

Circulating tumor cells as predictors of prognosis in metastatic breast cancer: clinical application outside a clinical trial

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ABSTRACT

Aims and background. Circulating tumor cells have a prognostic role in metastatic breast cancer. The aim of the study was to confirm the ability of circulating tumor cells, detected by the US Food and Drug Administration approved Cell Search assay, to predict the outcome of patients treated in a community general hospital.

Patients and methods. A prospective mono-institutional study was conducted at the Department of Medical Oncology at Spedali Civili, Brescia, Italy, from January 2009 to September 2010. A total of 93 consecutive patients with metastatic breast cancer were enrolled. Patients underwent a blood sample collection to detect circulating tumor cells at baseline and, subsequently, at the first follow-up examination (after 3-4 weeks from the beginning of a systemic therapy). A third sample was drawn at disease progression (at the beginning of a subsequent new course of therapy). The prognostic cutoff value of circulating tumor cells was fixed at 5 cells/7.5 ml of blood.

Results. At baseline, median overall survival and progression-free survival in the subgroup ≥ 5 circulating tumor cells/7.5 ml of blood were significantly shorter (5 months and 3 months, respectively) than in the subgroup with < 5 circulating tumor cells (8 months and 7 months, respectively) ($P = 0.003$ and $P < 0.001$). At the first follow-up, the subgroup with more than 5 circulating tumor cells/7.5 ml of blood had a median overall survival of 4 months *versus* 8 months in the subgroup with < 5 circulating tumor cells ($P < 0.001$) and a median progression-free survival of 3 months *versus* 7 months respectively ($P < 0.001$). At multivariate analysis, the level of circulating tumor cells at the first follow-up and at baseline remained significant as a predictor of progression-free and overall survival. The number of metastatic sites was significantly associated with overall and progression-free survival and correlated with the number of circulating tumor cells.

Conclusions. Our study confirms the role of circulating tumor cells as predictors of prognosis in metastatic breast cancer patients treated in general clinical practice.

Introduction

Metastatic breast cancer (MBC) patients have a median survival of 2-4 years and represent 6-10% of newly diagnosed breast tumors^{1,2}. Given the increased number of treatment options, there is the need to monitor treatment^{3,4}. Response evaluation to treatment is recommended and consists of clinical examination, radiological studies and circulating tumor markers⁵. All these approaches have low sensitivity and therefore new biomarkers are required to select better patients at high risk or those not responding to a specific therapy⁵.

Circulating tumor cells (CTC) from blood may play a role in the metastatic process, and their identification has a prognostic role in MBC⁶. The detection of ≥ 5 cells per 7.5 ml of blood before starting a systemic treatment is associated with a worse prognosis.

Key words: breast cancer, circulating tumor cell, metastasis.

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Moreover, the persistence or the elevation of CTC, at any time during therapy, is also indicative of treatment failure and correlates with disease progression^{7,8}. Assessment of CTC provides an earlier indication of disease status than conventional imaging and a more accurate correlation with survival and has the potential to influence the clinical management for patient with MBC^{9,10}. Currently, the detection of CTC is only possible in a few centers: validation of the protocol would be of interest to any community general hospital, evaluating the implementation of this new diagnostic tool.

The aim of the study was to confirm the prognostic role of CTC, detected by the US Food and Drug Administration approved Cell Search assay^{6,11}, in order to better define the outcome of patients treated in a community general hospital.

Patients and methods

Study design

A prospective mono-institutional study was conducted at the Department of Medical Oncology at Spedali Civili, Brescia, Italy, from January 2009 to September 2010. Its aim was to evaluate the prognostic value of CTC in MBC patients. The Cell Search System (Veridex, LLC, Raritan, NJ, USA) has been available at Spedali Civili since January 2009.

Principal inclusion criteria were histological diagnosis of breast cancer, age higher than 18 years, and metastatic disease. Prior adjuvant and/or any type or line of therapy was allowed.

A total of 108 consecutive women was enrolled in the study: two patient groups were analyzed separately according to ECOG performance status and treatment.

