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Meta-Analysis

META-ANALYSIS

# Circulating Tumor Cells in Breast Cancer Patients Treated by Neoadjuvant Chemotherapy: A Meta-analysis

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## Abstract

**Background:** We conducted a meta-analysis in nonmetastatic breast cancer patients treated by neoadjuvant chemotherapy (NCT) to assess the clinical validity of circulating tumor cell (CTC) detection as a prognostic marker.
**Methods:** We collected individual patient data from 21 studies in which CTC detection by CellSearch was performed in early breast cancer patients treated with NCT. The primary end point was overall survival, analyzed according to CTC detection, using Cox regression models stratified by study. Secondary end points included distant disease–free survival, locoregional relapse–free interval, and pathological complete response. All statistical tests were two-sided.
**Results:** Data from patients were collected before NCT (n = 1574) and before surgery (n = 1200). CTC detection revealed one or more CTCs in 25.2% of patients before NCT; this was associated with tumor size ($P < .001$). The number of CTCs detected had a detrimental and decremental impact on overall survival ($P < .001$), distant disease–free survival ($P < .001$), and locoregional relapse–free interval ($P < .001$), but not on pathological complete response. Patients with one, two, three to four, and five or more CTCs before NCT displayed hazard ratios of death of 1.09 (95% confidence interval [CI] = 0.65 to 1.69), 2.63 (95% CI = 1.42 to 4.54), 3.83 (95% CI = 2.08 to 6.66), and 6.25 (95% CI = 4.34 to 9.09), respectively. In 861 patients with full data available, adding CTC detection before NCT increased the prognostic ability of multivariable prognostic models for overall survival ($P < .001$), distant disease–free survival ($P < .001$), and locoregional relapse–free interval ($P = .008$).
**Conclusions:** CTC count is an independent and quantitative prognostic factor in early breast cancer patients treated by NCT. It complements current prognostic models based on tumor characteristics and response to therapy.

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Neoadjuvant chemotherapy (NCT) is a standard treatment for patients with large, nonmetastatic breast cancer and may allow breast-conserving surgery after tumor downsizing while decreasing the risk of subsequent relapse (1). In addition to these prominent clinical benefits, NCT provides a unique opportunity to study and quantify the cancer response to antineoplastic agents by assessment of the pathological response on the residual tumor at the time of surgery. Pathological complete response (pCR) rate has been thoroughly investigated as a potential surrogate of post-NCT survival, with heterogeneous results according to the various breast cancer subtypes (2,3). In this context, improving post-NCT survival prediction may lead to better evaluation of NCT efficacy and help to optimize adjuvant therapy.

Neoadjuvant therapy has two main modes of action, that is, shrinkage of the primary tumor and eradication of blood-borne tumor cell dissemination (4). Surprisingly, the second mode is totally ignored by current NCT assessments, despite the fact that cancer metastasis is the main cause of death in breast cancer and other solid tumors. The precise enumeration of circulating tumor cells (CTCs) in the peripheral blood of cancer patients as surrogate of the dissemination process is now possible after years of international standardization (5–7) and has demonstrated its clinical validity at metastatic stage in many cancer types (8–10). Moreover, experimental studies have shown that CTCs can induce tumors after xenografting into immunodeficient mice (11–13).

Here, we report the results of a meta-analysis bringing together individual patient data from the United States, Japan, and European countries. This large-scale multicenter meta-analysis investigated in nonmetastatic breast cancer patients treated by NCT whether CTC counts add statistically significant prognostic information on postneoadjuvant survival to the established assessment of primary tumor characteristics and pathological complete response.

## Methods

### Literature Review and Study Identification

The study protocol was set up by the study secretariat, and a search of Medline and major oncology congress abstracts was performed in February 2015 to find eligible studies (Supplementary Methods 1, available online). Direct contact was then established with all CTC analysis centers and cooperative research groups deemed to have eligible data. Inclusion criteria were published and unpublished studies conducted in nonmetastatic breast cancer patients treated by NCT; studies in which CTC count was performed at least once before surgery by the CellSearch system; studies with available survival data; studies declared and approved by an appropriate ethics committee; and patient accrual in or after January 2003 and before September 2015. Studies in which CTC count impacted patient management were not eligible.

### Data Collection

Each local investigator was responsible for collecting after informed consent and sharing individual anonymized data, which were centralized until April 28, 2016. The list of collected data is detailed in the study protocol (Supplementary Methods, available online). To take into account the heterogeneity between studies in terms of the number and timing of blood sampling and the duration of NCT, the protocol distinguished four different time points for CTC collection: five or fewer weeks prior to NCT initiation; one to eight weeks after NCT initiation; five or fewer weeks prior to surgery; one to 52 weeks after surgery. Data files were monitored manually for eligibility, and queries were sent to centers whenever necessary. Data were then merged into a centralized repository accessible only to the study secretariat and statisticians. No financial compensation was provided to individuals or participating centers. As per French law, this in silico study of anonymized data was declared to the *Commission Nationale de l'Informatique et des Libertés* (French data protection authority; CNIL number 1873422v0).

