Meta-Analysis of the Prognostic Value of Circulating Tumor Cells in Breast Cancer

Liling Zhang¹, Sabine Riethdorf², Gang Wu¹, Tao Wang¹, Kunyu Yang¹, Gang Peng¹, Junli Liu¹, and Klaus Pantel²

Abstract

Purpose: The prognostic value of circulating tumor cells (CTC) detected in breast cancer patients is currently under debate. Different time points of blood collections and various CTC assays have been used in the past decades. Here, we conducted the first comprehensive meta-analysis of published literature on the prognostic relevance of CTC, including patients with early and advanced disease.

Experimental Design: A comprehensive search for articles published between January 1990 and January 2012 was conducted; reviews of each study were conducted and data were extracted. The main outcomes analyzed were overall survival (OS) and disease-free survival (DFS) in early-stage breast cancer patients, as well as progression-free survival (PFS) and OS in metastatic breast cancer patients. Pooled hazard ratio (HR) and 95% confidence intervals (CIs) were calculated using the random and the fixed-effects models. Subgroup and sensitivity analyses were also conducted.

Results: Forty-nine eligible studies enrolling 6,825 patients were identified. The presence of CTC was significantly associated with shorter survival in the total population. The prognostic value of CTC was significant in both early (DFS: HR, 2.86; 95% CI, 2.19–3.75; OS: HR, 2.78; 95% CI, 2.22–3.48) and metastatic breast cancer (PFS: HR, 1.78; 95% CI, 1.52–2.09; OS: HR, 2.33; 95% CI, 2.09–2.60). Further subgroup analyses showed that our results were stable irrespective of the CTC detection method and time point of blood withdrawal.

Conclusion: Our present meta-analysis indicates that the detection of CTC is a stable prognosticator in patients with early-stage and metastatic breast cancer. Further studies are required to explore the clinical utility of CTC in breast cancer.

Introduction

Metastasis is the main cause of cancer-related death. However, even by currently available high-resolution imaging technologies, micrometastasis cannot be detected. Thus, in recent years much work has been entered into the detection and characterization of disseminated tumor cells (DTC) and circulating tumor cells (CTC). Through a pooled analysis accounting for 4,703 patients, Braun and colleagues (1) in 2005 reported that the presence of DTC in breast cancer patients is an independent predictor of poor prognosis. However, bone marrow sampling is an invasive procedure not easily accepted in the management of breast cancer. Thus, the focus in recent years has shifted to the detection of CTC in peripheral blood collections.

CTC are tumor cells detectable in blood that are released by primary tumors, recurrences, or metastases and that possess antigenic and genetic tumor-specific characteristics. The first report on tumor cells in bloodstream was attributed to Ashworth in 1869 (2). As then, various methods have been developed and optimized for their detection. Immunocytochemistry (ICC) and reverse transcriptase polymerase chain reaction (RT-PCR) are the 2 main approaches currently. Among various ICC methods, the CellSearch system is the only assay cleared by the U.S. Food and Drug Administration (FDA) for clinical use. This system uses ferrofluids coated with an antibody against the epithelial cell adhesion molecule (EpCAM) to magnetically enrich epithelial cells from whole blood. However, EpCAM-positive CTC are missed by CellSearch device and various new CTC assays have been developed in the recent years. Nevertheless, it is still unclear whether the existing CTC assays may detect irrelevant “bystander” cells and miss the metastasis-initiating cells.

Despite the technical advancements in CTC detection, the prognostic relevance of CTC in breast cancer remains
CTC can be detected in peripheral blood from early-stage and metastatic breast cancer patients, and different time points of blood collections and various CTC assays have been used in the past decades. Although with encouraging results from smaller single-center studies, the prognostic value of CTC in breast cancer patients remains uncertain because single trials may report conflicting results. A comprehensive meta-analysis may help resolve this controversy and give more accurate estimates of the average effect. The results of our meta-analysis showed that the presence of CTC was significantly associated with poorer survival in both early and metastatic breast cancer, which may help provide more convincing evidence to using CTC detection in future clinical practice.

