RESEARCH ARTICLE

Detection of Circulating Tumor Cells in Breast Cancer Patients: Prognostic Predictive Role

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Abstract

A determination of circulating tumor cell (CTC) effectiveness for prediction of progression-free survival (PFS) and overall survival (OS) was conducted as an adjunct to standard treatment of care in breast cancer management. Between November 2008 and March 2009, 22 metastatic and 12 early stage breast carcinoma patients, admitted to Ankara Oncology Training and Research Hospital, were included in this prospective trial. Patients' characteristics, treatment schedules and survival data were evaluated. CTC was detected twice by CellSearch method before and 9-12 weeks after the initiation of chemotherapy. A cut-off value equal or greater than 5 cells per 7.5 ml blood sample was considered positive. All patients were female. Median ages were 48.0 (range: 29-65) and 52.5 (range: 35-66) in early stage and metastatic subgroups, respectively. CTC was positive in 3 (13.6%) patients before chemotherapy and 6 (27.3%) patients during chemotherapy in the metastatic subgroup whereas positive in only one patient in the early stage subgroup before and during chemotherapy. The median follow-up was 22.0 (range: 21-23) and 19.0 (range: 5-23) months in the early stage and metastatic groups, respectively. In the metastatic group, both median PFS and OS were significantly shorter in any time CTC positive patients compared to CTC negative patients (PFS: 4.0 vs 14.0 months, Log-Rank p=0.013; and OS: 8.0 months vs. 20.5 months, Log-Rank p<0.001). OS was affected from multiple visceral metastatic sites (p=0.055) and higher grade (p=0.044) besides CTC positivity (log rank p<0.001). Radiological response of chemotherapy was also correlated with better survival (p<0.001). As a result, CTC positivity was confirmed as a prospective marker even in a small patient population, in this single center study. Measurement of CTC by CellSearch method in metastatic breast carcinoma cases may allow indications of early risk of relapse or death with even as few as two measurements during a chemotherapy program, but this finding should be confirmed with prospective trials in larger study populations.

Keywords: Breast cancer - circulating tumor cells - survival

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Introduction

Breast cancer is the second most common cause of cancer death in women after lung cancer in United States (Siegel et al., 2013). Metastatic breast cancer (MBC) is currently considered as an incurable disease but significant improvement in prognosis has been observed over the last decades (Siegel et al., 2013). Thus early detection of the recurrence of breast cancer is vital. Detection of Circulating Tumor Cells (CTC) was reported to be as an important prognostic marker from the results of 3 prospective randomised multicenter studies in breast, colon and prostate cancer (Cristofanilli et al., 2004; Cohen et al., 2008; De Bono et al., 2008). It is speculated that decrease in CTC numbers after initiation of chemotherapy, even after one cycle, may reflect treatment efficacy and good prognosis. In contrast, high numbers of CTC were claimed to be related to the treatment failure and worse prognosis (Miller et al., 2010). Importance of the early detection of treatment failure and early switching to the therapy before conventional clinical and radiological response evaluation has not been known, yet. Clinical studies of the impact of CTC counts in routine clinical practice are ongoing (Alemar and Schuur, 2013). Strategy of changing therapy versus maintaining therapy for metastatic breast cancer patients who have elevated circulating tumor cell levels after early treatment before classical radiological assessment were under study in a randomised prospective ongoing trials of Southwest Oncology Group (SWOG0500) and CirCe01 (France) trials (Bidard et al., 2012). Other ongoing trials STIC CTC METABREAST (France) and Endocrine Therapy

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Index (USA) assess the CTC guided hormone therapy vs. chemotherapy decision in M1 patients (Bidard et al., 2012). In two metastatic breast carcinoma cases, early changing to capecitabine plus lapatinib treatment after detection of doubling of CTC numbers on treatment resulted in near total decrease in CTC numbers (Camara et al., 2009). Ongoing trials DETECT III (M1 patients, Germany) and Treat CTC (cM0(i+)patients, European Organisation for Research and Treatment of Cancer/ Breast International Group) assess the use of anti-HER2 treatments in HER2-negative breast cancer patients selected on the basis of CTC charactersitics (Bidard et al., 2012). These ongoing trials may answer clinical questions about routine use of CTC in daily practice.

