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Research article

A real-world comparison of circulating tumor cells in breast cancer from China: Novel device, CTC counts and its overall survival

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ABSTRACT

Background: Both CellSearch and CellCollector have been accepted as the proper devices to capture CTC by domestic approval department. However, there is little article about the comparison between these two devices around the world. Herein, we conducted the real-world study to compare with these two devices and to re-verify the efficacy of CTC counts.

Methods: Patients who meet the following points should be included in the analysis. 1. Female, aged 18 years or older; 2. Eastern Cooperative Oncology Group (ECOG) score 0–2; 3. With at least one measurable tumor lesion; 4. Clear immunohistochemistry result; 5. Accept at least one CTC test. Patients were excluded in the analysis if they had a history of malignant tumors, incomplete follow-up information.

Results: 536 metastatic breast cancer patients who had been detected for CTC at least once by CellSearch or CellCollector were included in the analysis. CellCollector *in vivo* CTC detection technology has a higher detection rate than the CellSearch system (69.2% vs 57.4%, $P = 0.009$). However, the proportion of CTC≥5 detected by CellSearch was higher than CellCollector (37.4% vs 16.3%, $P < 0.001$). There was a statistically significant difference in overall survival of patients with CTC negative and CTC positive (mOS:49.8 months vs 26.9 months). After 4 weeks of treatment, when CTC decreased by more than 50%, there was a significant difference in survival between the two groups (40.1 months vs 25.8 months, HR = 0.588, 95% CI: 0.350–0.933). In addition, for HER2-positive patients, Patients with CTC HER2 positive had longer overall survival than patients with CTC HER2 negative (median OS: 26.7 months vs 17.3 month, HR = 0.528, 95% CI: 0.269–0.887).

Conclusions: Real-world data indicate that CTC is an independent prognostic factor, and CellCollector and CellSearch have their own advantages in CTC detection.

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1. Background

Circulating tumor cells (CTCs) are tumor cells that spontaneously or shed from the primary focus or metastasis into the peripheral blood circulation. The spread of tumor cells in peripheral blood is an important part of disease progression and distant metastasis, and is also the main cause of death of most cancer patients [1]. Although CTC detached from blood have been discovered since 150 years ago, it was not until Cellsearch (CS) was developed that specific detection and molecular examination of CTC have gained heightened attention. Afterwards, many researches have proven the strong associations between CTC counts and survival in metastatic breast cancer and many other cancers [2,3]. This may be explained by the fact that technology improvements may pose a tremendous challenge to enable more consistent CTC isolation [4].

The CellCollector (CC) is a new medical device with a 160 mm sterile steel wire of which the terminal 20 mm is covered with anti-EpCAM antibodies covalently coupled to a hydrogel layer [5]. The hydrogel layer will be put into the peripheral vein, within 30 min, to capture circulating tumor-like cell. Fluorescent staining and microscopic examination will be operated to identify whether the captured circulating tumor-like cell is CTC. We have conducted the first clinical registration study of CS in China [6]. The positive rate of CTC was 57.5% in this study. Afterwards, another registration study of CC has also been conducted by us in 2016 with a 74.8% sensitivity and 100% specificity [7].

Both CS and CC have been accepted as the proper devices to capture CTC by domestic approval department due to the studies conducted by us. In addition, from these two studies, we found some advantages in CC over CS system. First, although registration studies were performed in the different periods, we set the same inclusion criteria. It seemed that CC had a higher sensitivity than CS system in the similar enrolled populations. Second, we can use the CC system to carry out some testing like phenotypes and single cells sequencings, which is an essential part in precision medicine.

In our department, we continued our CTC testing by using CC or CS systems in real world. In this article, we conducted the real-world study to compare with these two devices and to re-verify the efficacy of CTC counts in overall survival. Considering less of patients received CTC testing by using both systems, we used propensity scores to eliminate the possible bias.

