Single circulating tumor cell detection and overall survival in nonmetastatic breast cancer

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Background: Circulation of cancer cells in the blood is a mandatory step for metastasis, but circulating tumor cells (CTC) have a low metastatic efficiency in preclinical animal models. In this prospective study, we reported the clinical outcome of nonmetastatic breast cancer patients according to CTC detection.

Patients and methods: In 115 nonmetastatic patients diagnosed with large operable or locally advanced breast cancer, we prospectively detected CTC using the CellSearch system before and after neoadjuvant chemotherapy in a phase II trial (REMAGUS02).

Results: At baseline, 23% of patients were CTC positive, but only 10% had >1 CTC/7.5 ml of blood. After a median follow-up of 36 months, CTC detection before chemotherapy was an independent prognostic factor for both distant metastasis-free survival (DMFS; \( P = 0.01 \), relative risk (RR) = 5.0, 95% confidence interval (CI) 1.4–17) and overall survival (OS; \( P = 0.007 \), RR = 9, 95% CI 1.8–45). CTC detection after chemotherapy was of less significance (\( P = 0.07 \) and 0.09, respectively). Moreover, CTC detection showed interesting characteristics as an individual predictive test for metastatic relapses (sensibility 55%, specificity 81%, and global accuracy 77%).

Conclusions: Detection of ≥1 CTC/7.5 ml before neoadjuvant chemotherapy can accurately predict OS. Our findings may change the clinical management of nonmetastatic breast cancer and indicate that the metastatic efficiency of CTC could be higher than previously reported.

Key words: breast cancer, circulating tumor cells, neoadjuvant chemotherapy, prognosis

Introduction

Hematogenous metastasis is a complex multistep process, involving local invasion, intravasation of cancer cells into blood vessels, circulation, recognition of a favorable distant microenvironment, extravasation, invasion of the extracellular matrix, dormancy, and subsequent secondary growth at a distant site [1]. These steps have been mainly described in preclinical laboratory models, particularly immunodepressed mice models. As i.v. injections of cancer cells generally lead to a small number of metastases in these models, circulating tumor cells (CTC) have been described as being metastasis inefficient [2], and researchers have focused their therapeutic experiments on subsequent steps of the metastatic process. Studies on disseminated tumor cells (DTC) in bone marrow (BM), conducted by our group and by others, have established that BM DTC detection is an independent prognostic factor in early breast cancer patients [3, 4]. However, BM homing might be restricted to a subset of cancer cells that express specific molecular features, as already demonstrated for lung or bone homing [5], and might be associated with a longer dormancy. This is supported by our previous observation that BM DTC were detected in only 60% of patients with overt metastases, these patients exhibiting a longer metastasis-free survival than BM DTC-negative patients [6]. Although these data indicated that CTC might be of less prognostic significance than BM DTC, we conducted a prospective multicenter detection of CTC in 115 nonmetastatic breast cancer patients who were treated by standard therapies in a neoadjuvant randomized phase II trial.

Our objective was to study whether CTC detection has a prognostic impact in these patients and to report test characteristics of CTC detection for predicting metastatic outcome at the individual level. In a preliminary study [7], we previously reported that CTC detection rates were low, with only 23% and 10% of patients displaying at least 1 and 2 CTC/7.5 ml before chemotherapy, respectively. CTC detection was not associated with any patient or tumor characteristic, except for young age (<50 years). CTC detection was also not associated with the primary tumor response to chemotherapy.
After a short 18-month median follow-up, we also reported that CTC positivity was associated with early distant metastasis-free survival (DMFS), by pooling patients who were CTC positive before ("pre-ChT") or after ("post-ChT") chemotherapy. However, these results were based on a small number of relapses and on a pooled analysis of pre-ChT and post-ChT data, whereas neither CTC positivity before chemotherapy nor CTC positivity after chemotherapy was significantly associated with clinical outcome. As the median follow-up of our cohort has now reached 36.4 months, clinical outcome was reanalyzed.

Figure 1. Circulating tumor cells (CTC) detection and survival. Distant metastasis-free survival (DMFS) and overall survival according to prechemotherapy CTC detection [(A) \( P = 0.007 \) and (B) \( P = 0.0006 \), respectively], postchemotherapy CTC detection [(C) \( P = 0.04 \) and (D) \( P = 0.02 \), respectively], and pre- and/or postchemotherapy CTC detection [(E) \( P = 0.004 \) and (F) \( P < 0.0001 \), respectively]. \( P \) values were obtained by the log-rank test (univariate analysis).
Table 1. CTC detection as a predictive test for metastatic relapse

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<th>CTC Detection</th>
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<td>Pre-ChT (%)</td>
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<td>Se</td>
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<td>Sp</td>
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Se, Sp, PPV, NPV, and GA of pre-ChT, post-ChT, and pre-ChT and/or post-ChT CTC detection as predictor of distant metastasis. For the purposes of comparison, these characteristics have also been reported for other commonly used prognostic factors: high grade, large T, cN+, triple neg, and pCR was achieved in 21 of 114 patients (18%).