Eligibility criteria
The studies were included in the meta-analysis if they reported survival data in breast cancer patients stratified by CTC status (presence/positive and absence/negative), provided sufficient data for determining an estimate of HR and a 95% confidence interval (CI), and enrolled more than 30 patients, and study patients did not overlap with patients in other included studies. Although no language restrictions were imposed initially, for the final analysis only articles written in English were included.

When more than 1 blood sample per patient was withdrawn, the results were excluded if survival data were not stratified by CTC status at each time point (e.g., persistent, increased, and decreased). We also excluded the studies in which histologic type of breast malignancies was inflammatory breast cancer or sarcoma.

We did not assign each study a quality score, because no such score has received general agreement for use in a prognostic meta-analysis, especially of observational studies. Instead, we conducted subgroup and sensitivity analyses because they are widely recommended.

Data extraction and outcomes
We recorded the following information about each eligible trial: author's names, journal and year of publication, number of patients analyzed, tumor stage, volume and timing of blood withdrawal, method of CTC detection, and cutoff value of CTC. We also recorded PFS, DFS, OS, survival curves, HR, and 95% CI if available. The HR was measured by comparing the CTC positive and CTC negative arms. For one study (21), the reported HR referred to the CTC negative arm rather than the CTC positive arm and was recalculated by taking reciprocal of HR to keep consistency with other trials.

When more than 1 blood sample per patient was withdrawn at different time points, we recorded results of each time point and classified these time points as "baseline," "mid-therapy," and "posttherapy." When more than 1 marker was used to detect CTC, and the HR for survival or the survival curve was reported for the different markers, we recorded all of these results as independent data sets.

The endpoints of clinical outcome analyzed were OS, PFS, and DFS. In patients with stage M0, the effect of CTC detection on DFS was evaluated, whereas in patients with stage M1 the respective endpoint was PFS. In both groups, the effect of CTC detection on OS was analyzed. Additional subgroup analyses were conducted with regard to the type of the detection method and the time point of blood withdrawal in relation to systemic therapy.

Statistical analysis
HR of each study was either directly collected from the original article or calculated as suggested by Parmar and colleagues (22). The pooled HR for survival was calculated by fixed and random-effects models. And if heterogeneity among the studies was observed, only random-effects estimates were presented.

Translational Relevance
CTC can be detected in peripheral blood from early-stage and metastatic breast cancer patients, and different time points of blood collections and various CTC assays have been used in the past decades. Although with encouraging results from smaller single-center studies, the prognostic value of CTC in breast cancer patients remains uncertain because single trials may report conflicting results. A comprehensive meta-analysis may help resolve this controversy and give more accurate estimates of the average effect. The results of our meta-analysis showed that the presence of CTC was significantly associated with poorer survival in both early and metastatic breast cancer, which may help provide more convincing evidence to using CTC detection in future clinical practice.

Materials and Methods

Search strategy
A comprehensive search of Medline and ISI Web of Knowledge was done between January 1990 and January 2012 as the major reports delivered before 1990 focused on the technical issues. The search strategy included the following keywords variably combined: "circulating tumor cell (s)," "breast cancer," "breast neoplasm," "micrometastasis," and "prognosis." Furthermore, reference lists of retrieved articles and review articles were reviewed manually to implement our search. Only studies published in peer-reviewed journals were included, data from letters and meetings abstracts were not eligible.

Data extraction and outcomes
We recorded the following information about each eligible trial: author's names, journal and year of publication, number of patients analyzed, tumor stage, volume and timing of blood withdrawal, method of CTC detection, and cutoff value of CTC. We also recorded PFS, DFS, OS, survival curves, HR, and 95% CI if available. The HR was measured by comparing the CTC positive and CTC negative arms. For one study (21), the reported HR referred to the CTC negative arm rather than the CTC positive arm and was recalculated by taking reciprocal of HR to keep consistency with other trials.

When more than 1 blood sample per patient was withdrawn at different time points, we recorded results of each time point and classified these time points as "baseline," "mid-therapy," and "posttherapy." When more than 1 marker was used to detect CTC, and the HR for survival or the survival curve was reported for the different markers, we recorded all of these results as independent data sets.

