RESEARCH ARTICLE

Prognostic Significance of Circulating Tumor Cells and Serum CA15-3 Levels in Metastatic Breast Cancer, Single Center Experience, Preliminary Results

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Abstract

Background: Breast cancer is the second leading cancer causing death in women. Circulating tumor cells are among the prognostic factors while tumor markers are of diagnostic value and can be used for follow-up. The aim of this study was to investigate the correlation between the prognostic significance of the serum CA15-3 levels, number of circulating tumor cells and histopathological tumor factors. Materials and Methods: Thirty patients recently diagnosed with breast cancer were included in the study. Number of circulating tumor cells and serum CA15-3 level were assessed when metastasis was detected and diagnostic value was assessed. Presence of associations with estrogen and progesterone receptors, c-erbB2, Ki-67 proliferation index and histological grade were also evaluated. Results: Median overall survival of the patients with serum CA15-3 levels of >108 ng/dl was 19 months whereas for those with a low serum level it was 62 months. Median overall survival for CTC ≥5vs CTC<5 patients was 19 months and 40 months respectively. The difference between the two groups was statistically significant. Conclusions: Prognostic significance of the CTC count and CA15-3 levels in metastatic breast cancer patients was demonstrated.

Keywords: Circulating tumor cells - metastatic breast cancer - ca15-3 - Turkey

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Introduction

Breast cancer (BC) is the second leading cancer causing death in women (Siegel et al., 2012). Despite the widespread use of the screening methods, 5% of the cases were diagnosed at the advanced stage. Spread of the BC cells native of the primary location through circulation is one cause leading to metastasis (Jacob et al., 2007; Fehm et al., 2008). Presence of the circulating tumor cells (CTC) was first demonstrated in 1869 (Asworth, 1869). Age at diagnosis, hormone receptor status, c-erbB2 status, lymph node metastasis are among prognostic factors of BC (Andreopoulou et al., 2008). Another prognostic factor described for the BC is the number of CTC (Cristofanilli et al., 2004; Budd et al., 2006; Hayes et al., 2006; Cristofanilli et al., 2007; Harris et al., 2007; Dawood et al., 2008; De Giorgi et al., 2009). Moreover it is suggested that CTC may be predictively effective for the evaluation of the response to the treatment (Tewes et al., 2009). Besides, antigens found in serum which are bound to tumor are of importance for their ability to reveal tumor biology and tumor burden. Those tumor markers are widely used for early detection of the cancer, disease follow-up and detection of the recurrence (Bosl et al., 1977; Fisher et al., 1997; Rustin et al., 2003; Duffy et al., 2001; Parker et al., 2004). The most widely used parameter in BC patients is CA-15-3 since CA15-3 concentrations are increased in 10% of patients with stage I, 20% with stage II, 40% with stage III, and 75% with stage IV BC.

In this study we planned to investigate prognostic significance of the CTC, CA15-3 levels and to determine whether a correlation exists between pathological tumor properties and CTC count and CA15-3 level.

Materials and Methods

30 patients followed up in our outpatient oncology clinic who were recently diagnosed with metastatic BC were included in this study. Their participation took place after an informed consent has been taken from every patient. Patients with a creatinine clearance of <50% according to Cockcroft formula, those with demonstrated infections, those underwent to blood transfusion in the last week and pregnant women were excluded. Peripheral

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venous blood samples were taken for CTC count and CA15-3 levels before palliative treatment was started. Data on estrogen receptor (ER), progesterone receptor (PR), c-erbB2, Ki-67 proliferation index, p53, histological grade which possess pathological tumor properties were recorded. Patients assessed for ER, PR, c-erbB2 and p53 were grouped as positive or negative. For Ki-67 proliferation index, patients were divided into two groups as those between 1-10% and those with >10%. According to histological grade they are classified into two groups as grade 2 and grade 3. For the CA15-3 level and CTC, patients are grouped into two as normal level (0-38 ng/ml) or high level and CTC ≥5/7,5 ml peripheral blood or CTC<5/7,5 ml peripheral blood respectively. Whether an association between CTC and CA15-3 levels exists, moreover whether CA15-3 levels and CTC counts correlate with pathological tumor properties and effects of CTC counts and CA15-3 levels on overal survival (OS) were all evaluated.