Ninety-three patients with ECOG performance status between 0 and 2 were enrolled before the beginning of a new line of systemic therapy. These patients underwent blood sample collection to ascertain CTC at baseline and, subsequently, at the first follow-up examination (usually 3-4 weeks after the beginning of treatment). A third sample was drawn after radiological and/or clinical and/or biochemical evidence of disease progression (at the beginning of a subsequent new course of therapy). An association between CTC and patient characteristics was evaluated.

Patient evaluation consisted of imaging studies (CT, PET-CT, chest X-ray, abdominal ultrasound) and biochemical analyses and was performed at the beginning, at mid-therapy and at the end of treatment (at different intervals, depending on treatment and schedule).

Fifteen patients with terminal disease and ECOG performance status 3 or 4 underwent a blood sample collection to detect CTC at the beginning of supportive care.

Blood was also drawn from a control group of 10 healthy donors, who had no known history of cancer or illness.

The local ethics committee approved the study, and all patients enrolled provided written informed consent for inclusion in the study.

Isolation and enumeration of CTC

The Cell Search System (Veridex, LLC, Raritan, NJ, USA) was used for the isolation and enumeration of CTCs^{11,12}. Blood samples were drawn into 10 ml EDTA Vacutainer tubes, maintained at room temperature and analyzed within a maximum of 72 h after collection. CTC were defined as nucleated cells, expressing cytokeratins 8, 18 and 19 and lacking CD45.

In the MBC setting, the prognostic cutoff value of CTC was fixed at 5 cells/7.5 ml of blood, with a poor prognosis indicated by ≥ 5 CTC/7.5 ml of blood and a good prognosis defined as < 5 CTC/7.5 ml of blood^{6,12}.

Statistical analysis

The aim of the study was to confirm the prognostic value of CTC in an unselected cohort of patients. Kaplan-Meier estimates of survival were based on number of CTC at baseline and at the first follow-up, and curves were compared using logrank testing. Progression-free survival and overall survival were defined as the time between the date of the first blood sample and the date of clinical progression or death or the last follow-up examination, respectively.

A third blood sample was collected from those patients who experienced disease progression to treatment. In this subgroup, overall survival¹ was calculated from the date of the blood sample to the date of the last follow-up examination or death.

Statistical significance was defined as $P < 0.05$. Multivariate analysis was conducted using the Cox regression method. Statistical analysis was conducted using SPSS software (SPSS, Chicago, IL, USA, version 17).

Results

Patient characteristics

From January 2009 to September 2010, a total of 93 consecutive patients with ECOG performance status 0-2 were enrolled in the study before starting a systemic treatment. Median age was 58 years (range, 33-80). Most of the patients had been pretreated, and 40% of them were starting a third line of therapy. Sixty-three patients (68%) received chemotherapy, 13 (14%) hormone therapy and 17 (18%) trastuzumab (combined with chemotherapy or hormone therapy). The most frequent chemotherapy regimen chosen was a taxane monotherapy (37%). Seventy-seven patients had visceral metastasis and 23 had 3 or more organs involved. Patient and clinical characteristics at baseline are listed in Table 1.

Forty-four patients had CTC ≥ 5 cells/7.5 ml at baseline, 41 at the first follow-up examination, and in 83% of cases, CTC were over the cutoff level even at baseline.

Table 1 - Patient characteristics at baseline according to CTC level (performance status ECOG 0-2)