Recent study with 236 metastatic breast cancer, claimed that nomograms which relied on CTC counts as a continous covariate, induced the use of web based tool for estimating survival, supporting treatment plan and clinical trial stratification in first line MBC (Giordano et al., 2012). The presence and detection of CTC is rarer in early breast cancer. Larger study populations and longer follow up time is needed to show clinical benefit of CTC detection. Thus prospective randomised study data is limited especially in early breast cancer. Ongoing Treat CTC trial combines the prognostic information of CTC in adjuvant setting and the promise of adjuvant trastuzumab given to HER2-negative patients in past studies (Bidard et al., 2012).

CellSearch (Veridex, LLC, Raritan, NJ) is a widely used semi-automatic commercial system that relies on immunomagnetic capture of CTCs using epitelyal cell adhesion molecule (EpCAM) which is expressed on the surface of epitelial malignancies, followed by positive selection with cytokeratin and negative selection of leukocytes. This method has been approved by US Food and Drug Association (FDA) and European Union (CE) for detection of CTCs in breast, colon and prostate cancer but results of prospective randomised studies are awaited for routine use as a standard of care if this detection yielded progression free survival (PFS) and overall survival (OS) (Harris et al., 2007; Bidard et al., 2012).

It was reported in 177 metastatic breast cancer who had CTC≥5 detected by Cellsearch method at baseline, had less PFS and OS compared to patients of whom baseline CTC<5 (7 months vs 3 months and 22 months vs 10 months, respectively) (Cristofanilli et al., 2004). They also claimed that the presence of high levels of CTC 3-5 weeks after the initiation of chemotherapy herald treatment failure (Cristofanilli et al., 2004). Reproducibility of CellSearch method was reported between 80-82% and the incidence of CTC as 70% in a study of 92 metastatic breast cancer patients (Riethdorf et al., 2007). Statistically shorter PFS and OS were reported in patients who had basal CTC≥5 than CTC<5 at the time of 14th week of treatment in a prospective randomised study of metastatic breast cancer (Hayes et al., 2006). The difference between operators were also reported to detect CTC was 0.7 % in a randomised study of 138 metastatic breast cancer patients (Budd et al., 2006). They also concluded that CTC detection was more sensitive, reliable and reproducible method than conventional radiological methods for the determination of survival in earlier treatment time (Budd et al., 2006). It was reported that CTC count was a significant predictive factor for overall survival (OS) in all immunohistochemically defined molecular subtypes (Munzone et al., 2012). In a larger study, 468 MBC patients were divided into three subgroups based on immunohistochemical staining of the primary tumor: hormone receptor postive, Her2-positive and triple negative groups. This study confirmed independent prognostic value of CTC detection but lack to show difference between primary tumor-based molecular subgroups and impact of CTC status on survival (Wallwiener et al., 2013). Circulating cell-free DNA carrying tumor-specific alterations (circulating tumor DNA) has been investigated and compared with cancer antigen 15-3 (CA 15-3) and circulating tumor cells in a recent study (Dawson et, al., 2013). They concluded that circulating tumor DNA levels showed a greater dynamic range, and greater correlation with changes in tumor burden, than did CA 15-3 or circulating tumor cells. Among the measures tested, circulating tumor DNA provided the earliest measure of treatment response in 10 of 19 women (53%) (Dawson et, al., 2013). There was also evedence that CTC in breast cancer exhibit dynamic changes in epithelial and mesenchymal composition and reversible shifts between these cell fates accompanied each cycle response to therapy and diasease progression in a recent study (Yu et al., 2013).

Detection of CTC may give knowledge about microscopic disease and this in turn yielded prognostic clue of the disease state. The detection of CTCs may be involved in the staging of metastatic breast cancer patients. Better classification of high risk patients later may be resulted in better use of targeted therapies finally causing improvement in personalized treatment. By the detection of CTCs and markers on CTCs may lead to cure of metastatic breast cancer in the future. The effectiveness of CTC determination on PFS and OS in breast cancer management as an adjunct to standard care of treatment was evaluated in the presenting study.

