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Circulating Tumor Cells in Metastatic Breast Cancer: A Prognostic and Predictive Marker

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Abstract

The role of circulating tumor cells (CTCs) as a marker for disease progression in metastatic cancer is controversial. The current review will serve to summarize the evidence on CTCs as a marker of disease progression in patients with metastatic breast cancer. The immunohistochemistry (IHC)-based CellSearch® is the only FDA-approved isolation technique for quantifying CTCs in patients with metastatic breast cancer. We searched PubMed and Web of Knowledge for clinical studies that assessed the prognostic and predictive value of CTCs using IHC-based isolation.

The patient outcomes reported include median and Cox-proportional hazard ratios for overall survival (OS) and progression-free survival (PFS). All studies reported shorter OS for CTC-positive patients versus CTC-negative. A subset of the selected trials reported significant lower median PFS for CTC-positive patients. The reported trials support the utility of CTC enumeration for patient prognosis. But further studies are required to determine the utility of CTC enumeration for guiding patient therapy. There are three clinical trials ongoing to test this hypothesis. These studies, and others, will further establish the role of CTCs in clinical practice. (*J Patient-Centered Res Rev.* 2014;1:85-92.)

Keywords

biomarker, advanced breast cancer, circulating tumor cells, progression, outcome

Introduction

Breast cancer is the leading non-skin cancer diagnosed in females in the United States, with more than 200,000 new cases reported per year.¹ Metastatic breast cancer (MBC) can present at initial diagnosis or after recurrence. While

treatments for MBC are not considered curative, development of targeted biologic therapies and chemotherapy have significantly increased survivals.^{2,3} Current prognostic factors for MBC include Eastern Cooperative Oncology Group patient performance status, the site of metastatic disease, the number of disease sites, estrogen receptor status, progesterone receptor status, human epidermal growth factor receptor-2 (HER2/neu) expression, progression-free interval, prior adjuvant therapy, and prior therapy for MBC.⁴⁻⁷ In addition to these initial tumor features, various techniques are used to measure tumor response or disease progression while on anticancer treatments. These include various imaging modalities that detect site of disease, quantify tumor volume,^{8,9} and detect glucose uptake.¹⁰⁻¹³ Unfortunately, imaging studies fail to capture tumor heterogeneity, are expensive and time consuming, and because they are done sporadically, may not provide timely detection of therapeutic resistance.¹⁴⁻¹⁶ Because of these shortcomings, various assays have been developed to measure blood-based biomarkers including CA15-3 and CA27.29, carcinoembryonic antigen (CEA) and CA-125.¹⁷⁻¹⁹ However, only 50% to 60% of MBCs have a positive tumor marker to follow, and prospective studies validating their clinical utility are still limited.²⁰⁻²³

More recently, circulating tumor cells (CTCs) isolated from blood have been tested as a new prognostic tool and as markers of disease progression.²⁴⁻⁴² Using CTCs as a biomarker affords the advantage of capturing cells that are biologically relevant to the metastatic process.^{43,44} CTCs are a rare population of cells of epithelial origin detectable in the blood of cancer patients.^{24,26} The presence of CTCs in the blood was documented more than a century ago by T. R. Ashworth, an Austrian pathologist who first reported this type of cell.⁴⁵ Through the years, researchers have used various techniques for isolating CTCs including microfluidics,⁴⁶⁻⁵⁰ antibody-coated magnetic beads combined with immunohistochemistry (IHC)^{27,51,52} and multiplex polymerase chain reaction (PCR).⁵³⁻⁵⁵ Using these techniques, researchers have reported worse

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prognoses with higher CTC counts in patients with breast,^{27-29,31,32,34,35,40,54,56-59} colon,^{25,60,61} prostate^{30,46,62,63} and lung cancer.⁶⁴⁻⁶⁷