The endpoints of clinical outcome analyzed were OS, PFS, and DFS. In patients with stage M0, the effect of CTC detection on DFS was evaluated, whereas in patients with stage M1 the respective endpoint was PFS. In both groups, the effect of CTC detection on OS was analyzed. Additional subgroup analyses were conducted with regard to the type of the detection method and the time point of blood withdrawal in relation to systemic therapy.

Statistical analysis
HR of each study was either directly collected from the original article or calculated as suggested by Parmar and colleagues (22). The pooled HR for survival was calculated by fixed and random-effects models. And if heterogeneity among the studies was observed, only random-effects estimates were presented.
Heterogeneity between studies was evaluated with the Cochran’s Q test as well as the I² index. Potential causes of heterogeneity were explored by meta-regression analyses. Publication bias was evaluated using the funnel plot and the Egger’s and Begg’s test. The effect of publication bias on the pooled effect was assessed by the “trim-and-fill” method. To evaluate the stability of the results, a 1-way sensitivity analysis was conducted. The scope of this analysis was to evaluate the influence of individual studies by estimating the average HR in the absence of each study. All statistical tests were conducted with the software STATA version 10.0.

**Results**

**Characteristics of identified studies**

Seven hundred thirty-three records were identified by the primary computerized literature search. However, after screening of the titles and abstracts, 672 studies were excluded because they were either duplicate, review articles, laboratory studies, written in non-English, or irrelevant to the current study. Sixty-one articles were further reviewed in detail. Twelve studies were further excluded because of multiple publications, no survival data, or small sample size. In refs. 23 and 24, although the patients came from the same population, the detection of CTC was conducted at “posttherapy” and “baseline,” respectively. Therefore, both studies were included, but the number of patients was only extracted from the larger one. Finally, 49 studies (6–21, 23–55) were identified as eligible for inclusion in the meta-analysis (Fig. 1).

The included 49 studies encompassed 6,825 breast cancer patients. Early stage (M0, n = 2,993; refs. 11–13, 17–19, 23–25, 28–31, 34, 35, 37, 39, 43, 51) and metastatic (M1, n = 3,069) patients (6–10, 20, 21, 27, 33, 36, 40–42, 44, 45, 47–50, 52–54) were enrolled in 19 (38.8%) and 22 (44.9%) studies, respectively. The remaining 8 studies (16.3%) had pooled patients with I–IV stages (refs. 14–16, 26, 32, 38, 46, 55; M0, M1; n = 763). The main features of the eligible studies are summarized in Table 1.

**Global Analysis of CTC Effects on Survival**

HRs for PFS/DFS were available in 35 studies (6, 9–14, 16–20, 23–32, 34, 35, 37, 39, 41, 43, 44, 48–51, 53, 54) accounting for 4,978 patients. In 12 studies (6, 13, 16, 17, 19, 32, 34, 35, 37, 39, 41, 48), more than 1 HR was extracted from each study because multimarkers or multi-time points were used to detect CTC in these studies and HRs were reported separately.

The estimated pooled HR for all studies showed a significantly increased risk of disease progression in patients with CTC positivity (HR, 2.07; 95%CI, 1.80–2.39; P = 0.00; df = 48; random effects). As the heterogeneity among studies was significant (P < 0.001, I² = 85.5%), random-effects model was applied. To explore potential sources of heterogeneity, we conducted meta-regression considering following covariates: publication year, sample size, detection...
Table 1. Characteristics of studies included in the meta-analysis