CTC, circulating tumor cells; pre-ChT, prechemotherapy; post-ChT, postchemotherapy; high grade, tumor histoprognostic grade 3; large T, tumor size T3 or T4 before chemotherapy; cN+, node positive before chemotherapy (clinical evaluation, cN1 or cN2); triple neg, triple-negative (hormone receptors and HER2) phenotype; pCR, pathological complete response (Le Chevallier criteria class 1 or 2); Se, observed sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; GA, global accuracy.

patients and methods

patients and treatments

Pre-ChT and/or post-ChT blood samples were obtained from a subgroup of patients included in the phase II randomized REMAGUS 02 trial from four different institutions. CTC detection was done whenever possible, and no attempt was made in this study to define a target statistical power. All samples were obtained with the patient’s written informed consent. Neither patients nor clinicians were informed of the results of CTC analysis. Eligibility criteria for the study were female patients aged between 21 and 65 years with histologically proven nonmetastatic invasive breast carcinoma (stages II and III), with tumors ineligible for breast-conserving surgery (diameter >3 cm, central, multifocal), or with risk factors making neoadjuvant chemotherapy the preferred treatment (i.e. ipsilateral lymph node involvement, rapid growth rate). The routine diagnostic work-up included mammography, breast magnetic resonance imaging, tumor biopsy with frozen sample, chest X-rays, abdominal ultrasound, bone scan, blood sampling, and clinical examination. HER2 status was considered positive if the HercepTest result was 3+. In doubtful cases (2+), a fluorescence in situ hybridization analysis was done. The cut-off used to define hormone receptor positivity was 10% of stained cells. All patients received four cycles of epirubicin-cyclophosphamide every 3 weeks followed by four cycles of docetaxel with or without trastuzumab, according to randomization, for HER2-positive tumor patients and with or without cetuximab for HER2-negative tumor patients. Surgery was followed by local and regional radiotherapy when indicated. All patients with HER2-positive tumors received adjuvant trastuzumab for 1 year, whereas all patients with hormone receptor-positive tumors received adjuvant tamoxifen or aromatase inhibitors.

CTC detection

Samples were maintained at room temperature and processed within 72 h after collection. All evaluations were done with no knowledge of the patient’s clinical status. The standardized CellSearch technique has been reported previously [8]. Briefly, 7.5 ml of blood is drawn on CellSave tubes. The CellSearch system is used to isolate and count CTC. It consists of a semi-automated sample preparation system and is used with the CellSearch Epithelial Cell Kit. The procedure enriches the sample for cells from leukocytes. Identification and counting of CTC are done with the CellSpotter Analyzer, a semiautomated fluorescence-based microscopy system that allows computer-generated reconstruction of cellular images. CTC are defined as nucleated cells lacking CD45 and expressing cytokeratin 8,18,19-phycoerythrin). Identification and counting of CTC are done with the CellSpotter Analyzer, a semiautomated fluorescence-based microscopy system that allows computer-generated reconstruction of cellular images. CTC are defined as nucleated cells lacking CD45 and expressing cytokeratin.

statistical methods

DMFS and overall survival (OS) were defined as the time elapsed between the date of initial diagnosis and the date of distant metastatic relapse or the date of death, respectively. Survival curves were plotted according to the Kaplan–Meier method. Statistical significance between survival curves was assessed using the log-rank test. Multivariate analysis was done by the Cox proportional hazards model with prognostic factors with P value <0.10 at univariate analysis. For all analyses, a P value of <0.05 was considered to be statistically significant. This report was written in accordance with the REporting of tumor MARKer Studies guidelines [9].

results

Among 115 patients, 14 (12%) metastatic relapses have occurred and 9 of these patients (8%) have died. In univariate analysis (log rank), pre-ChT detection of CTC (n = 95) was significantly associated with both DMFS (P = 0.007) and OS (P = 0.0006) (Figure 1A and B). Post-ChT detection (n = 85) was also significantly associated with both DMFS (P = 0.04) and OS (P = 0.02) (Figure 1C and D) (log rank). Finally, pooling pre-ChT and post-ChT detection (i.e. each patient with at least 1 CTC detected before and/or after chemotherapy classified as CTC positive), CTC detection was also significantly associated with both DMFS (P = 0.004) and OS (P < 0.0001) (Figure 1E and F) (log rank).

The characteristics of CTC detection and other prognostic factors as individual prognostic tests for metastatic relapse, shown in Table 1, were in line with these results, showing a global accuracy of >75% for CTC detection, whenever assessed. Univariate and multivariate analyses for OS and DMFS showed that pre-ChT CTC detection was a strong independent prognostic factor for both DMFS (P = 0.01,
relative risk (RR) = 5.0, 95% confidence interval (CI) 1.4–17] and OS (P = 0.007, RR = 9, 95% CI 1.8–45), along with tumor size and triple-negative phenotype (Table 2). In multivariate analysis, post-ChT CTC detection was found to be of less significance for both DMFS (P = 0.07) and OS (P = 0.09). Not surprisingly, when pooled, the two CTC detections (pre- and/or post-ChT) were significantly associated with DMFS (P = 0.009, RR = 4.3, 95% CI 1.4–12) and OS (P = 0.07, RR = 18, 95% CI 2.2–142).

conclusions

Taken together, these results show the following: (i) pretreatment CTC detection is an independent, strong prognostic factor for OS in nonmetastatic breast cancers during neoadjuvant chemotherapy; (ii) even a single CTC detected in 7.5 ml of blood is associated with the subsequent development of metastases in humans, demonstrating that no threshold is required for CTC detection by the CellSearch system, at least in the neoadjuvant setting; and (iii) CTC might metastasize more efficiently than previously thought [10]. Finally, other neoadjuvant trials combined with CTC detection, such as the GEPARQUATTRO trial conducted by German Breast Group, are needed to confirm our results. New bio-clinical prognostic indices, taking into account CTC detection, should also be developed and validated by pooling future trials.

funding

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disclosure

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references