Prognostic Relevance of Circulating Tumor Cells in Molecular Subtypes of Breast Cancer

Prognostische Relevanz zirkulierender Tumorzellen in molekularen Subtypen des Mammakarzinoms

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Abstract

Circulating tumor cells (CTCs) can be detected in the peripheral blood of breast cancer patients with early and metastatic disease. Recent data suggest that immune pathologic characteristics between the primary tumor, metastatic colonies and CTCs are discordant and that CTCs possess an independent phenotype that is associated with prognosis and treatment efficacy. Large scale gene expression analysis has provided the possibility to stratify breast cancer according to the gene expression fingerprint of primary tumor tissue into five intrinsic molecular subtypes which can be associated with different clinical outcome. As a consequence of the different prognostic power of primary tumors' characteristics and CTCs several groups have started to investigate if CTCs might be disseminated differentially within these breast cancer subtypes. They determined the CTC number in immunohistochemical subtypes to validate if CTCs may provide differential and more specific prognostic information within each subtype. This review provides an overview of the outcome of some recently published data gathered from early and metastatic breast cancer.

Zusammenfassung

Zirkulierende Tumorzellen (CTC) können im Blut von Mammakarzinom-Patientinnen in der adjuvanten sowie metastatischen Situation detektiert werden. Bezüglich des Immunphänotyps können sich CTC sowohl von den Metastasen als auch vom Primärtumor unterscheiden. Moderne Genexpressionsanalyse lässt Mammakarzinome in 5 molekulare Subtypen mit unterschiedlichem biologischem und klinischem Verhalten unterteilen. Aufgrund der unterschiedlichen prognostischen Bedeutung zirkulierender Tumorzellen und der prädiktiven Faktoren beim Mammakarzinom wurde das Phänomen der Tumorzelldissemination innerhalb der verschiedenen Mammakarzinom-Subtypen bereits von vielen Gruppen analysiert. Die CTC-Anzahl wurde bei Tumoren mit unterschiedlicher Immunhistochemie bestimmt und auf ihre prognostische Aussagekraft in Abhängigkeit von der Biologie des Primärtumors hin untersucht. Dieser Review bietet einen Überblick über aktuelle Daten im adjuvanten und metastasierten Setting.

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Abbreviations

BCSS	breast cancer-specific survival
CK	cytokeratin
CTC	circulating tumor cell
DFS	disease-free survival
EMT	epithelial-mesenchymal transition
ER	estrogen receptor
FISH	fluorescence in situ hybridization
HR	hormone receptor
IHC	immunohistochemistry
MBC	metastatic breast cancer
MFS	metastasis-free survival
OS	overall survival
PFS	progression-free survival
TN	triple-negative

Introduction

Heterogeneity of breast cancer

Breast cancer should not be considered as a single heterogeneous disease but as a conglomerate of heterogeneous diseases consisting of a plethora of different molecular histopathologic subtypes, clinical outcomes and responses to therapies. Currently, for patients with operable breast cancer the status of routine pathologic parameters deter-

mined in the primary tumor such as tumor size, lymph node status, endocrine receptor status, and the HER2 status are used to estimate risk and to give recommendations for adjuvant therapy. Improvements in determining these pathologic parameters as well as adjusted and targeted therapies have resulted in better prediction and prognosis, however, a significant proportion of cancer patients may be overtreated.

Approximately 20% of breast cancer patients who initially present with a localized disease will progress to metastatic breast cancer (MBC) and die of metastases [1]. Clinical management of patients with MBC is much less comprehensively structured. Its treatment is frequently based on expression profiles of the primary tumor. However, basing treatment decisions solely on the morphological features of the primary tumor potentially ignores many biological features of the metastases that may affect outcome [2, 3].

It is unclear whether different subclones of a heterogeneous primary tumor will metastasize to different organs, or if the expression of biomarkers within the metastasis will change due to adjuvant treatment or targeted therapy [4]. Many authors have noted a discrepancy between primary tumor and metastatic sites with regard to HER2 and hormone receptor (HR) status [5–8]. Routine biopsies of metastatic lesions are strongly recommended but sometimes hard to provide or somewhat dangerous for the patient. Nevertheless serial reevaluation of metastatic disease with regard to HER2 and hormone receptor status would be interesting from a scientific standpoint and could help to optimize treatment decisions.

These unsatisfying situations, both in the primary and in the metastatic disease, warrant to tune our available prognostic and predictive tests such as the information on intrinsic breast cancer subtypes and the presence of circulating tumor cells (CTCs) to potentially prevent patients from receiving unnecessary and/or ineffective treatment but experiencing the treatment-related side effects.

