Society of Medical Education & Research

2024, Volume 4, Issue 2, Page No: 66-80
ISSN: 3108-4834

Archive of International Journal of Cancer and Allied Science

FTH1-Expressing Circulating Tumor Cells as Predictors of Neoadjuvant Chemotherapy Response in Non-Metastatic Breast Cancer: A Prospective Cohort Study

Laura Beatrice Conti[1]*, Martina Giulia Rinaldi[1], Alessandro Fabbri[2], Nicolo Romano[2], Davide Lorenzo Greco[3], Simone Paolo Marchetti[3]

[1]Department of Oncology, University of Florence, Florence, Italy.
[2]European Institute of Oncology (IEO), Milan, Italy.
[3]Department of Clinical Medicine, University of Pisa, Pisa, Italy.

***E-mail** ✉ l.conti.unifi@outlook.com

Abstract

The link between specific circulating tumor cell (CTC) phenotypes or genotypes and the effectiveness of neoadjuvant chemotherapy (NAC) has not been clearly established. This investigation aimed to determine whether FTH1 gene–related CTCs (F-CTC), with or without epithelial–mesenchymal transition (EMT) markers, as well as their temporal variations, are associated with NAC outcomes in patients with non-metastatic breast cancer. A total of 120 patients with non-metastatic breast cancer scheduled for NAC were included. Detection of the FTH1 gene and EMT markers in CTCs was performed before NAC (T0), after two chemotherapy cycles (T1), and prior to surgery (T2). Binary logistic regression was applied to analyze the associations between different CTC subtypes and rates of pathological complete response (pCR) and breast-conserving surgery (BCS). The presence of ≥1 F-CTC in peripheral blood at T0 was identified as an independent predictor of pCR in patients with HER2-positive disease (odds ratio [OR] = 0.08, 95% confidence interval [CI], 0.01–0.98, P = .048). A decrease in F-CTC counts at T2 independently predicted a higher likelihood of BCS (OR = 4.54, 95% CI, 1.14–18.08, P = .03). Elevated F-CTC levels before NAC were associated with an unfavorable response to NAC. Continuous assessment of F-CTC may assist clinicians in tailoring individualized NAC strategies and facilitating BCS in patients with non-metastatic breast cancer.

Keywords: Circulating tumor cells, Ferroptosis, Ferritin heavy chain, Neoadjuvant chemotherapy, Pathological complete response, Breast-conserving surgery

Introduction

Since 2020, breast cancer has been the most frequently diagnosed malignancy worldwide [1]. Each year, over 600,000 deaths are attributed to breast cancer, largely as a result of metastatic relapse [2]. Even after successful treatment of early-stage disease, minimal residual disease may persist, serving as a potential origin for later metastasis. Circulating tumor cells (CTCs) are malignant cells released from the primary tumor into the bloodstream [3]. Their adverse impact on long-term survival in non-metastatic breast cancer has been well documented [4–6]. Nevertheless, the association between CTCs and pathological complete response (pCR) following neoadjuvant chemotherapy (NAC) remains ambiguous, mainly because earlier detection methods lacked sufficient sensitivity [7, 8]. Recent advances in high-sensitivity liquid biopsy techniques have enabled the identification of diverse CTC phenotypes and genomic characteristics, improving insight into their heterogeneity [9, 10] and offering valuable tools for evaluating NAC efficacy.

During dissemination, CTCs frequently undergo epithelial–mesenchymal transition (EMT), characterized by reduced expression of epithelial markers and

Access this article online https://smerpub.com/

Received: 10 April 2024; **Accepted:** 21 August 2024

How to cite this article: Conti LB, Rinaldi MG, Fabbri A, Romano N, Greco DL, Marchetti SP. FTH1-Expressing Circulating Tumor Cells as Predictors of Neoadjuvant Chemotherapy Response in Non-Metastatic Breast Cancer: A Prospective Cohort Study. Arch Int J Cancer Allied Sci. 2024;4(2):66-80. https://doi.org/10.51847/a6F8KUtMpS

increased expression of mesenchymal markers. According to EMT status, CTCs are categorized as epithelial CTCs (E-CTC), mesenchymal CTCs (M-CTC), or hybrid epithelial/mesenchymal CTCs (E/M-CTC) [9, 11]. Prior studies have reported associations between EMT-related CTCs and tumor progression or long-term outcomes [11, 12]. However, the effects of E-CTC, M-CTC, E/M-CTC, total CTC counts, or their longitudinal changes on NAC efficacy in non-metastatic breast cancer have not yet been systematically explored.

