Article published online: 2022-08-22

Original Article

Breast Cancer

Role of Circulating Tumor Cells in Determining Prognosis in Metastatic Breast Cancer

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South Asian J Cancer 2023;12(1):62–67.

Abstract

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Background Circulating tumor cells (CTCs) in the peripheral blood may play a major role in the metastatic spread of breast cancer. This study was conducted to assess the role of CTCs to determine the prognosis in terms of survival in metastatic breast cancer patients.
Methods This prospective study of 36 patients was conducted at the Hospital from April 2016 to May 2018. Details of each patient related to the demographic profile, tumor type, treatment, and follow-up information were recorded. The number of CTCs in the peripheral blood was measured by Celsee PREP 400 sample processing system and Celsee Analyzer imaging station.
Results There was a positive correlation between the number of site of metastasis with number of CTCs (p-value < 0.001). In the patients with clinical/partial response, a significant reduction in the number of CTCs after 1 month of therapy was observed (p-value $= 0.003$). When the number of CTCs at baseline and 6 months were compared with the positron emission tomography response at 6 months, a statistically significant difference in CTCs in patients having partial response after 6 months was observed (p-value $= 0.001$). On comparison with the responder groups, a statistically significant reduction in CTCs at baseline and 6 months was observed (p-value $= 0.001$). Patients with CTCs less than 5 and more than or equal to 5 after 1 month of treatment had a mean progression-free survival of 11.1 months and 7.5 months (p-value $= 0.04$) and a mean overall survival of 11.6 and 9.6 months (p-value $= 0.08$), respectively.
Conclusion Assessment of CTCs provides a more quantifiable response than radiographic evaluation and at a much earlier time point and is also a better predictor of survival.

Keywords
- circulating tumor cells
- metastatic breast cancer
- response
- survival
- prognosis

* Equal Contribution.

DOI **https://doi.org/10.1055/s-0042-1753477** ISSN **2278-330X**

How to cite this article: Dhaka S, Tripathi R, Doval DC,et al. Role of Circulating Tumor Cells in Determining Prognosis in Metastatic Breast Cancer. South Asian J Cancer 2023;12(1):62–67.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Introduction

Breast cancer is the most frequently diagnosed cancer worldwide accounting for 23% of the total cancer cases and 14% of the cancer deaths.[1] Different prognostic factors for breast cancer have been identified over the years with an aim to identify patients with an unfavorable prognosis and to improve the treatment strategies according to the individual risk (recurrence and mortality).[2,3]

Circulating tumor cells (CTCs), the breakaway cancer cells in the peripheral blood detached from the solid primary tumor, may survive in the peripheral blood for several years and play a major role in the metastatic spread of breast cancer. Around 2.5 and 0.01% CTCs have been reported to form micrometastases and macrometastasis, respectively.[4] CTCs with the use of multiple sampling provide a so-called real-time "liquid biopsy" that greatly helps in treatment selection and optimization.[5–8] In addition to TNM staging, CTCs may help in predicting disease progression in lymph node negative patients and thereby help in decision making regarding the need for therapy and to monitor treatment efficacy. In breast cancer, measurable CTCs after first line of therapy can help in making early changes in treatment and a second-line therapy can be chosen.[9,10] Early metastatic relapse has been correlated with the identification of CTCs in the peripheral blood in breast cancer and has also been identified as the predictors of progression-free survival (PFS) and overall survival (OS).[11,12] Enumeration and analysis of CTCs can distinguish between high- and low-risk profiles for PFS and OS.[13] Genetic and molecular analyses of CTCs provide insight in the metastatic process and the effectiveness of therapy.[10]

This study was conducted to assess the role of CTCs to determine the prognosis in metastatic breast cancer by correlating with patient's outcome after chemotherapy in terms of survival in metastatic breast cancer patients.

Methods

This prospective study was conducted in the Department of Medical Oncology and Department of Pathology at our hospital from April 2016 to May 2018. This study was approved by the Institutional Review Board (Ethics committee) of our hospital. Written informed consent was taken from all the patients before the initiation of this study. Thirty-six patients with clinically or radiologically proven newly diagnosed metastatic breast cancer with no history of prior treatment for cancer were included in the study. Patients with Eastern Cooperative Oncology Group (ECOG) score performance status 3 and 4 and any associated comorbidity or malignancy were excluded from the study.