2. Methods

2.1. Study population and data collection

This is a real-world study that included metastatic breast cancer patients whose CTCs were detected with CS system or CC platform. Patients met the following points should be included in the analysis. 1. Female, aged 18 years or older; 2. Eastern Cooperative Oncology Group (ECOG) score 0–2; 3. With at least one measurable tumor lesion; 4. Clear immunohistochemistry result; 5. Received at least one CTC test. All patients meeting those criteria should be included to avoid possible selection bias. Patients were excluded in the analysis if they had an incomplete follow-up information. Patients with definite pathological diagnosis of breast cancer should be treated according to the established plans and standards at that time.

Data were collected from Chinese society of Clinical oncology breast cancer (CSCO BC) database. Use of the CSCO BC database was approved by the Ethics Board of the Affiliated Hospital of Qingdao University (QYFYKYLL 221311920). Written informed consent was waived from participants. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

2.2. Detection of CTC using CS

7.5 ml peripheral blood was collected and stored in the CellSave tube, and then CTC was captured and isolated using the CS circulating epithelial cell kit. CTC positive was defined as EpCAM, DAPI and CK staining positive, while CD45 staining negative, and the cell diameter was >4 μm.

2.3. Detection of CTC using CellCollector

The sampling needle was punctured into the peripheral blood of the cubital vein through a 20G indwelling needle, to ensure that the functional area was exposed to the peripheral blood, and was left in the body for 30 min of incubation. Tumor cells are specifically captured by binding to EpCAM antigen on the tumor cell surface. Upon completion of CTC collection, the CC captured with CTC was stained and identified according to the requirements of the staining kit. Negative (NK92 cells) and positive (SK-BR-3 cells) controls were stained simultaneously. Staining analysis was performed with CD45 (Exbio, clone MEM-28-Alexa647) antibody, cytokeratin CK7/19/panCK antibody (EXBIO Praha, clone A53-B/A2-Alexa488), and nuclear staining with Hoechst33342 (Sigma), combined with cytological morphology to identify whether it is a tumor cell. Patients with one or more CTC are judged to be CTC positive. Of note, these markers have different sensitivity and specificity, and they may result in “False positives”. To distinguish such “False positives”, we genotyped against a subset of CTCs while counting; single-cell sequencing was also performed in previous studies; These methods can better distinguish between “False positives”.

2.4. Outcome assessment

The primary outcome was overall survival (OS), calculated from date of first CTC testing to death from any cause. Secondary outcomes were progression free survival (PFS) and the CTC detection rates between two devices. PFS was calculated from date of

salvage therapy receiving first CTC testing to progression, or death from any cause. Patients who were alive without an event as of the analysis cutoff date were censored at the last study follow-up date. CTC detection rate was calculated with the following formula: Detection rate = (patients with positive CTCs/total patients) *100%.

2.5. Data analysis

We used $\chi 2$ or Fisher's exact tests to assess associations between the presence of CTC and primary tumor characteristic. We used Fisher's exact test when any one of the observed frequencies in the two-by-two contingency table was <5. We also used Kaplan-Meier and Cox proportional hazards regression to estimate hazard ratio (HR) and 95% confidence interval (CI) for the relationship between CTC numbers and OS or PFS. Results were considered significant at $p < 0.05$. Statistical analyses were performed using SPSS 21.0 System.

To address the imbalance of potential confounders between the two groups, we matched treatment groups using propensity scores matching (PSM) [8]. The propensity score was estimated as the predicted probability of a patient being in the CC or CS group from a logistic regression model. Patients in CS group were matched 1:1 to that in CC group by using the greedy matching method.

3. Results

A total of 536 eligible patients received CTC testing from September 2010 to October 2017 in our department, including 364 cases received CS detection and 172 cases received CC detection. The clinical characteristics of the patients were shown in Table 1. Patients were diagnosed from July 1989 to November 2016. More than half of patients were lymph node positive both in CS and CC group (70.1% and 76.0% respectively, $p = 0.171$). However, more patients in CS group were stage IV metastatic breast cancer compared with these in CC group (14.3% vs. 8.1%, $p < 0.01$). Patients in CS group also had higher rates in HER2 positive (75.5% vs 45.9%, $p < 0.001$) and visceral metastasis (74.2% vs 62.8%, $p = 0.007$) subtypes when compared with that in CC group.

