Feasibility of circulating tumor cells (CTCs) combined with medical imaging examinations to screen for cancer and improve diagnosis in non-metastatic invasive breast cancer patients: a cohort study

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Abstract

Background

Previous study has reported that circulating tumor cells (CTCs) could serve as a diagnostic biomarker in breast cancer (BC) screening. However, the differential efficacy of routine examination including ultrasound (US), mammogram (MG), magnetic resonance imaging (MR), and breast-specific gamma imaging (BSGI) and CTCs is unknown. This study aimed to combine CTCs with other BC screening imaging modalities to screen for cancer and enhance diagnostic potency in non-metastatic BC patients.

Methods

102 treatment-naive non-metastatic BC patients were enrolled in this study between December 2017 and November 2018. All these patients received CTC detection and at least one medical imaging examination. Correlations of CTC enumeration with patients' clinicopathological characteristics and medical imaging examinations were evaluated.

Results

CTC detection rates (average CTC counts) in stage I-III BC patients were 92.9% (2.1), 87.2% (2.4) and 100% (4.2), respectively. CTCs counts were positively associated with cancer stage ($p = 0.0084$) and tumor size ($p = 0.0301$). CTCs counts were more correlated with US than MR, and showed least correlation with MG. CTCs counts were not associated with molecular subtypes of BC nor breast-specific gamma imaging (BSGI) results, indicating that CTC enumeration cannot be used to reflect molecular signatures of BC. When both cut-off values for CTCs and Breast Imaging-Reporting and Data System (BI-RADS) were used, false negative rates (FNR) of CTCs, US, MG and MR in BC detection in this study were 22.5%, 15.8%, 30.6% and 12.8%, respectively. Combination of CTC with US, MG or MR decreased FNR of BC detection to 7.9%, 8.3% and 5.8%, respectively. False negatives are more common in early stage patients, and in patients with smaller tumors.

Conclusion

CTCs counts can be used as a diagnostic aid in BC screening and early diagnosis. CTCs counts were more relevant to US than MR or MG. Conjugation of CTCs counts would improve the diagnostic potency of medical imaging examinations for BC detection, especially for MG in Chinese women.

Background

In China, there were 304,000 newly diagnosed cases of breast cancer (BC) and approximately 69,900 women died from BC in 2015 [1]. According to the World Health Organization, although the mortality rates

of BC in developed countries are decreasing, incidence and mortality rates of BC in developing countries such as China and India are still increasing [2]. “Early detection, early treatment” means that patients would have better survival outcomes if the tumors were diagnosed earlier. So various methods have been proposed as BC early diagnosis and screening tools, including breast palpation, medical imaging methods and blood-based assay.

Diagnostic imaging modalities recommended by American Joint Committee on Cancer (AJCC) for BC screening include mammography (MG), ultrasound (US) and magnetic resonance imaging (MR). MG uses low dose X-rays to examine lesions in the breast, allowing to view small calcification points, tumor in situ, and the structure of the breast, which are signs of early cancer lesions. Studies showed that overall sensitivity of MG for cancer screening was 87% [3]. Breast US is a non-invasive examination that uses high-frequency acoustic reflection to reveal tissue inside the breast. Breast US is often used in conjunction with MG to increase the sensitivity of BC detection for women at average risk. US can help us identifying whether the lumps found in MG is solid or filled with fluid. Although US is more accurate than MG in differentiating breast masses or cysts, it can not detect the small calcification points and is less sensitive to detect tumors which is less than 5 mm with diameter or deep in the breast. MR is often used in conjunction with MG for high-risk women BC screening. In this article, MR means enhancing-MR for all patients. MR has no radiation exposure, and can provide excellent images with high contrast and resolution under appropriate conditions. MR is the most sensitive diagnostic tool for breast diseases, because it can be performed in any direction without the influence of tissue overlap or breast composition. MR is often used in the diagnosing and staging process rather than screening of BC. However, MR is more expensive than MG and US. Therefore, MG and US are the two most common used BC screening modalities in practice in China. Breast specific gamma imaging (BSGI) is a physiologic and molecular approach to breast imaging using a high resolution, small-gamma camera and a tracer agent called ^{99m}Tc-Sestamibi (MIBI) which we have investigated in our previous study[4].

Circulating tumor cells (CTCs) are tumor cells which have detached from the primary tumor or metastatic sites into circulation. Studies have reported the prognostic value of CTC in BC. Therefore, in the 8th edition of the AJCC cancer guidelines, it is written that CTC can be used as a prognostic biomarker in BC to predict patients’ survival outcomes. Patients with metastatic breast cancer (MBC) usually have more CTC and BC patients with more CTC usually have shorter progression free survival and overall survival [5–6]. Moreover, CTC can reflect the tumor burden, and can be used as a monitoring biomarker to assess patients’ response to treatment and tumor recurrences [7]. Previous study with CytoSorter® (Hangzhou Watson Biotech, Hangzhou, China) in BC has shown that CTCs could be used as a diagnostic aid in early diagnosis and screening of BC [8].