Details of each patient related to the demographic profile, investigations, tumor type, histopathology details, treatment, and follow-up information were recorded. Pathological diagnosis of the patients was confirmed using core needle biopsy. Distant metastasis was diagnosed with appropriate imaging modality like positron emission tomography-computed tomography (PET-CT), X-ray chest or CT chest, ultrasonography abdomen or CT abdomen and if needed, bone scintigraphy. All patients underwent PET scan after 3 months and after completion of systemic therapy, that is, 6 months after starting systemic therapy.

The number of CTCs in the peripheral blood was measured before giving chemotherapy and subsequently after first cycle and 6 months of starting chemotherapy. The integrated CTCs analysis system consists of Celsee PREP 400 sample processing system (for performing single cell analysis of CTCs directly from blood samples) and Celsee Analyzer imaging station.[14]

A novel microfluidic slide at the core of the Celsee PREP confirmed the isolation of CTCs in the respective compartments.[14] Following the cell capturing, cells were stained first with DAPI (4',6-diamidino-2-phenylindole), a fluorescent nuclear stain, while nucleated cells were stained with the primary antibody cocktail, anti-PanCK and anti-CD45. The antibody against CD45 was used as a marker for background leukocytes. Filters for DAPI (nucleus), pan cytokeratin [PanCK], an epithelial marker, and CD45, a leukocyte marker were used in the standard assay. A DAPI +, PanCK +, and CD45-cell was classified as a CTC.[10,11]

The comparison of the baseline tumor burden to the tumor size and burden after palliative chemotherapy by imaging was done to assess the clinical response to chemotherapy. The Response Evaluation Criteria in Solid Tumors guidelines version 1.1 was used[15] to quantitatively assess the clinical response to chemotherapy. The primary outcome was survival. Response to therapy and survival were calculated at the end of 1 year.

Statistical analysis was done using SPSS version 20 for Windows (SPSS Inc, Chicago, Illinois, United States). Chi-squared test, Fisher's exact test, Mann–Whitney test, and Wilcoxon rank-sum test were used as applicable (p-value <0.05 was considered as significant). The correlation between CTCs and metastasis was assessed using Pearson's correlation coefficient. Kaplan–Meier method was employed for the survival analysis.[16]

Results

Altogether, 36 patients were included in the study and the mean age was 50 years (range: 34–76 years). The distribution of pre- and postmenopausal women was similar in the study group (50% each). In terms of performance status, the majority of the patients were ECOG1 (50%). A total of 56 and 47% patients were estrogen receptor/progesterone receptor positive and human epidermal growth factor receptor 2 (HER-2) positive, respectively. On histology, around 55% patients were classified as low or moderate grade. The demographic profile of the patients is depicted in ►Table 1.

All the patients were in the phase of initiating their first line of therapy for metastatic breast cancer. Docetaxel along with epirubicin was the most commonly used regimen in this study (42%) followed by docetaxel, carboplatin, and trastuzumab with or without pertuzumab in HER-2/neu positive patients. The rest of the patients were given paclitaxel alone, docetaxel alone or paclitaxel and trastuzumab. Before evaluation at 1 year, eight patients were withdrawn from the study. Three of these patients were lost to follow-up, four

Table 1 Patient demographics

Characteristics	*n* = 36	%
Mean age (years)	50.1	–
Menopausal status		
Premenopausal	18	50
Postmenopausal	18	50
Performance status		
ECOG0	10	28
ECOG1	18	50
ECOG2	8	22
ER/PR status		
Positive	20	56
Negative	16	44
HER2 status		
Positive	17	47
Negative	19	53
Histology		
Low or moderate grade	20	55
High grade	16	45

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; *n*, number; PR, progesterone receptor.

died and one had unexpected severe cardiac symptoms and stopped the treatment.