Materials and Methods

Between November 2008 and March 2009, 22 metastatic and 12 early stage breast carcinoma patients, admitted to Ankara Oncology Training and Research Hospital and gave informed consent to participate, were included in this prospective trial. This study was approved by the local ethical committee of the hospital. Patients' characteristics, treatment schedules and survival data were evaluated. Physical examination, staging and routine radiological assessment were done regularly for the evaluation of response without any intervention. CTC was detected by CellSearch method before and 9-12 weeks after the initiation of chemotherapy. Study was not supported by any firm or foundation, CTC kits were donated to Ankara Oncology Training and Research Hospital for demonstration of CellSearch Method from the local distributor were readily used.

Patients were included in this study if they were Eastern Cooperative Oncology Group (ECOG) performance status

was between 0-2. Metastatic patients and non-metastatic patients who started a new chemotherapy regimen entered in this study. Staging was done by TNM system (Edge et al., 2010). Chemotherapy responses were evaluated by using revised response evaluation criteria in solid tumours (RECIST) version 1.1 (complete response, partial response, stable disease and progression) (Eisenhauer et al., 2009). CTC was detected by CellSearch method before and 9-12 weeks after the initiation of chemotherapy. For processing, a 7.5 ml venous blood sample was dropped to 10 ml 'Cellsave Tube' (Veridex LLC, Raritan, NJ). For isolation and counting of CTCs, CellSearch System (Veridex LLC, Raritan, NJ, USA) was used which was defined before (Cristofanilli et al., 2004). Results were expressed as number of cells per 7.5 ml blood sample (Cristofanilli et al., 2004). Isolation and counting of CTC was done by an independent operator without knowing patients data as defined before previous studies (Kagan et al., 2002). Cut-off value equal or greater than 5 cells per 7.5 ml blood sample was considered to be ctc positive as defined before (Kagan et al., 2002). CTC results were not used as a part of staging or treatment decision plan troughout the entire study.

Statistics

Overall survival (OS) was calculated from the date of diagnosis and death for any reason or the date of last contact. Progression free survival (PFS) was calculated from the date of first treatment until disease progression. Cut-off value equal or greater than 5 was considered to be ctc positive as defined before. Fisher's exact test was used to compare patient characteristics, CTC distribution and tumor factors between the populations. The survival of the patients was estimated using the Kaplan-Meier method. Long-rank test was used to compare and analyse the survival data. The determination of independent prognostic factors influencing survival was performed by Cox proportional hazard model. The 95% confidence interval was calculated for all hazard ratios (HRs) in Cox regression analysis. A p value less than 0.05 was considered to be statistically significant. For stastistical analysis, SPSS for Windows, version 15.0 software (SPSS Inc, Chicago, Illionis, USA) was used.

Results

Patients' characteristics

Between November 2008 and March 2009, 22 metastatic and 12 early stage breast carcinoma patients, admitted to Ankara.Oncology Training and Research Hospital and gave informed consent to participate, were included in this prospective trial. Patients' characteristics, treatment schedules and survival data were evaluated. Physical examination and routine radiological assessment were done regularly for the evaluation of response. CTC was detected twice by CellSearch method before and 9 to 12 weeks after the initiation of chemotherapy.

All patients were female. Median age was 48.0 (range: 29-65) and 52.5 (range: 35-66) in early stage and metastatic groups, respectively (Table 1). Treatment schedules and first site of metastasis was shown in Table 2.

CTC was positive (≥5) in 3 (13.6%) patients before chemotherapy and 6 (27.3%) patients at anytime during chemotherapy in the metastatic group whereas CTC was positive in only one patient in early stage group before and during chemotherapy (Table 3). Clinical and radiological

Table 1. Basic Characteristics' of Early Stage and Metastatic Breast Cancer Patients

Characteristics		Metastatic Patients n (%)	Early-stage Patients n (%)
Menopausal Status	Premenopausal	15 (68.2)	7 (58.3)
	Postmenopausal	7 (31.8)	5 (41.7)
Family History	Present	4 (18.2)	1 (8.3)
	Absent	18 (81.8)	11 (91.7)
Breast Operation	Present	19 (86.4)	12 (100)
	Absent	3 (13.6)	0 (0.0)
Stage at Diagnosis	I-II	10 (45.5)	7 (58.3)
	III-IV	12 (54.5)	5 (41.7)
Grade	Unknown	9 (40.9)	1 (8.3)
	II	4 (18.2)	5 (41.7)
	III	9 (40.9)	6 (50.0)
Estrogen Receptor	Positive	12 (54.5)	6 (50.0)
	Negative	10 (45.5)	6 (50.0)
Progesterone Receptor	Positive	12 (54.5)	7 (58.3)
	Negative	10 (45.5)	5 (41.7)
c-erbB2 Status	Positive	11 (50.0)	7 (58.3)
	Negative	11 (50.0)	5 (41.7)