<table>
<thead>
<tr>
<th>First author of study (ref.), y</th>
<th>NO. of patients</th>
<th>Sampling time</th>
<th>Stage</th>
<th>Detection method</th>
<th>Detection rate, %</th>
<th>Cutoff of CTC&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Outcome</th>
<th>HR estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stathopoulou (25), 2002</td>
<td>148</td>
<td>Baseline</td>
<td>M</td>
<td>RT-PCR</td>
<td>30</td>
<td>—</td>
<td>DFS, OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Gaforio (26), 2003</td>
<td>92</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;, M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Other ICC</td>
<td>62</td>
<td>1 CTC/10 mL</td>
<td>PFS, OS</td>
<td>Data extrapolated</td>
</tr>
<tr>
<td>Weigelt (27), 2003</td>
<td>94</td>
<td>Mid-therapy</td>
<td>M</td>
<td>RT-PCR</td>
<td>31</td>
<td>—</td>
<td>PFS, OS</td>
<td>Data extrapolated</td>
</tr>
<tr>
<td>Xenidis (28), 2003</td>
<td>161</td>
<td>Mid-therapy</td>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>RT-PCR</td>
<td>27</td>
<td>—</td>
<td>DFS</td>
<td>Data extrapolated</td>
</tr>
<tr>
<td>Giatromanolaki (29), 2004</td>
<td>100</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;</td>
<td>RT-PCR</td>
<td>33</td>
<td>—</td>
<td>DFS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Pierga (14), 2004</td>
<td>114</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;, M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Other ICC</td>
<td>25</td>
<td>1 CTC/3 × 10&lt;sup&gt;5&lt;/sup&gt; MNC</td>
<td>DFS, OS</td>
<td>Data extrapolated</td>
</tr>
<tr>
<td>Masuda (30), 2005</td>
<td>98</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;</td>
<td>RT-PCR</td>
<td>18</td>
<td>—</td>
<td>DFS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Benoy (15), 2006</td>
<td>148</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;, M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>RT-PCR</td>
<td>15, 20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Budd (7), 2006</td>
<td>138</td>
<td>Mid-therapy</td>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Cellsearch</td>
<td>25</td>
<td>5 CTC/7.5 mL</td>
<td>OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Hayes (6), 2006</td>
<td>177</td>
<td>Baseline</td>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Cellsearch</td>
<td>49</td>
<td>5 CTC/7.5 mL</td>
<td>PFS, OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Ntoulia (18), 2006</td>
<td>101</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;</td>
<td>RT-PCR</td>
<td>14</td>
<td>—</td>
<td>DFS, OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Wiedswang (31), 2006</td>
<td>318</td>
<td>Posttherapy</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;, M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Other ICC</td>
<td>10</td>
<td>1 CTC/1 × 10&lt;sup&gt;6&lt;/sup&gt; MNC</td>
<td>DFS, OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Pierga (14), 2004</td>
<td>114</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;, M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Other ICC</td>
<td>25</td>
<td>1 CTC/3 × 10&lt;sup&gt;5&lt;/sup&gt; MNC</td>
<td>DFS, OS</td>
<td>Data extrapolated</td>
</tr>
<tr>
<td>Masuda (30), 2005</td>
<td>98</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;</td>
<td>RT-PCR</td>
<td>18</td>
<td>—</td>
<td>DFS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Benoy (15), 2006</td>
<td>148</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;, M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>RT-PCR</td>
<td>15, 20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Budd (7), 2006</td>
<td>138</td>
<td>Mid-therapy</td>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Cellsearch</td>
<td>25</td>
<td>5 CTC/7.5 mL</td>
<td>OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Hayes (6), 2006</td>
<td>177</td>
<td>Baseline</td>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Cellsearch</td>
<td>49</td>
<td>5 CTC/7.5 mL</td>
<td>PFS, OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Ntoulia (18), 2006</td>
<td>101</td>
<td>Baseline</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;</td>
<td>RT-PCR</td>
<td>14</td>
<td>—</td>
<td>DFS, OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Wiedswang (31), 2006</td>
<td>318</td>
<td>Posttherapy</td>
<td>M&lt;sub&gt;0&lt;/sub&gt;, M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Other ICC</td>
<td>10</td>
<td>1 CTC/1 × 10&lt;sup&gt;6&lt;/sup&gt; MNC</td>
<td>DFS, OS</td>
<td>Reported in text</td>
</tr>
</tbody>
</table>

(Continued on the following page)
method, time point of blood withdrawal, and tumor stage (M0 vs. M1). At univariate analysis, the explanatory variable that influenced HR estimates was the tumor stage only (P = 0.001; Table 2).