In this study, lymph nodes were the most common site of metastasis (86%) followed by lungs, liver, bones, brain, skin and soft tissue, pleura and adrenal. Twenty-two patients had three or more metastasis. In all the 36 patients, the mean number of CTCs were 13.8 with range varying from 0 to 48. At baseline, 27 (75%) patients had CTCs more than or equal to 5. There was a positive correlation between the number of site of metastasis with number of CTC (*p*-value < 0.001) (►Fig. 1).

In terms of the clinical efficacy determined at 3 months, 4 (11%) patients had complete response (CR), 19 (54%) patients had partial response (PR), 7 (20%) patients had stable disease (SD), 5 (15%) patients had progressive disease (PD), and one patient could not be evaluated as he was lost to follow-up (►Table 2). In the patients with CR, the mean number of CTCs was 21 (median: 17) before starting the treatment that decreased to 4.25 after one cycle of chemotherapy (*p*-value $= 0.14$). However, in the patients with PR, after one cycle of chemotherapy the mean number of CTCs significantly decreased to 6.3 from 12.9 (*p*-value $= 0.001$). When both the responder groups (CR and PR) were considered together, a significant reduction in the number of CTCs after 1 month of therapy was observed (*p*-value $= 0.003$). In comparison to the baseline values, the patients with PD showed a significant increase in their CTCs at 1 month.

Similarly, when the number of CTCs at baseline and 6 months were compared with the PET response at 6 months, a statistically significant difference in CTCs in patients having PR after 6 months was observed (*p*-value $= 0.001$). When the patients with CR and PR were combined together, a statistically significant reduction in their CTCs at baseline and 6 months was observed (*p*-value $= 0.001$) (►Table 3).

When taking a cutoff of more than or equal to 5 CTCs, 16 patients with CR or PR had more than 5 CTCs at baseline that further decreased to 6 patients at 6 months. Similarly, three patients with PD had more than or equal to 5 CTC at baseline that increased to four patients at 6 months (►Table 4).

The patients were grouped on the basis of the number of CTCs (<5, ≥ 5) for calculating the survival. No statistical difference was observed in terms of PFS when compared at

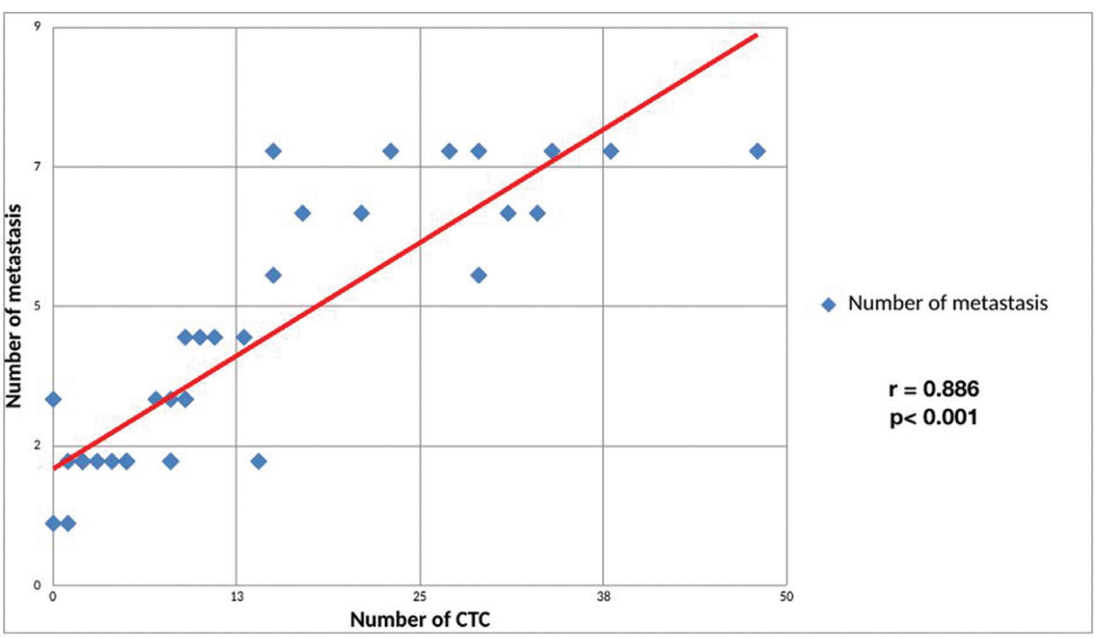

Fig. 1 Correlation of number of circulating tumor cells (CTC) at baseline with number of sites of metastasis in each patient.