Beyond phenotypic classification, the genetic features of CTCs have gained increasing attention. Ferroptosis represents a non-apoptotic, iron-dependent mode of cell death that is closely linked to iron metabolism and has been implicated in the development of several cancers, including breast cancer [13]. A defining feature of ferroptosis is the involvement of redox-active iron [14]. Key regulatory genes of ferroptosis include FTH1, GPX4, ACSL4, SLC7A11, and TFRC. FTH1 plays a central role in iron homeostasis by controlling iron storage and release, thereby maintaining cellular redox balance. Its overexpression has been observed in various malignancies and is thought to influence ferroptosis. Elevated FTH1 mRNA and protein expression have been reported in tumor stem cells from HER2/Neu transgenic mice [15], while increased nuclear FTH1 staining in triple-negative breast cancer has been linked to poor prognosis [16]. Excessive reactive oxygen species (ROS) can trigger ferroptosis [17, 18], thereby restraining tumor cell growth. Iron-dependent lipid peroxide accumulation, driven by intracellular redox-active iron via the Fenton reaction, is a key event in this process. FTH1 overexpression in tumor cells, including those in breast cancer [19], may enhance antioxidant defenses by stabilizing high ROS levels [20] and may contribute to resistance to chemotherapy [21]. Conversely, suppression of FTH1 in cells undergoing EMT promotes ferroptosis through increased ROS generation [22, 23]. Despite these findings, it remains uncertain whether FTH1-positive CTCs, alone or combined with EMT markers, or their changes during NAC, are related to treatment efficacy in non-metastatic breast cancer.

Therefore, this study sought to evaluate the relationships between various CTC subtypes, their dynamic alterations, and NAC effectiveness in patients with non-metastatic breast cancer.

Materials and Methods

Study design

This work was conducted as a prospective cohort study with an observational design at a single medical center. Approval was granted by the Clinical Research Ethics Committee of Sun Yat-sen Memorial Hospital (SYSEC-KY-KS-2021-103), and the study protocol was registered with the Chinese Clinical Trials Registry (ChiCTR2100046262). Between May 2021 and January 2022, participants were systematically recruited from the Breast Cancer Center of Sun Yat-sen Memorial Hospital after providing written informed consent. Women aged ≤70 years with a first diagnosis of non-metastatic breast cancer and no history of prior treatment were eligible for inclusion. Additional inclusion criteria required: (1) clinical eligibility for NAC; (2) an Eastern Cooperative Oncology Group performance status score of 0–1; and (3) adequate hematopoietic function together with preserved liver and kidney function. Patients were excluded if they had: (1) inflammatory breast cancer or (2) coexisting malignant diseases (eg, thyroid cancer).

Data collection

At enrollment, all patients underwent a comprehensive diagnostic workup, including clinical examination, breast ultrasonography, mammography, magnetic resonance imaging (MRI), pathological confirmation by biopsy, routine laboratory analyses, and evaluation of major organ function. Neoadjuvant chemotherapy protocols consisting of anthracycline-based agents combined with paclitaxel were determined by the chief physician in line with NCCN recommendations. For individuals with human epidermal growth factor receptor 2 (HER2)-positive tumors, dual-targeted therapy was incorporated into the treatment regimen. Clinical assessments and laboratory testing were repeated at every chemotherapy cycle. Immediately prior to surgical treatment, breast ultrasound, mammography, and MRI were performed again to reassess tumor size and calcification patterns. Surgical management included breast-conserving surgery (BCS) or mastectomy, with or without reconstructive procedures, all performed by the chief physician. To decrease the incidence of positive resection margins and limit the need for secondary operations, a modified intraoperative cavity margin processing technique was adopted at our center [24]. After surgery, patients were monitored for recurrence and survival outcomes at 3-month intervals **(Figure 1b).**