Both CTCs and medical imaging examinations could be used as a screening method for BC. Therefore, the aims of this work were: (1) to correlate CTCs with medical imaging examinations, such as US, MG, MR or BSGI, and (2) to compare their sensitivities in BC detection. Non-metastatic BC patients, who have performed CTC detection and medical imaging examinations before biopsy and treatment, were enrolled

in this study. Correlations of CTCs with patients' clinicopathological characteristics and medical imaging examinations were evaluated to answer our questions.

Methods

Patients

In total, 102 non-metastatic BC patients were enrolled in this study from December 2017 to November 2018, who had performed CTC detection and medical imaging examinations. Inclusion criteria was as follows: (1) patients aged between 18 and 75 years old; (2) patients had CTC detection before biopsy and treatment; (3) patients had no pathological proof of metastatic BC; (4) patients received US, MG, MR, or breast-specific gamma imaging (BSGI) examinations before treatment. Exclusion criteria was as follows: (1) patients had other malignant tumors or other malignant diseases within 5 years prior to enrollment; (2) patients had no medical imaging examination. Among the 102 BC patients, 101 had US, 84 had MG, 91 had MR, while 42 had BSGI examinations. Patients' clinicopathological characteristics, including age, histological type, grade, hormone receptors, human epidermal growth factor receptor 2 (HER2) and the clinical stage at diagnosis, were collected. This study followed the principles established in the Declaration of Helsinki and was approved by the ethics committee of Second Affiliated Hospital of Zhejiang University School of Medicine. Written informed consent was obtained from each participant.

CTCs detection

CytoSorter[®] epithelial cells detection kit was used for CTC enrichment. CTC detection procedure was described as in the previous study [8]. Once the CTC enrichment was finished, the CytoChipNano microfluidic chip was removed from CytoSorter[®] and proceed to the immunofluorescence staining of PanCK-FITC, CD45-PE and DAPI. An OPPNO immunofluorescence microscopy (DSY5000X, OPPNO, Chongqing, China) was used to identify CTCs by searching for PanCK-FITC⁺, CD45-PE⁻, and DAPI⁺ cells.

Medical imaging examinations

US examination was performed with IU Elite[®] (Philips Healthcare, Best, Netherlands). MG examination was performed using Selenia[®] (Hologic, Santiago, USA). Breast MR examination was performed on a 1.5 T system with Aera[®] (Siemens, München, Germany). BSGI examination was performed with Dilon 6800 Gamma Camera (Dilon Technologies, Newport News, VA). Examination results were evaluated by an experienced radiologist according to the American College of Radiology (ACR) BI-RADS assessment categories. BI-RADS is a risk assessment of likelihoods of BC, with a score ranging between 0 and 6. Categories 1–6 represent negative, benign, probably benign, suspicious or indeterminate abnormality, highly suggestive of malignancy, and known cancer, respectively. Category 4 is subdivided into 3 sub-categories: 4A for a low suspicion of malignancy, 4B for a moderate suspicion of malignancy, and 4C for a high suspicion of malignancy. Breast density was visually estimated according to BI-RADS classification and classified as follows: a = almost entirely fat (less than 25% of breast comprising glandular tissue); b = having scattered fibroglandular densities (25–50% of breast comprising glandular

tissue); c = heterogeneously dense (51–75% of breast comprising glandular tissue); and d = extremely dense (more than 75% of breast comprising glandular tissue). Detailed examination procedure for BSGI was described as in the previous study [4].

Statistical analysis

Statistical analyses were performed using Prism 6.0 (Graphpad, La Jolla, CA, USA). Student t test was used for continuous variables, as appropriate. The χ^2 test and Fisher's exact test were used for the comparison of categorical parameters. One-way analysis of variance (ANOVA) was performed to calculate the differences among multiple groups. A two-sided p value less than 0.05 was considered statistically significant.

Results

Comparison of CTCs with BC patients' clinicopathological features

Studies have shown that CTC could reflect tumor burden in BC [5, 8]. 102 BC patients, including 42 stage I, 47 Stage II and 13 stage III, with a mean average age of 53.8 years were enrolled in this study. As shown in Table 1, the CTC detection rates (average CTC counts) for stage I-III BC patients were 92.9% (2.1), 87.2% (2.4) and 100% (4.2), respectively. To determine whether CTCs were associated with BC patients' clinicopathological characteristics, statistical analyses were performed among different groups of BC patients based on their age, AJCC stages, tumor size, lymph node metastasis, molecular subtypes and histology grades. Results showed that CTCs were correlated with AJCC stage ($p = 0.0084$) and tumor size ($p = 0.0301$), but not with age, lymph node metastasis, molecular subtype, or histology grade. If BC patients were grouped first by the molecular subtype, no correlation of CTCs with AJCC stage would be observed in each molecular subtype group (data not shown).

