Pooled Analysis of the Prognostic Relevance of Circulating Tumor Cells in Primary Breast Cancer

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Abstract

Purpose: Although unequivocal evidence has shown the prognostic relevance of circulating tumor cells (CTC) in the peripheral blood of patients with metastatic breast cancer, less evidence is available for the prognostic relevance of CTCs at the time of primary diagnosis.

Experimental Design: We conducted a pooled analysis of individual data from 3,173 patients with nonmetastatic (stage I–III) breast cancer from five breast cancer institutions. The prevalence and numbers of CTCs were assessed at the time of primary diagnosis with the FDA-cleared CellSearch System (Janssen Diagnostics, LLC). Patient outcomes were analyzed using meta-analytic procedures, univariate log-rank tests, and multivariate Cox proportional hazard regression analyses. The median follow-up duration was 62.8 months.

Introduction

Dissemination of tumor cells from the primary tumor into the bloodstream is a critical step in tumorigenesis, and is considered a precursor of distant metastases. High-resolution imaging technologies often cannot detect the spread of early tumor cells and occult micrometastases (circulating tumor cells, CTCs, in blood peripheral blood (as few as 1 CTC per 106 leukocytes), facilitating their use as a surrogate marker of minimal residual disease (MRD) and enabling clinical researchers to perform real-time monitoring of disease progression and treatment responses through repeated blood sampling. Numerous published studies demonstrate that CTC prevalence predicts disease recurrence and survival in patients with metastatic breast cancer (MBC; refs. 2–6), and the persistence of CTCs after treatment has been shown to predict lack of responses to therapy in metastatic settings (7–9).

Recent advances in isolation and enrichment methods make it possible to detect and enumerate extremely rare CTCs in the peripheral blood (as few as 1 CTC per 107–108 leukocytes), facilitating their use as a surrogate marker of minimal residual disease (MRD) and enabling clinical researchers to perform real-time monitoring of disease progression and treatment responses through repeated blood sampling. Numerous published studies demonstrate that CTC prevalence predicts disease recurrence and survival in patients with metastatic breast cancer (MBC; refs. 2–6), and the persistence of CTCs after treatment has been shown to predict lack of responses to therapy in metastatic settings (7–9).

The use of CTCs as a prognostic and predictive marker might be more important for patients with early breast cancer than for patients with MBC, for whom treatment is palliative in nature, and preliminary prospective clinical trials suggest that the presence of CTCs at the time of primary diagnosis could predict early disease recurrence and reduced survival (10–15).

Herein, using CTC results obtained using the FDA-cleared CellSearch System, we present the first large pooled analysis of the prognostic value of the presence of CTCs at the time of primary diagnosis in patients with non-MBC. The primary aim of this study was to evaluate whether CTCs could serve as an independent prognostic factor of disease recurrence and survival, using individual patient data. In addition, the large number of individual patient data available allowed us to conduct subgroup analyses.

Results: One or more CTCs were detected in 20.2% of the patients. CTC-positive patients had larger tumors, increased lymph node involvement, and a higher histologic tumor grade than did CTC-negative patients (all P < 0.002). Multivariate Cox regressions, which included tumor size, nodal status, histologic tumor grade, and hormone receptor and HER2 status, confirmed that the presence of CTCs was an independent prognostic factor for disease-free survival [HR, 1.82; 95% confidence interval (CI), 1.47–2.26], distant disease-free survival [HR, 1.89; 95% CI, 1.49–2.40], breast cancer–specific survival (HR, 2.04; 95% CI, 1.52–2.75), and overall survival (HR, 1.97; 95% CI, 1.51–2.59).

Conclusions: In patients with primary breast cancer, the presence of CTCs was an independent predictor of poor disease-free, overall, breast cancer–specific, and distant disease-free survival.

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Translational Relevance

Circulating tumor cells (CTC) that are shed from the primary tumor into the bloodstream are thought to be responsible for tumor progression through initiation of metastatic growth in distant organs. Thus, CTCs detected in the peripheral blood of cancer patients have the potential to function as an easily accessible marker with high prognostic and predictive value. Our study shows the strong independent prognostic effect of CTCs on disease-free survival and overall survival in primary breast cancer patients. To our knowledge, it is both the first pooled analysis and largest study on the prognostic relevance of CTCs in primary breast cancer with CTC assessments being based on the only FDA-approved CTC detection method. Our data complement the results of a recently published pooled analysis showing an independent prognostic effect of CTCs in metastatic breast cancer, suggesting that CTCs can serve as valuable prognostic markers in all stages of breast cancer.

with sample sizes yielding statistically robust results to determine whether the prognostic value of CTCs varied among subgroups, and to identify patients who might be particularly responsive to additional adjuvant therapies.