The HRs for OS were available in 40 studies (6–24; random effects; Fig. 2B). The heterogeneity among studies was significant (I² = 65.2%), and publication bias existed (P = 0.03; Supplementary Fig. S1B). The trim-and-fill analysis revealed that 6 studies might be missing and that if these were published, the adjusted HR would be 2.28 (95% CI, 1.71–3.03; P = 0.00; df = 34; random effects).

The HRs for OS were available in 13 studies with early-stage breast cancer. The estimated pooled HR showed that the presence of CTC is a significantly increased risk of disease recurrence in early-stage patients (HR, 2.86; 95% CI, 2.19–3.75; P = 0.00; df = 28; random effects; Fig. 2A). The heterogeneity among studies was significant (P = 0.00; I² = 65.2%), and publication bias existed (P = 0.03; Supplementary Fig. S1A). The trim-and-fill analysis revealed that 2 studies might be missing and that if these were published, the adjusted HR would be 2.66 (95% CI, 2.13–3.32; P = 0.00; df = 19; fixed effects).

One-way sensitivity analysis confirmed the stability of our results (Supplementary Fig. S2A and B).

### CTC impact on survival in early-stage breast cancer

The HRs for DFS were available in 19 studies with early-stage breast cancer. The estimated pooled HR showed that the presence of CTC is a significantly increased risk of disease recurrence in early-stage patients (HR, 2.66; 95% CI, 2.13–3.32; P = 0.00; df = 28; random effects; Fig. 2A). The heterogeneity among studies was significant (P = 0.00; I² = 65.2%), and publication bias existed (P = 0.03; Supplementary Fig. S1A). The trim-and-fill analysis revealed that 2 studies might be missing and that if these were published, the adjusted HR would be 2.66 (95% CI, 2.13–3.32; P = 0.00; df = 19; fixed effects).

One-way sensitivity analysis confirmed the stability of our results (Supplementary Fig. S2A and B).

### Table 1. Characteristics of studies included in the meta-analysis (Cont’d)

<table>
<thead>
<tr>
<th>First author of study (ref.), y</th>
<th>NO. of patients</th>
<th>Sampling time</th>
<th>Stage</th>
<th>Detection method</th>
<th>Detection rate, %</th>
<th>Cutoff of CTC</th>
<th>Outcome</th>
<th>HR estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pierga (53), 2011</td>
<td>267</td>
<td>Baseline</td>
<td>M1</td>
<td>CellSearch</td>
<td>44</td>
<td>5 CTC/7.5 mL</td>
<td>PFS, OS</td>
<td>Reported in text</td>
</tr>
<tr>
<td>Reinholz (64), 2011</td>
<td>86</td>
<td>Baseline</td>
<td>M1</td>
<td>RT-PCR</td>
<td>56–75; 23–38</td>
<td>PFS, OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serrano (55), 2011</td>
<td>85</td>
<td>Posttherapy</td>
<td>M0, M1</td>
<td>Other ICC</td>
<td>63, 46, 43, 31</td>
<td>OS</td>
<td>Data extrapolated</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ref., reference; CTC, circulating tumor cells; MNC, mononuclear cells; RT-PCR, reverse transcriptase polymerase chain reaction; ICC, immunocytochemistry; OS, overall survival; PFS, progression-free survival; DFS, disease-free survival.

aDetection rates were for markers CK-19 and mammaglobin, respectively.
bThe cutoff value was 4 CTC/sample in early-stage patients, whereas the cutoff value was 13 CTC/sample in metastatic patients.
cHR for OS in early breast cancer was extrapolated from survival curves.
dDetection rates were for markers CK-19, mammaglobin and HER2, respectively.
eDetection rates were for markers CK-19, mammaglobin at baseline, respectively.
fDetection rates were for markers CK-19 and mammaglobin at baseline, respectively.
gDetection rates were for the study time intervals of 1 to 5 months, more than 5 to 12 months, more than 12 to 24 months, and more than 24 to 50 months, respectively.