Table 1
Correlation of CTCs with BC patients' clinicopathological features

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTC Count (Range)	P
AJCC Stage						
I	42	56 (57)	39	92.9	2.1 (0–7)	0.0084
II	47	52.1 (52)	41	87.2	2.4 (0–13)	
III	13	53.1 (55)	13	100	4.2 (2–15)	
TNM T Stage						
T1	64	54.2 (55.5)	58	90.6	2.2 (0–13)	0.0301
T2	32	52.1 (50)	30	93.8	3.1 (0–15)	
T3	6	59 (57.5)	5	83.3	2.3 (0–5)	
TNM N Stage						
N0	62	54.9 (56)	56	90.3	2.2 (0–7)	0.0712
N1	31	52.7 (54)	28	90.3	2.9 (0–15)	
N2	6	46.7 (43)	6	100	2 (2)	
N3	3	58.3 (55)	3	100	5.3 (3–9)	
Lymph Node Metastasis						
Yes	40	52.3 (54)	37	92.5	3 (0–15)	0.1673
No	62	54.9 (56)	56	90.3	2.2 (0–7)	
Molecular Subtypes						
Luminal A	34	53.9 (55)	29	85.3	2.3 (0–9)	0.8058
Luminal B	36	52.5 (54.5)	34	94.4	2.9 (0–15)	
HER2 OE	12	53.8 (54.5)	12	100	2.4 (1–5)	
TN	20	55.3 (54.5)	18	90	2.1 (0–5)	
Histology Grade(1 = low; 2 = medium; 3 = high) (n = 63)						
1	6	55.2 (56.5)	6	100	2.2 (1–3)	0.9832

BC = breast cancer; n = number of patients; CTCs = circulating tumor cells; AJCC = American Joint Committee on Cancer; TNM = tumor-node-metastasis; OE = over-expression; TN = triple negative.

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTC Count (Range)	<i>P</i>
2	40	54.6 (56.5)	37	92.5	2.5 (0–15)	
3	17	53.5 (53)	16	94.1	2.2 (0–5)	
BC = breast cancer; n = number of patients; CTCs = circulating tumor cells; AJCC = American Joint Committee on Cancer; TNM = tumor-node-metastasis; OE = over-expression; TN = triple negative.						

Comparison of CTCs with US in BC

101 out of 102 patients had US examination. Results of US were grouped according to the tumor size estimated by US or by BI-RADS as shown in Table 2. Based on the tumor size T , five categories were defined – 1a, 1b, 1c, 2 and 3, representing patients with $T \leq 0.5$ cm, $0.5 \text{ cm} < T \leq 1$ cm, $1 \text{ cm} < T \leq 2$ cm, $2 \text{ cm} < T \leq 5$ cm, and $T > 5$ cm, respectively. CTC enumerations were correlated with US when BC patients were grouped into 1, 2 and 3 or into 1a + 1b, 1c, 2 and 3 ($p = 0.0322$ or 0.0309). CTC enumerations were correlated with US as well when BC patients were grouped by BI-RADS into 3, 4a, 4b, 4c, 5 and 6 or 3, 4, 5, and 6 ($p = 0.0213$ or 0.0464). CTC enumeration showed a statistical significant difference between patients with BI-RADS score $\geq 4b$ and with $\leq 4a$. Taken together, CTCs were positively associated with US examination.

Table 2
Correlations of CTCs with ultrasonography in BC

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTCs Count (Range)	<i>P</i>
US (n = 101)						
1a	3	58.3 (57)	3	100	1.7 (1–2)	0.0639
1b	8	55.6 (57)	7	87.5	1.6 (0–3)	
1c	38	51 (51)	36	94.7	2.3 (0–7)	
2	49	55.2 (55)	43	87.8	2.8 (0–15)	
3	3	56.3 (60)	3	100	4	
1a + 1b	11	56.4 (57)	10	90.9	1.6 (0–3)	0.0309
1c	38	51 (51)	36	94.7	2.3 (0–7)	
2	49	55.2 (55)	43	87.8	2.8 (0–15)	
3	3	56.3 (60)	3	100	4	
1	49	52.2 (53)	46	93.9	2.1 (0–7)	
2	49	55.2 (55)	43	87.8	2.8 (0–15)	0.0322
3	3	56.3 (60)	3	100.0	4	
US BI-RADS (n = 101)						
3	4	56 (48.5)	2	50.0	1.3 (0–3)	0.0213
4a	12	50.1 (52.5)	11	91.7	1.8 (0–6)	
4b	25	50.2 (49)	23	92.0	1.8 (0–3)	
4c	29	55.7 (55)	27	93.1	3.1 (0–15)	
5	30	56.9 (57)	28	93.3	2.9 (0–9)	
6	1	32	1	100	2	0.0464
3	4	56 (48.5)	2	50.0	1.3 (0–3)	
4	66	52.6 (52)	61	92.4	2.4 (0–15)	
5	30	56.9 (57)	28	93.3	2.9 (0–9)	
6	1	32	1	100	2	

CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; US = ultrasonography; BI-RADS = breast imaging reporting and data system.

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTCs Count (Range)	<i>P</i>
≤ 4a	16	51.6 (56)	13	81.3	1.7 (0–6)	0.0193
≥ 4b	85	54 (54)	79	92.9	2.6 (0–15)	
CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; US = ultrasonography; BI-RADS = breast imaging reporting and data system.						