Currently, CellSearch® (Veridex LLC, Raritan, NJ) is the only U.S. Food and Drug Administration (FDA)-approved technique for quantifying CTCs in patients with metastatic cancer. The CellSearch® isolation is a multistep process involving an initial CTC enrichment stage using magnetic particles recognizing the epithelial cell adhesion molecule (EpCAM). This enrichment concentrates CTCs from 7.5 ml of blood to 300 µl. The cell concentrate is stained for epithelial cell markers (cytokeratin-8, -18, -19), nucleic acid dye 4, 2-diamidino-2-phenylindole dihydrochloride (DAPI), and a leukocyte-specific marker (CD45). The stained cells are imaged and analyzed using semiautomated image analysis. In this assay, a CTC is defined as a DAPI positive, cytokeratin positive and CD45 negative cell. The positive threshold is set at 5 CTCs per 7.5 ml of blood. The positive selection using EpCAM-coated ferromagnetic beads enables binding of multiple ferromagnetic particles to a single cell through the use of biotin/avidin chemistry, thereby amplifying the magnetic load. This chemistry increases CTC capture and sensitivity, but leads to nonspecific binding to leukocytes. The cell isolate is stained, enabling one to distinguish CTCs from leukocytes and cell debris.

Independent research has previously validated the analytical performance of the CellSearch® system.⁶⁸ The inter- and intra-assay variability are very low at <5%. Furthermore, shipping and storage up to 72 hours has minimal effects on CTC detection, if samples are stored at room temperature. Longer durations of storage increase unassigned events, cells requiring identification by the user, and the time required for analysis. The recovery efficiency of cancer cell lines diluted in healthy blood is reported to be 80%.⁶⁸

One limitation of the CellSearch® method is the positive selection of CTCs using EpCAM-antibody coated beads. While a significant portion (80%-100%) of breast cancers express EpCAM,⁶⁹⁻⁷¹ studies indicate lower EpCAM expression in CTCs and disseminated tumor cells.⁷² Furthermore, there is decreased EpCAM expression with epithelial-mesenchymal transition.⁷³ This suggests that selection of CTCs based on EpCAM expression may reduce CTC capture. The avidin/biotin chemistry utilized by CellSearch® is designed to overcome this decrease in EpCAM antigen expression by amplifying the magnetic load per antigen.

A second weakness of CellSearch® is the use of specific cytokeratins to identify CTCs. Downregulation of cytokeratins occurs with epithelial-mesenchymal transformation.^{74,75} The possible reduction in expression of cytokeratins by CTCs is addressed in CellSearch® by using pan-cytokeratin antibodies.

In the 2007 American Society for Clinical Oncology (ASCO)²⁰ update of recommendations for tumor biomarkers in breast cancer, the available evidence for CTC measurements in patients with advanced breast cancer was reviewed. The 2007 ASCO guidelines recommended against use of CTC measurements pending further evidence and validations. However, several studies regarding the role of CTCs as a prognostic factor or as a marker of disease progression have been published since the ASCO review. One meta-analysis has addressed the use of PCR-based techniques for quantifying the presence of CTCs as a biomarker.⁵⁶ But PCR-based techniques fail to give information on the morphology and other features of the tumor cells present in the peripheral blood and there is no FDA-approved technology for PCR-based techniques (Table 1). No reviews or meta-analyses have addressed the use of IHC-based CTC isolation techniques as a marker for patients with metastatic breast cancer.

Table 1. Advantages and disadvantages of current CTC isolation techniques

	Approach	Advantage	Disadvantage
IHC-based	Cell search	Cell morphology	Multistep
	CellSearch	Cell enumeration	Positive selection
	CTC chip	Multiple stainings	with EpCAM
	Filter-based µCell concentrator	Localization of stainings	
PCR-based	CK19	Multiplex	High background
	Mammoglobin		Limited to CTC specific markers
	CEA		Low cell number
			No cell enumeration

CEA, carcinoembryonic antigen; CTC, circulating tumor cell; CK19, cytokeratin-19; EpCAM, epithelial cell adhesion molecule; IHC, immunohistochemistry; PCR, polymerase chain reaction.