### Statistical Analysis

The meta-analytical fixed effect model for time-to-event metadata used to obtain an overall hazard ratio was a Cox model stratified by study (14,15). The primary end point of this study was overall survival, defined as the time from NCT initiation to death from any cause. Distant disease–free survival was defined as the time from NCT initiation to distant recurrence or death from any cause, whichever came first. Locoregional relapse–free interval was defined as the time from NCT initiation to locoregional relapse; patients who died or patients with no documented evidence of locoregional relapse were censored. We explored the heterogeneity in regards to CTC count distribution across centers (Supplementary Methods, available online) in addition to the heterogeneity in regards to CTC detection on survival. Between-study heterogeneity in regards to CTC detection on survival was assessed using chi-square and $I^2$ statistics. Results were obtained based on a Cox regression model, stratified by study, with the following covariates: CTC (using the two or more CTCs threshold) and interaction between CTC and study. $I^2$ represents the proportion of total variation in study estimates that is due to heterogeneity rather than sampling error. $I^2$ values vary between 0% and 100%; $I^2$ values of less than 30% correspond to low heterogeneity. Fisher exact tests with Monte Carlo approximation and Kruskal-Wallis tests were used to investigate associations of population characteristics with CTC count. The landmark method was used to assess the prognostic effects of the last CTC count (before surgery) and pathological complete response, with the landmark set at surgery; in these analyses conducted in patients with no event prior to surgery, the abovementioned survival times were calculated from the date of surgery. The Kaplan-Meier method was used to estimate survival curves. Cubic restricted splines of 2 degrees of freedom were used to model potential nonlinear effects of continuous CTC in the Cox regression model.

To assess the added value of CTC count to clinicopathological variables, likelihood ratio (LR) statistics in Cox regression models stratified by study were used to estimate the added value of CTCs to clinicopathological models, which included a list of prespecified variables known to be prognostic in this setting (age <50 vs >50 years, tumor size T1–2, T3–4, T4d, lymph node status cN0 vs cN1–3, tumor grade 1–2 vs 3, tumor subgroup hormone receptor–positive/human epidermal growth factor receptor–negative [HER2-], HER2+, triple-negative). To control for overfitting of clinicopathological models and to allow unbiased estimates of the added value of CTCs, the data set was randomly divided 500 times into a training and validation series using a previously described method (4). Clinicopathological models were fitted to the training series, and we calculated the

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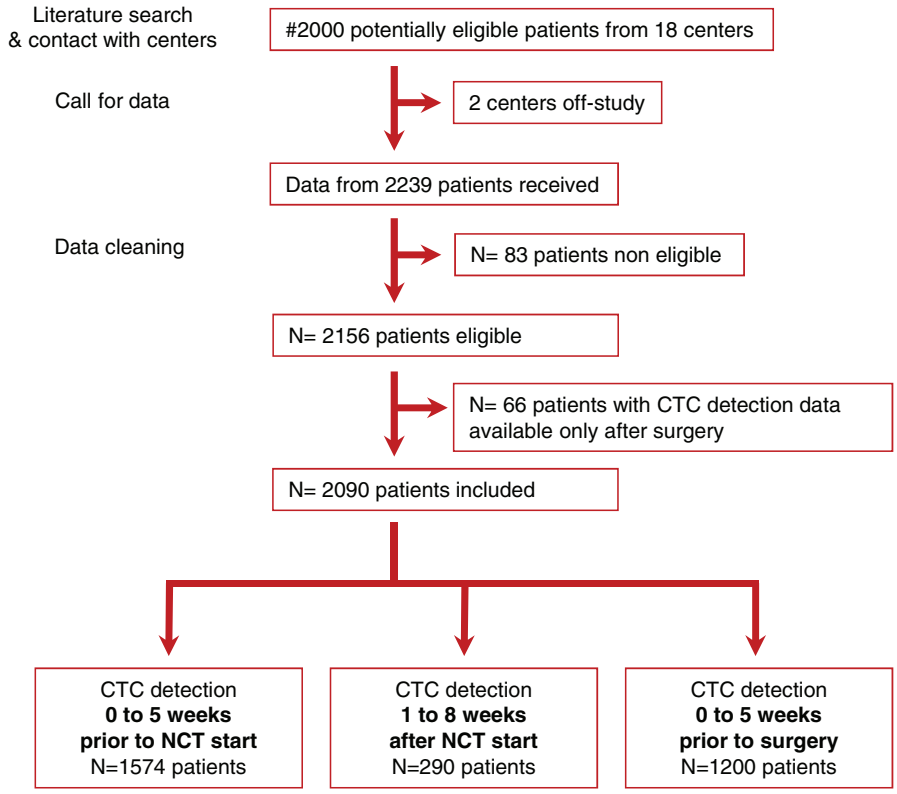