Characteristics (ECOG PS 0-2)		No. patients (%)	No. CTC <5 at baseline (%)	No. CTC ≥5 at baseline (%)	P
Age at enrollment (yr)	≤58	50 (53)	27 (54)	23 (46)	0.85
	>58	43 (46)	22 (51)	21 (49)	
Stage IV at diagnosis	Positive	18 (19)	11 (61)	7 (39)	0.14
	Negative	75 (81)	38 (51)	37 (49)	
Estrogen (Er) and progesterone (PgR) receptors	Er+ and/or PgR+	85 (91)	43 (51)	42 (49)	0.10
	Both negative	8 (9)	6 (75)	2 (25)	
Her-2	Positive	18 (19)	13 (72)	5 (28)	0.12
	Negative	75 (81)	36 (48)	39 (52)	
Ki-67	Low <15%	34 (36)	21 (62)	13 (38)	0.5
	Intermediate-high ≥15%	53 (57)	24 (45)	29 (55)	
	Unknown	6 (7)	4 (67)	2 (33)	
Disease progression	Visceral	77 (83)	40 (52)	37 (48)	0.12
	Non-visceral	16 (17)	9 (56)	7 (44)	
No. metastatic sites	1	24 (26)	18 (75)	6 (25)	0.03
	≥2	69 (74)	31 (45)	38 (55)	
Previous CT lines	0	26 (28)	15 (58)	11 (42)	0.11
	1	21 (23)	15 (71)	6 (29)	
	≥2	46 (49)	19 (41)	27 (59)	

ECOG PS, performance status; CTC, circulating tumor cells.

At the first follow-up (3-4 weeks after enrollment), a blood sample was not taken from 3 patients: 2 of them had died and the number of CTC at baseline was 14 and 155, respectively.

At disease progression, a third blood sample was drawn from 41 patients.

Median follow-up was 7 months (range, 1-20). Forty-eight patients died and 70 showed disease progression during the follow-up period. For the entire group of 93 patients, the overall median progression-free survival was 5 months (range, 1-19) and the median overall survival was 7 months (range, 1-20).

Fifteen patients with terminal disease and ECOG performance status 3 or 4 were analyzed separately. In this subgroup, all patients had more than 5 cells/7.5 ml of blood, and the median number of CTC was 670 (range, 18-9993). None were alive at the time of analysis, and median overall survival was 13 days (range, 8-30). Patient characteristics are listed in Table 2.

Specificity of the assay was tested analyzing each 7.5 ml of blood from 10 healthy donors. No CTC were found in these control samples.

Outcome in the subgroup with ECOG performance status 0-2

As regards the association between clinical response and CTC level at baseline, among 62 patients who experienced disease progression at clinical evaluation, 39 patients (63%) had CTC ≥5 at baseline and 40 patients (65%) at first follow-up.

The Kaplan-Meier curves, in Figures 1 and 2, show the

Table 2 - Patient characteristics with terminal illness (performance status ECOG 3 and 4)

Variables (ECOG PS 3-4)		No. patients (%)
Estrogen (Er) and progesterone (PgR) receptors	Er+ and/or PgR+	14 (93.3)
	Both negative	1 (6.7)
Her-2	Positive	1 (6.7)
	Negative	14 (93.3)
Disease progression	Visceral	15 (100)
	Non-visceral	0 (0)
No. of metastatic sites	1	1 (6.7)
	2	5 (33.3)
	≥3	9 (60)
Previous chemotherapy lines	0	3 (20)
	1	2 (13.3)
	≥2	10 (66.7)

ECOG PS, performance status.

association between overall survival or progression-free survival and CTC level at baseline (CTC1°). At baseline, the patient subgroup with CTC ≥5/7.5 ml of blood had a significantly inferior overall survival (median, 5 months; 95% confidence interval (CI), 3 to 6.9; $P = 0.003$) and progression-free survival (median, 3 months; 95% CI, 2.1 to 3.8; $P < 0.001$) compared to the group with CTC <5/7.5 ml of blood (median overall survival, 8 months; 95% CI, 6 to 9.9) (median progression-free survival, 7 months; 95% CI, 6.1 to 7.8).