In the last decade progress in large scale molecular characterization of cancer tissues has extended our knowledge on heterogeneity of e.g. breast cancer to new levels [9]. In their seminal work Perou and coworkers provided the basis to associate the phenotypic diversity of primary breast tumors with corresponding gene expression profiles and enabled to classify breast cancers into four – later on five – different molecular subtypes of breast cancer: estrogen receptor (ER)positive (ER+)/luminal-A or -B, basal-like, HER2+, and normal breast [10, 11]. The existence of these five molecular subtypes has later been confirmed in independent datasets [12] and besides that they have been associated with significant differences in distant spread patterns, independent of conventional clinical–pathologic variables [13].

These classified molecular subtypes which were originally based on the differential expression of a set of genes have recently been transferred into a surrogate clinico-pathologic characterization based on ER/PR expression, HER2 status, proliferation or histologic grade using immune histochemical (IHC) techniques [14, 15]. This prompted experts taking part in the 2011 St. Gallen Consensus Conference to introduce this surrogate definition of intrinsic breast cancer subtypes (luminal A-like, luminal B-like [HER2-], luminal B-like [HER2+], HER2, and basal-like) into clinical use to better define the categories of breast cancers to be treated [16].

The concept of circulating tumor cells

The theory on hematogenous spread of cancer was developed by several researchers in 19th century [17]. It is now widely accepted that solid tumors are able to shed single tumor cells into the blood circulation where they are dispersed throughout the body [18–20]. Those CTCs may suffer different fates: they go into apoptosis, fall into a dormant state (dormancy) or they survive and finally transmigrate into secondary organ sites to persist and to eventually outgrow to metastases [21, 22]. This transient nature of a single CTC in the peripheral blood makes it an attractive candidate to take a screenshot of the current expression of therapeutically relevant markers in the potentially harmful cell population in a minimally-invasive liquid biopsy format. Highly sensitive methods based on expression of surface proteins or their physical characteristics have been developed in recent years to detect single CTC for diagnostic purposes. One such method is utilized by the CellSearch System (Veridex, LLC, Warren, NJ, USA), a device approved by the US Food and Drug Administration (FDA) in 2004 for the detection of CTCs in the peripheral blood of patients with MBC. Another approach is to perform RT-PCR measurement of cytokeratin (CK) mRNA expression levels (CK19, CK20) which are used as surrogate for the presence of CTCs in the peripheral blood and which have been shown to correlate with disease outcome and the results from CellSearch [23, 24]. The great hope regarding the CTCs' clinical relevance is that by characterizing their individual phenotype and/or genotype clinicians will be able to target the Achilles heel of these CTCs which are finally responsible for metastasis in order to improve prognosis and prediction.

Prognostic Relevance of CTCs

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Metastatic breast cancer

In advanced breast cancer the CTCs' prognostic value has clearly been demonstrated – particularly their detection before treatment as an independent predictor of progression-free survival (PFS) and overall survival (OS) [25–27]. Moreover, a substantial decrease in the CTC count is an early marker of individual response to treatment and thus CTC screening provides an easy-to-perform alternative method to monitor success of a given therapy [28].

Since CTC levels appear to reflect response to treatment early in the course of a new regimen, they might potentially guide therapy decisions in metastatic breast cancer. This issue has been addressed by two currently ongoing clinical trials: SWOG 0500 from the Southwestern Oncology Group and CirCe01 at the Institut Curie, France [29, 30]. In CirCe01, patients in the advanced metastatic setting (3rd line and higher) with insufficient CTC decrease after start of new therapy line will change to another regimen, which will be, again, evaluated by early CTC changes. In the SWOG 0500 phase III trial, patients with persistently elevated CTC counts after one cycle of first-line chemotherapy were randomized between continuation of treatment until clinical progression or to early switch to another regimen [30]. Both studies were designed to clarify whether CTC changes may detect chemoresistance earlier than classical radiological methods and save patients with metastatic disease from the adverse effects of an inefficient chemotherapy regimen.