| CTC data available | Survival | Other data available | N patients |
|---|---|---|---|
| 0-5 weeks prior to NCT start | OS | - | 1574 |
| | | Baseline characteristics | 1362 |
| | | Baseline characteristics and pCR | 1332 |
| | DDFS | - | 1574 |
| | | Baseline characteristics | 1362 |
| | | Baseline characteristics and pCR | 1323 |
| | LRFI | - | 1574 |
| | | Baseline characteristics | 1362 |
| | | Baseline characteristics and pCR | 1322 |
| 0-5 weeks prior to surgery | OS | - | 1186 |
| | | Baseline characteristics | 1071 |
| | DDFS | - | 1186 |
| | | Baseline characteristics | 1064 |
| | LRFI | - | 1186 |
| | | Baseline characteristics | 1063 |
| 0-5 weeks prior to NCT start and during NCT (either after NCT start or prior to surgery) | OS | Baseline characteristics and pCR | 861 |
| | DDFS | Baseline characteristics and pCR | 856 |
| | LRFI | Baseline characteristics and pCR | 855 |

**Figure 1.** Study flow chart. Overall, 2030 patients had circulating tumor cells (CTCs) detected prior to neoadjuvant chemotherapy (NCT) start and/or prior to surgery. Because of the lower number of collected data, results with CTC detection at one to eight weeks after NCT start are not reported here. CTC = circulating tumor cells; DDFS = distant disease–free survival; LRFI = locoregional relapse–free interval; NCT = neoadjuvant chemotherapy; OS = overall survival.

average increases in LR statistic ($\chi^2$ LR value and associated *P* value) and concordance index on the validation series with 95% confidence intervals (CIs) based on the percentiles of the 500 resamples. Logistic regression models were used to investigate associations with pCR status. *P* values of less than .05 were considered statistically significant, and all statistical tests were two-sided. SAS (version 9.3) and R (version 3.3.2) were used for statistical analyses.

## Results

### Included Data

Data for 2156 patients from 16 centers and 21 studies were collected; this report focuses on 2030 patients with CTC detection before NCT and/or before surgery (Figure 1; Supplementary Figure 1 and Supplementary Table 1, available online). The

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**Table 1.** Patient characteristics and circulating tumor cell detection before neoadjuvant chemotherapy

| Characteristics | No. of patients (%) | ≥1 CTCs threshold | | | |
|---|---|---|---|---|---|
| | | 0 CTCs No. (%) | ≥1 CTCs No. (%) | *P* | Continuous CTC count *P* |
| Age, y | 1574 (100) | | | .24* | .19† |
| ≤50 | 843 (53.6) | 746 (88.5) | 97 (11.5) | | |
| >50 | 731 (46.4) | 629 (86.0) | 102 (14.0) | | |
| Tumor size | 1547 (100) | | | <.001‡ | <.001† |
| cT1 | 122 (7.9) | 99 (81.1) | 23 (18.9) | | |
| cT2 | 770 (49.8) | 598 (77.7) | 172 (22.3) | | |
| cT3 | 343 (22.2) | 260 (75.8) | 83 (24.2) | | |
| cT4a–c | 108 (7.0) | 77 (71.3) | 31 (28.7) | | |
| cT4d | 204 (13.2) | 120 (58.8) | 84 (41.2) | | |
| Lymph nodes | 1567 (100) | | | .05* | .02† |
| cN0 | 656 (41.9) | 507 (87.3) | 149 (22.7) | | |
| cN1–3 | 911 (58.1) | 664 (72.9) | 247 (27.1) | | |
| Tumor grade | 1371 (100) | | | .51* | .43† |
| I | 65 (4.7) | 52 (80.0) | 13 (20.0) | | |
| II | 702 (51.2) | 514 (73.2) | 188 (26.8) | | |
| III | 604 (44.1) | 443 (73.3) | 161 (26.7) | | |
| Tumor subgroup | 1570 (100) | | | .23* | .12† |
| HER2+ | 365 (23.2) | 277 (75.9) | 88 (24.1) | | |
| HR+ HER2- | 800 (51.0) | 607 (75.9) | 193 (24.1) | | |
| HR- HER2- | 405 (25.8) | 290 (71.6) | 115 (28.4) | | |

*P values were calculated using a two-sided Fisher exact test. CTC = circulating tumor cell; HER2 = human epidermal growth factor receptor 2; HR+ = hormone receptor positive; HR- = hormone receptor negative.
†P values were calculated using a two-sided Kruskal-Wallis test.
‡P values were calculated using a two-sided Fisher exact test with Monte Carlo sampling.

blood volume screened for CTC was 7.5 mL for 1670 patients (77.5%, 19 studies), 15 mL for 460 patients (21.3%, one study: GueparQuinto), and 30 mL for 26 patients (1.2%, one study: UMMC2003-045). We took into account the absolute number of CTCs detected, as 1) there were no statistically significant differences in CTC detection rate between patients with one blood tube and those with more than one blood tube screened at each time point; and 2) as reported beyond, only modest evidence of heterogeneity was found in this meta-analysis.