Figures 3 and 4 show Kaplan-Meier curves for the levels of CTC at the first follow-up (CTC2°). In the subgroup of 41 patients with more than 5 CTC/7.5 ml of blood,

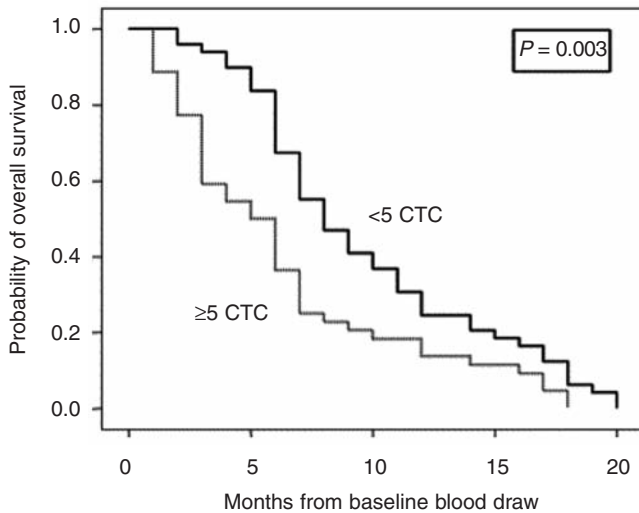


Figure 1 - Overall survival (OS) stratified by circulating tumor cell (CTC) levels at baseline (CTC1°).

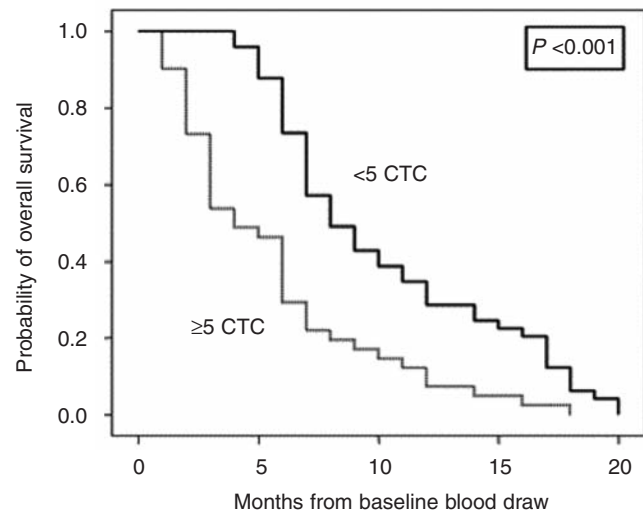


Figure 3 - Overall survival (OS) stratified by circulating tumor cell (CTC) levels at the first follow-up (CTC2°).

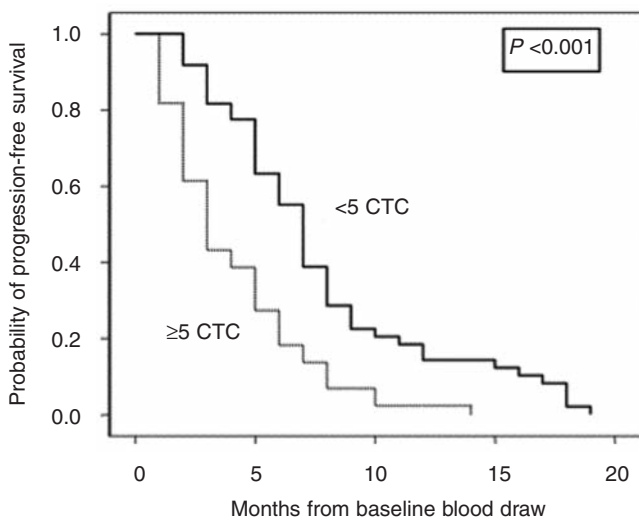


Figure 2 - Progression-free survival (PFS) stratified by circulating tumor cell (CTC) levels at baseline (CTC1°).