Table 2. First site(s) of Metastasis and Therapy Received by Early Stage and Metastatic Breast Cancer Patients

	Metastatic Patients (n=22) n (%)	Non-Metastatic Patients (n=12) n (%)
Adjuvant Chemotherapy		
Present	16 (72.7)	12 (100)
Absent	6 (27.3)	0 (0.0)
Type of Adjuvant Chemotherapy		
Antracycline based	10 (45.5)	6 (50.0)
Taxane-trastuzumab	6 (27.3)	6 (50.0)
Adjuvant Chemotherapy Cycle		
3-4	9 (40.9)	3 (25.0)
6-9	13 (59.1)	9 (75.0)
Adjuvant Hormonotherapy		
Present	11 (50.0)	9 (75.0)
Absent	11 (50.0)	3 (25.0)
Adjuvant Radiotherapy		
Present	9 (40.9)	9 (75.0)
Absent	13 (59.1)	3 (25.0)
Metastastic Site (s)		
Local-Regional	7 (31.8)	-
MultipleVisceral Metastatic Sites	15 (68.2)	-
Palliative Chemotherapy		
1st Line	10 (45.5)	-
2nd Line	8 (36.4)	-
3rd Line	4 (18.2)	-
Paliative Chemotherapy Type		
Antracycline	4 (18.2)	-
Taxane-trastuzumab	5 (22.7)	-
Capecitabine-platin-taxane	4 (18.2)	-
Platin-gemcitabine. etoposide	5 (22.7)	-
Other	4 (18.2)	-

treatment response evaluation results were shown in Table 3.

Survival analysis

The median follow-up was 22.0 (range: 21-23) and 19.0 (range: 5-23) months in the early stage and metastatic groups, respectively.

In the metastatic group, according to initial CTC measurements, both median PFS and OS were significantly shorter in initial (before chemotherapy) CTC positive patients compared to initial CTC negative patients (PFS: 3.0 vs 13.0 months, Log-Rank p<0.001; and OS: 6.0 months vs. 19 months, Log-Rank p<0.001, figure 1 and 3). In the metastatic group, according to CTC measurements (between 9-12 weeks of chemotherapy) both median PFS and OS were significantly shorter in anytime CTC positive patients compared to anytime CTC negative patients (PFS: 4.0 vs 14.0 months, Log-Rank p=0.013; and OS: 8.0 months vs. 20.5 months, Log-Rank p<0.001, Figure 1B, 2B).

In metastatic group both PFS and OS were affected from multiple visceral metastatic sites (p=0.002 and p=0.055 respectively, table-4), higher grade (p=0.015 and p=0.044 respectively, table-4), initial CTC positivity (log rank p<0.001 and p<0.001 respectively, Table 4) and anytime CTC positivity (p=0.013 and p<0.001 respectively) and also radiological response of chemotherapy during chemotherapy cycles (both p<0.001, Table 4).

Both PFS and OS were not different according to stage of the tumor, ER, PR and CerbB2 status, age, ECOG performance status, adjuvant chemotherapy, radiotherapy or hormonotherapy. Since data about the grade of pathological specimens of the 9 patients were missing, correlation between grade and PFS or OS, was not considered to be clinically significant. Because of the

Table 3. Treatment Responses of Study Population According to Clinical, Radiological and Circulating Tumor Cell Change

		Metastatic Patients (n=22) n (%)	Non-Metastatic Patients (n=12) n (%)
ECOG Status	1	21 (95.5)	12 (100.0)
	2	1 (4.5)	0 (0.0)
ECOG Status (mid-term evaluation)			
	1	19 (86.4)	12 (100.0)
	2	3 (13.6)	0 (0.0)
Outcome at the end of study			
	Exitus	12 (54.5)	0 (0.0)
	Alive	10 (45.5)	12 (100.0)
Progression	Present	18 (81.8)	1 (8.3)
	Absent	4 (18.2)	11 (91.7)
CTC Status (Measured at any time)			
	Positive (≥5)	6 (27.3)	1 (8.3)
	Negative (<5)	16 (72.7)	11 (91.7)
CTC Change	Negative to Negative	16 (72.7)	11 (91.7)
	Positive to Positive	3 (13.6)	0 (0.0)
	Negative to Positive	3 (13.6)	0 (0.0)
	Positive to Negative	0 (0.0)	1 (8.3)
Radiological Evaluation (Mid-term)			
	Stabile Disease	11 (50.0)	No Recurrence
	Regression	6 (27.3)	No Recurrence
	Partial Regression	4 (18.2)	No Recurrence
	Progression	1 (4.5)	1 (8.3)

