed that HER2 expression in the primary tumor was a significant risk factor for a greater CTC number, while no clear associations were observed between CTC number and hormonal receptor expression or triple negative breast cancer.

There were some limitations to this meta-analysis. First, some MBC patients received chemotherapy or endocrinotherapy of varying strategies before blood was sampled for CTC detection; this may have caused heterogeneity in the results among patients. Second, our meta-analysis was based on published literature; thus, individual

patient data could not be obtained, which could have further improved the accuracy of our results. Third, some of our data analyses were based on relatively small numbers of patients because of missing information in several studies. This was particularly true for the analysis of the association between CTC number and treatment response, the results for which showed significant heterogeneity. Fourth, more than 85 % of the studies included in this meta-analysis were conducted in the USA or Europe, which could have generated publication bias.

In conclusion, the results of this meta-analysis further support the prognostic value of CTCs in MBC. Additionally, HER2 expression in the primary tumor, but not hormonal receptor expression in the primary tumor or TNBC, was associated with a greater CTC number. Furthermore, our results also suggested that a CTC number of ≥ 5 is associated with a worse treatment response. Moreover, several reports revealed that the CTC phenotype is not in accordance with that of the primary tumor and that CTCs with various phenotypes possess distinct metastatic potentials [36, 38]. Therefore, in consideration of the limitations of this study and to understand the clinical utility of CTC detection in breast cancer, large interventional or observational studies based on CTC subtypes should be performed in the future to generate more accurate results.

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest were disclosed.

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