Table 2 Comparison of PET scan at 3 months with number of CTCs at baseline and after 1 month of systemic therapy

PET scan 3 months $n = 35$	n (%)	CTC baseline			CTC 1 month			p-Value
		Mean	Median	Range	Mean	Median	Range	
Complete response	4 (11%)	21	17	2–48	4.25	4.5	0–8	0.14
Partial response	19 (54%)	12.9	9	0–34	6.3	4	0–14	0.001
Responders (CR + PR)	23 (65%)	14.3	10	0–48	5.9	4	0–14	0.003
Stable disease	7 (20%)	17.6	11	1–38	17.2	13	0–44	0.83
Progressive disease	5 (15%)	7	5	3–15	18.2	18	12–25	0.02

Abbreviations: CR, complete response; CTC, circulating tumor cells; N/n, number; PET, positron emission tomography; PR, partial response.

Table 3 Comparison of PET scan at 6 months with number of CTCs at baseline and after 6 months of systemic therapy

PET scan at 6 months $n = 28$	Number of patients	CTC baseline			CTC 6 months			p-Value
		Mean	Median	Range	Mean	Median	Range	
Complete response	4	21	17	2–48	2.75	2	0–7	0.12
Partial response	16	13.6	9.5	0–34	3.1	2	0–13	0.001
Responders (CR + PR)	20	15.1	11.5	0–48	3	2	0–13	0.001
Stable disease	3	12.3	9	1–27	8.7	6	0–20	0.17
Progressive disease	5	16.8	11	0–38	21.2	23	0–43	0.11

Abbreviations: CR, complete response; CTC, circulating tumor cells; N/n, number; PET, positron emission tomography; PR, partial response.

Table 4 Comparison of patients with more than or equal to 5 CTCs at baseline, after 1 month and after 6 months of systemic therapy with PET scan at 6 months

Treatment efficacy at 6 months	n	CTC ≥5 at baseline	CTC ≥5 at 1 month	CTC ≥5 at 6 months
Complete response	4	3	2	1
Partial response	16	13	8	5
Stable disease	3	2	2	2
Progressive disease	5	3	3	4
Total	28	21	15	12

Abbreviations: CTC, circulating tumor cells; n, number; PET, positron emission tomography.

baseline between the two groups (►Fig. 2). Patients with baseline CTCs less than 5 and more than or equal to 5 had a mean PFS of 9.8 and 8.6 months, respectively (p-value = 0.37). However, statistically significant difference was noted when these groups were compared at 1 month after treatment. Post 1 month of treatment, the patients with CTCs less than 5 and more than or equal to 5 had a mean PFS of 11.1 and 7.5 months, respectively (p-value = 0.04). In terms of OS, there was no statistically significant difference in the patients with CTCs less than5 and CTCs more than or equal to 5 either at baseline or after 1 month of systemic therapy. Patients with baseline CTCs less than 5 had a mean OS of 10.2 months, whereas the patients with baseline CTCs more than or equal to 5 had a mean OS of 10.4 months (p-value = 0.98) (►Fig. 3). After 1 month of treatment, patients with CTCs less than 5 and more than or equal to 5 had a mean OS of 11.6 and 9.6 months, respectively (p-value = 0.08).

Discussion

This study reports the data from 36 patients of metastatic breast cancer treated with palliative systemic therapy and investigated the role of CTCs and other clinicopathological parameters (number of metastasis, hormonal status, and response to therapy) as predictive and prognostic factors in these patients. The role of the number of CTCs in predicting early response to treatment and survival was also evaluated.