Materials and Methods

Data collection

Breast cancer centers known to have conducted studies involving the determination of CTCs in the peripheral blood of primary breast cancer patients using the CellSearch System were personally contacted by the first author (W. Janni) and asked if they would be willing to provide individual patient data for a pooled analysis. Fully anonymized individual patient data for the pooled analysis were provided by five academic breast cancer units: Enschede (the Netherlands), Houston (TX), Munich (Germany), Paris (France), and Tübingen (Germany); hereafter, all studies will be referred to by their corresponding cities. Some of the patient cohort data were published previously [Enschede (10), Houston (12), Munich (14), and Paris (13)], and additional information regarding the data collection, patients, and treatments is available in these original publications. Because of differences in the inclusion and/or exclusion criteria, inclusion of additional patients, or updated follow-up information, the patient numbers and survival results reported herein might differ from those presented in the original publications.

Patients

Potentially eligible patients were diagnosed with histologically confirmed, operable, stage I–III invasive breast cancer without the evidence of metastatic disease (tumor stage sizes T1–T4, nodal stages N0–N3, and metastasis stage M0). Ineligible patients were those with no valid data on CTC presence at the time of primary diagnosis as assessed using the FDA-cleared CellSearch System (see Supplementary Fig. S1 in the Supplementary Appendix). Informed consent was obtained from all patients prior to blood collection for CTC analyses. All studies were approved by the responsible ethical boards and conducted in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research.

The primary tumor stages at diagnosis were classified according to the criteria defined by the revised American Joint Committee on Cancer and International Union against Cancer TNM classification system (16). Histologic grading was classified according to Black's nuclear grading system (ref. 17; Houston) or the Elston–Ellis modification of the Scarff–Bloom–Richardson grading system (ref. 18; all others). Tumors were defined as hormone receptor positive if the percentage of cells with immunohistochemical nuclear staining for estrogen, progesterone, or both was 10% or higher. Tumors with strong (3+) immunohistochemical membranous staining were defined as HER2-positive; tumors with moderate (2+) membranous staining were classified as HER2-positive only if an additional FISH analysis yielded a positive result.

Detection of CTCs

Presence and number of CTCs were analyzed using the standardized, semiautomatic CellSearch system, which has been described in detail previously (19, 20). Briefly, blood samples were collected in CellSave tubes (Janssen Diagnostics) and were centrifuged to separate the solid blood components from the plasma. After the immunomagnetic capture and enrichment of epithelial cell adhesion molecule (EpCAM)-positive cells via antibody-coated ferrofluid nanoparticles, the EpCAM-enriched cells were stained with phycoerythrin (PE)-conjugated antibodies C11 and A53-B/A2 specific for cytokeratins 8, 18, and 19 (epithelial cell markers), an allophycocyanin-conjugated mAb (H30) specific for CD45 (leucocyte marker), and the fluorescent nucleic acid dye 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). CTCs were identified and counted using a semiautomated fluorescence-based microscope system that generated images of the stained cells; the CTCs were defined as cytokeratin-positive and CD45-negative nucleated cells >4 μm in size.

Blood samples for CTC status evaluations were collected prior to neoadjuvant chemotherapy (Paris) or at the time of primary surgery (Enschede, Houston, Munich, and Tübingen). One 7.5-mL blood sample per patient was used for CTC evaluations in Houston, Paris, and Tübingen, whereas four 7.5-mL blood samples per patient were analyzed separately in Enschede. In Munich, 30 mL of peripheral blood was collected per patient and was pooled and concentrated to a final volume of 7.5 mL prior to the CTC analysis (14). Patients were assessed as CTC-positive if at least 1 CTC was detected, regardless of the initial blood volume used for analysis. For statistical analyses involving the CTC numbers, the results for the four 7.5-mL blood samples per patient that had been collected in Enschede were pooled to obtain an average number of CTCs per 7.5 mL of blood.

