Changing Levels of Circulating Tumor Cells in Monitoring Chemotherapy Response in Patients with Metastatic Breast Cancer

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Abstract. *Background: The detection of >5 circulating tumor cells (CTCs)/7.5 ml blood in patients being treated for metastatic breast cancer (MBC) has recently been shown to be predictive for therapy efficacy. The aim of this study was to investigate whether changing CTC levels during the course of chemotherapy treatment would also be useful in monitoring response to treatment. Patients and Methods: CTC levels were determined in 58 MBC patients at the beginning and after 3 cycles of chemotherapy. Changes in CTC level (either negative CTCs (<5 CTCs/7.5 ml blood) turning positive, vice versa, or a change of ±25%) were correlated to radiologic Response Evaluation Criteria In Solid Tumors (RECIST) criteria, as well as serum CA 15-3 measurements, and were evaluated for their capability to predict survival. Results: Changing CTC levels significantly correlated with response to therapy as measured by radiologic RECIST criteria (p<0.001), and serum CA 15-3 level changes (p=0.017). Patients with decreasing CTC levels survived significantly longer than patients with increasing CTC levels (17.67±5.90 months versus 4.53±0.54 months, p<0.001). Conclusion: The observation of changes in CTC level during the course of chemotherapy is useful in monitoring therapy efficacy and is correlated with overall survival. Further prospective trials should investigate the clinical usefulness of determining changes in CTC level during chemotherapy of MBC.*

Modern adjuvant therapy has improved prognoses of women suffering from breast cancer. However, about 40% of all cases will relapse, making breast cancer the second leading cause of cancer death among women in the US (1). As metastatic breast cancer (MBC) is incurable, the use of chemo-, hormonal or targeted therapy to treat MBC has palliative purpose. In this setting, it is of great importance to monitor individual therapy response, as with regard to toxicity and disease progression, ineffective treatment should be avoided. This is usually realized by examining clinical parameters, radiologic response and the measurement of serum tumor markers (TM), such as CA 15-3 (2).

Recently, circulating tumor cells (CTCs) have attracted interest as a promising tool to monitor therapy response in women being treated for MBC. The detection of CTCs in the peripheral blood of MBC patients was reported to have an independent prognostic value (3). During the course of therapy, elevated CTCs may be predictive of response to treatment (4). Assessment of CTCs was suggested to be an earlier and more reproducible indicator of disease status than routine imaging methods. A semi-automatic method that enables reliable detection and enumeration of CTCs in blood is the US Food and Drug Administration (FDA)-approved CellSearch assay (Veridex LLC, Raritan, NJ, USA) (5). However, additional validation is needed to confirm the clinical value of this test (6).

We investigated whether changing CTC levels during chemotherapy i) correlated with therapy response, as measured by radiographic Response Evaluation Criteria In Solid Tumors (RECIST) criteria (7), ii) compared CTC measurements with the "classical" TM CA 15-3 and iii) evaluated the ability of these approaches to predict survival.

Patients and Methods

Patients. Patients receiving chemotherapy treatment for MBC between 01/2008 and 12/2009 at the Department of Gynecology/Obstetrics, Tuebingen (Germany) were routinely monitored by CTC counts, TM and radiographic assessment. Before starting a new treatment, patients received a baseline blood draw for CTC and CA 15-3 evaluation, as well as standard imaging studies. Reassessment of disease status was conducted after three cycles of chemotherapy (9-12 weeks, depending on treatment type and schedule) with the same modalities used at baseline. Standard RECIST criteria were used to determine patient

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Table I. *Patient demographics*.

	n	<5 CTCs n (%)	Decreasing CTCs or NC n (%)	Increasing CTCs or NC n (%)	*p*-Value
Total	58	24 (41.4)	24 (41.4)	10 (17.2)	
Type of therapy					
CHT alone	43	18 (41.9)	17 (39.5)	8 (18.6)	
CHT plus target therapy	15	6 (40.0)	7 (46.7)	2 (13.3)	0.850
Line of therapy					
1st	22	12 (54.4)	9 (40.9)	1 (4.5)	
≥ 2nd	36	12 (33.3)	15 (41.7)	9 (25.0)	0.097
ER/PR status					
ER positive	47	19 (40.4)	20 (42.6)	8 (17.0)	1.000
PR positive	35	15 (42.9)	15 (42.9)	5 (14.3)	0.825
Her2 status					
Positive	17	6 (35.3)	2 (11.8)	9 (52.9)	
Negative	41	18 (43.9)	15 (36.6)	8 (19.5)	0.592
Site of metastasis					
Visceral	48	20 (41.7)	19 (39.6)	9 (18.8)	
Non-visceral	10	4 (40.0)	5 (50.0)	1 (10.0)	0.903
Status at last follow-up					
Alive	35	19 (54.3)	15 (42.9)	1 (2.9)	
Dead	23	5 (21.7)	9 (39.1)	9 (39.1)	0.001