The mean CTCs count at the beginning of the study in all the 36 patients was 13.8 (0–48). At baseline, 27 (75%) patients had CTCs more than or equal to 5 that has been used as a cutoff in the previous studies.[9,17] In a study by Cristofanilli et al, it was observed that 49% patients with metastatic breast disease had more than or equal to 5 CTCs; however, none of the 145 normal subjects and 200 subjects

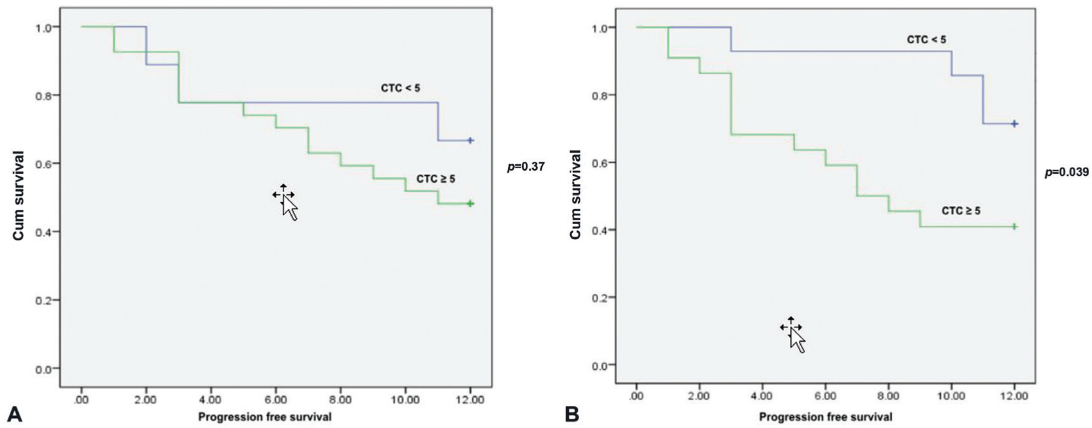

Fig. 2 Kaplan–Meier graph showing progression-free survival when compared between the two groups with circulating tumor cell (CTC) less than 5 and CTC more than or equal to 5 at (**A**) baseline (**B**) 1 month of treatment.

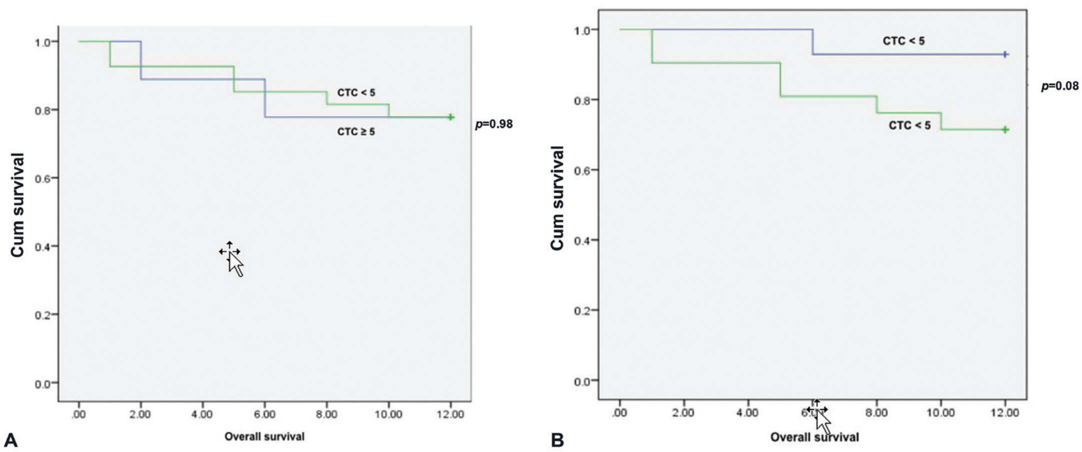

Fig. 3 Kaplan–Meier graph showing overall survival when compared between the two groups with circulating tumor cell (CTC) less than 5 and CTC more than or equal to 5 at (**A**) baseline and (**B**) 1 month of treatment.