The first results of the SWOG 0500 trial, presented at the San Antonio Breast Cancer Symposium 2013 and now published in the Journal of Clinical Oncology, show that switching to another che-

motherapy regimen does not improve survival in patients with elevated CTC counts after one cycle of initial chemotherapy [30]. The SWOG 0500 trial confirmed also that patients with low baseline CTC levels perform best, reaching a median overall survival of 35 months, followed by patients whose CTC levels decrease during treatment (23 months) and patients with persistently high CTC levels (13 months). Which patients need first-line chemotherapy or – in case of HR-positive disease – only endocrine treatment will be addressed by the French STIC CTC Metabreast trial (NCT01710605) and the USA/Canada-based COMETI-P2 trial (NCT01701050). In the STIC CTC trial, patients were randomized between clinician choice and CTC-driven choice; patients in the "CTC arm" will be stratified to chemo- or endocrine treatment based on their CTC counts (high levels: chemotherapy, low levels: endocrine therapy). Treatment in the standard arm will be based on clinicians' decision. The COMETI-P2 study is a phase II trial evaluating the feasibility of the CTC-Endocrine Therapy Index. This index is based on the expression of four markers (estrogen receptor, Bcl2, HER2, and Ki67) assessed on isolated CTCs by immunocytofluorescence (CellSearch®) and was designed to predict clinical response to endocrine treatment in metastatic setting [31].

Early breast cancer

Presence of CTCs has been reported in 10–60% of patients with stage I–III breast cancer by various types of detection assays [32, 33], e.g. CK19 mRNA amplification [23] or the CellSearch (Veridex, Raritan, NJ) method [34]. Recently, Rack et al. published the results from the German SUCCESS trial; blood samples from 2026 early average-to-high risk breast cancer patients before chemotherapy and 1492 patients after chemotherapy were analyzed [33]. The presence of CTCs was strongly associated with shorter disease-free survival (DFS) and OS. Further, patients with at least 5 CTCs/30 ml blood were at highest risk for disease recurrence. Patients with CTC persistence after chemotherapy had significantly worse DFS and OS as well. These new data from a large clinical trial support previous findings from the REMAGUS 02 trial which reported that the presence of one or more CTCs before the start of systemic chemotherapy is an independent predictor of both metastasis-free survival (MFS) and OS in patients with stage II and III breast cancer [35]. In congruence with these findings are data from smaller studies [36, 37].

Prognostic Relevance of CTCs According to Breast Cancer Subtype

Recent data suggest that the phenotypes and several clinicopathologic characteristics are discordant among the primary tumor, metastatic cells, and isolated tumor cells [5–7, 38, 39]. This indicates that it may be the presence of CTCs or even the phenotype/genotype of an individual CTC which is associated with breast cancer prognosis and treatment response in the first place [40]. Therefore, detection, (molecular) characterization, and the clinical role of CTCs in different subtypes of breast cancer are currently investigated in several research projects.

Metastatic breast cancer

In the first study addressing this question, Giordano et al. retrospectively analyzed 517 MBC patients for the presence of CTCs [41] (**Table 1**). Subtypes of primary tumors were determined by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) and CTCs were enumerated by CellSearch before start of a new systemic treatment. The authors classified patients with at least one positive hormone receptor (HR; ER or PR ≥ 1%) as HR+. 206 (40%) patients had ≥ 5 CTCs at baseline blood draw, 311 (60%) had < 5 CTCs. Regarding overall distribution of CTCs in different subtypes, a larger proportion of HR+/HER2-negative patients had ≥ 5 CTCs than did patients with other subtypes of tumor – a finding which does not overlap with previously published results from similar but smaller population of MBC patients [42–44]. The prognostic power of the CTC count appeared to be most valuable in HR+ and TN breast cancers and least valuable in HER2-positive cancers pretreated with targeted therapy. HER2-positive MBC patients with ≥ 5 CTCs had a PFS and an OS similar to patients with < 5 CTCs which indicates that CTCs are strongly predictive for survival in all HER2-negative MBC subtypes who had not been treated with targeted therapy.

A second study using CellSearch to determine the CTC count in MBC patients was published by Munzone et al. [45]. This group retrospectively classified 203 patients into intrinsic subgroups defined by IHC of 5 biomarkers (ER, PR, HER2, Ki-67, and grade), according to the recent St. Gallen guidelines, and determined their CTC status. All patients had CTCs enumerated before starting a new treatment as standard of care. Most patients (74%) were pretreated for metastatic disease and had more than one metastatic site (66%). In total, 92 patients had ≥ 5 CTCs/7.5 ml blood and again at multivariable analysis, CTC count was a statistically significant predictor for PFS and OS confirming the CTCs' prognostic power in MBC. When stratifying the tumors into the intrinsic subgroups the results are confirming CTCs as strong predictor of survival in different MBC subtypes: CTCs were

Table 1 CTCs and molecular subtypes in metastatic breast cancer.