Statistical analysis

Associations between the presence of CTCs and both baseline patient characteristics and established prognostic factors were evaluated using the t test for continuous variables, the Cochran–Armitage test for trends in the ordered categorical variables of tumor stage, nodal stage, and grading, and the χ² test for all other categorical variables.

We performed pooled analyses separately for four different survival endpoints defined according to the Standardized Definitions for Efficacy End Points (STEEP) criteria (21). Overall survival (OS) included death from any cause as an event. To calculate breast cancer–specific survival (BCSS), only death due to breast cancer–related causes (e.g., metastasis-dependent organ failure or
breast cancer progression) was considered an event. Disease-
free survival (DFS) included invasive disease recurrence, second
primary tumors, and death from any cause as events; all noninvasive in situ cancer events were excluded. To calculate
distant disease-free survival (DDFS), only distant recurrence
(metastasis and second primary tumors) and death from any
cause were regarded as events. Ipsilateral or regional disease
recurrences and contralateral breast cancers were excluded from
analysis. All time-to-event intervals were measured from time
of primary diagnosis to date of the event. If no endpoint was
reached, data were censored at the date of the last follow-up. All
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reached, data were censored at the date of the last follow-up. All
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recurrences and contralateral breast cancers were excluded from
analysis. All time-to-event intervals were measured from time
of primary diagnosis to date of the event. If no endpoint was
reached, data were censored at the date of the last follow-up. All
median follow-up times were calculated using the reverse
Kaplan–Meier method (22).

For all four survival endpoints (OS, BCSS, DFS, DDFS), the
univariate HR and 95% confidence intervals (CI) for disease
recurrence or death were initially calculated for each of the five
centers on the basis of the individual patient data, using CTC
positivity (yes/no) as the sole variable. Next, a summary estimate
of the HRs and 95% CIs for each of the survival endpoints was
obtained through a meta-analytic approach on the basis of
random-effects models. If the HR for a survival endpoint could
not be calculated for a single study because of a lack of events in
CTC-negative or CTC-positive patients, the study was subsequent-
ly excluded from the meta-analytic HR calculation for the survival
endpoint. This was the case for the OS and BCSS endpoint
calculations, in which the Tübingen data had to be excluded
because of missing events among the CTC-positive patients.

Interstudy heterogeneity was assessed with the Q-test, and sensi-
tivity analyses were conducted by computing meta-analytic HRs
and CIs that were calculated following the omission of one study
at a time.

Univariate significance values of the study variables were deter-
mined according to Kaplan–Meier estimates and log-rank tests.
The simultaneous effects of multiple covariates on survival end-
points were calculated using Cox proportional hazards regression
models stratified by study center. Because of the missing values
with regard to clinicopathologic variables included in the multi-
variate models, 96 patients had to be excluded from all multi-
variate analyses. Our initial model included tumor grade (G1, G2,
G3), histologic type (ductal, lobular, other), tumor stage (T1, T2,
T3, T4), nodal stage (N0, N1, N2, N3), hormone receptor status
(positive, negative), HER2 status (positive, negative), menopaus-
ual status (premenopausal, postmenopausal), and hormone recep-
tor status, or HER2 status (Table 1). Patients with CTCs more often received neoadjuvant and/or adjuvant chemotherapy than did patients without CTCs, where-
as no association was identified between the presence of CTCs
and endocrine therapy, HER2-targeted therapy, or radiotherapy
(Table 1).

Meta-analysis

Data on the number of patients and follow-up duration as well
as on the number of events for the four study endpoints OS, BCSS,
DFS, and DDFS by center are shown in Supplementary Table S1 of
the Supplementary Appendix. The meta-analytic summary esti-
mate for the OS HR according to the presence of CTCs was 2.444
(95% CI, 1.811–3.298; P < 0.001). The HRs calculated for the
single studies ranged from 1.538 (Houston) to 4.212 (Paris), and
were significant for all studies, except Houston. No significant
interstudy heterogeneity was observed (Q-test, P = 0.344). Sensi-
tivity analyses confirmed that the exclusion of any one study did
not markedly affect the HR or confidence interval summary estimates
(HR estimate range, 2.273–2.623; all P < 0.003). Regarding BCSS,
the HR summary estimate was 2.540 (95% CI, 1.910–3.378; P < 0.001). The single-study HRs ranged from 1.734 (Houston)
to 4.212 (Paris) and, again, were significant for all studies, except Houston. The Q-test revealed no significant heterogeneity among
the studies (P = 0.561), and the sensitivity analyses revealed that
the HR and corresponding CI summary estimate, which were
calculated after the removal of any one study at a time, remained
virtually unchanged (HR estimate range, 2.435–2.693; all
P < 0.001).