response to treatment. To correlate CTC level profiles during chemotherapy with radiologic response and other variables, an increase or decrease of at least 25% from CTC level at baseline was defined as a significant change, as described previously (8). This threshold of 25% was also used to determine changes in the CA 15-3 profiles.

CTC measurement. Blood samples were drawn into 7.5-ml CellSave tubes (Veridex, Warren, NJ, USA), maintained at room temperature and processed within 72 h after collection. The CellSearch Epithelial Cell Test (Veridex, Warren, NJ, USA) was used for enrichment and enumeration of CTCs as described elsewhere (5). CTCs expressing the epithelial cell adhesion molecule (EpCAM) were briefly immunomagnetically enriched and labeled by 4',6-diamidino-2-phenylindole (DAPI) staining and monoclonal antibodies specific for leukocytes (anti-CD45-allophycocyan) and epithelial cells (anti-cytokeratin-phycoerythrin). CTCs are defined as CD45-negative, cytokeratin-positive nucleated cells and were enumerated by trained operators. A blood sample was positive when at least 5 CTCs were present, as published previously (3).

CA 15-3 measurement. Serum CA 15-3 concentrations were determined using a sequential chemiluminescent sandwich immunoassay on the ADVIA Centaur System (Siemens Healthcare Diagnostics, Eschborn, Germany). A cut-off level of 33 U/ml was used, as described previously (9).

Statistical analysis. Statistical analyses were carried out using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). Survival was calculated by the Kaplan-Meier method and compared by the log-rank test. Categorical variables were compared using Chi-squared test and Fisher's exact test. Continuous variables were compared using two-sided unpaired Students *t*-test. All *p*-values are reported.

Results

Patient characteristics. A total of 58 patients receiving chemotherapy for advanced MBC between 01/2008 and 12/2009 were eligible in this retrospective analysis. Thirty-six patients (62%) had ≥second-line treatment for MBC and 15 patients (25.9%) received chemotherapy in combination with targeted therapy. Patient characteristics are shown in detail in Table I. The average follow-up time was 13.2 months (range 7.8-18.6 months). At the time of analysis, 23 patients (39.7%) had died and the median overall survival from follow-up blood draw was 17.67 months. The average interval between baseline and follow-up blood draw was 65 days (range 52.2-79.8 days). At baseline/follow-up, 31/20 patients (53.4%/ 27.6%) had ≥5 CTCs/7.5ml blood. Twenty-four (41.4%) patients had <5 CTCs at both blood draws, 24 patients (41.4%) had a decreasing CTC level and for 10 patients (17.2%), the CTC level increased or did not change (NC).

Correlation of CTC changes during chemotherapy and therapy response. Changing CTC levels during chemotherapy significantly correlated to therapy response as measured by radiologic RECIST criteria ($p<0.001$, Table II). Sixteen patients (27.6 %) had progressive disease (PD). In these patients, the CTC level increased by an average of 24.3 (±17.4)/7.5 ml blood (Figure 1). Using 5 CTCs/7.5 ml blood as a threshold, 13 patients with PD had elevated CTCs during at least one blood draw; 5 of these had a decreasing

Table II. *Correlation of changes in CTC levels and response to chemotherapyl as measured by radiologic RECIST criteria and serum CA 15-3.*

		CTC level profile			Mean absolute CTC change/7.5 ml blood
	n	<5 CTCs n (%)	Decreased CTCs or NC n (%)	Increased CTCs or NC n (%)	
Total	58	24 (41.4)	24 (41.4)	10 (17.2)	
Radiologic response					
PD	16	3 (18.8)	5 (31.3)	8 (50.0)	+24.3 (±17.4)
PR	30	13 (43.3)	16 (53.3)	1 (3.3)	+7.4 (±13.7)
SD	12	8 (66.7)	3 (25.0)	1 (8.3)	–25.5 (±10.2)
p-Value				<0.001	0.022
CA 15-3 serum profile					
Below cut-off	18	11 (61.1)	7 (38.9)	0 (0.0)	–13.4 (±7.0)
Decreasing	16	7 (43.8)	8 (50.0)	1 (6.3)	–6.0 (±4.5)
Increasing	15	3 (20.0)	5 (33.3)	7 (46.7)	+6.1 (±7.4)
Unchanged	9	3 (33.3)	4 (44.4)	2 (22.2)	–7.0 (±11.9)
p-Value				0.017	0.180