## CTC Detection and Clinicopathological Characteristics

Among the 1574 patients with CTC count available before NCT, CTC count ranged from 0 to 559 (median = 0 CTCs; third quartile = 1 CTC): 398 (25.2%), 199 (12.6%), and 93 (5.9%) patients had one or more CTCs, two or more CTCs, and five or more CTCs detected, respectively (Supplementary Tables 2–4, available online). Patient characteristics and their association with CTC detection before NCT are shown in Table 1 (and in Supplementary Tables 2–5, available online). A prominent association was observed between CTC count and tumor size before NCT ($P < .001$), as higher detection rates were observed in inflammatory (cT4d) breast cancers. This association was expected, and the study protocol was designed to distinguish inflammatory from noninflammatory breast cancers. After excluding T4d cancers from the analysis, no statistically significant associations were observed between CTC detection rates and baseline characteristics (with the exception of tumor size and five or more CTCs, $P = .049$) (Supplementary Tables 6–9, available online). In-depth modeling of CTC count distribution supported the homogeneity assumption across centers (Supplementary Figures 2 and 3, available online).

Among 1200 patients with CTC count available before surgery, CTC count was lower than the baseline count ($P < .001$) and ranged from 0 to 51 (median = 0 CTCs; third quartile = 0 CTCs): 181 (15.1%), 64 (5.3%), and 12 (1.0%) patients had one or more CTCs, two or more CTCs, and five or more CTCs detected, respectively (Supplementary Tables 10–12, available online). No statistically significant association was observed between CTC detection before surgery and patient characteristics (Supplementary Table 10–13, available online).

## CTC Detection and Pathological Complete Response

Patients achieving a pathological complete response (defined as ypT0/isN0 in >90% of patients) (Supplementary Table 1, available online) had a better overall survival (multivariable analysis; hazard ratio [HR] = 0.19, 95% CI = 0.10 to 0.31, $P < .001$), distant metastasis–free survival (HR = 0.25, 95% CI = 0.16 to 0.38, $P < .001$), and locoregional relapse–free interval (HR = 0.49, 95% CI = 0.29 to 0.80, $P = .004$).

In the population of patients without inflammatory cancers, a slightly higher rate of pathological complete response was observed in patients with no CTC compared with patients with one or more CTCs before NCT (24.2%, 95% CI = 21.7 to 26.9 vs 17.4%, 95% CI = 13.3 to 22.2, $P = .01$) (Supplementary Table 14, available online). However, this association between pathological complete response and CTC detection was not observed at other cutoffs and time points (Supplementary Tables 14–17, available online). Multivariable logistic regression models did not find any statistically significant impact on pathological complete response of CTC detection before NCT or before surgery (data not shown).

## CTC Detection and Postneoadjuvant Outcome

With a median follow-up of 59.1 (range = 0.9–125.7, interquartile range = 39.3–70.2) months, 301, 418, and 157 overall survival,