The summary estimate for the DFS HR in association with the presence of CTCs was 2.080 (95% CI, 1.688–2.563; P < 0.001). The single-study HRs ranged from 1.896 (Tübingen) to 2.706
(Paris) and were significant for all studies, except Tübingen. There was no significant heterogeneity among the studies
(Q-test, P = 0.899), and sensitivity analyses showed that exclusion of any one study at a time had no marked effect on

Prognostic Role of CTCs in Primary Breast Cancer

Prevalence of CTCs and associations with clinical parameters

A total of 3,173 patients with stage I–III invasive breast cancer
and known CTC statuses were included in our pooled analysis
(Supplementary Fig. S1 in the Supplementary Appendix). Median
age was 54 years (range, 21–91 years), and median follow-up
duration was 62.8 months. At least one CTC was detected in 640
(20.2%) of the patients; the numbers of detected CTCs ranged
from 1 to 827. Data regarding patient number, age, and enroll-
ment period as well as presence and number of CTCs from the five
centers are presented in Supplementary Table S1 of the Supple-
imentary Appendix.

The presence of CTCs was associated with a larger tumor size,
increased lymph node involvement, unfavorable histologic
grade, and lobular tumor type, whereas no significant associ-
atation was identified between CTC presence and menopausal
status, hormone receptor status, or HER2 status (Table 1).
the HR or confidence limit summary estimates, which were calculated from the remaining studies (HR estimate range, 2.018–2.321; all \( P < 0.001 \)). The results of the DDFS meta-analysis were similar to those obtained for the other three survival endpoints. The HR summary estimate associated with the presence of CTCs was 2.196 (95% CI, 1.737–2.776; \( P < 0.001 \)), with single-study HRs ranging from 1.916 (Munich) to 2.920 (Houston). Again, there was no significant heterogeneity among the studies (Q-test, \( P = 0.664 \)), and the HR summary estimates determined from the sensitivity analyses, in which single studies were excluded from the analysis one at a time, ranged from 2.099 to 2.801 (all \( P < 0.001 \)).
Survival, disease recurrence, and CTC status

Overall, 238 of 3,173 patients (7.5%) died during follow-up, and 93 (39.1%) of these patients presented with CTCs at the time of primary diagnosis. The presence of CTCs significantly predicted shorter OS in both the univariate analysis (HR, 2.413; 95% CI, 1.859–3.131; log-rank test: $P < 0.001$; Fig. 1A), and in the multivariate Cox proportional hazard models after controlling for tumor grade and stage, nodal stage, hormone receptor, and HER2 receptor expression (HR, 1.974; 95% CI, 1.505–2.590; $P < 0.001$; Table 2). The prognostic value of CTCs for OS was evident not only when comparing patients with and without CTCs, but also when using CTC cut-off values ranging from 1 (0–1 CTC vs. > 1 CTC) to 20 (0–20 CTCs vs. > 20 CTCs), as revealed in univariate analyses using log-rank tests (all $P < 0.04$; Fig. 2A).

Breast cancer-specific deaths were reported for 198 patients (83.2% of all recorded deaths). Patients with CTCs were more likely to die from breast cancer than were patients without CTCs (HR, 2.606; 95% CI, 1.962–3.460; log-rank test: $P < 0.001$; Fig. 1B), and the independent prognostic value of CTCs was confirmed in a multivariate Cox regression analysis (HR, 2.042; 95% CI, 1.519–2.746; $P < 0.001$; Table 2). Similar to OS, the prognostic value of CTCs for BCSS was significant for all CTC

Figure 1.
Kaplan–Meier plots of survival according to presence of CTCs at the time of primary diagnosis. OS (A), BCSS (B), DFS (C), and DDFS (D). HR denotes the HR, and $P$ values refer to log-rank tests.
Table 2. Multivariate HRs (cox proportional hazards regression model, stratified for study center) for OS, BCSS, DFS, and DDFS in a pooled analysis of early breast cancer patients