Although both groups had a median of 1 CTC count, the detection rates vary. In CS group, 57.4% (209/364) of patients were CTC positive ranged from 0 to 2740. This proportion came to 37.4% (136/364) when calculated 5 or more CTCs as positive. In CC group, 69.2% (119/172) of patients had CTCs ranged from 0 to 128, significantly higher than that in CS group ($p = 0.009$). However, only 16.3% (28/172) of patients had 5 or more CTCs, much lower than that in CS group ($p < 0.001$).

Considering the imbalance in clinical characteristics, the propensity score model was matched according to clinical M status, HER2 status, visceral metastasis, and the number of treatment lines. After propensity score matching, for 1:1 matching, a total of 320 patients were included (Fig. 1). Patients in CS group had a 58.1% positive rate compared with 70.6% of that in CC group ($p = 0.02$). The positive rate of CTC was much higher in these older patients or the lower tumor burden in the early stage (Fig. 2).

The clinical characteristics was shown in Table 1. We analyzed the association between the CTC counts and long-term outcomes in

Table 1
Clinical characteristics of patients received CTC.

characteristic	Original patients			Patients after PSM		
	Cellsearch (n = 364)	Cellcollector (n = 172)	p	Cellsearch (n = 160)	Cellcollector (n = 160)	p
age			0.607			0.090
≤45 y	197 (54.1%)	89 (51.7%)		99 (61.9%)	84 (52.5%)	
> 45 y	167 (45.9%)	83 (48.3%)		61 (30.1%)	76 (47.5%)	
Menstruation			0.329			1.000
premenopausal	191 (52.5%)	89 (51.7%)		94 (58.8%)	94 (58.8%)	
postmenopausal	173 (47.5%)	83 (48.3%)		66 (41.2%)	66 (41.2%)	
T stage			0.756			1.000
T ≤ 5 cm	277 (76.7%)	117 (78.0%)		128 (80.0%)	128 (80.0%)	
T > 5 cm	84 (23.3%)	33 (22.0%)		32 (20.0%)	32 (20.0%)	
N stage			0.171			0.058
N0	109 (29.9%)	37 (24.0%)		50 (31.2%)	35 (21.9%)	
N1-3	255 (70.1%)	117 (76.0%)		110 (68.8%)	125 (78.1%)	
M stage			0.044			0.841
M0	312 (85.7%)	158 (91.9%)		147 (91.9%)	146 (91.2%)	
M1	52 (14.3%)	14 (8.1%)		13 (8.1%)	14 (8.8%)	
Hormonal receptor			0.455			0.074
HR positive	184 (50.5%)	81 (47.1%)		90 (56.2%)	74 (46.2%)	
HR negative	180 (49.5%)	91 (52.9%)		70 (43.8%)	86 (53.8%)	
HER2 stage			< 0.01			0.823
HER2 positive	275 (75.5%)	79 (45.9%)		81 (50.6%)	79 (49.4%)	
HER2 negative	89 (24.5%)	93 (54.1%)		79 (49.4%)	81 (50.6%)	
Visceral metastasis			0.007			1.000
yes	270 (74.2%)	108 (62.8%)		102 (63.8%)	102 (63.8%)	
no	94 (25.8%)	64 (37.2%)		58 (36.2%)	58 (36.2%)	
Therapy lines			0.044			0.692
≤2nd line	165 (45.3%)	94 (54.7%)		121 (75.6%)	124 (77.5%)	
> 2nd line	199 (54.7%)	78 (45.3%)		39 (24.4%)	36 (22.5%)	

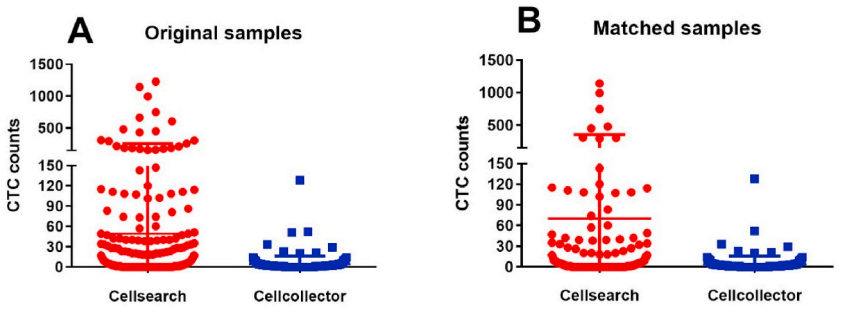


Fig. 1. CTC distributions in different groups before and after PSM.