The current review will serve to summarize published articles addressing the use of IHC-based CTC isolation as a marker in patients with metastatic breast cancer. The aim of this review is to assist medical oncologists and oncology nurse practitioners in understanding the potential use of CTCs in clinical practice, and to provide guidance for future research needs on CTCs as a marker in patients with MBC.

Methods

Literature Search

The databases PubMed and Web of Science were systematically searched for all relevant articles reporting human studies published from January 2001 to September 2013 (past 12 years). The Medical Subject Headings (MeSH terms)/keywords used included “circulating tumor cells,” “neoplastic cells, circulating,” “breast neoplasms” or “breast cancer,” and “prognostic*” or “outcome*.” Reviewed publications were limited to articles. The reference list was checked for relevant articles that contained retrospective or prospective studies of patients with metastatic breast cancer.

Inclusion and Exclusion Criteria

The search results were then screened according to the following inclusion criteria: (1) outcome measurements reported had to include either median progression-free survival (PFS) or overall survival (OS) or both; (2) measurements had to include CTC enumeration defined as EpCAM-positive, DAPI-positive, cytokeratin-positive and CD45-negative cells; (3) circulating tumor cells had to be isolated from venous blood.

Articles were excluded if they reported clinical trials involving patients that received surgery during the course of the study. The article search for this review did not include unpublished literature, conference abstracts, or dissertations. No language restrictions were placed on this search, but following the initial review of English abstracts, no non-English articles were selected. The references of selected articles were searched by hand for additional studies, but no unique publications were identified by this search.

Data Extraction

The first author (FMH) screened and retrieved the literature list using the mentioned criteria. The reviewer (FMH) was not blinded to the article title or authors. The data extracted from each article included CTC characteristics, median PFS, and OS. Within the selected articles, radiologists were blinded to the patient clinical progression and CTC characteristics.

Data Analysis

The selected studies were assessed unblinded by the authors using the critical appraisal of prognostic test questionnaire from the Center for Evidence Based Medicine (CEBM) at University of Oxford (www.cebm.net). The critical appraisal questionnaire does not provide a numeric score, but it contains a set of questions appraising the validity of the clinical trial, with three possible answer options, “yes,” “no” or “unclear.” An article was included in this review if reviewers answered

“no” or “unclear” to only one CEBM appraisal question and “yes” to all other appraisal questions (Table 2). Since the study appraisal did not provide a numeric value, intrarater reliabilities were not analyzed.

Statistical Analysis

We recorded the reported median and Cox hazard ratio values for PFS and OS, in addition to their 95% confidence intervals.

Results

The current review only included clinical studies that reported PFS, OS, or both in patients with MBC. The initial search with “breast neoplasms” and “neoplastic cells, circulating” or other keywords representing breast cancer and CTCs produced a broad selection of articles from PubMed and Web of Science. The additional keywords “outcome*” and “prognosis*” were added, producing 164 articles from PubMed and 41 from Web of Science (searched in September 2013). After further screening of the abstracts, 17 articles were selected based on the inclusion and exclusion criteria. The main reason for exclusion included: studies involving patients with nonmetastatic breast cancer, clinical trials using non-IHC based CTC isolation, and studies not reporting PFS or OS. From the selected articles, none were excluded based on their CEBM questionnaire.

All of the selected articles with the exception of one study used the FDA-approved CellSearch® technique for isolating CTCs (Table 2). While other approaches have been developed and tested in research settings, the articles reporting these techniques did not meet the inclusion and exclusion criteria for this review. Gradilone et al.⁵⁴ reported the only study implementing a different CTC identification method. This study used magnetic bead selection coupled with multiplex PCR to study CTC gene expression of chemotherapy resistance proteins.

Studies using CellSearch® to enumerate CTCs reported differences in median PFS or OS at the cut-off of 5 CTCs/7.5 mL blood (Tables 3 and 4).^{27,29,31,32,34,35,57,76-80} All studies reported decreased OS of CTC-positive versus CTC-negative patients. These reported measurements included changes in median OS and the Cox proportional hazard ratio. A subset of the selected trials reported lower median PFS between CTC-positive and CTC-negative patients. One study reported a significant difference in OS between the CTC-positive and CTC-negative patients, but reported a limited difference in PFS.⁷⁶ The inconsistency of OS and PFS in this trial may be due to the limitations of PFS as a marker for OS.