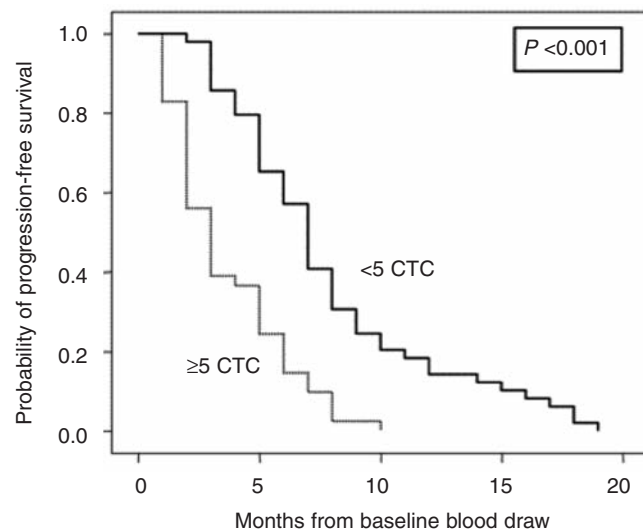


Figure 4 - Progression-free survival (PFS) stratified by circulating tumor cell (CTC) levels at the first follow-up (CTC2°).

median overall survival and progression-free survival were significantly shorter (4 months, 95% CI, 2.6 to 5.3 months; 3 months, 95% CI, 2.1 to 3.8) than the subgroup with less than 5 CTC (8 months, 95% CI, 6 to 9.9; and 7 months; 95% CI, 6.1 to 7.8).

Outcome at disease progression in the subgroup with ECOG performance status 0-2

At disease progression, a blood sample was collected from 41 patients. Median overall survival from the beginning of enrollment in the study was 9 months (range, 4-20), whereas overall survival¹ (from the time of the third blood sample drawn to the last follow-up/death) was 3 months (range, 1-12): 22 patients (54%) died dur-

ing the study. At the time of progression, CTC were higher than 5 cells/7.5 ml in 28 cases (68%). The value of CTC (CTC3°) was statistically correlated to overall survival (overall survival from the beginning of enrollment in the study to the last follow-up/death) ($P = 0.04$) and to overall survival¹ (from the third blood sample to the last follow-up/death) ($P = 0.004$) (Figure 5).

Univariate and multivariate analysis in the subgroup with ECOG performance status 0-2

At univariate analysis, only the number of metastatic sites and the level of CTC at baseline and at the first follow-up were associated significantly with overall and progression-free survival. At multivariate analysis, the

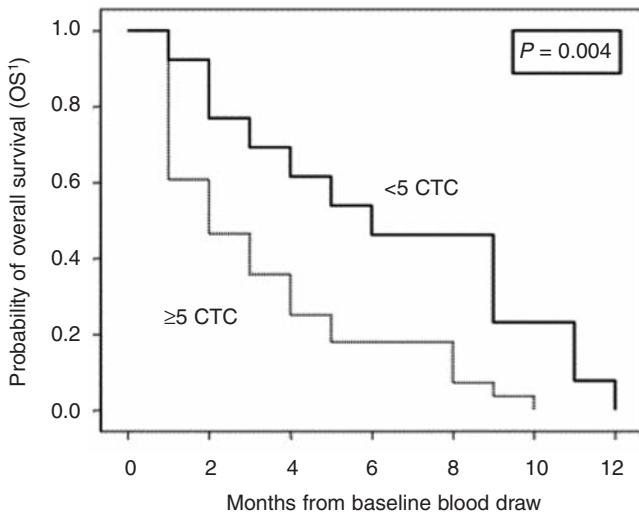


Figure 5 - Overall survival (OS) from the time of the third blood drawn, according to CTC levels (\geq or $<5/7.5$ ml of blood) (CTC³).

number of CTC at baseline and at the first follow-up were strongly associated to progression-free survival and overall survival (Tables 3 and 4).

ECOG performance status was closely associated to overall survival and progression-free survival, evaluating the entire population ($P < 0.001$).

Discussion

MBC is a heterogeneous disease, and the main goal of systemic treatment is the prolongation of survival, reduction of tumor burden and palliation of symptoms¹. The choice of treatment is based on prognostic and predictive factors, and its therapeutic efficacy must be balanced with side effects and quality of life^{2,4}.