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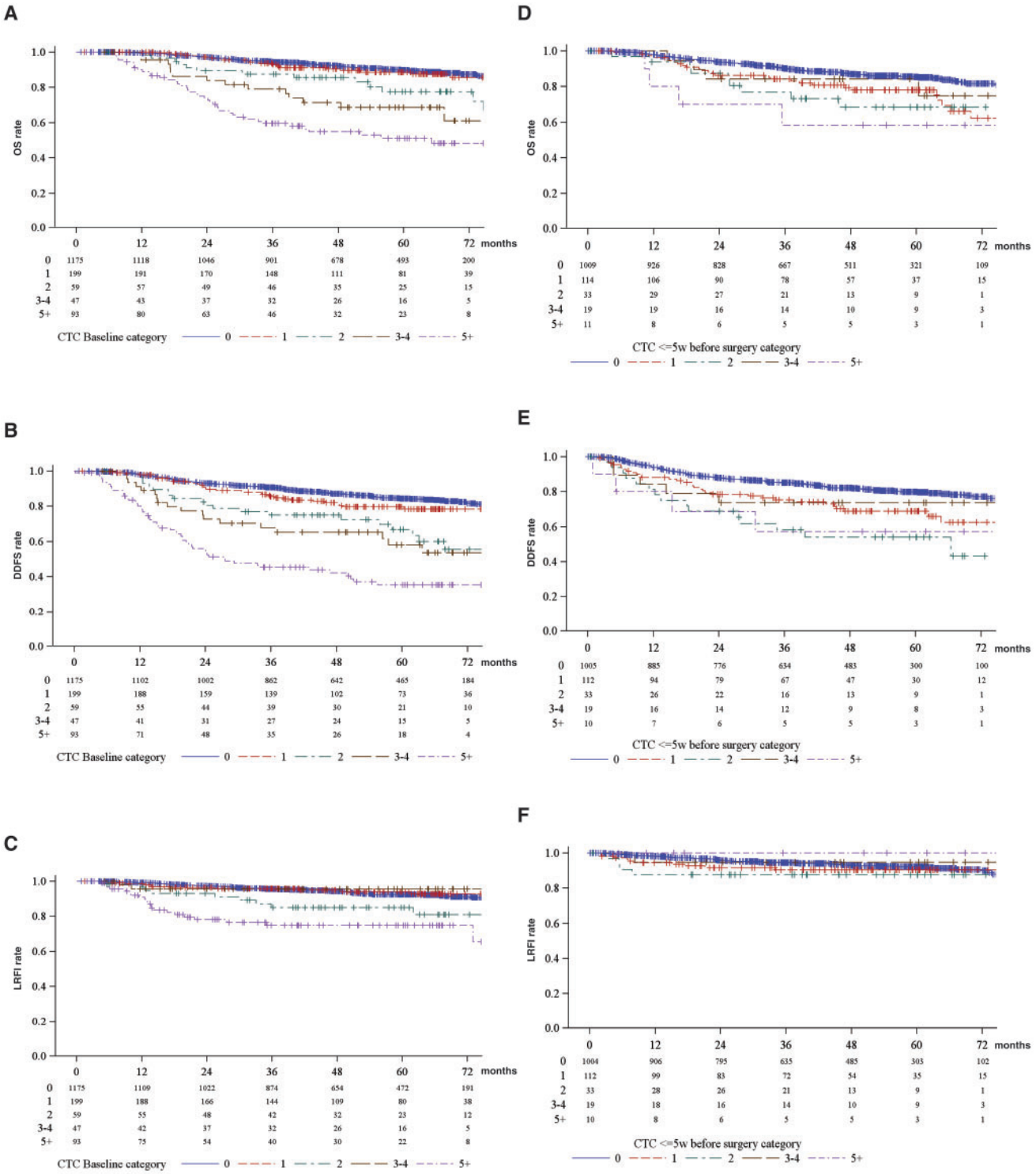


**Figure 2.** Survival curves according to circulating tumor cell (CTC) count. Survival curves and the corresponding number of patients at risk are displayed according to CTC count before neoadjuvant chemotherapy (NCT): zero CTCs, one CTC, two CTCs, three or four CTCs, five or more CTCs. *P* values were obtained by stratified log-rank tests and were two-sided. **A)** Overall survival (OS) and CTC count before NCT (in months; $P < .001$). **B)** Distant disease–free survival (DDFS) and CTC count before NCT (in months; $P < .001$). **C)** Locoregional relapse–free interval (LRFI) and CTC count before NCT (in months; $P < .001$). **D)** OS and CTC count before surgery (in months; univariate stratified $P = .002$). **E)** DDFS and CTC count before surgery (in months; $P < .001$). **F)** LRFI and CTC count before surgery (in months; $P = .51$). CTC = circulating tumor cell; DDFS = distant disease–free survival; LRFI = locoregional relapse–free interval; OS = overall survival.

distant disease–free survival, and locoregional relapse–free interval events were observed, respectively. CTC count before NCT was a statistically significant prognostic factor in univariate analyses for overall survival ($P < .001$), distant disease–free survival ($P < .001$), and locoregional relapse–free interval ($P < .001$) (Figure 2, A–C). Patients with one, two, three to



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**Table 2.** Multivariable survival analyses*

| Variable | OS | | DDFS | | LRFI | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | P† | HR (95% CI) | P† | HR (95% CI) | P† |
| Age, y | | .04 | | .004 | | .08 |
| ≤50 | 0.72 (0.53 to 0.99) | | 0.69 (0.53 to 0.89) | | 0.70 (0.47 to 1.04) | |
| >50 | 1.00 (reference) | | 1.00 (reference) | | 1.00 (reference) | |
| Tumor size | | .001 | | .0003 | | .48 |
| T1–T2 | 1.00 (reference) | | 1.00 (reference) | | 1.00 (reference) | |
| T3–T4 | 1.60 (1.11 to 2.31) | | 1.56 (1.15 to 2.12) | | 1.16 (0.70 to 1.89) | |
| T4d | 2.85 (1.51 to 5.11) | | 2.65 (1.54 to 4.37) | | 1.69 (0.66 to 3.74) | |
| Lymph nodes | | <.001 | | <.001 | | .02 |
| cN0 | 1.00 (reference) | | 1.00 (reference) | | 1.00 (reference) | |
| cN1–3 | 1.94 (1.39 to 2.76) | | 1.63 (1.07 to 2.54) | | 1.63 (1.07 to 2.54) | |
| Tumor grade | | .72 | | .94 | | .05 |
| 1–2 | 1.00 (reference) | | 1.00 (reference) | | 1.00 (reference) | |
| 3 | 1.06 (0.76 to 1.50) | | 0.99 (0.75 to 1.31) | | 1.53 (0.99 to 2.38) | |
| Tumor subgroup | | <.001 | | <.001 | | <.001 |
| HR+ HER2- | 1.00 (reference) | | 1.00 (reference) | | 1.00 (reference) | |
| HER2+ | 1.29 (0.80 to 2.04) | | 1.05 (0.72 to 1.51) | | 2.31 (1.28 to 4.19) | |
| HR- HER2- | 3.92 (2.65 to 5.84) | | 2.14 (1.55 to 2.96) | | 4.35 (2.51 to 7.69) | |
| CTC count before NCT | | <.001 | | <.001 | | <.001 |
| 0–1 | 1.00 (reference) | | 1.00 (reference) | | 1.00 (reference) | |
| ≥2 | 3.93 (2.81 to 5.45) | | 3.73 (2.82 to 4.90) | | 3.02 (1.88 to 4.75) | |