Comparison of CTCs with MG, MR and BSGI in BC

84, 91 and 42 out of 102 patients had MG, MR and BSGI examinations, respectively. Breast tissue composition category was used to measure the density of breast tissue. Comparisons of CTCs with MG, MR and BSGI were shown in Tables 3, 4 and 5, respectively. There was no correlation of CTCs with MG, MG breast composition categories or BI-RADS. No correlation was found as well between CTCs and MR, MR breast composition categories, BI-RADS, time-signal intensity curves (TIC). Neither, no correlation was found as well between CTCs and BSGI. As a result, CTC enumeration was not associated with MG, MR, or BSGI examination.

Table 3
Correlations of CTCs with mammography in BC

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTCs Count (Range)	<i>P</i>
MG (n = 43)						
1a	1	62	1	100	3	0.568
1b	5	57.2 (59)	3	60	1.4 (0–3)	
1c	13	54.9 (56)	12	92.3	2.4 (0–6)	
2	22	56.6 (55)	20	90.9	2.2 (0–9)	
3	2	60 (60)	1	50	2 (0–4)	
1a + 1b	6	58 (60)	10	166.7	1.7 (0–3)	0.7507
1c	13	54.9 (56)	36	276.9	2.4 (0–6)	
2	22	56.6 (55)	43	195.5	2.2 (0–9)	
3	2	60 (60)	3	100	2 (0–4)	
1	19	55.9 (56)	46	242.1	2.2 (0–6)	0.7807
2	22	56.6 (55)	43	195.5	2.2 (0–9)	
3	2	60 (60)	3	150.0	2 (0–4)	
MG Breast Tissue Composition Categories (n = 82)						
a	8	62.9 (61)	8	100	2.4 (1–4)	0.6793
b	12	60.8 (59)	11	91.7	2.6 (0–9)	
c	52	51.3 (50)	47	90.4	2.6 (0–15)	
d	10	49 (48)	10	100	3.1 (2–7)	
a + b	20	61.7 (60.5)	19	95	2.5 (0–9)	0.8808
c + d	62	50.9 (50)	57	91.9	2.7 (0–15)	
MG BI-RADS (n = 72)						
2	4	44.3 (43)	4	100	2 (1–3)	0.8762
3	10	51.3 (52.5)	9	90	2.1 (0–3)	
4a	8	54.8 (55)	6	75	1.8 (0–4)	

CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; MG = mammography; BI-RADS = breast imaging reporting and data system.

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTCs Count (Range)	<i>P</i>
4b	16	51.4 (51)	16	100	3.2 (1–15)	0.9709
4c	29	56.8 (59)	26	89.7	2.6 (0–9)	
5	5	59 (58)	5	100	2.2 (1–3)	
2	4	44.3 (43)	4	100	2 (1–3)	
3	10	51.3 (52.5)	9	90	2.1 (0–3)	
4	53	54.9 (56)	48	90.6	2.6 (0–15)	0.2841
5	5	59 (58)	5	100	2.2 (1–3)	
≤ 4a	22	51.3 (51.5)	19	86.4	2 (0–4)	
≥ 4b	50	55 (56)	47	94	2.7 (0–15)	
CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; MG = mammography; BI-RADS = breast imaging reporting and data system.						

Table 4
Correlations of CTCs with magnetic resonance imaging examination in BC

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTCs Count (Range)	<i>P</i>
MR (n = 83)						
1a	2	58.5 (58.5)	2	100	1.5 (1–2)	0.1843
1b	4	50.5 (52)	3	75	2.8 (0–6)	
1c	36	53.7 (54)	31	86.1	1.8 (0–4)	
2	35	51.7 (49)	33	94.3	2.6 (0–9)	
3	6	57.2 (59.5)	6	100	2.2 (1–4)	
1a + 1b	6	53.2 (56.5)	5	83.3	2.3 (0–6)	0.167
1c	36	53.7 (54)	31	86.1	1.8 (0–4)	
2	35	51.7 (49)	33	94.3	2.6 (0–9)	
3	6	57.2 (59.5)	6	100	2.2 (1–4)	
1	42	53.6 (54.5)	36	85.7	1.9 (0–6)	
2	35	51.7 (49)	33	94.3	2.6 (0–9)	0.0876
3	6	57.2 (59.5)	6	100	2.2 (1–4)	
MR Breast Tissue Composition Categories (n = 91)						
a	3	58 (66)	1	33.3	2.4 (1–4)	0.683
b	27	60.3 (60)	24	88.9	2.6 (0–9)	
c	52	51.2 (50)	49	94.2	2.6 (0–15)	
d	9	45.1 (43)	9	100	3.1 (2–7)	
a + b	30	60.1 (60)	25	83.3	2.5 (0–9)	0.4016
c + d	61	50.3 (48)	58	95.1	2.7 (0–15)	
MR BI-RADS (n = 86)						
3	6	52.8 (53.5)	5	83.3	2.3 (0–6)	0.292
4a	5	53.4 (57)	4	80	1.2 (0–2)	
4b	15	51.9 (49)	15	100	2.2 (1–7)	

CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; MR = magnetic resonance imaging; BI-RADS = breast imaging reporting and data system; TIC = time-signal intensity curves.