### Table 2. Results of meta-regression analysis exploring source of heterogeneity with progression-free/disease-free and overall survival.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>PFS/DFS Univariate analysis</th>
<th>OS Univariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection method</td>
<td>Coefficient SE P</td>
<td>Coefficient SE P</td>
</tr>
<tr>
<td>Time point of sampling</td>
<td>−0.21 0.12 0.08</td>
<td>−0.13 0.09 0.15</td>
</tr>
<tr>
<td>Stage of breast cancer</td>
<td>−0.41 0.12 0.001</td>
<td>−0.25 0.10 0.01</td>
</tr>
<tr>
<td>Sample size</td>
<td>−0.001 0.001 0.25</td>
<td>0.0003 0.0006 0.64</td>
</tr>
<tr>
<td>Publication year</td>
<td>−0.07 0.04 0.07</td>
<td>−0.03 0.03 0.27</td>
</tr>
</tbody>
</table>

NOTE: The dependent variable is the lnHR for PFS/DFS or OS from each study. Weights have been assigned according to the estimated variance of the lnHR. SE, standard error of the coefficient.
CTC impact on survival in metastatic breast cancer

HRs for PFS were available in 12 studies with metastatic breast cancer. The estimated pooled HR showed CTC positivity was associated with a significantly increased risk of progression (HR, 1.78; 95% CI, 1.52–2.09; \( P = 0.00; df = 15 \); random effects; Fig. 2C). The heterogeneity among studies was moderate (\( P = 0.00; df = 15 \); random effects). Publication bias existed (\( P_{Begg} = 0.82; P_{Egger} = 0.02; \) Supplementary Fig. S1C). The trim-and-fill analysis revealed that 7 studies might be missing and that if these were published, the adjusted HR would be 1.48 (95% CI, 1.25–1.74; \( P = 0.00; df = 22 \); random effects).

The HRs for OS were available in 19 studies with metastatic breast cancer. The estimated pooled HR showed CTC presence was associated with a significantly increased risk of death (HR, 2.33; 95% CI, 2.09–2.60; \( P = 0.00; df = 22 \); fixed effects; Fig. 2D). The heterogeneity among studies was absent (95% CI, 1.25–1.74; \( P = 0.00; df = 22 \); random effects).

One-way sensitivity analysis confirmed the stability of our results (Supplementary Fig. S2C and D).

Influence of sampling time point and detection method

As shown in Table 3, subgroup analyses stratified by detection method and time point of blood sampling confirmed that the CTC presence was a strong prognosticator in all subgroups except the subgroup designated "other ICC." The prognostic value of CTC for PFS/DFS was significant in the "RT-PCR" subgroup (HR, 2.58; 95% CI, 1.99–3.35) and the "CellSearch" subgroup (HR, 1.85; 95% CI, 1.53–2.25), whereas it was not significant in the subgroup "other ICC" (HR, 1.11; 95% CI 0.95–1.29; Fig. 3A). In the subgroup "other ICC," Serrano’s study was overweighted (87.01%), which may influence the pooled result. Therefore, pooled analysis was conducted again omitting this study, and the results showed that the subgroup HR did not cross the 1.0 line (HR, 2.42; 95% CI, 1.62–3.62), thereby also indicating the significant prognostic impact of CTC.
detection in this subgroup. The analysis of OS as endpoint reveals similar results (Fig. 3B).

Discussion

The present analysis is based on a large pool of clinical studies (6,825 patients) and substantially differs from the other 2 meta-analysis published in 2011 [56, 57], which considered smaller series, and evaluated studies analyzing blood samples only by RT-PCR. Here, we identified 49 studies that assessed the prognostic value of CTC detection by RT-PCR as well as the CellSearch system or other ICC methods. It provides evidence that the presence of CTC in peripheral blood is significantly associated with poorer prognosis both in early-stage and metastatic breast cancer. The pooled results are fairly stable and not influenced by the CTC detection method and time point of blood withdrawal (Table 3). In addition, this meta-analysis of pooled data