CTC detection: Peripheral venous blood samples were taken into 10 ml CellSave Preservative Tube before treatment was initiated. 7.5 ml of whole blood was mixed with 6.5 ml of buffer, and centrifuged for 10 minutes at 2450 rpm. CTC count was carried out in the first 72 hours by immunfluorescence using CellTracks®AutoPrep system (Allard et al., 2004).

CA15.3 detection: For serum CA15-3 levels, peripheral venous blood samples were taken into 8.5 ml blood vacuum collection tubes with gel and centrifuged for 10 minutes at 3000 rpm. The analysis was carried out on AdviaCentaur using chemiluminescence technique.

Statistical analysis

SPSS ver16 was used for statistical analysis. Between group comparisons were carried out using nonparametric spearman correlation test, Man-Whitney u test, chi-square test. You den index was also used for CA15-3 cut-off value.

Results

Thirty patients with metastatic BC were included in the study. Their demographics and respective findings are summarized in Table 1. Median age was 52 (30-65). Twenty one patients were presented with invasive ductal carcinoma histology, 2 patients had invasive lobular carcinoma, 1 patient had mucinous carcinoma, 1 patient had inflammatory carcinoma and 5 patients had mixed type breast cancer histology. Sixteen patients were ER positive whereas 14 patients were negative while 18 patients were PR positive and 12 patients were PR negative, 14 patients were assessed as p53 positive while 16 were p53 negative and finally 15 patients were c-erbB2 positive while 15 patients were negative. Of the 15 patients evaluated for Ki-67 proliferation 7 had a value of 1-10% and 8 had >10%. Seventeen patients had a CTC count of ≥5/7.5 ml (5-111/7.5ml). The mean value was calculated as 25.94 ±31.44/7.5 ml. CTC count of 13 patients varied between 0-4/7.5 cc. The mean CTC value observed in this group was 0.84±0.98/7.5 ml. CA 15-3 levels of the 25 patients were above the normal range while they fell between the normal range in 5 patients. High levels of CA15.3 observed in 25 patients were between 40-1748 ng/dl (mean value 284±383.44). At the time of metastasis, a correlation was demonstrated between the CTC count and the change in CA15.3 levels using Spaerman correlation analysis (p=0.006). 76.9% of those with CTC<5 was p53 negative while 23.1 of them were positive. 35.3% of the patients with CTC≥5 were p53 negative and 64.7 of them were p53 positive. A statistically significant relationship was demonstrated between p53 positivity and increased CTC value as well as p53 negativity and decreased CTC value (p=0.024). A statistically significant relationship was observed between decreased CTC value and grade 2 histology (p=0.04). No correlation was demonstrated between cerbB2 status, hormonal status and CTC count. 14 patients with bone metastasis, 13 patients with visceral metastasis and 3 patients with bone and visceral metastasis were included in the study. No correlation was observed between metastatic site and CTC count (p=0.237). CA15.3 value of the all 17 patients with CTC≥5 were above the normal values (p 0.005). Median OS of the whole patient group included in the study was found to be 25 months (95%CI; 17.3-32.6) (Figure 1). Median OS of the patients with CTC<5 was 40 months (95%CI; 22.4-57.5) while it was 19 months in those with CTC≥5 (95%CI; 16.3-21.6) (Figure 2). The difference between the median OS of CTC<5 and CTC≥5 patients was found to be statistically significant (p:0.035 Log rank test). No correlation was revealed between CA15.3 value and pathological properties of the tumor. Cut-off value of the CA15.3 calculated according to You den index was 108 ng/dl thus median OS in patients with CA15.3<108 ng/dl was 62 months (95%CI; 13.8-110.1) and in those with CA15.3≥108 ng/dl median OS was 19 months (95%CI; 12.4-25). The difference between the two groups was statistically significant (p:0.015, log rank test) (Figure 3). This significance was also retained in multivariate analysis (Table 2). 73.3% of the patients were lost during follow-up.