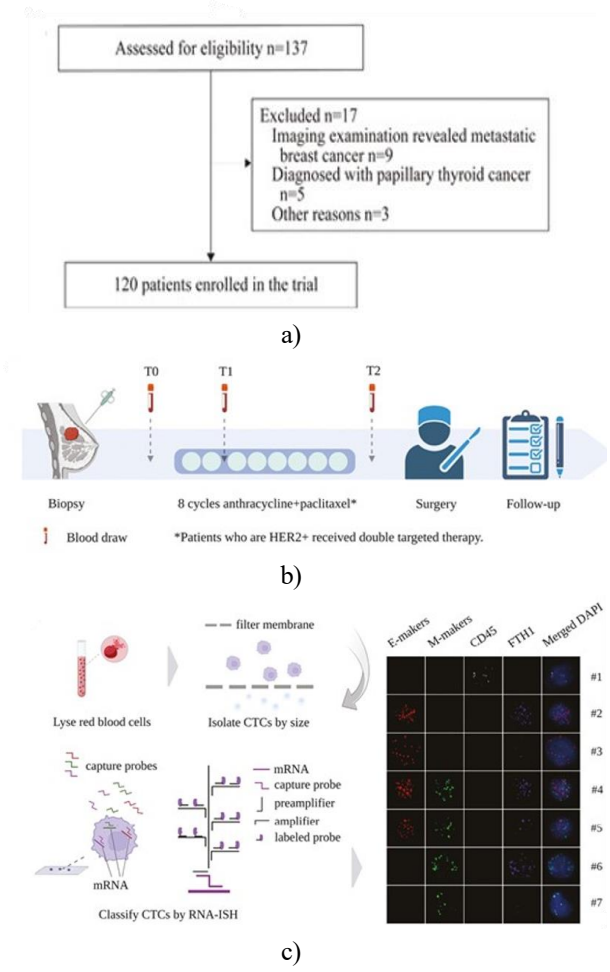


Figure 1. Overview of patient selection, experimental framework, and circulating tumor cell (CTC) detection methodology.

(a) Flow diagram illustrating the patient screening and enrollment process.

(b) Schematic outline of the study protocol. Histopathological classification was confirmed in all cases through biopsy of the primary breast lesion. Neoadjuvant chemotherapy comprised 8 cycles of anthracycline-based treatment combined with paclitaxel; patients with HER2-positive tumors additionally received dual-targeted therapy. Surgical management was undertaken after completion of chemotherapy, followed by scheduled postoperative telephone follow-up. Peripheral blood sampling was performed at three predefined stages: T0 (prior to initiation of NAC), T1 (after completion of 2 chemotherapy cycles), and T2 (immediately before surgery).

(c) Diagram depicting the CTC identification and classification procedure. After red blood cell lysis, CTCs were enriched by membrane filtration and phenotypically characterized using RNA in situ hybridization (RNA-ISH). Specific biomarker-associated fluorescent signals were examined under fluorescence microscopy. Cell population #1 represented leukocytes expressing CD45 only. Cell populations #2 and #3 corresponded to epithelial CTCs (E-CTC) with epithelial marker expression. Cell populations #4 and #5 indicated hybrid epithelial/mesenchymal CTCs (E/M-CTC) expressing both marker types. Cell populations #6 and #7 denoted mesenchymal CTCs (M-CTC) expressing mesenchymal markers. Purple FTH1 signal dots exceeding 9 were observed in cell groups #2, #4, and #6, identified CTCs with elevated FTH1 expression, categorized as F-CTCE, F-CTCE/M, and F-CTCM, respectively.

Panels **Figures 1b and 1c** were generated using BioRender.com, with formal permission obtained.

During the study, 5 mL of peripheral venous blood was obtained from each participant for CTC analysis at three time points: before NAC (T0), following 2 chemotherapy cycles (T1), and prior to surgical intervention (T2).

Outcomes

The primary study endpoint was the pathological complete response (pCR) rate, defined as the absence of residual invasive carcinoma in both breast tissue and axillary lymph nodes within postoperative specimens following NAC.