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTCs Count (Range)	<i>P</i>
4c	30	53.6 (54.5)	27	90	2.8 (0–15)	0.7037
5	15	52.8 (54)	15	100	2.5 (1–5)	
6	15	54.1 (54)	13	86.7	2.4 (0–5)	
3	6	52.8 (53.5)	5	83.3	2.3 (0–6)	
4	50	53.5 (54)	46	92	2.5 (0–15)	
5	15	52.8 (54)	15	100	2.5 (1–5)	
6	15	54.1 (54)	13	86.7	2.4 (0–5)	
≤ 4a	11	53.1 (57)	9	81.8	1.8 (0–6)	0.0941
≥ 4b	75	52.8 (54)	70	93.3	2.5 (0–15)	
MR TIC (n = 91)						
1	22	52.4 (54)	20	90.9	2.7 (0–15)	0.84
2	38	53.1 (53)	36	94.7	2.3 (0–7)	
3	27	55.5 (56)	23	85.2	2.5 (0–9)	
CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; MR = magnetic resonance imaging; BI-RADS = breast imaging reporting and data system; TIC = time-signal intensity curves.						

Table 5
Correlations of CTCs with breast-specific gamma-imaging in BC

Group	n	Average (Median) Age (years)	CTC Positive	CTC Detection Rate (%)	Average CTC Count (Range)	<i>P</i>
BSGI Score (1.82n) (n = 42)						
1	6	54.5 (53.5)	6	100	2.3 (2–3)	0.84
2	30	53.5 (54)	30	100	2.6 (1–13)	
3	6	52 (52)	6	100	2 (2–15)	
CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; BSGI = breast-specific gamma-imaging						

Comparison of CTCs with medical imaging examinations with cut-off settings

Previous studies have shown that when CTC cut-off was set to 2 the greatest diagnostic potency in BC diagnosis can be achieved [8]. Therefore, next we would like to know whether CTCs would be more relevant to medical imaging examinations when the cut-off values were used. First we compared CTCs

and medical imaging examinations with patients' clinicopathological characteristics when cut-off values were used. As shown in Table 6, CTC and MG positive rates were not associated with TNM stage and molecular subtypes. US positive rates were correlated with AJCC stage and lymph node metastasis. MR positive rate was only correlated with AJCC stage. Correlation of CTC positive rates with US, MG and MR are shown in Table 7–9, respectively. CTC positive rate showed a significant correlation with US when patients were divided into with BI-RADS score $\geq 4b$ and $\leq 4a$. However, CTC positive rate was not correlated with MR nor MG. Based on the *P* value, CTCs were more correlated with US than MR, and showed least correlation with MG.

Table 6
Correlations of CTCs and medical imaging examinations with BC patients' clinicopathological features with cut-off setting*

Characteristics	<i>P</i>			
	CTC (n = 102)	US (n = 101)	MG (n = 72)	MR (n = 76)
AJCC Stage	0.0964	0.0438	0.3332	0.0422
TNM T Stage	0.2417	0.1923	0.1668	0.116
TNM N Stage	0.3921	0.0865	0.6971	0.422
Lymph Node Metastasis	0.3269	0.0157	0.2609	0.0747
Molecular Subtypes	0.4713	0.9188	0.8881	0.088
CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; US = ultrasonography; MG = mammography; MR = magnetic resonance imaging.				
* CTCs is set to 2, while medical imaging BI-RADS is set to 4b.				

Table 7
Correlations of CTCs with ultrasonography in BC with cut-off setting*

Groups	n	Proportion (%)	CTCs ≥ 2		CTCs < 2		P
			n	Proportion (%)	n	Proportion (%)	
US (n = 101)							
1a	3	3	2	66.7	1	33.3	0.5181
1b	8	7.9	5	62.5	3	37.5	
1c	38	37.6	32	84.2	6	15.8	
2	49	48.5	37	75.5	12	24.5	
3	3	3	3	100	0	0	
1a + 1b	11	10.9	7	63.6	4	36.4	0.3589
1c	38	37.6	32	84.2	6	15.8	
2	49	48.5	37	75.5	12	24.5	
3	3	3	3	100	0	0	
1	49	48.5	39	79.6	10	20.4	0.5768
2	49	48.5	37	75.5	12	24.5	
3	3	3	3	100	0	0	
US BI-RADS (n = 101)							
3	4	4	2	50	2	50	0.0368
4a	12	11.9	6	50	6	50	
4b	25	24.8	18	72	7	28	
4c	29	28.7	25	86.2	4	13.8	
5	30	29.7	27	90	3	10	
6	1	1	1	100	0	0	0.1574
3	4	4	2	50	2	50	
4	66	65.3	49	74.2	17	25.8	
5	30	29.7	27	90	3	10	

CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; US = ultrasonography; BI-RADS = breast imaging reporting and data system.