Author	n (≥ 5 CTC)	Method	Frequency bias	All subtypes	Lum A	Lum B-like		HER2+	TN
						HER2−	HER2+		
Giordano (2012)	517 (206)	CS	HR+/HER2−	PFS, OS	PFS, OS		n. s. (OS p = 0.084)	n. s.	OS
Munzone (2012)	203 (92)	CS	HR+/HER2−	PFS, OS	PFS, OS	OS	OS	OS	n. s. (OS p = 0.06)
Wallwiener (2013)	468 (205)	CS	ER+	PFS, OS	PFS, OS		OS		PFS, OS

CS: CellSearch™; HR: hormone receptor; PFS: progression free survival; OS: overall survival; n. s.: not significant

mostly found in patients with luminal-A/luminal-B/HER2-negative subtypes. The CTC count was a significant prognostic factor for OS in all subtypes, except for triple negative patients (borderline significance). In the HR+/HER2-negative patients CTC count was a significant prognostic factor for PFS.

The most recent data come from a large prospective multicenter study including nine German breast cancer centers. Therein Wallwiener et al. investigated blood samples from 486 MBC patients [46]. The study design was very similar to the one followed by Giordano et al. [41] with the molecular subtypes being determined by immunohistochemical staining of the primary tumor. CTC enumeration was carried out before initiation of a new therapy using the CellSearch system obtaining 205 CTC-positive patients. Like Giordano et al., the authors classified patients which were positive for estrogen and/or progesterone as HR+ and classified the patients into three subgroups

1. HR+/HER2−,
2. HER2+, or
3. HR−/HER2− (triple negative).

As already observed by Giordano et al. [41], CTC-positivity was significantly more frequent in ER+ patients and the CTC status did not differ significantly among subtypes of MBC. Additionally, Wallwiener et al. observed a higher rate of CTC-positivity in patients with both bone and visceral metastases compared to those with either bone or visceral metastases. When stratified by the molecular subtype, baseline CTC count was predictive for OS in all subgroups. Regarding PFS, the CTC status was a prognostic factor in HR+/HER2− and HR−/HER2− patients. Wallwiener et al. separated the patients with HER2+ primary tumors according to their pretreatment with trastuzumab and found out that the CTC status of untreated HER2+ patients was prognostic for OS [46]. No impact on OS was observed in HER2+ patients with previous trastuzumab treatment. No prognostic effect of CTC for PFS was observed in both groups.

Regarding the prognostic significance of CTC in MBC subtypes there seem to be two common themes. In general CTCs are more often found in luminal A (HR+/HER2−) and luminal B/HER2− tumors compared with HER2+ and basal-like tumors. This may be a consequence of the clinical practice that HER2+ MBC patients are treated with HER2-targeted therapy (trastuzumab, lapatinib) [45]. Alternatively, it is known that cells of basal-like molecular breast cancer subtype may shift to low EpCAM expression and increased expression of mesenchymal markers such as vimentin, epidermal growth factor receptor (EGFR) and epithelial-mesenchymal-transition (EMT) compared to breast cancers with a luminal subtype [47, 48]. Since CellSearch™ is a purely EpCAM-based capture system it may identify less basal-like cells. This may bias their frequency in a way that the number of basal-like CTCs might be underestimated [49, 50]. Secondly, in most studies, CTC detection did not predict clinical outcome in women with HER2+ MBC [41]. Fittingly, Munzone et al. observed that patients with 0 CTCs/7.5 mL blood at baseline and all subtypes, except for HER2+, seemed to perform better than CTC-positive patients [45]. Further studies demonstrated a marked decrease in CTC count at follow-up if MBC patients had received biological therapies such as trastuzumab or bevacizumab [42, 44]. In the studies published by Munzone et al. [45] and Giordano et al. [41] most of the HER2+ breast cancer patients have received anti-HER2 treatment which may have eliminated CTCs with HER2 amplification or overexpression and may thereby have reduced the prognostic value of CTC enumeration in this subtype. In support, Georgoulias et al. [51] showed that trastuzumab eliminates chemotherapy-resistant CTCs and reduces the risk of disease recurrence in early breast cancer patients. In the study from Wallwiener et al. the number of patients treated with trastuzumab was quite low (6.5%), whereas CTC detection had no impact on survival if patients had already received trastuzumab treatment [46]. CTC detection was a predictor of OS in the subgroup of initially HER2+ patients who had not been treated with trastuzumab. This supports the suggestion that HER2-directed therapy reduces the prognostic value of CTC enumeration.