Our study confirmed the prognostic role of CTC^{6,8,9}; the detection of ≥ 5 CTC per 7.5 ml of blood before starting treatment is both a strong and independent predic-

Table 4 - Multivariate Cox regression analysis at first follow-up. The table shows variables with statistically significant correlation with progression-free survival (PFS) and overall survival (OS)

Variable	Hazard ratio (95% CI), PFS	P, PFS	Hazard ratio (95% CI), OS	P, OS
CTC at first follow-up (≥ 5 vs <5)	3.22 (1.96-5.31)	<0.001	2.76 (1.73-4.39)	<0.001
No. of metastatic sites (≥ 2 vs 1)	1.81 (1.09-3.02)	0.02	1.74 (1.06-2.86)	0.02
Her-2 (positive vs negative)	1.76 (0.99-3.11)	0.051	1.96 (1.10-3.49)	0.02

CTC, circulating tumor cell.

tor of worse progression-free survival and overall survival in MBC. In addition, the serial assessment of CTC levels, over the course of treatment, provides an estimate of treatment response and clinical outcome^{6,7}. After the beginning of a new therapy, 67% (n = 62) of patients experienced disease progression and 65% had more than 5 CTC/7.5 ml of blood at the first follow-up examination. The increase in CTC during treatment could convert the outcome from favorable to unfavorable, as revealed by the fall in median progression-free survival (3 months) and overall survival (4 months) in our cohort of patients. At multivariate analysis, the level of CTC at the first follow-up and at baseline remained significant as a predictor of progression-free survival and overall survival.

CTC enumeration maintained the same prognostic value independently of the line of therapy. Most patients had been pretreated and 40% were starting a third-line therapy. In the same patient, the CTC count could be reassessed at the beginning of a new therapy, as well. Overall survival was affected by the increase in CTC at any point in time (as represented in the subgroup defined CTC³).

The palliative effect of metastatic treatment was obtained through the reduction of tumor burden¹. The level of CTC is correlated to the number of metastatic sites, and both affected independently the outcome of our cohort of patients. Their detrimental effects may also combine, thus worsening the outcome ($P = 0.038$). This is more evident in the subgroup of patients with terminal illness, where a higher CTC number is associated with advanced disease: blood and solid organs are invaded by cancer cells as in leukemia. The correlation between the number of metastatic sites and the number of CTC suggests that CTC may be used in staging advanced disease.

CTC detection is suitable for routine assessment of metastatic breast cancer, as demonstrated by the analysis of our cohort of unselected patients, outside a clinical trial. Peripheral blood is an ideal source for the de-

Table 3 - Multivariate Cox regression analysis at baseline. The table shows variables with statistically significant correlation with progression-free survival (PFS) and overall survival (OS)

Variable	Hazard ratio (95% CI), PFS	P, PFS	Hazard ratio (95% CI), OS	P, OS
CTC at baseline (≥ 5 vs <5)	2.02 (1.29-3.17)	0.002	1.60 (1.04-2.45)	0.03
N. of metastatic sites (≥ 2 vs 1)	1.76 (1.05-2.94)	0.03	1.59 (0.96-1.54)	0.06

CTC, circulating tumor cells.

tection of CTC because of the noninvasive sampling procedure¹¹. The aim of our study was demonstration of the application of the assay in clinical practice. In particular, CTC enumeration was included as part of risk assessment of MBC patients behind clinical, pathological and biochemical parameters^{1,2}: the presence of CTC may predict the presence of a more aggressive disease. At the moment, measurement of CTC should not be used to influence treatment decisions, and additional validations are needed to confirm their clinical value⁵. CTC count should be the object of clinical trial to investigate their role for the optimization and personalization of treatments, stratifying patients who are starting a new therapy. However, CTC detection does not modify clinical management of patients with terminal disease, where the utility of the technique is limited.

In conclusion, our study provides evidence of the role of CTC as predictors of prognosis in the metastatic setting. Many questions regarding how the metastatic process is executed remain as yet unanswered¹³, and one of the main objectives is to identify how CTC establish metastases. A better understanding of their biology^{14,15}, as well as the availability of more specific and sensitive techniques to identify and characterize these CTC better, might lead to more accurate risk assessment and may also provide new therapeutic targets.

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