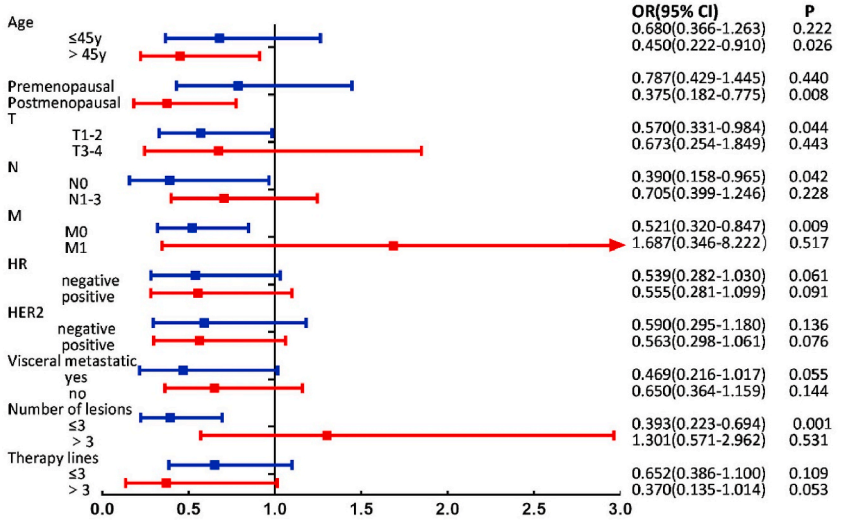
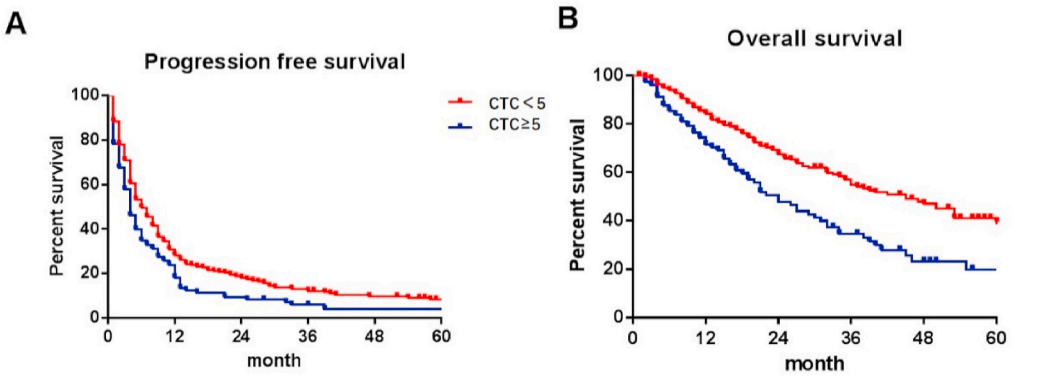


Fig. 2. CTC detection in different devices after propensity score matching.

these patients with a median follow-up time of 20 months. For patients with 5 or more CTCs, the median PFS was 4.3 months, while it was 6.2 months for patients with CTC < 5 (HR = 0.725, 95% CI: 0.534–0.851) (Fig. 3A). The association between CTC counts and overall survival were also significant, the median OS was 44.8 months for CTC < 5 compared with 21.6 months for CTC≥5 (HR =



Group	N	Events	Median PFS	HR(95%CI)
CTC < 5	372	289 (77.7%)	6.2mo	0.725(0.534-0.851)
CTC≥5	164	134 (81.7%)	4.3mo	

Group	N	Events	Median PFS	HR(95%CI)
CTC < 5	372	121 (32.5%)	44.8mo	0.531(0.340-0.658)
CTC≥5	164	73 (44.5%)	21.6mo	

Fig. 3. PFS and OS of CTCs when using 5 as boundary.

0.531, 95% CI: 0.340–0.658; Fig. 3B).