Table 2. Summary of reviewed studies, isolation method and review based on Oxford prognostic checklist

Author	Year	# of participants	Method	Follow-up time	Oxf Prog checklist	Isolation method
Cristofanilli et al. ²⁷	2004	177	Prospective	9 months	All yes	CellSearch
Budd et al. ³⁵	2006	138	Prospective	Median 28 months	All yes	CellSearch
Hayes et al. ²⁹	2006	177	Prospective	30 months	All yes	CellSearch
Cristofanilli et al. ⁵⁷	2007	151	Retrospective	Median 12.7 months	Yes, except patient stage at enrollment is not mentioned	CellSearch
Nole et al. ⁴¹	2008	60	Prospective	Median 34 weeks	All yes	CellSearch
De Giorgi et al. ³¹	2009	115	Retrospective	40 months after mid-therapy	Yes, no separation of other prognostic groups	CellSearch
Liu et al. ³⁴	2009	81	Prospective	Median 13.3	Yes, no separation of other prognostic groups	CellSearch
Bidard et al. ³⁷	2010	67 patients (4 nonoperable locoregional relapse)	Prospective	Median 8.8 months	All yes	CellSearch
De Giorgi et al. ³²	2010	195	Retrospective	36 months	All yes	CellSearch
Botteri et al. ⁵⁹	2010	80	Prospective	Median 28 months	All yes	CellSearch
Gradilone et al. ⁵⁴	2011	42	Prospective	Median 24 months	All yes	EpCAM-coated beads (CELLlection Dynabeads) + Multiplex PCR
Harkopf et al. ³⁶	2011	58	Retrospective	Median 13.2 months	Yes, except no separation of different prognostic determinants	CellSearch
Giuliano et al. ³³	2011	235	Retrospective	Median 18 months	All yes	CellSearch
Muller et al. ⁷⁶	2011	253	Prospective	Median 11 months	All yes	CellSearch
Pierga et al. ⁸⁰	2012	267	Prospective	Median 14.9 months	All yes	CellSearch
Giordana et al. ⁷⁷	2012	517	Retrospective	Median 24.6 months	All yes	CellSearch
Wallwiener et al. ⁷⁹	2013	486	Prospective	Median 11.1 months	All yes	CellSearch

EpCAM, epithelial cell adhesion molecule; PCR, polymerase chain reaction.

Several studies compared CTC counts with imaging modalities including fluorodeoxyglucose positron emission tomography (PET) and computed tomography (CT) imaging. Multivariate analysis of CTC enumerations indicated that CTCs provide additional prognostic information that is unavailable with imaging studies.^{29,32,57} Furthermore, baseline CTC enumeration better predicted median OS compared to PET-based techniques.³¹ Interestingly, the CTC counts were dependent on the site of metastasis, in addition to the level of metastatic involvement. For example, patients with bone and visceral metastases had higher CTC counts compared to patients with bone-only metastasis.³²

Most studies did not control for type of anticancer therapy. The therapies used in the reviewed articles included various endocrine therapies targeting the hormone receptors, agents targeting the HER2 and vascular endothelial growth factor receptors, and traditional chemotherapy. In one reviewed

study,³⁷ the cut-off of 3 CTCs per 7.5 ml of blood was associated with a shorter time-to-disease-progression in patients with MBC receiving the antiangiogenic drug bevacizumab.³⁷ Angiogenesis is a necessary step for intravasation of CTCs into the blood; therefore, the lower cut-off in this study for CTC-positive samples may be due to the impairment of angiogenesis by bevacizumab. The ability of CTCs to predict a prognosis, independent of patient therapy, further supports their use as a biomarker.