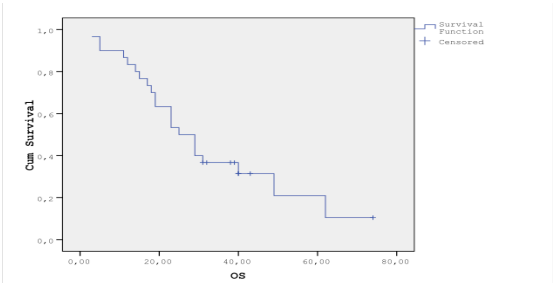

Figure 1. Median Overall Survival

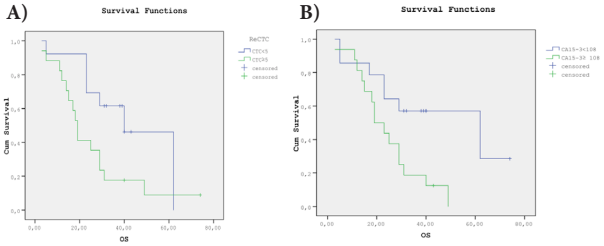

Figure 2. Median Overall Survival. A) CTC<5 versus CTC≥5, B) CA 15-3 <108 vs CA15-3≥108

Table 1. Patients Characteristics

		n	%
Patient number		30	
Age	Median	51	
	Range	30-65	
Histologic subtype	Invasive ductal carcinoma	21	70
	Miksed type carcinoma	5	16,8
	Invasive lobular carcinoma	2	6,6
	Others	2	6,6
Estrogen receptor	Positive	16	53
	Negative	14	47
Progesterone receptor	Positive	18	60
	Negative	12	40
P53	Positive	14	47
	Negative	16	53
cerbB2	Positive	15	50
	Negative	15	50
Ki-67 proliferation index (n=15)			
	%1-10	7	23
	>%10	8	26
CTC	≥5	17	56
	<5	13	44
CA15.3	Normal	5	16,8
	High	25	83,2
Histologic grade	2	22	73
	3	8	27
Metastasis site	Bone	14	46,7
	Visceral	13	43,3
	Visceral + Bone	3	10

Table 2. Multivariate Analysis

Risk Factor	Categories Compared	Hazar Ratio	95% CI	p
CA15.3	<108 and ≥108	0.377	(0.143-0.995)	**<0.049**
HG	2 and 3	0.122	(0.37-0.410)	**0.001**
Age		0.945	(0.899-0.993)	**0.026**

Discussion

In this study, CTC which is one of the prognostic factors in BC was evaluated in women diagnosed with breast cancer before treatment has been initiated, when metastasis is detected. Tumor marker CA15.3 was also measured in peripheral blood. Whether a relationship exists between CTC and CA15.3 level and pathological tumor properties and metastasis type was assessed. A correlation was observed between CTC count and the change in CA15.3 level. Moreover, increased CTC count and increased CA15.3 level were found to be related to patient survival. In clinical practice, use of CA15.3 level is not recommended for screening, diagnose and primary treatment monitoring for breast cancer (Harris et al., 2007). But in a study presented by Colomer et al., the disease free survival of patients with a postoperative CA15.3 level of <35 U/ml (which is the upper limit) was 25 months whereas in those with a high CA15.3 level it appeared to be 18.3 months. The authors also reported that disease free survival of the patients with CA15.3 levels of 40 U/ml was half of those exhibiting normal levels of CA15.3 (Colomer et al., 1989). Another study by Tampellini et al. demonstrated that median OS of the patients whose CA15.3 value was ≤30 kilounits at the time of metastasis was longer than those with higher CA15.3 level (Tampellini et al., 1997). On the other hand a study conducted by De La Lande et al. revealed that the time between the diagnose of breast cancer and detection of high levels of CA15.3 is prognostically significant (De La Lande et al., 2002). Appearance of high levels of tumor markers such as CEA and/or CA15.3 found in serum before symptoms of breast cancer appear or metastasis detected physically or radiologically is focus of interest. High levels of tumor markers are usually detected in 40-50% of patients with BC 3-18 months before the radiologic detection of metastasis (Ruibal et al., 1987; Kallioniemi et al., 1988; Colomer et al., 1989; Safi et al., 1989). In our present study CA15.3 level of the majority of the patients (83.2%) was above the normal range at the time when metastasis was detected using conventional methods and this was consistent with literature. The cut-off value calculated was 108 ng/dl and the median OS of the patients whose levels were below this cut-off value was determined as 62 months, while in those with high levels this survival was 19 months. Difference between these two durations was statistically significant and it is still obvious in multivariate analysis.