Table 4. Factors Related to Progression Free Survival and Overall Survival

Variables	P Value
Progression Free Survival	
Initial CTC (≥5 vs <5)	<0.001
CTC at anytime (≥5 vs <5)	0.013
Metastasis Site (Locoregional vs Multiple Site)	0.002
Grade (Grade II vs III)	0.015
Radiological Response (Regression vs Progression)	<0.001
Overall Survival	
Initial CTC (≥5 vs <5)	<0.001
CTC at anytime (≥5 vs <5)	<0.001
Metastasis Site (Locoregional vs MultipleVisceral Metastatic Sites)	0.055
Grade (Grade II vs III)	0.044
Radiological Response (Regression vs Progression)	<0.001

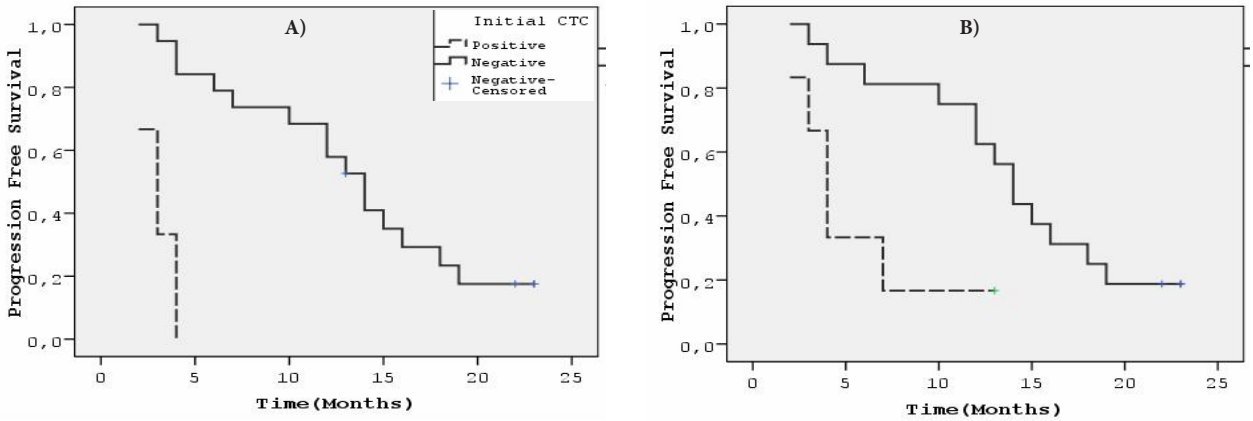

Figure 1. Kaplan-Meier Survival Curves Showing Progression Free Survival in Metastatic Breast Cancer Patients According. A) Initial CTC Status (positive vs negative): Ordinate (Y) represents cumulative progression free survival and axis (X) represents time (months). Progression Free Survival of patients (dotted line) who were initially CTC positive (CTC≥5) was shorter than patients (straight line) who were initially CTC negative (CTC<5). (Progression free survival 3,0 months vs 13,0 months; Log-Rank p<0,001). **B) CTC Status at Any Time of the Study (positive vs negative):** Ordinate (Y) represents cumulative progression free survival and axis (X) represents time (months). Progression Free Survival of patients (dotted line) who were CTC positive at any time of the study (CTC≥5) was shorter than patients (straight line) who were initially CTC negative (CTC<5). Progression free survival 4.0 months vs 14.0 months; Log-Rank p=0.013