In addition, multiple trials evaluated CTCs at specific study intervals, and reported significant differences in outcomes based on CTC status changes during treatment, suggesting the role of CTCs as a marker of disease progression. Hayes et al. reported similar median OS between baseline CTC negative participants and patients that converted from CTC positive to CTC negative during the study.²⁹ Furthermore, all studies reported that in response to therapy, there was a

Table 3. Median progression free survival (PFS) values for CTC-positive and CTC-negative patients, and Cox proportional hazard ratios (HR) based on univariate analysis

Author	Year	Median PFS CTC<5, CTC≥5 (month)	95% CI PFS CTC<5, CTC≥5 (month)	Cox HR
Cristofanilli ²⁷	2004	7.0, 2.7	(5.8-8.9),(2.1-4.4)	1.76
Nole ⁴¹	2008	>11.5, 5.1	N/A	2.5
Liu ³⁴	2009	5.1, 3.2	(3.1-6.7),(2.5-6.2)	1.4
Gradilone ⁵⁴	2011	16.3, 9.2	N/A	N/A
Giuliano ³³	2011	12.0, 7.0	(9.6-14.3),(5.8-8.2)	1.72
Muller ⁷⁶	2011	10.9, 9.3	(9.4-12.5),(7.8-10.9)	N/A
Pierga ⁸⁰	2012	16.0, 8.2	N/A	1.9
Giordano ⁷⁷	2012	6.3, 5.8	(5.3-7.3),(5.0-6.7)	1.23
Wallwiener ⁷⁹	2013	7.6, 4.8	(5.9-9.3),(3.9-5.6)	1.82

CI: confidence interval; CTC: circulating tumor cell.

Table 4. Median overall survival (OS) for CTC-positive and CTC-negative patients, and Cox proportional hazard ratio (HR) based on univariate analysis

Author	Year	Median OS CTC<5, CTC≥5 (month)	95% CI OS CTC<5, CTC≥5 (month)	Cox HR	95% CI (Cox HR)
Cristofanilli ²⁷	2004	>18, 10.1	N/A,(6.2-14.6)	4.26	N/A
Budd ³⁵	2006	22.6, 8.5	(20.1 to >25.0),(6.2-15.1)	3.18	(2.0-5.9)
Hayes ²⁹	2006	21.9, 10.9	(20.1 to >25),(6.4-15.1)	2.45	(1.6-3.7)
Cristofanilli ⁵⁷	2007	29.3, 13.5	N/A	2.3	(1.3-4.1)
De Giorgi ³¹	2009	28.0, 6.2	(17.2 to >18.2), (5.9-15.7)	1.3	(0.7-2.6)
De Giorgi ³²	2010	34.8, 18.5	(23.5 to >40),(13.6-85.0)	2.2	(1.4-3.5)
Hartkopf ³⁶	2011	>24, 9.8	N/A, (7.3-12.3)	N/A	N/A
Giuliano ³³	2011	40.1, 21.9	(34.9-45.4),(15.6-28.4)	2.5	(1.6-3.9)
Muller ⁷⁶	2011	20.1, 14.0	(18.8-21.5),(12.8-21.5)	2.48	(1.5-4.4)
Pierga ⁸⁰	2012	>33, 19.8	N/A	2.4	(1.1-5.4)
Giordano ⁷⁷	2012	32.4, 18.3	(25.3-39.5),(15.5-21.1)	2.1	(1.7-2.7)
Wallwiener ⁷⁹	2013	>23, 18.01	N/A	4.8	(2.9-7.8)

CI: confidence interval; CTC: circulating tumor cell.

significant drop in absolute CTC counts and the number of CTC positive patients.^{27,31,36} Based on these earlier results, a clinical trial was initiated by the Southwest Oncology Group (SWOG S0500; NCT00382018, www.clinicaltrials.gov) to test whether women with MBC and with elevated CTCs after one course of first-line chemotherapy would benefit from a switch in chemotherapy. This would differ from the current clinical standards, because the switch in therapy would occur prior to identifiable clinical progression based on standard clinical or imaging criteria. The preliminary results of this trial have now been reported and the primary endpoint of improved median OS was not met.⁸¹ In addition to the SWOG trial, a second trial (CirCè01) has been initiated to further characterize the role of CTCs as predictive markers of response to therapy. The CirCè01 is a phase III multicenter clinical trial using changes in CTC counts to

guide the choice of third-line chemotherapy for MBC. In this trial, CTCs will be measured prior to the addition of a new chemotherapy drug and, if there is little change in CTC counts after two weeks, a new chemotherapy will be initiated (NCT01349842). The final results from these trials will serve to determine the utility of changes in CTC enumeration on guiding patient therapy, and will better establish the role of CTCs in clinical practice.