<table>
<thead>
<tr>
<th>Survival endpoint</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>OS (n = 233 events) CTCS</td>
<td></td>
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<tr>
<td>Positive vs. negative</td>
<td>1.974 (1.505–2.590)</td>
<td>&lt;0.001</td>
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<tr>
<td>Tumor grade</td>
<td></td>
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<tr>
<td>G2 vs. G1</td>
<td>1.137 (0.540–2.391)</td>
<td>0.735</td>
</tr>
<tr>
<td>G3 vs. G1</td>
<td>2.440 (1.644–3.712)</td>
<td>0.018</td>
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<tr>
<td>Tumor stage</td>
<td></td>
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<tr>
<td>T2 vs. T1</td>
<td>1.847 (1.323–2.580)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T3 vs. T1</td>
<td>3.882 (2.429–6.204)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T4 vs. T1</td>
<td>3.165 (1.730–5.878)</td>
<td>&lt;0.001</td>
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<tr>
<td>Nodal stage</td>
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<tr>
<td>N1 vs. N0</td>
<td>2.549 (1.771–3.667)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N2 vs. N0</td>
<td>5.839 (2.529–13.622)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N3 vs. N0</td>
<td>6.738 (4.275–10.621)</td>
<td>&lt;0.001</td>
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<tr>
<td>Hormone receptor status</td>
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<tr>
<td>Positive vs. negative</td>
<td>0.381 (0.282–0.514)</td>
<td>&lt;0.001</td>
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<tr>
<td>HER2 status</td>
<td></td>
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<tr>
<td>Positive vs. negative</td>
<td>0.593 (0.426–0.825)</td>
<td>0.002</td>
</tr>
<tr>
<td>BCSS (n = 193 events) CTCS</td>
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<td></td>
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<tr>
<td>Positive vs. negative</td>
<td>2.042 (1.519–2.746)</td>
<td>&lt;0.001</td>
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<tr>
<td>Tumor grade</td>
<td></td>
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<tr>
<td>G2 vs. G1</td>
<td>1.347 (0.532–3.407)</td>
<td>0.530</td>
</tr>
<tr>
<td>G3 vs. G1</td>
<td>3.291 (1.310–8.265)</td>
<td>0.011</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
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<tr>
<td>T2 vs. T1</td>
<td>2.233 (1.516–3.289)</td>
<td>&lt;0.001</td>
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<tr>
<td>T3 vs. T1</td>
<td>5.090 (3.025–8.565)</td>
<td>&lt;0.001</td>
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<td>T4 vs. T1</td>
<td>3.217 (1.613–6.416)</td>
<td>&lt;0.001</td>
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<td>Nodal stage</td>
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<tr>
<td>N1 vs. N0</td>
<td>2.759 (1.844–4.128)</td>
<td>&lt;0.001</td>
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<tr>
<td>N2 vs. N0</td>
<td>3.894 (2.451–6.185)</td>
<td>&lt;0.001</td>
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<tr>
<td>N3 vs. N0</td>
<td>7.526 (4.575–12.383)</td>
<td>&lt;0.001</td>
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<td>Hormone receptor status</td>
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<tr>
<td>Positive vs. negative</td>
<td>0.353 (0.255–0.488)</td>
<td>&lt;0.001</td>
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<tr>
<td>HER2 status</td>
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<tr>
<td>Positive vs. negative</td>
<td>0.633 (0.444–0.902)</td>
<td>0.011</td>
</tr>
<tr>
<td>DFS (n = 387 events) CTCS</td>
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<tr>
<td>Positive vs. negative</td>
<td>1.822 (1.470–2.258)</td>
<td>&lt;0.001</td>
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<tr>
<td>Tumor grade</td>
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<tr>
<td>G2 vs. G1</td>
<td>1.584 (0.869–2.887)</td>
<td>0.153</td>
</tr>
<tr>
<td>G3 vs. G1</td>
<td>3.076 (1.686–4.814)</td>
<td>&lt;0.001</td>
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<tr>
<td>Tumor stage</td>
<td></td>
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<tr>
<td>T2 vs. T1</td>
<td>1.429 (1.126–1.814)</td>
<td>0.003</td>
</tr>
<tr>
<td>T3 vs. T1</td>
<td>2.246 (1.542–3.273)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T4 vs. T1</td>
<td>2.915 (1.825–4.857)</td>
<td>&lt;0.001</td>
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<tr>
<td>Nodal stage</td>
<td></td>
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<tr>
<td>N1 vs. N0</td>
<td>1.935 (1.480–2.529)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N2 vs. N0</td>
<td>2.660 (1.929–3.670)</td>
<td>&lt;0.001</td>
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<tr>
<td>N3 vs. N0</td>
<td>5.359 (3.793–7.375)</td>
<td>&lt;0.001</td>
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<tr>
<td>Hormone receptor status</td>
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<tr>
<td>Positive vs. negative</td>
<td>0.551 (0.435–0.698)</td>
<td>&lt;0.001</td>
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<tr>
<td>HER2 status</td>
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<tr>
<td>Positive vs. negative</td>
<td>0.701 (0.546–0.907)</td>
<td>0.005</td>
</tr>
<tr>
<td>DDFS (n = 304 events) CTCS</td>
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<tr>
<td>Positive vs. negative</td>
<td>1.888 (1.485–2.401)</td>
<td>&lt;0.001</td>
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<tr>
<td>Tumor grade</td>
<td></td>
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<tr>
<td>G2 vs. G1</td>
<td>1.610 (0.775–3.345)</td>
<td>0.202</td>
</tr>
<tr>
<td>G3 vs. G1</td>
<td>3.604 (1.738–7.475)</td>
<td>&lt;0.001</td>
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<tr>
<td>Tumor stage</td>
<td></td>
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<td>T2 vs. T1</td>
<td>1.657 (1.254–2.191)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T3 vs. T1</td>
<td>2.585 (1.683–3.971)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T4 vs. T1</td>
<td>3.276 (1.951–5.499)</td>
<td>&lt;0.001</td>
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(Continued on the following column)