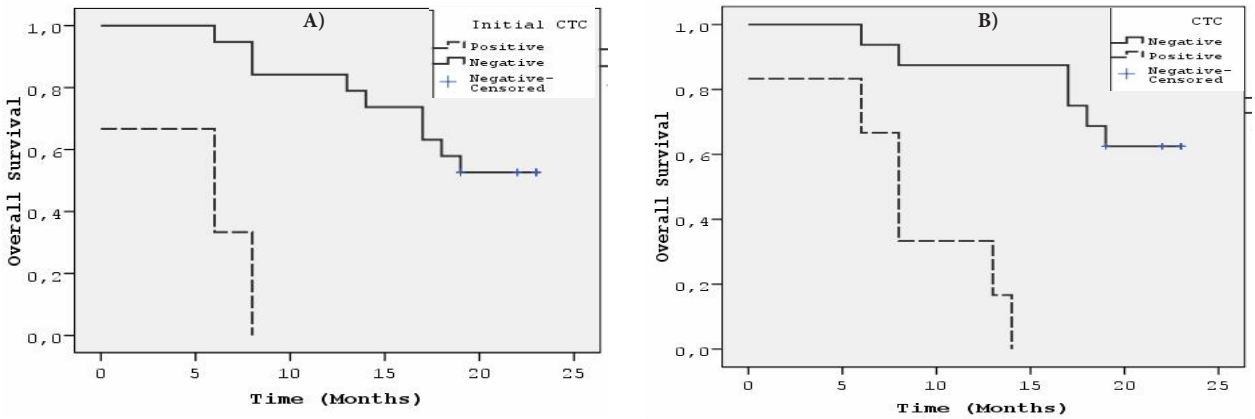

Figure 2. Kaplan-Meier Survival Curves Showing Overall Survival in Metastatic Breast Cancer Patients According. A) Initial CTC Status (positive vs negative). Ordinate (Y) represents cumulative overall survival and axis (X) represents time (months). Overall Survival of patients (dotted line) who were initially CTC positive (CTC≥5) was shorter than patients (straight line) who were initially CTC negative (CTC<5). (Overall survival 6.0 months vs 19.0 months; Log-Rank p<0,001). **B) CTC status at Any Time of the Study (positive vs negative).** Ordinate (Y) represents cumulative overall survival and axis (X) represents time (months). Overall Survival of patients (dotted line) who were initially CTC positive (CTC≥5) was shorter than patients (straight line) who were CTC negative at any time of the study(CTC<5). (Overall survival 8.0 months vs 20.5 months; Log-Rank p<0,001), CTC: Circulating Tumor Cell

limited number of patients in adjuvant treatment group, survival analysis was not performed, at all.

Discussion

The incidence of CTC in breast cancer was reported to be 12-50% in early setting and 25-80% in advanced and metastatic settings depending on the methods used in different clinical studies (Riethdorf S et al.,2008; Franken et al.,2012). In our study CTC was positive (≥5) in 3 (13.6%) patients before chemotherapy and 6 (27.3%) patients during chemotherapy in the metastatic group whereas CTC was positive in only one patient in early stage group before and at anytime during chemotherapy. There was only one recurrence in the adjuvant patient group, that gave an impression that this patient group had unintendly low risk for recurrence. However, this was a speculative result because of small number of patients and relatively short follow-up time in the adjuvant setting. It was recommended that more than a single cut-off point for positivity of CTC should be used for better risk classification especially in adjuvant trials because there has been no defined threshold point for CTC positivity in this group (Tibbe et al., 2007). It was reported that as CTC levels increased as a continous variable, there was a non linear risk of death in a study of 80 metastatic breast cancer patients (Botteri et al., 2010).

In the metastatic group in our study, both median PFS and OS were significantly shorter in CTC positive patients compared to CTC negative patients according to initial or anytime CTC measurements. It was previously reported similiar results in a randomised prospective multicenter study in 177 metastatic breast cancer (Cristofanilli et al., 2004). In a subgroup analysis of 83 metastatic breast cancer who had received first line chemotherapy further showed that OS was shorter in basal CTC positive and control CTC positive women at the 4th week of treatment than CTC negative women (Cristofanilli et al., 2005). They also concluded that CTC was an independent prognostic factor for prediction of progression free survival and overall survival (Cristofanilli et al., 2005). Similiar randomised study confirmed this results by detecting CTC positivity at baseline (before chemotherapy) and at the 14th week of chemotherapy (Hayes et al., 2006). Researchers claimed that CTC was correlated better with hematogenous dissemination rather than locally invasive disease (Nakagawa et al., 2007). They also believed that non metastatic breast cancer cases who was CTC positive, had greater risk of early distant metastasis than CTC negative breast cancers of same stage (Nakagawa et al., 2007). Prognostic significance of CTCs in metastatic breast cancer patients also confirmed in 185 newly diagnosed cases retrospectively (Dawood et al., 2008). CTC positivity (CTC≥5) was found to be an independent parameter and relative risk of death was 3.64 (CI: 2.11-6.30) (Dawood et al., 2008). They proposed that CTC positivity should be involved in staging of breast cancer and this high risk patients were a candidate for clinical trials of selected targeted therapies in order to eliminate CTCs (Dawood et al., 2008). Recently, CTC detection compared with serum tumor markers in a large prospective trial in first line chemotherapy for 267 MBC patients (Pierga et al., 2012). They confirmed that treshold of CTC≥5 was statistically significant for PFS and OS on multivariate analysis independently from serum tumor marker (Pierga et al., 2012).