CTC level, 7 had an increasing CTC level and in 1 patient the CTC level remained unchanged. In 30 patients (51.7%), radiologic follow-up revealed a partial remission (PR). Within this group, the CTC level decreased by an average of 25.5 (±10.2)/7.5 ml blood (Figure 1). Elevated CTCs were detected during at least one blood draw in 17 out of the 30 patients with PR; 16 of these had decreasing and 1 patient had an increasing CTC level. Of the 12 patients with stable disease (SD), 4 had CTC levels above threshold during at least one blood draw; 3 of these patients had a decreasing CTC level and 1 patient had increasing CTCs. The mean CTC increase within this group was 7.4 (±13.7)/7.5 ml blood (Figure 1).

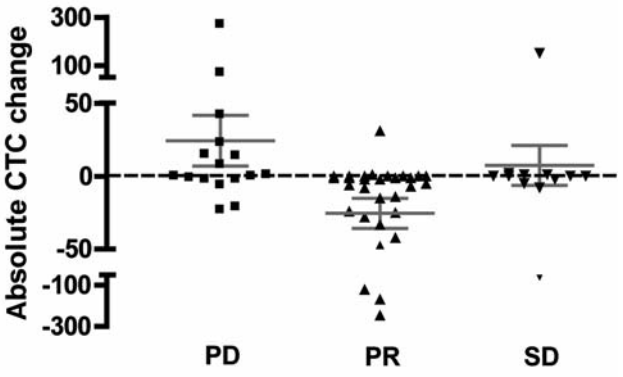

Figure 1. *Absolute CTC changes during cycles 1-3 of chemotherapy. Therapy response was measured by radiologic RECIST criteria (PD=progressive disease, PR=partial remission, SD=stable disease). Dots represent absolute CTC changes as calculated by the difference between the CTC level at follow-up minus the CTC level at baseline. Horizontal lines represent the mean the CTC change within each group with standard errors as indicated. Absolute CTC changes significantly correlated to therapy response (p=0.022).*

Correlation of CTC changes and CA 15-3 changes during chemotherapy. CTC level changes significantly correlated to CA 15-3 changes during chemotherapy (p=0.017, Table II). In 40 patients (68.97%), elevated CA 15-3 serum levels were detected at any time-point. A decreasing CA 15-3 serum level was found in 16 of these patients, while 15 patients had an increasing serum level. Of the 16 patients with a decreasing CA 15-3 serum level, 9 patients had elevated CTCs during at least one blood draw; 8 of these patients had a decreasing and 1 patient had an increasing CTC level. Of the 15 patients with increasing CA 15-3 serum levels, 12 patients were found to have elevated CTCs during at least one blood draw; the CTC level decreased in 5 and increased in 7 patients.

Prognostic value of CTC changes. Different approaches to monitor chemotherapy response (*i.e.* CTCs, radiologic RECIST criteria and CA 15-3) were compared as predictors for patient survival (Figure 2). We found that in patients with CTCs below threshold (<5 CTCs / ml blood) at both blood draws, median overall survival (OS) was significantly longer than in patients that had elevated CTCs during at least one blood draw (not defined (n.d.) as the survival curve does not fall to 50 % vs. 9.77±2.51 months, p=0.002). Furthermore, absolute changes in CTC levels (*i.e.* decreasing *vs.* increasing CTC levels) significantly predicted OS (17.67±5.90 months *vs.* 4.53±0.54 months, p<0.001). With regard to the fact that changes in CTC levels may