* CTCs is set to 2, while US BI-RADS is set to 4b.

Groups	n	Proportion (%)	CTCs ≥ 2		CTCs < 2		<i>P</i>
			n	Proportion (%)	n	Proportion (%)	
6	1	1	1	100	0	0	0.0029
$\leq 4a$	16	15.8	8	50	8	50	
$\geq 4b$	85	84.2	71	83.5	14	16.5	
CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; US = ultrasonography; BI-RADS = breast imaging reporting and data system.							
* CTCs is set to 2, while US BI-RADS is set to 4b.							

Table 8
Correlations of CTCs with mammography in BC with cut-off setting*

Correlations of CTCs with mammography in DC with cut-off setting							
Groups	n	Proportion (%)	CTCs ≥ 2		CTCs < 2		P
			n	Proportion (%)	n	Proportion (%)	
MG (n = 43)							
1a	1	2.3	1	100	0	0	0.5951
1b	5	11.6	3	60	2	40	
1c	13	30.2	11	84.6	2	15.4	
2	22	51.2	14	63.6	8	36.4	
3	2	4.7	1	50	1	50	
1a + 1b	6	14	4	66.7	2	33.3	0.5421
1c	13	30.2	11	84.6	2	15.4	
2	22	51.2	14	63.6	8	36.4	
3	2	4.7	1	50	1	50	
1	19	44.2	15	78.9	4	21.1	0.4672
2	22	51.2	14	63.6	8	36.4	
3	2	4.7	1	50	1	50	
MG Breast Tissue Composition Categories (n = 82)							
a	8	9.8	6	75	2	25	0.4119
b	12	14.6	9	75	3	25	
c	52	63.4	42	80.8	10	19.2	
d	10	12.2	10	100	0	0	
a + b	20	24.4	17	85	5	25	0.5244
c + d	62	75.6	52	83.9	10	16.1	
MG BI-RADS (n = 72)							
2	4	5.6	3	75	1	25	0.3062
3	10	13.9	9	90	1	10	

CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; MG = mammography; BI-RADS = breast imaging reporting and data system.

* CTCs is set to 2, while MG BI-RADS is set to 4b.

Groups	n	Proportion (%)	CTCs ≥ 2		CTCs < 2		<i>P</i>
			n	Proportion (%)	n	Proportion (%)	
4a	8	11.1	4	50	4	50	0.8715
4b	16	22.2	14	87.5	2	12.5	
4c	29	40.3	24	82.8	5	17.2	
5	5	6.9	4	80	1	20	
2	4	5.6	3	75	1	25	
3	10	13.9	9	90	1	10	0.2657
4	53	73.6	42	79.2	11	20.8	
5	5	6.9	4	80	1	20	
$\leq 4a$	22	30.6	16	72.7	6	27.3	
$\geq 4b$	50	69.4	42	84	8	16	
CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; MG = mammography; BI-RADS = breast imaging reporting and data system.							
* CTCs is set to 2, while MG BI-RADS is set to 4b.							

Table 9

Correlations of CTCs with magnetic resonance imaging examination in BC with cut-off setting*

Groups	n	Proportion (%)	CTCs ≥ 2		CTCs < 2		P
			n	Proportion (%)	n	Proportion (%)	
MR (n = 83)							
1a	2	2.4	1	50	1	50	0.2827
1b	4	4.8	3	75	1	25	
1c	36	43.4	26	72.2	10	27.8	
2	35	42.2	30	85.7	5	14.3	
3	6	7.2	3	50	3	50	
1a + 1b	6	7.2	4	66.7	2	33.3	0.2044
1c	36	43.4	26	72.2	10	27.8	
2	35	42.2	30	85.7	5	14.3	
3	6	7.2	3	50	3	50	
1	42	50.6	30	71.4	12	28.6	0.1052
2	35	42.2	30	85.7	5	14.3	
3	6	7.2	3	50	3	50	
MR Breast Tissue Composition Categories (n = 91)							
a	3	3.3	1	33.3	2	66.7	0.1837
b	27	29.7	19	70.4	8	29.6	
c	52	57.1	43	82.7	9	17.3	
d	9	9.9	7	77.8	2	22.2	
a + b	30	33	20	66.7	10	33.3	0.1034
c + d	61	67	50	82	11	18	
MR BI-RADS (n = 88*)							
3	6	7	4	66.7	2	33.3	0.2351
4a	5	5.8	2	40	3	60	

CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; MR = magnetic resonance imaging; BI-RADS = breast imaging reporting and data system; TIC = time-signal intensity curves.

* CTCs is set to 2, while MR BI-RADS is set to 4b.