Meanwhile, when these patients were grouped using negative and positive, the CTC counts were also highly associated with overall survival. For these detected without any CTCs, their median OS was 49.8 months compared with 26.9 months in these with positive CTC counts (HR = 0.599, 95%CI: 0.452–0.795) (Fig. 4A). Multivariate analysis showed that HER2 status, CTC counts, number of treatment line and visceral metastasis were all important factors affecting OS (Table 2), while different testing platforms had little effects on OS.

A total of 41.8% (224/536) of patients received a second CTC testing after 4 weeks of their initial therapy. Patients without CTCs in the second CTC testing had a longer OS than these with CTCs (52.6 month vs 26.9month, HR = 0.496, 95% CI:0.314–0.784) (Fig. 4B). We conducted a grouping analysis based on the results of the CTC counts, and found that patients with twice negative CTCs had the best prognosis (median OS: 52.6 months), while those with twice positive CTCs had a median OS of 26.6 months (Fig. 4C). The decrease of CTC counts became statistical significance when CTC decreased by more than 50%, (40.1 months vs 25.8 months, HR = 0.588, 95% CI: 0.350–0.933) (Fig. 4D). In addition, for HER2-positive patients, Patients with CTC HER2 positive had longer overall survival than patients with CTC HER2 negative (median OS: 26.7 months vs 17.3 month, HR = 0.528, 95% CI: 0.269–0.887) (Supplementary Fig. 1).

4. Discussion

There are several implications in our study, first, patients in CC group had a superior detection rate while inferior detection numbers of CTCs compared with that in CS group. Second, five CTC is always used as boundary to distinguish the survival for breast cancer patients. Here in this study, patients with less than 5 CTCs had a definitely longer progression free survival and overall survival. However, it does not mitigate overall benefit from less CTCs. Patients with negative CTCs, whether in the first testing or in the second, were more likely to have a longer survival compared with these with positive CTCs. Additionally, the degree of CTC decrease is a key factor affecting CTC decreased by more than 50% showed substantial improvements in OS which indicated the benefit from dynamic CTC testing.

After the approval of CS by the Food and Drug Administration (FDA) and Chinese National Medical Products Administration (NMPA) for clinical use, a large number of studies have explored the value of CTC in cancer. The significance of CTC have been clinically recognized in efficacy monitoring [9–11], early diagnosis [12] and so on. However, the low content of CTC from CS contributed to as low as 20% of detection rate [13,14] in cancer patients which posed severe limits to clinical application of CTC. CC has been approved to greatly increase the detection rate of CTC. It is said that detection rate in early lung cancer has been about 50–60% [12,15], not along other solid tumor patients.

In this study, we found CC had superior detection rate of CTC than CS which is consistent with the comparative studies of the two