In our study, in metastatic group both PFS and OS were also affected from multiple visceral metastatic sites (p=0.002 and p=0.055 respectively) and higher grade (p=0.015 and p=0.044 respectively), but not affected from the stage of the tumor, ER, PR and CerbB2 status, age, ECOG performance status, adjuvant chemotherapy, radiotherapy or hormonotherapy. Since grade data of the 9 patients were missing, correlation between grade and PFS and OS, were not considered clinically significant.

Radiological response of chemotherapy during

chemotherapy cycles was also correlated with better survival in our study (both p<0.001). Similar result was reported in 138 metastatic breast cancer patients (Budd et al., 2006). They found that both positive basal CTC and positive CTC at 4th weeks of study and better correlated with outcome than conventional radiological evaluation at 10th weeks of the study (Budd et al., 2006).

It has not well known how frequently and in which period of chemotreapy cycle was necessary to detect CTCs for prediction of survival benefit of chemotherapy yet. Researchers found a correlation between radiological progression and CTC positive patients (CTC≥5) in a 3th-5th weeks and 7th-9th weeks of chemotherapy in a heavily pretreated 68 metastatic breast cancer study population (Liu et al., 2009). Others confirmed this results in a 119 metastatic breast cancer patient population in a prospective multicenter study (Nakamura et al., 2010). They found that 7 of 11 (63.6%) of patients, whose CTC level increased 100% after one cycle of chemotherapy, had progressive disease by imaging at first follow up. They concluded that CTCs were highly correlated with imaging results before and after chemotherapy (Nakamura et al., 2010). A meta-analysis of the prognostic value of CTC in breast cancer showed that between January 1990 and January 2012, eligible 49 studies enrolling 6,825 patients were conducted (Zhang et al., 2012). The prognostic value of CTC was significant in both early (DFS: HR 2.86; 95%CI 2.19-3.75; OS: HR, 2.78; 95%CI 2.22-3.48) and metastatic breast cancer (PFS: HR, 1.78; 95% CI, 1.52-2.09; OS: HR, 2.33; 95% CI, 2.09-2.60). Further subgroup analyses showed that this results were stable irrespective of the CTC detection method and time point of blood withdrawal (Zhang et al., 2012).

In our study, because of limited number of patients in adjuvant treatment group, survival analysis were not performed. Since it is a rarer entity for adjuvant breast cancer patient group there were studies claimed that only a single CTC number might be important (Slade et al., 2009). Even in metastatic cancer patients it was reported that detection and phenotyping of CTC was challenging (Coumans et al., 2012). A randomized study investigated prognostic effect of CTC in a neoadjuvant trial of 115 locally advanced breast cancer patients (Bidard et al., 2010). They found that there was a correlation between presence of CTC (CTC≥1, detected by CellSearch method) and a shorter time to metastasis (p=0.01) and a worse overall survival (p=0.007) during 36 months follow up (Bidard et al., 2010). Other study confirmed that persistence of disseminated tumor cells after neoadjuvant treatment for locally advanced breast cancer predicts poor survival (Mathiesen et al., 2012).

As a result, CTC positivity was confirmed as a prospective marker even in our small patient population, in this single center study. Measurement of CTC by CellSearch method in metastatic breast carcinoma may be an indicative of early risk of relapse or death and even as less as two times of measurement during the whole chemotherapy program may be enough to have a decision but this finding should be confirmed with prospective trials in larger study populations.

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