Table 3. Results of subgroup analyses for effects of CTC presence on progression-free/disease-free and overall survival

<table>
<thead>
<tr>
<th>Study selection</th>
<th>df</th>
<th>HR (95% CI; random effects)</th>
<th>Test for heterogeneity ($I^2$)</th>
<th>df</th>
<th>HR (95% CI; random effects)</th>
<th>Test for heterogeneity ($I^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Detection method</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT-PCR</td>
<td>27</td>
<td>2.58 (1.99–3.35)</td>
<td>67.7%</td>
<td>19</td>
<td>2.38 (1.98–2.85)</td>
<td>0.0%</td>
</tr>
<tr>
<td>CellSearch</td>
<td>12</td>
<td>1.65 (1.53–2.25)</td>
<td>56.3%</td>
<td>18</td>
<td>2.45 (2.10–2.85)</td>
<td>28.8%</td>
</tr>
<tr>
<td>Other ICC</td>
<td>7</td>
<td>1.11 (0.95–1.29)</td>
<td>71.8%</td>
<td>11</td>
<td>2.35 (1.49–3.86)</td>
<td>73.0%</td>
</tr>
<tr>
<td><strong>Time point of blood withdrawal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>33</td>
<td>2.39 (1.96–2.91)</td>
<td>64.7%</td>
<td>34</td>
<td>2.37 (2.10–2.67)</td>
<td>5.6%</td>
</tr>
<tr>
<td>Mid-therapy</td>
<td>6</td>
<td>1.98 (1.23–3.20)</td>
<td>89.6%</td>
<td>8</td>
<td>2.51 (1.86–3.40)</td>
<td>48.0%</td>
</tr>
<tr>
<td>Posttherapy</td>
<td>7</td>
<td>1.62 (1.03–2.54)</td>
<td>84.3%</td>
<td>6</td>
<td>2.03 (1.23–3.67)</td>
<td>69.0%</td>
</tr>
<tr>
<td>All</td>
<td>48</td>
<td>2.07 (1.80–2.39)</td>
<td>85.5%</td>
<td>50</td>
<td>2.45 (2.07–2.90)</td>
<td>70.8%</td>
</tr>
</tbody>
</table>

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

Figure 3. Influence of the CTC detection method on the prognostic impact of CTC. Subgroup analysis of the HR for survival in the total population of patients (M0 and M1 stages) depending on the presence or absence of CTC, stratified by the detection method. A, PFS/DFS. B, OS.
confirmed that the presence of CTC represents a significant risk factor for both PFS/DFS and OS, even after adjustment for publication bias.

The 2 main approaches for CTC detection are ICC and RT-PCR. One advantage of ICC methods over nucleic acid methods is the preservation of the cell during the process, which enables further characterization of CTC by additional ICC and molecular methods. Compared with ICC, RT-PCR seems to be more sensitive (58). In our analysis, the number of studies using RT-PCR to detect CTC is nearly equal to the number of studies using ICC. Particularly, among those studies using ICC methods, the CellSearch system was used in 18 studies (18/29, 62.1%). At present, the CellSearch system is the only assay cleared by the U.S. FDA for clinical use. In subgroup analysis, pooled HRs for PFS/DFS and OS are stably statistically significant in RT-PCR and CellSearch subgroups. In the “other ICC” subgroup, pooled HR was not statistically significant (HR, 1.11; 95% CI, 0.95–1.29), and we deduced that the heterogeneity might be the main cause for this finding. Omitting the overweighted study, adjusted pooled HR was 2.42 (95% CI, 1.62–3.62) and the heterogeneity disappeared. These results indicate that, although with different sensitivity and specificity, different detection systems are able to detect prognostically relevant CTC in breast cancer. However, it is desirable that future studies on the clinical utility of CTC use standardized detection methods.