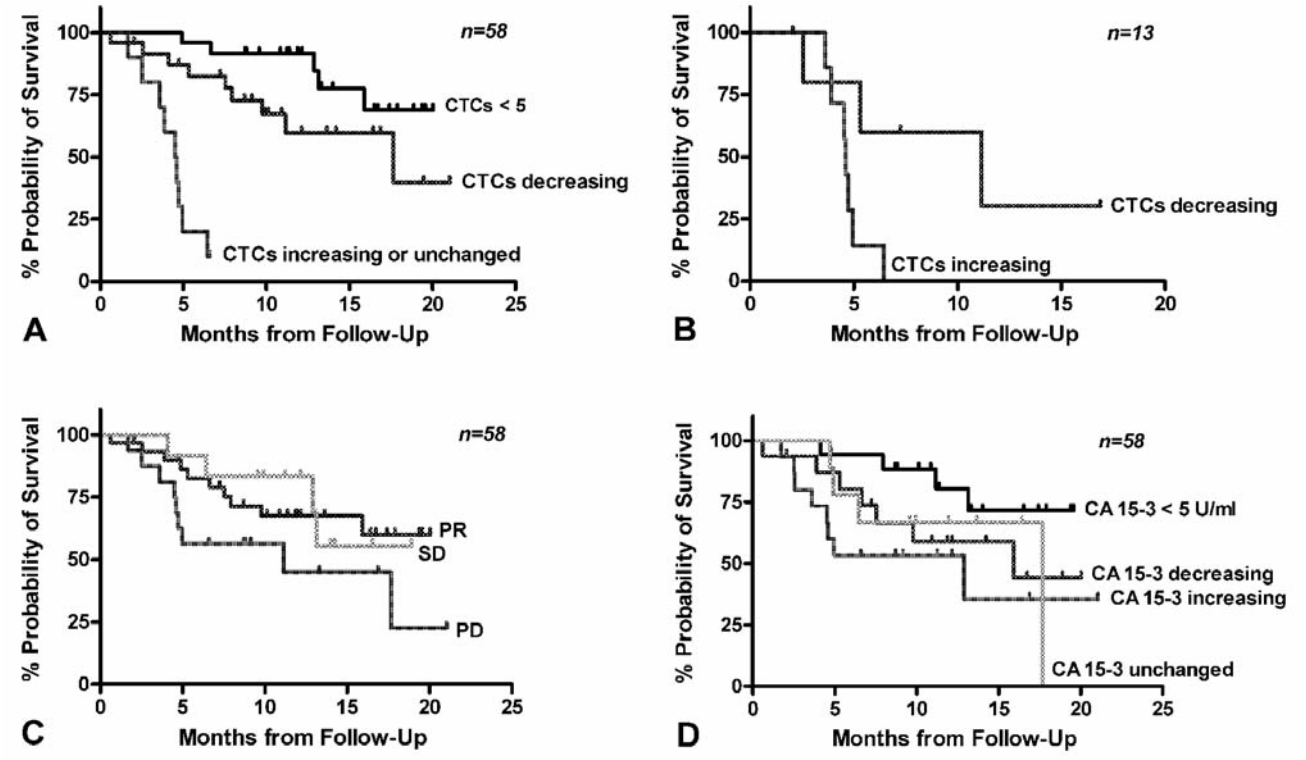

Figure 2. *Kaplan-Meier plots of overall survival (OS) according to chemotherapy response as monitored by CTCs (A, B), radiologic RECIST criteria (C) and CA 15-3 (D). Survival times were calculated from the time of follow up blood draw. (A) Median OS for patients with <5 CTCs/7.5 ml blood at both blood draws was not defined as the survival curve does not fall to 50% (n.d.), 17.67±5.90 months for patients with decreasing CTCs and 4.53±0.54 months for patients with increasing CTCs (p<0.001). (B) OS of the patients with elevated (>5 CTCs/7.5 ml blood) CTCs at both blood draws; median overall survival was 11.15±4.64 months for patients with decreasing CTCs and 4.59±0.86 months for patients with increasing CTCs (p=0.041). (C) Median OS for patients with stable disease (SD) or partial remission (PR) was n.d. and was 11.15±7.78 months for patients with progressive disease (PD) (p=0.179). (D) OS for patients with CA 15-3 serum levels <33 U/ml at both blood draws was n.d., and was 15.90±6.68 months for patients with decreasing CA 15-3 serum levels, 17.67 months for patients with unchanged CA 15-3 serum levels and 12.89±5.72 months for patients with increasing CA 15-3 serum levels (p=0.138).*

predict survival due to the proportion of patients changing from CTC-positive to -negative during therapy or *vice versa*, OS was calculated for patients that had elevated CTCs at both blood draws. As shown in Figure 2B, patients with decreasing CTC levels had a significantly prolonged median OS (11.15±4.64 months for patients with decreasing CTCs *vs*. 4.59±0.86 months for patients with increasing CTCs, p=0.041).