Conclusion

The discovery of circulating tumor cells and the development of different isolation methods has led to an expanding area of research. The prognostic value of baseline CTCs using the Veridex method at the defined 5 cells/mL cutoff in metastatic breast cancer is clear. Published studies have correlated CTC counts with MBC to tumor burden, PET and CT-based

staging, site of metastasis, level of metastatic involvement, OS and PFS.

Despite the prognostic significance of CTCs, their role as a predictive marker remains unknown. Randomized, controlled clinical trials that include therapeutic arms dependent on changes in CTC enumeration are required to establish the usefulness of this biomarker in managing patient care. The final results of the SWOG-S0500 and CirCè01 trials will serve to establish the role of CTCs as markers for quantifying disease response to therapy. At this time, routine clinical use as a predictive marker is not recommended.

An advantage of IHC-based CTC isolation techniques is the ability to quantify the expression and activity of specific biologic receptors. For example, some previous studies have quantified HER2, estrogen-receptor and progesterone-receptor expression in CTCs.⁸² It would be important to correlate the biomolecular characteristics of CTCs with patient outcome, and to utilize this information for designing therapeutic trials. A recently initiated clinical trial (DETECT III) plans to use CTC HER2 status in patients with metastatic breast cancer to guide therapy. In this trial, patients with HER2-negative tumor but HER2-positive CTCs will receive lapatinib with standard therapy or standard therapy alone. The estimated primary completion date for this trial is March 2016 with the primary outcome measure of PFS (NCT01619111). This trial will determine the utility of CTC biomolecular signatures for guiding patient therapy.

CTC analysis is limited by the availability of current isolation technologies and their cost. The development of other CTC isolation techniques including PCR and microfluidic-based methods can address these shortcomings.^{46-48,50,56,83} The ability to isolate CTCs at multiple points of treatment by simple blood draw is an important advantage of CTCs in comparison to biopsy and imaging techniques. Currently, various tumor markers, for example, CEA, CA15-3 and CA27.29, are used to assess response to therapy. The addition of CTC enumeration to a panel that includes these tumor markers can increase their overall sensitivity and specificity. Compared to the tumor markers, CTCs can potentially provide multiple biological measurements including expression of hormone receptors, HER2, insulin growth factor receptor, and the receptors kinase activity. To better measure these biological markers in a single CTC, further advances in single-cell analysis are required. This would fulfill the promise of CTC as a “liquid biopsy” of the tumor. But even with the evidence supporting the role of CTCs as a marker of disease, there are a subset of CTC negative MBC patients with poor outcomes.

Therefore, further research is required to understand the limitations of CTCs as a biomarker of disease progression. In conjunction with clinical trials, controlled biologic experiments are required to establish the relevance of CTCs to disease spread.

Treatment of patients with metastatic breast cancer requires a fine balance between administering therapies to control the malignancy with the goal of improving cancer-related symptoms and complications as well as survival, but at the same time limiting the negative impact on quality of life from the treatments. Often, clinicians are faced with the challenge of determining whether the treatment is benefiting the patient using limiting information derived from the patient’s symptoms, exam and basic laboratory results. Newer tools are clearly needed to aid in this decision making. Circulating tumor cells have the potential to be a noninvasive, early predictive biomarker to aid clinicians in their goal of maximizing benefit and limiting harms to patients with advanced breast cancer in the future.

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Conflicts of Interest

David J. Beebe, PhD, has an ownership interest in BellBrook Labs LLC.

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