Subgroup analyses

The univariate HRs for OS according to the presence of CTCs in various subgroups (plus both the number of patients and the number of events observed within each subgroup) are shown in Fig. 3. The HRs were 2.801 (95% CI, 1.989–3.944; P < 0.001) for patients with hormone receptor–positive tumors and 1.894 (95% CI, 1.261–2.845; P = 0.02) for patients with hormone receptor–negative tumors. The prognostic relevance of CTC status was similar in patients with HER2-negative tumors (HR, 2.388; 95% CI, 1.783–3.199; P < 0.001) and those with HER2-positive tumors (HR, 2.521; 95% CI, 1.294–4.807; P = 0.002). CTC status had significant prognostic relevance in triple-negative breast cancer patients (HR, 1.973; 95% CI, 1.254–3.104; P = 0.003), in patients with hormone receptor–positive/HER2-negative tumors (HR, 2.621; 95% CI, 1.784–3.850; P < 0.001) and in those with hormone receptor–positive/HER2-positive tumors (HR, 3.616; 95% CI, 1.649–7.928; P = 0.001); however, the CTC status was not significantly associated with prognosis in patients with hormone receptor–negative/HER2-positive tumors (HR, 1.594; 95% CI, 0.618–4.111; P = 0.331).

Presence of CTCs did not significantly predict OS in the subgroup of patients with nodal stage 0 disease (HR, 1.322; 95% CI, 0.705–2.478; P = 0.383), while it was significantly associated with OS in patients with nodal stage N1 (HR, 2.523; 95% CI, 1.623–3.923; P < 0.001), N2 (HR, 3.275; 95% CI, 1.340–4.211;
or N3 disease (HR, 2.186; 95% CI, 1.274–3.750; \( P = 0.004 \)). In addition, the presence of CTCs did not affect OS in low-risk patients, defined here as those with stage T1N0 primary tumors (HR, 1.030; 95% CI, 0.294–3.618; \( P = 0.963 \)). In contrast, the presence of CTCs had a highly significant effect on OS in high-risk patients, defined as those with primary tumors larger than 2 cm (T2–T4) and lymph node involvement (HR, 2.460; 95% CI, 1.784–3.390; \( P = 0.001 \); see Supplementary Fig. S3 in the Supplementary Appendix).