*Multivariable analyses on overall survival (n = 1362 patients, n = 184 events), distant disease–free survival (n = 1362 patients, n = 267 events), and locoregional relapse–free survival (n = 1362 patients, n = 110 events). Multivariable analyses taking into account CTC and T4d interaction, as well as CTC as a continuous variable, are displayed in Supplementary Material 8 (available online). CTC = circulating tumor cell; DDFS = distant disease–free survival; HER2 = human epidermal growth factor receptor 2; HR+ = hormone receptor positive; HR- = hormone receptor negative; LRFI = locoregional relapse–free interval; NCT = neoadjuvant chemotherapy; OS = overall survival.

†Two-sided *P* values correspond to Wald tests.

four, and five or more CTCs before neoadjuvant chemotherapy displayed hazard ratios of death (95% CI) of 1.09 (0.65 to 1.69), 2.63 (1.42 to 4.54), 3.83 (2.08 to 6.66), and 6.25 (4.34 to 9.09), respectively (Supplementary Table 18, available online). Hazard ratios for distant disease–free survival and locoregional relapse–free interval are displayed in Supplementary Table 18 (available online). The observed prognostic impact of CTC count did not change after removing T4d tumors from the analyses (Supplementary Table 19, available online). Using the fewer than two vs two or more CTCs categorization, the overall pooled estimate of having two or more CTCs vs fewer than two CTCs was given by a hazard ratio (95% CI) of 4.36 (3.19 to 5.90) for overall survival, 3.89 (3.00 to 5.03) for distant distant–free survival, and 3.30 (2.12 to 5.03) for locoregional relapse–free interval (Supplementary Table 18, available online). CTC count before surgery was also a statistically significant prognostic factor for overall survival and distant disease–free survival (Figure 2, D–F; Supplementary Tables 20 and 21, available online). There was only modest evidence for between-study heterogeneity of the prognostic effect of two or more CTCs on overall ($I^2 = 0\%$), distant disease–free ($I^2 = 0\%$), and locoregional relapse–free interval ($I^2 = 34.1\%$) in the whole patient population (Supplementary Table 22, available online). Multivariable analyses further confirmed CTC count before NCT as an independent prognostic factor for survival (Table 2; Supplementary Tables 23 and 24, available online).

### CTC Detection and Prognostic Models

The addition of baseline CTC count modeled as a binary variable (fewer than two vs two or more CTCs) to clinicopathological prognostic models (Table 2) resulted in statistically significant increases in prognostication of overall survival ($P < .001$), distant disease–free survival ($P < .001$), and locoregional relapse–free interval ($P = .008$) (Table 3); similar results were obtained when modeling CTC count as a continuous variable (splines) (Table 3). In 861 patients with full data available, further investigations showed that adding baseline CTC count to models including clinicopathological data and pathological complete response status also increased postneoadjuvant survival prognostication (Table 3). Regarding CTC detection during NCT, we found that it did not increase survival prognostication (Table 3).

## Discussion

This international meta-analysis shows that hematogenous cancer cell dissemination is affecting the outcome of treated patients and that this process cannot be predicted precisely enough by the current assessment of the primary tumor characteristics and response. Besides the diagnosis of inflammatory (T4d) breast cancer, CTC count was independent from any other baseline clinical or pathological characteristics. We also found that, in contrast to many other known clinicopathological prognostic factors, CTC detection (either before NCT or before surgery) was not associated with pathological complete response. We found that the best postneoadjuvant survival models included prespecified clinicopathological prognostic markers at baseline, CTC detection at baseline, and pathological complete response, demonstrating the relevance of such metastasis-associated biomarkers for outcome prediction. Although presurgery CTC detection demonstrated its prognostic significance, it did not improve the accuracy of an