Further, in the studies from Munzone et al. a high percentage (29%) of samples contained 1–4 CTCs – a group which is classified as "CTC-negative" in the other studies [45]. Interestingly, this intermediate group has a much higher prognostic power in HER2-negative tumors – meaning that already one CTC makes the difference between good and worse prognosis. In this subgroup, this intermediate CTC-group has the same survival probability as patients with > 5 CTC/7.5 ml blood. In contrast, for patients with HER2-positive tumors the survival probability of patients with 1–4 CTC/7.5 ml blood tends to be the same as for CTC-negative patients. Hypothetically, different cut-offs might be required for different molecular subtypes of the disease. It would be interesting to know about heterogeneity and individual metastatic potential of each CTC in these groups. This observation emphasizes the need for a thorough and standardized analysis of the CTC images provided by CellSearch™ system.

Early breast cancer

Data on CTCs in different subtypes of non-metastatic breast cancer are inconsistent. The largest dataset including 2026 patients was published recently by Rack et al. [33] (**Table 2**). No association was found between CTC-positivity and luminal, basal-like, or HER2-positive tumors. Presence of CTC was a strong predictor for worse clinical outcome; however, when the authors refined their investigation by adding the information about the tumors' intrinsic subtype the CTCs' prognostic power was restricted to the largest subgroup of patients with luminal tumors.

Contrary to these findings are the results from the study by Hwang et al.; the authors retrospectively evaluated 166 patients with operable breast cancer (stage I–IIIA) which had not been pre-treated [52]. After surgery patients with HER2-positive tumors did not receive adjuvant treatment with trastuzumab. CK20 mRNA-positive blood was detected in 37 of 166 patients (22.3%). These CK20 mRNA-positive patients had less favorable outcomes in terms of MFS and OS than patients with CK20 mRNA-negative patients. When the breast cancer samples were grouped into the molecular subtypes, luminal-A and luminal-B did not differ significantly in MFS and OS according to CTC-status. In contrast, patients with HER2-positive or TN disease and a CTC-positive status had shorter MFS and OS.

Another study published in 2007 by Ignatiadis et al. analyzing the expression analysis of CK19 mRNA in 444 patients produced similar results to those by Hwang et al.: The presence of CK19 mRNA-positive CTCs was associated with shorter DFS and OS in the TN and HER2-positive subgroups but not in the ER+/HER2− subgroup [23, 53]. There is no plausible explanation for this inconsistency between these studies.

Table 2 CTCs and molecular subtypes in early breast cancer.

Author	n (CTC pos.)	Method	Frequency bias	All subtypes	Lum A	Lum B-like		HER2+	TN
						HER2−	HER2+		
Rack (2014)	2 026* (435)	CS	none	DFS, BCSS, OS	DFS, OS	n. s.			n. s.
Ignatiadis (2007)	444* (181) I–III	RT-PCR (CK19)	none	DFS, OS	n. s.	n. s.	n. d.	DFS, OS	DFS, OS
Hwang (2012)	166 (37) I–IIIA	RT-PCR (CK20)	none	MFS, OS	n. s.	n. s.	n. s.	MFS,OS	OS (MFS: p = 0.051)

* All patients had average-to-high risk breast cancer and received chemotherapy. n. s.: not significant; CS: CellSearch™; BCSS: breast cancer-specific survival; DFS: disease-free survival; OS: overall survival; MFS: metastasis-free survival

Conclusion

In conclusion, the presented results strongly confirm the independent prognostic value of CTC enumeration in both early and metastatic breast cancer patients. CTC evaluation might provide further information that could be useful for individualizing breast cancer treatment. However, CTC positivity and clinical relevance of CTC detection vary between breast cancer subtypes. Interestingly, metastatic patients with HR+/HER2− tumors are most likely to present with CTCs in peripheral blood; the prognostic relevance of CTCs in this subtype seems to be the highest. In HER2-positive and triple-negative disease, data on prognostic significance of CTCs are inconclusive. Possibly, the role of CTC enumeration is strongly influenced by previous targeted treatment. From this point of view, current and future trials with therapies targeting specific CTC phenotypes and genotypes might have an impact on prognosis in patients with metastatic breast cancer; ongoing European and German clinical trials, such as TREAT CTC and DETECT III, may thus substantially improve our understanding of HER2-negative metastatic disease.

Conflict of Interest

None.

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