Groups	n	Proportion (%)	CTCs ≥ 2		CTCs < 2		P
			n	Proportion (%)	n	Proportion (%)	
4b	15	17.4	11	73.3	4	26.7	0.4116
4c	30	34.9	24	80	6	20	
5	15	17.4	14	93.3	1	6.7	
6	15	17.4	11	73.3	4	26.7	
3	6	6.8	4	66.7	2	33.3	
4	52	59.1	39	75	13	25	0.062
5	15	17	14	93.3	1	6.7	
6	15	17	11	73.3	4	26.7	
≤ 4a	11	12.8	6	54.5	5	45.5	
≥ 4b	75	87.2	60	80	15	20	
MR TIC (n = 91)							
1	22	24.2	16	72.7	6	27.3	0.7251
2	38	41.8	31	81.6	7	18.4	
3	27	29.7	21	77.8	6	22.2	
CTCs = circulating tumor cells; BC = breast cancer; n = number of patients; MR = magnetic resonance imaging; BI-RADS = breast imaging reporting and data system; TIC = time-signal intensity curves.							
* CTCs is set to 2, while MR BI-RADS is set to 4b.							

Comparing the false negative rates (FNR) of CTCs and medical imaging examinations in BC diagnosis, MR has the lowest FNR of 12.8%, followed by US with 15.8%, CTCs with 22.5% and MG with 30.6% as shown in Table 10. Conjugation CTCs with medical imaging examinations decreased the FNR of US, MG and MR to 7.9%, 8.3% and 5.8%, respectively. Combination of CTCs decreased significantly FNR of MG in BC diagnosis.

Table 10
False negative rates of different diagnostic methods in BC detection

Method	n	Cut-off	Positive	False Negative Rates (%)
CTCs	102	2	79	22.5
US	101	4b	85	15.8
MG	72	4b	50	30.6
MR	86	4b	75	12.8
CTCs + US	101	2 or 4b	93	7.9
CTCs + MG	72	2 or 4b	66	8.3
CTCs + MR	86	2 or 4b	81	5.8
BC = breast cancer; n = number of patients; CTCs = circulating tumor cells; US = ultrasonography; MG = mammography; MR = magnetic resonance imaging.				

Discussions

Previous study with CytoSorter® in BC showed that CTC could reflect tumor burden and could be used as a diagnostic aid in BC screening and early diagnosis [8]. First we compared CTCs with BC patients' clinicopathological features and found that CTCs were associated with tumor stage and tumor size, but not with lymph node metastasis, patients' age and tumor molecular subtype and histology grade. Latest study in the lab shows that mesenchymal type of CTCs in BC is more invasive and more related with lymph node and distant metastasis [9]. We used epithelial cell adhesion molecule (EpCAM) antibody to capture the epithelial types of CTCs, which may explain that CTCs are associated with tumor size, but not with lymph node involvement. Only confirmed BC patients were enrolled in this study. If CTC positive was defined as any patient with $CTC \geq 2$, CTC positive rates were not correlated with tumor stage, tumor size, lymph node involvement, and tumor molecular subtypes as shown in Table 6. CTC negative rates were 0 in stage III patients and in patients at N2 and N3 stages. But CTC negative rates were comparable in other group of patients. CTC detection rates (positive rates) in stage I and II BC were 92.9% (71.6%) and 87.2% (76%), respectively, which is in line with the previous finding that CTC can be used as an screening aid for early stage BC [8].

Next, we compared CTCs with other BC screening imaging methods, such as US, MG and MR. Our analysis showed CTCs were more associated with US than MR, and showed least correlation with MG. MG is an X-ray imaging to detect for presence of tumor or lump in breast, and is currently the most efficient screening method recommended by American Cancer Society (ACS) and AJCC to detect early BC. The sensitivity of MG depends on the patient's age and breast composition. Sensitivity is higher in women over 50 and with fatty breasts [10]. High breast density leads to the FNR of MG. In fact, MG had the highest FNR of 30.6% in this study, followed by US (15.8%) and MR (12.8%). Breast is a mixed structure, containing both dense tissue (i.e., glandular tissue and connective tissue, together known as

fibroglandular tissue) and fatty tissue. On a MG film, fatty tissue appears dark, whereas fibroglandular tissue appears as white areas. Tumors usually have similar density as fibroglandular tissue and therefore, it is harder to detect tumor in women with denser breasts with MG. FNR occur more frequently among younger women as well because younger women are more likely to have dense breasts [10]. As Oriental women usually have dense breasts, MG is less sensitive in Chinese females [11]. MG was not associated with any clinicopathological features of BC in this study, which may explain why CTCs were not correlated with the MG. MG FNR are surprisingly more observed in stage II patients (38.2%) and in patients at T2 stage (44%). MG FNR in other groups of BC patients were ranging from 15–30%.

US uses sound waves to make a computer picture of the inside of the breast. US can differentiate fluid-filled cysts from solid masses, therefore, it is most useful for looking at some breast changes, such as lumps. BC detection rate with US is comparable with MG, with a greater proportion of invasive and node-negative cancers among US detections [12, 13]. US usually cannot detect the small calcification points and is less sensitive to detect tumors less than 5 mm or deep in the breast. Since most BC are superficial tumors and Chinese women usually have dense breasts, US was more sensitive for BC detection than MG in our study. FNR of US was negatively associated with patients' TNM stage. US showed higher FNR in stage I patients, and in patients at T1 and N0 stage. US usually generates more false-positive results (FPR) [13], which cannot be confirmed in our study since no healthy volunteer or patients with benign breast diseases were enrolled in this study.