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**Table 3.** Assessment of added prognostic information of circulating tumor cells before and during neoadjuvant chemotherapy*

| Model 1 | Model 1 average c-index | Model 2 | Model 2 average c-index | Average c-index increase, model 2 – model 1 (95% CI) | ddf | Average increase $\chi^2$ (95% CI) | P† |
|---|---|---|---|---|---|---|---|
| Models with CTC count before NCT | | | | | | | |
| OS (n = 1362) | | | | | | | |
| CP | 0.736 | Model 1 + $CTC_{BC}$ (< or ≥2 CTC) | 0.767 | 0.031 (–0.003 to 0.063) | 1 | 28.9 (13.3 to 44.1) | <.001 |
| CP | 0.741 | Model 1 + $CTC_{BC}$ (splines) | 0.779 | 0.038 (0.004 to 0.070) | 2 | 30.0 (14.7 to 48.5) | <.001 |
| DDFS (n = 1362) | | | | | | | |
| CP | 0.666 | Model 1 + $CTC_{BC}$ (< or ≥2 CTC) | 0.705 | 0.039 (0.011 to 0.070) | 1 | 38.7 (21.9 to 58.6) | <.001 |
| CP | 0.664 | Model 1 + $CTC_{BC}$ (splines) | 0.709 | 0.045 (0.016 to 0.073) | 2 | 41.2 (24.2 to 63.7) | <.001 |
| LRFI (n = 1362) | | | | | | | |
| CP | 0.728 | Model 1 + $CTC_{BC}$ (< or ≥2 CTC) | 0.734 | 0.006 (–0.021 to 0.031) | 1 | 8.8 (2.4 to 19.2) | .008 |
| CP | 0.727 | Model 1 + $CTC_{BC}$ (splines) | 0.731 | 0.004 (–0.032 to 0.032) | 2 | 9.7 (1.9 to 20.7) | .003 |
| Models with CTC count before NCT and pCR (landmark analyses) | | | | | | | |
| OS (n = 1332) | | | | | | | |
| CP+ $CTC_{BC}$ (< or ≥2 CTC) | 0.771 | Model 1 + pCR | 0.809 | 0.037 (0.008 to 0.064) | 1 | 26.7 (13.1 to 44.0) | <.001 |
| CP+ $CTC_{BC}$ (splines) | 0.778 | Model 1 + pCR | 0.813 | 0.035 (0.002 to 0.065) | 1 | 18.8 (7.9 to 36.1) | <.001 |
| CP + pCR | 0.774 | Model 1 + $CTC_{BC}$ (< or ≥2 CTC) | 0.809 | 0.035 (0.002 to 0.063) | 1 | 30.3 (14.7 to 48.8) | <.001 |
| CP + pCR | 0.774 | Model 1 + $CTC_{BC}$ (splines) | 0.813 | 0.039 (0.004 to 0.072) | 2 | 30.5 (14.4 to 47.8) | <.001 |
| DDFS (n = 1323) | | | | | | | |
| CP+ $CTC_{BC}$ (< or ≥2 CTC) | 0.698 | Model 1 + pCR | 0.746 | 0.048 (0.019 to 0.072) | 1 | 27.9 (13.0 to 45.0) | <.001 |
| CP+ $CTC_{BC}$ (splines) | 0.704 | Model 1 + pCR | 0.751 | 0.047 (0.016 to 0.070) | 1 | 21.8 (10.0 to 37.3) | <.001 |
| CP + pCR | 0.703 | Model 1 + $CTC_{BC}$ (< or ≥2 CTC) | 0.746 | 0.044 (0.012 to 0.068) | 1 | 36.3 (19.7 to 55.4) | <.001 |
| CP + pCR | 0.703 | Model 1 + $CTC_{BC}$ (splines) | 0.751 | 0.048 (0.018 to 0.074) | 2 | 37.1 (21.1 to 57.1) | <.001 |
| LRFI (n = 1322) | | | | | | | |
| CP+ $CTC_{BC}$ (< or ≥2 CTC) | 0.726 | Model 1 + pCR | 0.728 | 0.002 (–0.042 to 0.031) | 1 | 4.90 (0.30 to 13.1) | .03 |
| CP+ $CTC_{BC}$ (splines) | 0.724 | Model 1 + pCR | 0.726 | 0.003 (–0.041 to 0.029) | 1 | 2.6 (0.01 to 9.61) | .10 |
| CP + pCR | 0.729 | Model 1 + $CTC_{BC}$ (< or ≥2 CTC) | 0.728 | –0.001 (–0.031 to 0.024) | 1 | 9.1 (1.74 to 18.7) | .002 |
| CP + pCR | 0.729 | Model 1 + $CTC_{BC}$ (splines) | 0.726 | –0.003 (–0.037 to 0.021) | 2 | 8.7 (1.7 to 18.9) | .02 |
| Models with CTC count before NCT, pCR, and CTC count during NCT (landmark analyses) | | | | | | | |
| OS (n = 861) | | | | | | | |
| CP+$CTC_{BC}$+pCR | 0.804 | Model 1 + $CTC_{DC}$ (< or ≥2 CTC) | 0.806 | 0.002 (–0.016 to 0.013) | 1 | 3.1 (0.0 to 11.4) | .08 |
| CP+$CTC_{BC}$+pCR | 0.798 | Model 1 + $CTC_{DC}$ (splines) | 0.796 | –0.003 (–0.038 to 0.080) | 2 | 5.0 (0.2 to 12.9) | .02 |
| DDFS (n = 856) | | | | | | | |
| CP+$CTC_{BC}$+pCR | 0.758 | Model 1 + $CTC_{DC}$ (< or ≥2 CTC) | 0.760 | 0.002 (–0.013 to 0.014) | 1 | 3.1 (0.0 to 9.7) | .08 |
| CP+$CTC_{BC}$+pCR | 0.756 | Model 1 + $CTC_{DC}$ (splines) | 0.755 | –0.001 (–0.016 to 0.007) | 2 | 2.7 (0.02 to 10.8) | .09 |
| LRFI (n = 855) | | | | | | | |
| CP+$CTC_{BC}$+pCR | 0.717 | Model 1 + $CTC_{DC}$ (< or ≥2 CTC) | 0.707 | –0.011 (–0.074 to 0.006) | 1 | 0.9 (0.0 to 3.6) | .34 |
| CP+$CTC_{BC}$+pCR | 0.725 | Model 1 + $CTC_{DC}$ (splines) | 0.715 | –0.010 (–0.047 to 0.006) | 2 | 0.6 (0.0 to 2.8) | .44 |