**Discussion**

Our study is the first pooled analysis to demonstrate that CTC positivity at the time of primary early breast cancer diagnosis is an independent and highly significant prognostic marker of OS, DFS, BCSS, and DDFS in non-MBC with the highest level of evidence. Our study also adds important new data not reported in previous studies on the prognostic value of CTCs in early breast cancer in terms of a comprehensive analysis of the prognostic role of CTCs in various breast cancer subgroups and a detailed evaluation of the prognostic impact of CTCs using a broad range of different CTC cut-off values. Importantly, there was no significant heterogeneity among the five centers, and sensitivity analyses showed that the large patient cohort from Munich did not bias the results, as the presence of CTCs remained a significant prognostic factor with respect to all four survival endpoints even after excluding the Munich data from the analysis.

As shown by the subgroup analyses, the prognostic value of CTCs varied among breast cancer subtypes. CTC positivity was not a significant prognostic factor of outcome in low-risk patients with T1N0 tumors, suggesting that early-stage breast cancers detected...
decisions regarding the appropriateness of dose-intensi
tivity for CTCs in this group, and CTC presence might direct
value of CTCs in high-risk patients suggests a potential clinical
importance of CTCs. In contrast, the signifi
cance of the CTCs. Presence of CTCs significantly predicted
outcome in patients with hormone receptor
negative tumors. It is of considerable interest to assess whether there is an
optimal cut-off point with regard to the prognostic impact of CTCs. In accordance with previously reported studies (3), a cut-off
value of five CTCs has been used to evaluate the prognostic role of CTCs in metastatic settings. However, this fixed cut-off value has
been criticized on the basis of biologic, technical, and statistical
reasons (25–28), and recent data indicate that there is a direct
relationship between CTC number and outcome (2, 29, 30). Given that CTCs are detected at much lower frequencies in
nonmetastatic compared with MBC, most studies to date have
used a cut-off point of ≥1 CTC to assess the prognostic role of
CTCs.

Because of the limited number of non-MBC patients with multiple CTCs, no information regarding the prognostic value
of CTCs with respect to different cut-off points was previously
available. Our large pooled analysis revealed significant prognostic
relevance for CTCs independent of the selected cut-off points
(cut-off values ranged from 1 to 20 CTCs; corresponding HRs ranged from 2.413 to 4.480 for OS and from 2.040 to 3.463 for
DFS), indicating that the presence of at least one CTC yields
relevant prognostic information.

Limitations of this study include the difference in blood
volumes used for the CTC analyses among the contributing
centers. However, CTC positivity rates did not differ significantly
among four of the five participating centers ($\chi^2$ test, degrees of
freedom = 3, $P = 0.143$). The only exception was the Tübingen
study, which reported a considerably lower CTC positivity rate
than the other centers (Supplementary Table S1). This discrepancy
was probably due to the fact that the patient cohort from
Tübingen had higher frequencies of N0 (72.8%) and T1 tumors
(66.8%) compared with the other centers. A more general

<table>
<thead>
<tr>
<th>Hormone-receptor status (HRS)</th>
<th>CTC-negative</th>
<th>CTC-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2-negative</td>
<td>115/1,953(5.9%)</td>
<td>74/488 (15.2%)</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>28/543 (5.2%)</td>
<td>18/145 (12.4%)</td>
</tr>
<tr>
<td>Combined hormone-receptor and HER2 status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2-negative/HER2-negative</td>
<td>53/412 (12.9%)</td>
<td>29/115 (25.2%)</td>
</tr>
<tr>
<td>HER2-negative/HER2-positive</td>
<td>15/191 (7.9%)</td>
<td>6/52 (11.5%)</td>
</tr>
<tr>
<td>HER2-positive/HER2-negative</td>
<td>62/1,540 (4.0%)</td>
<td>45/373 (12.1%)</td>
</tr>
<tr>
<td>HER2-positive/HER2-positive</td>
<td>13/955 (3.7%)</td>
<td>12/93 (12.9%)</td>
</tr>
</tbody>
</table>

Nodal status

| N0   | 39/1,136 (3.4%) | 13/249 (5.2%) |
| N1   | 52/997 (5.2%)   | 32/233 (13.7%)|
| N2   | 29/208 (10.8%)  | 20/89 (22.5%) |
| N3   | 25/122 (20.5%)  | 28/68 (41.2%) |