Although MG and US are currently the mostly used imaging techniques for BC detection in practice, the low sensitivity and specificity of these two imaging tools resulted in a demand for new imaging modalities and breast MR has become increasingly important in the detection and delineation of BC [13, 14]. A breast MR uses magnets and radio waves to take pictures of the breast. Breast MR is the most sensitive method for detection of BC and has more diagnostic value on pretherapeutic BC staging, monitoring of primary systemic therapies and solving problematic diagnostic situations where direct biopsy is not possible [14]. MR has the lowest FNR of 12.8% in our study. Similar to US, most MR FNR were observed in stage I BC patients, and in patients at T1 and N0 stage.

Medical imaging modalities showed different FNR in patients with different molecular subtypes of tumor. MR and US showed lowest FNR in human epidermal growth factor receptor 2 (HER2) over-expression BC patients, while MG had a similar FNR in all 4 molecular subtypes. The general limitation of imaging modalities for BC screening is that most methods cannot detect tumors smaller than certain size. That is why US and MR showed higher FNR in early stage BC (stage I) and in patients with smaller tumor (T1). Previous study in the lab showed that MR has the highest sensitivity of BC screening in Chinese women, followed by US and then MG [4]. But in Stage I BC patients, CTC, US, MG, and MR showed similar FNR ranging from 26–28%.

BSGI is a molecular breast imaging procedure that shows the metabolic activity of breast lesions [15]. MR-TIC can reflect the hemodynamic features of a specific lesion, especially about the vascular structure around tumor. CTCs were not associated with BSGI nor MR-TIC. Bansal et al used flow

cytometry to detect CTCs in 114 BC patients and found that CTC positivity did not show any correlation with the tumor immunohistochemical profile [16]. Taken together, CTC enumeration cannot be used as a biomarker to reflect the molecular signature of BC. CTCs are heterogeneous. Some are epithelial type, and some are mesenchymal type. Some may express stem-cell markers, such as CD44, or other proteins, such as PD-L1 or HER2. Therefore, profiling of CTCs is considered as a promising tool to reveal the molecular signature of BC, allowing for a better personal treatment rearrangement.

Our results show CTCs are more associated with US than MR or MG. Comparing the consistence between CTCs and medical imaging examinations in BC detection, CTCs show the highest consistence of 70.3% with US, followed by 68.2% with MR and 58.3% with MG, which may explain why CTCs are more correlated with US than MR, and show least correlation with MG. Interpretation of medical imaging examinations require a radiologist's expertise. An inexperienced expert might misinterpret the imagings to generate false results. Compared to CTCs, medical imaging examinations might be considered as a diagnostic tool with more human error bias.

Lastly, we liked to know whether conjugation of CTC would decrease FNR of imaging examinations for BC detection as well. Table 10 showed that combination of CTC with medical imaging examination decreased their FNR for BC detection, especially for MG. FNR of MG decreased from 30.6–8.3%, even more than in conjugation with US. Medical imaging examinations combine with CTCs counts would improve the diagnostic potency for BC detection.

Conclusions

Results of this work showed that CTCs detected by CytoSorter® system could be used as a diagnostic aid to assist in early diagnosis and screening of BC. CTCs were more relevant to US than MR or MG. Conjugation of CTCs would improve the diagnostic potency of medical imaging modalities for BC detection, especially for MG in Chinese women. Still more studies on larger patient population should be conducted to confirm our findings and to elucidate the clinical value of utilizing CTCs as a diagnostic, therapeutic and prognostic indicator in BC.

Abbreviations

American Cancer Society (ACS)

American College of Radiology (ACR)

American Joint Committee on Cancer (AJCC)

breast cancer (BC)

Breast Imaging-Reporting and Data System (BI-RADS)

breast-specific gamma imaging (BSGI)

circulating tumor cells (CTCs)

epithelial cell adhesion molecule (EpCAM)

false negative rates (FNR)

false-positive results (FPR)

human epidermal growth factor receptor 2 (HER2)

mammogram (MG)

magnetic resonance imaging (MR)

^{99m}Tc-Sestamibi (MIBI)

metastatic breast cancer (MBC)

One-way analysis of variance (ANOVA)

time-signal intensity curves (TIC)

ultrasound (US)

Declarations

Ethics approval and consent to participate

This study followed the principles established in the Declaration of Helsinki and was approved by the ethics committee of Second Affiliated Hospital of Zhejiang University School of Medicine which had obtain JCI certification. Written informed consent was obtained from individual or guardian participants.

Consent for publication

Not applicable.

Availability of data and material

All data analysed during this study are included in this published article.

Competing interests

The authors declared that they had no competing interests.

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Authors' contributions

Conception and design: X S and J H. Acquisition of data: XY J, ZG C, K W, XY Y, WZ C, JX J, Z W. Analysis and interpretation of data: X S, ZG Z, Y C, WH F and J H. Writing of the manuscript: X S, WH F, ZG Z. Review of the manuscript: All authors have read and approved the manuscript.

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