Most studies assessed the clinical significance of CTC detected at baseline and showed that the presence of CTC at this time point was an independent prognostic factor. How about CTC detected during or after systemic therapy? Our present results indicate that CTC detection at mid-therapy or posttherapy cannot be only used for monitoring therapeutic effects but has also prognostic relevance. In our meta-analysis, CTC detected before, during and after systemic therapy showed similar prognostic value (Table 3), suggesting that the presence of CTC at different time points is a strong prognostic indicator, which is not significantly influenced by systemic therapy. The fact, that CTC detection at baseline (before therapy) is as predictive for an unfavorable outcome as the detection during or after therapy, suggests that most CTC might be rather resistant to the current forms of therapy in breast cancer. However, we could not evaluate the effects of therapy on the prognostic value of CTC, which is an important parameter in prognostic studies (59). The reason for this drawback of our study is that the therapeutic regimens were various among most studies included in this meta-analysis.

The prognosis of patients with early-stage and metastatic breast cancer is obviously quite different. Therefore, most published studies researched these 2 patient cohorts separately. However, in this meta-analysis, there are 8 studies that analyzed patients without separation into M₀ and M₁ stages. Meta-regression analysis revealed that it was tumor stage that was the main source of heterogeneity for this meta-analysis. When these 8 studies were omitted, heterogeneity was decreased or disappeared, which confirmed that early-stage and metastatic breast cancer patients should be analyzed separately to obtain more accurate results.

Some limitations of this meta-analysis need to be discussed. First, our meta-analysis is based on data from trials whose results have been published, and we did not obtain updated individual patient data. Use of individual patient data may further enhance the accuracy and reduce the uncertainty of the estimates. Second, significant heterogeneity was found in our study. Although at meta-regression, only tumor staging was significantly associated with HR estimates, variability in definitions of end point, measurements, and experimental design may also contribute to the heterogeneity. Therefore, validation of the prognostic power of CTC should be conducted through large multicenter prospective studies based on homogeneous populations. Third, publication bias is a concern. We tried to identify all relevant data, but it is unavoidable that some data could still be missing. Missing information may reflect negative results that could reduce the prognostic power of CTC. However, we conducted the trim-and-fill analysis and found that even if these missing studies were published the association of CTC and poorer survival was still significant.

In conclusion, the present results support the notion of a strong prognostic value of CTC in early-stage and metastatic breast cancer patients. In the future, the detection of CTC at different time points before and during systemic therapy might serve as a tool to guide treatment in cancer patients. To achieve clinical utility of CTC in breast cancer, more intervention trials in which therapy decision making is based on CTC results need to be initiated, such as the ongoing multicenter 80500 trial led by the Southwest Oncology Group and the German DETECT-III trial focusing on anti-HER2 therapy with lapatinib in patients with HER2-positive CTC.

Disclosure of Potential Conflicts of Interest

K. Pantel receives an honorarium from Speakers Bureau. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: L. Zhang, G. Wu, P. Klaus

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Yang

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Zhang, S. Riethdorf, T. Wang, K. Yang, G. Peng, J. Liu, P. Klaus

Writing, review, and/or revision of the manuscript: L. Zhang, S. Riethdorf, G. Wu, T. Wang, K. Yang, G. Peng, J. Liu, P. Klaus

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Zhang, T. Wang, G. Peng, J. Liu

Study supervision: G. Wu, P. Klaus

Acknowledgments

The authors thank Dr. Daniel F. Hayes for his critical comments on the manuscript, and thank statistician Dr. Sheng Wei at Department of Epidemiology and Statistics for his critical support with statistics.

Grant Support

This work was supported by National Natural Science Foundation of China 81101573 (L. Zhang). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 23, 2012; revised July 31, 2012; accepted August 8, 2012; published OnlineFirst August 20, 2012.
Meta-Analysis of Circulating Breast Cancer Cells

References


2. Ashworth TA. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Aust Med J 1869;14:146–9.


Meta-Analysis of the Prognostic Value of Circulating Tumor Cells in Breast Cancer

Liling Zhang, Sabine Riethdorf, Gang Wu, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-1587

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2012/08/20/1078-0432.CCR-12-1587.DC1

Cited articles
This article cites 59 articles, 14 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/18/20/5701.full#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/18/20/5701.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.