Risk group

| Low-risk (T1/N0) | 13/603 (2.2%) | 3/120 (2.5%) |
| High-risk (T234/N123) | 86/829 (10.4%) | 66/266 (24.8%) |

Total

| Deaths/women | 145/2,533 (5.7%) | 93/640 (14.5%) |

Figure 3. Forest plot of OS and comparison of patients with and without CTCs in various subgroups. The black diamonds indicate the HRs (CTC positive vs. CTC negative) for the subgroup analyses, and the white diamond represents the overall HR for the pooled analysis including all 3,173 patients (for better comparison also indicated by the dashed vertical line). The size of the diamonds is proportional to the sample size (number of patients) in the groups, and the horizontal lines indicate the corresponding 95% CIs for the HRs. The solid vertical line represents an HR of 1.0 (i.e., no difference in survival between CTC-positive and CTC-negative patients).
and the presence of EpCAM-positive CTCs as detected using the EpCAM expression (37). However, EpCAM-positive CTCs are tumors, as these are characterized by low or even nonexistent epithelial marker expression, which is accompanied by a corresponding loss of organ metastasis (34, 35). EpCAM-negative CTCs have been detected in the blood of patients with different subtypes of MBC (34, 35), while patients with a baseline CTC count below 5 CTC/7.5 mL presumably at a higher risk receive chemotherapy, which patients with a baseline CTC count equal to or higher than 5 CTC/7.5 mL receive endocrine therapy. The aim of the French CirCe01 trial (Circulating Tumor Cells to Guide Chemotherapy for Metastatic Breast Cancer; NCT01349842) is to evaluate repeatedly over several lines of chemotherapy whether patients whose CTC count does not decrease after the first treatment cycle benefit from a change of chemotherapy regimens. COMET P2 (NCT 01701050) is an American observational trial on ER-positive, HER2-negative MBC patients that aims to determine a CTC-Endocrine Therapy Index (CTC-ETI) to predict whether patients either will respond favorably to a new endocrine therapy or will be resistant to endocrine treatment and thus need a chemotherapeutic treatment. The results of these trials will be crucial for evaluating the potential for CTC implementation in clinical breast cancer management.

In conclusion, this large pooled analysis provides strong evidence for the independent prognostic relevance of CTCs in primary breast cancer patients, and can thus be regarded as proof-of-principle for the use of CTCs as prognostic markers in early breast cancer. The next step toward clinical application of CTCs is incorporating CTC phenotypes or changes in CTC numbers during treatment to facilitate the selection of appropriately targeted and individualized therapy that will hopefully improve survival and quality of life in patients with non-MBC. Future technologies for CTC detection with enhanced sensitivity that also incorporate stem cell and EMT markers might further improve the value of CTCs as prognostic and predictive tool in early breast cancer.

Disclosure of Potential Conflicts of Interest

W.J. Janni reports receiving commercial research grants from AstraZeneca, Chugai, GlaxoSmithKline, Janssen Diagnostics, Novartis, Pfizer, Roche, and Sanofi-Aventis. B. Rack reports receiving commercial research grants from Janssen Diagnostics, Lilly, Novartis, and Sanofi Aventis; and is a consultant/advisory board member for Novartis. L.W.M.M. Terstappen reports receiving commercial research grants from Janssen Diagnostics. J.-Y. Pierga reports receiving commercial research grants from Chugai, Pfizer, Roche, and Teva; and is a speaker on behalf of colorectal cancer. P.A. Fasching reports receiving commercial research grants from AstraZeneca, Celgene, and Novartis; and is a speaker. P.A. Fasching reports receiving commercial research grants from AstraZeneca, Celgene, GlaxoSmithKline, Nanostring, Novartis, Pfizer, Roche, and Teva; and is a speaker. P.A. Fasching reports receiving commercial research grants from GlaxoSmithKline, Nanostring, Novartis, Pfizer, Roche, and Teva; and is a speaker. P.A. Fasching reports receiving commercial research grants from AstraZeneca, Celgene, GlaxoSmithKline, Nanostring, Novartis, Pfizer, Roche, and Teva; and is a speaker.

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References


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Administrative, technical, or material support: W.J. Janni, B. Rack, A. Lucci

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