The first comprehensive prospective study conducted by Cristofanilli et al. on the prognostical significance of CTC count showed that in metastatic disease, CTC count assessed before the treatment and during the first visit after the treatment had affected disease free survival and OS. A total of 177 heterogeneously treated metastatic BC, including 83 patients receiving first line chemotherapy were enrolle (Cristofanilli et al., 2004). In a study by Dawood et al. assessing CTC count before chemotherapy in metastatic BC cases, CTC was found to be <5 in 61.6% and ≥5 in 38.4% of the patients. Median OS were 28.3 months and 15 months respectively. In-between difference was statistically significant (p<0.0001). Relatively better survival was irrespective of hormone receptor, her2 status and the treatment given (anthracycline, taxane, hormonal therapy) (Dawood et al., 2008). Giuliano et al evaluated prognostic and predictive significance of CTC count in metastatic BC patients receiving first line systemic treatment. Two hundred and thirty five patients were retrospectively evaluated. A correlation was found between the basaline CTC count and survival times. In multivariate analysis the basaline CTC count was confirmed as an independent predictor of PFS and OS (Giuliano et al., 2011). On the other hand, Takudome et al evaluated the basaline CTC count in heavily treated metastatic BC patients. In that study, only 28 heterogeneous patients were enrolled. The basaline CTC count did not contribute significantly to determine the prognosis (Tokudome et al., 2011). Consoli et al assessed CTC count as predictor of prognosis in metastatic BC. A total of 93 patients with metastatic BC were prospectively enrolled. CTC counts were detected at basaline and at the first follow-up examination. At multivariate analysis, the CTC count at basaline and at the first follow-up was found to be as a predictor of PFS and OS (Consoli et al., 2011). In the study by Bidard et al assessing CTC count and serum tumor markers in metastatic BC patients treated by first line chemotherapy, CTC count and CA15.3 level

were statistically significant for PFS (Bidard et al., 2012). Pierga et al assessed the prognostic and predictive value of CTC count compared with serum tumor markers in metastatic BC patients received by first line chemotherapy. In multivariate analysis, basaline CTC count was associated with an independent prognostic factor for PFS and OS. Patients with elevated basaline CTC count were likely to have at least one elevated serum tumor markers (CEA or CA15.3) (Pierga et al., 2012). As consistent with literature, median OS durations were found to be 40 months and 19 months for patients with low and high CTC counts respectively in our study. The difference between the two durations was statistically significant. CTC was ≥5 in 5% of the patients while 44% of the patients had a CTC count of <5. In an analysis conducted by Hayes et al. (2006) relatively high CTC counts observed before the initiation of palliative chemotherapy and during follow-up visits were found to be prognostically significant for PFS and OS. Effect of CTC count on median PFS and median OS was found to be irrespective of histology, hormone receptor status, HER2 status, de novo metastatic/recurrent disease status. Moreover when CTC was continuously monitored, CTC count observed as <5 at any visit was related to prolonged median PFS and median OS (Hayes et al., 2006). Retrospective studies comparing CTC response and response assessment using PET CT in metastatic breast cancer patients showed that CTC count was found to be <5 in 2/3 of the cases where a radiologic response was detected using PET CT; both radiologic response was assessed using PET CT and CTC count was calculated as >5 in 16% of the patients included in the study. It is demonstrated that CTC count and PET CT response would predict OS. Median OS of the patients with a low post-chemotherapy CTC count appeared to be longer than patients whose CTC count is >5, irrespective of radiologic response (Budd et al., 2006). In a study where response to chemotherapy was assessed every three months using CTC count and PET CT in metastatic disease, CTC response was consistent with the radiologic response evaluated by PET CT in 67% of the measurements. CTC response was determined as an independent risk factor for OS in multivariate analysis (De Giorgi et al., 2009). A relationship between CTC count and metastatic site was not observed in this study.

As literature points out, studies assessing the correlation between the CTC count and pathological tumor properties were usually conducted in patients diagnosed with operable breast cancer. In these studies the correlation between the CTC count and pathological tumor properties such as tumor size, hormonal status, HER2 status, nodal involvement characteristics, grade and histological type was evaluated. Only the HER2 status appeared to be correlated with CTC count (Rack et al., 2008; Lang et al., 2009). In our study a correlation between immunhistochemical q53 positivity and CTC count was detected when metastasis was observed. A correlation between CTC count and CA15.3 level was demonstrated as well.

In this study prognostic significance of the CTC count in metastatic BC patients was consistent with literature. CA15.3 level which is still not recommended by guidelines in routine practice, was demonstrated to be prognostically significant above the cut-off value of 108 ng/ml. Our next study plan would cover assessment of radiologic response to the treatment in metastatic breast cancer patients and assessment of the correlation between the CTC count and the change in CA15.3 level.

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