with benign breast disorders had more than or equal to 5 CTCs.[9] Similarly, another recent study from Japan showed that 32% patients with metastatic breast disease had more than or equal to 5 CTCs in the peripheral blood.[17] Fehm et al and Hayes and Smerage have shown a prevalence of more than or equal to 5 CTCs in metastatic breast cancer to the tune of 60 to 70%.[18,19] In our study, a higher percentage of patients showing increased CTCs may be due to the delay in presentation and late stage presentation as pointed out by various Indian studies due to the lack of proper awareness and screening programs.[20] At 3 months, complete clinical response was observed in 4/35 (11%) patients and partial clinical response in 19/35 (54%) patients. SD and PD were seen in 7/35 (20%) and 5/35 (15%) patients, respectively. Those patients with complete clinical response did not show a significant decrease in their mean CTCs count; however, when the patients with complete and PR were included, this difference was statistically significant. Similarly, when the mean CTCs at baseline were compared with the mean CTCs at 6 months in patients showing PR at 6 months after systemic therapy, a significant decrease in mean CTCs was observed. This points out toward the inference that the difference in the CTCs at baseline and 1 month can predict the clinical response at 3 and 6 months. There was no difference in PFS at baseline or before starting systemic therapy in patients with less than 5 CTCs in comparison to the patients with more than or equal to 5 CTCs; however, this difference was statistically significant when the PFS was compared in both the groups after 1 month of systemic therapy. Although statistically there was no difference in OS in patients with less than 5 CTCs in comparison to those with more than or equal to 5 CTCs at baseline or at 6 months, the present data suggests that the CTC status after the treatment may be a prognostic marker. In addition, CTCs were useful to estimate the treatment efficacy as a predictive marker. These results suggest that keeping a track on the number of CTCs may contribute in predicting the efficacy of the treatment. In particular, the prognosis of radiologically responding patients (SD and PR) was divided into good and unfavorable prognosis groups according to the number of CTCs. Similarly, radiologically nonresponding patients (PD) were also divided into these two groups depending on the number of CTCs. Furthermore, in this report, radiological disease progression patients with more than or equal to 5 CTCs demonstrated a significantly worse prognosis than the patients with CTCs less than 5.[21]

Many a times, in the absence of measurable disease, it becomes extremely difficult to ascertain treatment response radiologically. Also, in the patients in whom the lesions are difficult to assess, it becomes complex and a cause of concern for the treating physician, and hence, the assessment of CTCs might be a useful predictor of treatment efficacy in addition to the tumor markers.

This study brings to light an important finding that especially in metastatic breast cancers, the frequency of CTCs before initiating a new therapy and at follow-up may be very useful predictors of PFS. This finding could not be replicated in terms of OS, although a trend was observed that may be due to the small sample size of the patients. A reliable estimate of disease progression and survival earlier than the estimations made with the use of traditional imaging methods (3–4 weeks vs. 10–12 weeks after the initiation of therapy, respectively) using the CTCs levels cutoff point of more than or equal to 5 could be made.

The results of this study can be utilized in both standard care and clinical research and can spare the patient from life-threatening toxicities with more effective therapeutic options for the patient[22] however, this needs to be prospectively assessed in clinical trials designed to investigate this question. CTCs may prove to be an effective tool in assessing the response of novel therapeutic agents apart from its clinical utility.[23] CTCs could also become a validated end point for prospective clinical trials.

In conclusion, the number of CTCs has a biological as well as clinical importance in breast cancer patients with metastatic disease. Assessment of CTCs provides a more quantifiable response than radiographic evaluation and at a much earlier time point than the radiologic studies and are also a better predictor of survival than the radiographic response. In future, CTCs may become an integral part of the diagnosis and management strategies in breast cancer.

Ethical Approval

This study was approved by the Institutional Review Board.

Informed Consent

Written informed consent was taken from all the patients before the initiation of this study.

Funding

None.

Conflict of Interest

None.