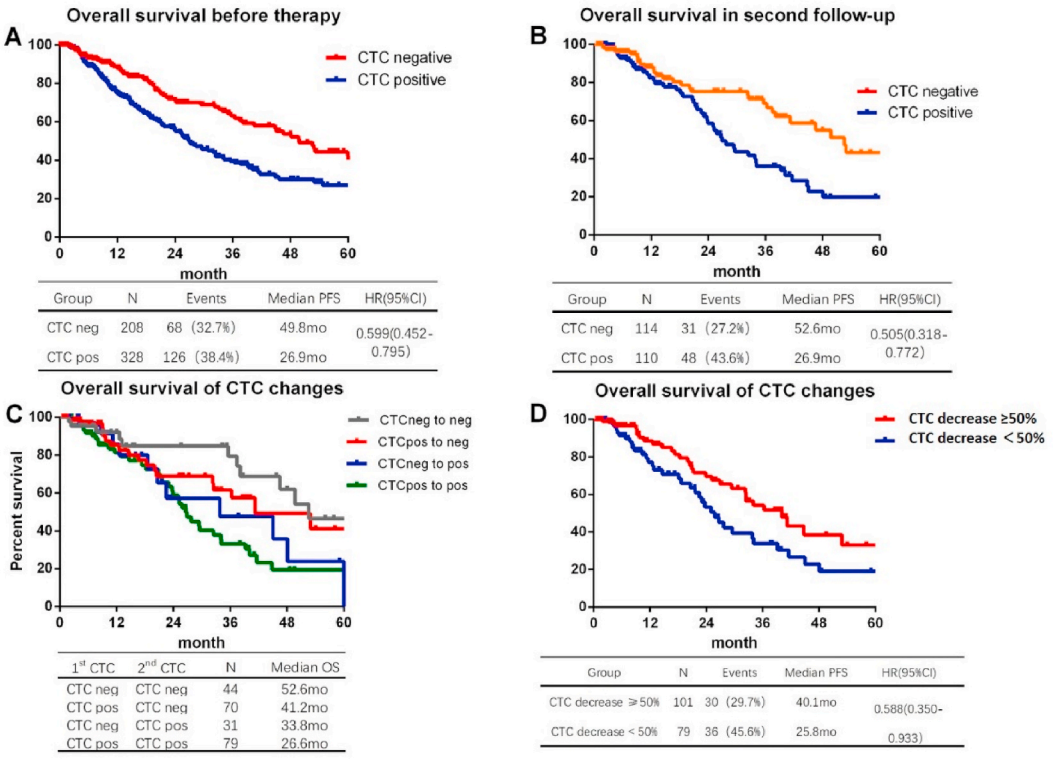


Fig. 4. Overall survival of CTCs.

Table 2
Multivariate analysis of overall survival.

Factors	Groups	HR	95%CI
Different technologies	Cellsearch vs. Cellcollector	0.696	0.471–1.028
Ages	≤45 y vs. > 45 y	1.020	0.707–1.471
Menopause	Premenopausal vs. postmenopausal	0.801	0.554–1.158
Clinical T stage	T1-2 vs. T3-4	0.951	0.673–1.343
Clinical N stage	N0 vs. N1-3	1.049	0.749–1.469
Clinical M stage	M0 vs. M1	1.133	0.742–1.731
Hormone receptor	Negative vs. Positive	0.778	0.577–1.051
HER2 status	Negative vs. Positive	0.469	0.337–0.654
Visceral metastasis	No vs. Yes	1.529	1.032–2.266
Number of lesions	≤3 vs. > 3	1.847	1.341–2.544
Therapy lines	≤3rd vs. > 3rd line	2.258	1.658–3.077
CTC counts	Negative vs. Positive	1.573	1.148–2.156

techniques [16–19]. We also observed that the CC had a higher detection rate, but a much lower numbers of CTCs. There may be two possible reasons for this situation: firstly, CC passively captures the flowing CTC by introducing the antibody coated rod into the blood vessel, thereby bypassing the separation process. CTC must be close to the antibody on the wire to bind. However, the capture of CTC by this device is moderate, possibly because there is no mechanism to explain how flowing cells are attracted to the rod [20]. Secondly, although previous studies have indicated that CC has a higher blood flow after long-term filtration in the body, Dizdar's study found through calculation that the volume monitored by CC is only 0.3–18 ml, which may be the reason for the low CTCs count in our study's CC detection technology [21]. Previous studies have found that the number of CTC is an independent predictor of prognosis in metastatic breast cancer, and its number and reproducibility will change with the efficacy of drug treatment in repeated samples [22], this result was further confirmed in this study.

To eliminate the possible bias, a propensity score matching was used [23]. CC still had a high detection rate after balancing the four factors with significant differences in visceral metastasis, number of treatment lines, HER2 status, and newly diagnosed metastasis status. The higher CTC detection rate makes it possible to monitor CTC changes. Patients with older ages, lower clinical TNM stages and small number of tumor lesions are more likely to be positive when using CC platform. We searched several articles in pubmed to support our findings. A randomized controlled study from Germany compared the detection rates of CS and CC, and found no significant difference in the total number of CTC detections between the two methods [21]. In early patients with nonmetastatic (M0), CC was more likely to detect CTC than CS. Another large, randomized, controlled study of breast cancer patients found that the number of CTCs detected was associated with bone metastases [24]. However, there is currently relatively little research on the correlation between CTC detection rate and age, and more research results are needed to further explore their relationship. Furthermore, CS also have the advantages in terms of more than 5 CTCs. A further study is needed to find whether the high positive rate while low numbers will affect the clinical applications.