Patients with CA 15-3 serum levels below threshold (33 U/ml) at both blood draws also survived significantly longer than patients with elevated serums level at at least one blood draw (n.d. *vs*. 15.90±3.351 months, p=0.042). However, decreasing *vs*. increasing CA 15-3 serum levels did not influence patient survival (15.90±6.68 months *vs*. 12.89±5.72 months, p=0.138). Moreover, response to chemotherapy as measured by radiologic RECIST criteria did not significantly predict OS (n.d. for PR and SD, 11.15±7.78 months for PD, p=0.179).

Discussion

The prognostic value of CTCs in MBC patients has been proven by large studies. The presence of >5 CTCs/7.5 ml blood at the beginning of a new therapy is strongly associated with reduced OS (3, 10). Moreover, the prognostic value of CTC determination is maintained at subsequent follow-up time points; converting an elevated CTC level to a level of <5 CTCs/7.5 ml blood prolongs survival (4). This prompted us to investigate whether any change in CTC level would be useful to routinely monitor response to chemotherapy treatment. For this purpose a change in CTC level was considered significant when either CTC-negative cases (<5 CTCs/7.5 ml blood) became positive or *vice versa*, respectively, or when a change of ±25% was detected. This value has been described for various TMs by others (8, 11).

CTC level changes were highly correlated to objective therapy response as measured by radiologic RECIST criteria.

Additionally, of the patients with increasing serum CA 15-3, a significant proportion had increasing CTCs. Similarly to previous reports, we found no correlation between the number of CTCs and the number of sites of disease, indicating that the number of CTCs is not representative for tumor burden (12). Moreover, there was no correlation between the total number of CTCs and time to death. However, in an individual patient, CTC level changes were useful to predict overall survival. This was even true for patients with positive CTCs that failed to turn negative under chemotherapy. In line with previous reports, CTC level changes were found to be more powerful surrogates of OS than radiologic RECIST criteria (12).

The presented data indicate that CTC level changes are useful to monitor chemotherapy response. This has relevance for the clinical practice of treating MBC, as palliative chemotherapy is routinely changed when non-tolerable toxicity or signs of disease progression occur. Assessment of chemotherapy efficacy is thus essential and currently performed by radiology, clinical examination and determination of serum TMs (13). However, these approaches have certain limitations. First, imaging modalities often require several cycles of therapy before providing reliable information on disease status (14). In this study, CTC determination was performed at the time point of radiographic follow-up, i.e. after 3 cycles of therapy. Interestingly, Budd et al demonstrated that the assessment of CTCs is capable of monitoring response to therapy as early as 4 weeks after the initiation of therapy (12). Moreover, radiologic methods are expensive and inconvenient, whereas CTC determination using the CellSearchSystem can be conducted by a simple blood test. Second, a patient may not have clinically or radiographically measurable disease. Particularly in those patients, more reliable methods to determine disease progression are needed. In this context, Budd et al demonstrated that the presence of CTCs also predicts OS in non-measurable MBC (15). Third, the "classical" serum TMs like CA 15-3, have limited specificity and sensitivity. Rising tumor marker levels may occur during the first 4-6 weeks of a new therapy (16) and tumor markers can be elevated due to nonmalignant conditions. They should therefore be used in conjunction with diagnostic imaging, history, and physical examination (6, 17). In contrast to serum TMs, CTC levels were found not to be elevated in nonmalignant conditions (18). Furthermore, our results demonstrate that changing CTC levels in response to chemotherapy are a more powerful predictor of survival than changing serum CA 15-3 levels.

In the future, therapy of malignant diseases will be more and more individualized. Therefore, the evaluation of predictive markers plays an important role to optimize tumor therapy. The phenotype of the primary tumor is, however, often different from the phenotype of metastatic disease (19). In addition, to monitor therapy response and to determine prognosis, CTCs can be used as "real time biopsies" in MBC patients. Re-evaluating therapeutic targets on CTCs holds great promise to enable a more individualized and optimized anti-metastatic therapy in cancer patients (20).

In conclusion, the data presented in this report indicates that changing CTC levels during chemotherapy are useful to monitor therapy efficacy. This complements previous observations that changing positive to negative CTCs in the course of a therapy correlates with mortality and disease progression (4). The signification of switching a therapy due to elevated CTCs is currently addressed in a randomized prospective trial, SWOG S0500, led by the Southwest Oncology Group (21). Further prospective trials should investigate the clinical usefulness of determining CTC level changes.

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