*For each analysis, the number of included patients corresponds to the number of patients with available data. Clinical and pathological characteristics were not statistically different among the different patient populations included in these analyses. CI = confidence interval; CP = baseline clinicopathological model; CTC = circulating tumor cells; $CTC_{BC}$ = CTC count before NCT; $CTC_{DC}$ = CTC count during NCT, using the highest value of CTC detection either after NCT start or prior to surgery; ddf = degree of freedom; DDFS = distant disease–free survival; LRFI = locoregional relapse–free interval; NCT = neoadjuvant chemotherapy; OS = overall survival; pCR = pathological complete response.
†Two-sided likelihood ratio test P value corresponds to the average increase in $\chi^2$.

optimized prognostic model taking into account CTC detection at baseline.

In addition to the expected occurrence of distant metastases, CTC counts were also correlated to locoregional relapse. Although we cannot exclude that this unexpected finding might be simply a "bystander" effect of identifying more aggressive tumors, it could also suggest that the spread of breast cancer cells in the body might be also associated with a "self-seeding" mechanism (ie, breast cancer cells recirculate from distant sites back to the primary site), which has been demonstrated so far only in experimental models (4) and with bone marrow disseminated tumor cells (16).

CTC count was a quantitative marker, as each CTC detected added a quantum of poor prognosis. In our clinical validity analyses, survival curves displayed a statistically significant survival difference starting from two CTCs detected. Interestingly, the two other thresholds that were tested in the current meta-analysis, namely one or more and five or more CTCs, were also able to distinguish a high-risk population. Any future adjuvant trial based on CTC detection should have to define a threshold compatible with its target population size (five or more CTCs being a rare event), taking into account that one CTC was detected in some healthy individuals with the applied CTC assay (5) and that the low CTC count observed in nonmetastatic breast cancer patients follows a Poisson's distribution of rare events—blurring the line between zero and one CTCs detected in a single tube of blood (17). The same reason might explain why the longitudinal follow-up of CTC count and changes appeared of lower clinical relevance than previously reported in metastatic breast cancer patients (8).

The main limitation of this study is related to the technical aspects underlying CTC detection by the CellSearch technique.



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Biologically, this US Food and Drug Administration–cleared technique captures CTC by their epithelial cell surface antigens, the expression of which is decreased in some cancer cells during the epithelial-to-mesenchymal transition (EMT) (18). However, abundant evidence supporting the clinical validity of this CTC detection technique in breast cancer had been reported (19); moreover, tumor cells with an intermediate EMT phenotype detected by CellSearch appear to be metastasis-initiator cells (17), which might explain why small incremental increases in CTC counts were associated with increased risks of relapse in the present analysis.

Taken together, while the validity of NCT trials is impaired by the limited correlation between primary tumor response and postneoadjuvant survival, the enumeration of CTC improves postneoadjuvant survival prediction. NCT being also used in patients with other tumor entities where blood-borne dissemination of cancer cells plays also an important role (eg, rectal cancer) (20), the current report might stimulate future clinical studies in NCT-treated patients and therefore has implications beyond breast cancer.

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