It is said that patients with 5 or more CTCs could contribute to a notable reduction of survival in breast cancer. Our results are highly consistent with this assume. Five or more CTCs remained an independent poor prognostic factor, and patients with CTC≥5 had shorter PFS and OS than patients with CTC<5. Meanwhile, we still found that the less CTCs patients had, the better their overall survival would be. This is also proposed that in clinical, the prognosis of CTC<5 may not be necessarily better, but the prognosis of patients with positive CTC is definitely worse. In this study, we find the positive CTCs, which is not necessarily need to be 5 CTCs, can also predict the overall survival of patients. This also favors the application of CC in clinical due to the high detection rates compared with CS. Previous studies have explored that the detection rate of CC technology in breast cancer patients is on average between 41% and 70% [21,25,26]. Although the sensitivity of CC count is lower than that of CS count, the correlation between CTC count detected by CC count and clinical prognosis in breast cancer patients is still very clear. In this manuscript, we performed a separate analysis of HER2-positive breast cancers. It can be seen that the positive rate of CTCs detected by CC was 50.6% after propensity score. We subsequently performed a prognostic analysis and found that CTC HER2-positive patients had longer overall survival than CTC HER2-negative patients.

Dynamic monitoring of CTC during treatment can better reflect the patient's response to treatment and its prognosis. A number of studies have shown that patients with persistent CTC positive before and after treatment have the worst prognosis, and those with negative CTC after treatment have a better prognosis. Our results are comparable to those in the pivotal trials [27–29]. Furthermore, there is a common consensus that CTC decrease could reflect the improvement of survival whatever the degree, however, we found only these with 50% of CTC decrease could indicate the future survival benefit. Several reasons might explain such phenomenon. First, in this study, patients received second CTC testing only after their first cycle of therapy, a short term period may not reflect the real dynamic changes of CTCs. Second, the small fluctuation of CTC counts may not reflect the real changes of tumor burden and may contribute to the inevitable bias. Only these with obviously CTC changes can show the survival benefits.

As a retrospective real-world study, there were also some limitations. First, all the comparative data are from different patients. The results may be more intuitive and accurate when using the two techniques on the same patient. Second, as a real-world data, there is an inevitable selection bias. Although a PSM was done to mimic important attributes of randomized clinical trials, it cannot adjust for patient characteristics that are not measured and included in the model [30]. The loss of data during the PSM contribute to a relatively smaller study population which may influence result interpretation. Finally, the volume of CTC monitoring needs to be clarified by

algorithms. Our study only conducted strict statistics on the CTC counts, without calculating and analyzing the volume. This is also an issue that we need to pay attention to in our future research. Thus, further investigations and follow-ups will be performed to resolve the aforementioned issue and to determine the literal implications of the CTCs in breast cancer patients.

Ethic statement

The study complies with all regulations.

Data were collected from Chinese society of Clinical oncology breast cancer (CSCO BC) database. This study was reviewed and approved by the Ethics board of The Affiliated Hospital of Qingdao University, with the approval number: QYFYKYLL 221311920. All patients or their proxies provided informed consent to participate in the study.

Data availability statement

Access to the dataset supporting the conclusion of this article can only legally be accessed on acceptance from the Chinese Society of Clinical Oncology breast cancer database collaborative group. Interested parties can contact the corresponding author for further information. We confirm that others would be able to access these data in the same manner as the authors. We also confirm that the authors do not have any special access privileges that others would not have.

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CRediT authorship contribution statement

Feng Li: Writing – review & editing, Data curation. **Jianbin Li:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Funding acquisition, Formal analysis, Data curation. **Yang Yuan:** Writing – review & editing, Resources, Data curation. **Huiqiang Zhang:** Writing – review & editing, Resources, Data curation. **Shaohua Zhang:** Writing – review & editing, Resources, Data curation. **Li Bian:** Writing – review & editing, Resources, Data curation. **Tao Wang:** Writing – review & editing, Resources, Data curation. **Zefei Jiang:** Writing – review & editing, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29217.

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