References

1 Agarwal G, Ramakant P. Breast cancer care in India: the current scenario and the challenges for the future. Breast Care (Basel) 2008;3(01):21–27

2 Schmidt M, Fasching PA, Beckmann MW, Kölbl H. Biomarkers in breast cancer - an update. Geburtshilfe Frauenheilkd 2012;72 (09):819–832

3 Melcher C, Scholz C, Jäger B, Hagenbeck C, Rack B, Janni W. Breast cancer: state of the art and new findings. Geburtshilfe Frauenheilkd 2012;72(03):215–224

4 Park Y, Kitahara T, Urita T, Yoshida Y, Kato R. Expected clinical applications of circulating tumor cells in breast cancer. World J Clin Oncol 2011;2(08):303–310

5 Mikulová V, Kološtová K, Zima T. Methods for detection of circulating tumour cells and their clinical value in cancer patients. Folia Biol (Praha) 2011;57(04):151–161

6 Mostert B, Sieuwerts AM, Martens JWM, Sleijfer S. Diagnostic applications of cell-free and circulating tumor cell-associated miRNAs in cancer patients. Expert Rev Mol Diagn 2011;11(03): 259–275

7 Deng G, Herrler M, Burgess D, Manna E, Krag D, Burke JF. Enrichment with anti-cytokeratin alone or combined with anti-EpCAM antibodies significantly increases the sensitivity for circulating tumor cell detection in metastatic breast cancer patients. Breast Cancer Res 2008;10(04):R69

8 Ignatiadis M, Georgoulias V, Mavroudis D. Circulating tumor cells in breast cancer. Curr Opin Obstet Gynecol 2008;20(01):55–60

9 Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351(08):781–791

10 Balic M, Lin H, Williams A, Datar RH, Cote RJ. Progress in circulating tumor cell capture and analysis: implications for cancer management. Expert Rev Mol Diagn 2012;12(03):303–312

11 Lianidou ES, Markou A. Circulating tumor cells in breast cancer: detection systems, molecular characterization, and future challenges. Clin Chem 2011;57(09):1242–1255

12 Sun YF, Yang XR, Zhou J, Qiu SJ, Fan J, Xu Y. Circulating tumor cells: advances in detection methods, biological issues, and clinical relevance. J Cancer Res Clin Oncol 2011;137(08):1151–1173

13 Reinholz MM, Kitzmann KA, Tenner K, et al. Cytokeratin-19 and mammaglobin gene expression in circulating tumor cells from metastatic breast cancer patients enrolled in North Central Cancer Treatment Group trials, N0234/336/436/437. Clin Cancer Res 2011;17(22):7183–7193

14 Riahi R, Gogoi P, Sepehri S, et al. A novel microchannel-based device to capture and analyze circulating tumor cells (CTCs) of breast cancer. Int J Oncol 2014;44(06):1870–1878

15 Edge S, Byrd DR, Compton CC, Fritz AG, Greene F, Trotti A. AJCC Cancer Staging Manual. 7th edition. New York: Springer; 2010: 347–376

16 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–481

17 Tokudome N, Ito Y, Takahashi S, et al. Detection of circulating tumor cells in peripheral blood of heavily treated metastatic breast cancer patients. Breast Cancer 2011;18(03):195–202

18 Fehm T, Müller V, Aktas B, et al. HER2 status of circulating tumor cells in patients with metastatic breast cancer: a prospective, multicenter trial. Breast Cancer Res Treat 2010;124(02): 403–412

19 Hayes DF, Smerage J. Is there a role for circulating tumor cells in the management of breast cancer? Clin Cancer Res 2008;14(12):3646–3650

20 Thakur NA, Humne AY, Godale LB. Delay in presentation to the hospital and factors affecting it in breast cancer patients attending tertiary care center in Central India. Indian J Cancer 2015;52 (01):102–105

21 Budd GT, Cristofanilli M, Ellis MJ, et al. Circulating tumor cells versus imaging–predicting overall survival in metastatic breast cancer. Clin Cancer Res 2006;12(21):6403–6409

22 Harbeck N, Penault-Llorca F, Cortes J, et al. Breast cancer. Nat Rev Dis Primers 2019;5(01):66

23 Krebs MG, Hou JM, Ward TH, Blackhall FH, Dive C. Circulating tumour cells: their utility in cancer management and predicting outcomes. Ther Adv Med Oncol 2010;2(06): 351–365