Secondary endpoints included the rate of breast-conserving surgery (BCS) and event-free survival (EFS). The BCS rate was calculated as the proportion of patients undergoing BCS among all patients who proceeded to surgery. EFS was defined as the duration from initial diagnosis to the occurrence of disease progression, including local recurrence, distant metastasis, development of a second primary malignancy, or death from any cause [25].

Isolation and classification of CTCs

CTC enrichment and phenotypic identification were carried out using the CanPatrol platform in combination with a tricolor RNA-ISH approach.

Prior to CTC enrichment, erythrocytes were eliminated using a red blood cell lysis solution composed of 154 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA. Samples were centrifuged using a TDZ5-WS centrifuge and subsequently passed through an 8-μm pore-size membrane (Millipore, Billerica, USA) under vacuum

pressure generated by a SurExam pump (Guangzhou, China).

All procedures were conducted in 24-well culture plates (Corning, NY, USA). A multiplex RNA-ISH assay based on branched DNA (bDNA) amplification—incorporating capture probes, preamplifier sequences, amplifier sequences, and labeled probes—was employed to detect and enumerate CTCs. The marker panel included four epithelial markers (EpCAM and CK8/18/19), two mesenchymal markers (vimentin and twist), a leukocyte marker (CD45), and the FTH1 gene marker. Nuclear counterstaining was performed using 4′,6-diamidino-2-phenylindole (DAPI). Fluorescence microscopy (100× oil immersion objective, Olympus BX53, Tokyo, Japan) revealed red fluorescence for epithelial markers, green fluorescence for mesenchymal markers, white fluorescence for leukocyte markers, and purple fluorescence indicating FTH1 gene expression **(Figure 1c).** The mean number of FTH1 signal dots detected per CTC across all patients was 9.4 (±9.5). CTCs exhibiting ≥9 FTH1 signal points were classified as FTH1-associated CTCs (F-CTC). Correspondingly, epithelial, hybrid epithelial/mesenchymal, and mesenchymal CTCs with high FTH1 expression were designated as F-CTCE, F-CTCE/M, and F-CTCM, respectively.

Statistical analyses

Baseline patient features were summarized as absolute numbers and corresponding percentages for categorical variables. Comparisons of baseline characteristics between patients who achieved pCR and those who did not were carried out using the chi-square test or Fisher's exact test, depending on data distribution. To assess the ability of different CTC subtypes measured at T0 to predict pCR, receiver operating characteristic (ROC) curves were generated and the area under the curve (AUC) was calculated. Optimal cutoff points for each CTC category were established based on the maximum Youden index. The cutoff values applied for total CTCs, E-CTC, E/M-CTC, and M-CTC were 4, 2, 3, and 1, respectively. For FTH1-related CTC subtypes, the cutoff value was uniformly set at 1 for F-CTC, F-CTCE, F-CTCE/M, and F-CTCM. Changes in CTC levels at T1 and T2 were evaluated relative to baseline (T0); a value below the defined cutoff was categorized as a decrease, whereas values remaining at or above the cutoff were classified as no decrease. Associations between CTC status at T0 and pCR, as well as between CTC status changes at T1 or T2 and pCR or BCS, were examined using chi-square or Fisher's exact tests. Potential predictors of pCR and BCS were first screened using univariate binary logistic regression and subsequently assessed using multivariate binary logistic regression. Variables meeting a significance threshold of 0.05 in univariate analyses were entered into the multivariate models. Event-free survival (EFS) was analyzed using Kaplan–Meier survival estimates, and differences between groups were evaluated with the log-rank test to determine the impact of baseline CTC status at T0 and longitudinal CTC changes at T1 and T2.

All analyses were two-sided, with $P < .05$ indicating statistical significance. Statistical processing was performed using IBM SPSS Statistics, version 26.

Results and Discussion

Clinical Characteristics of Patients

Between May 2021 and the end of the enrollment period, 137 patients were assessed for eligibility. Seventeen individuals were excluded, including 9 patients with metastatic breast cancer, 5 patients diagnosed with papillary thyroid carcinoma, and 3 patients who declined participation due to concerns related to the COVID-19 pandemic. Ultimately, 120 patients were enrolled prospectively in the study, as detailed in **Figure 1a**.

Postoperative pathological evaluation classified 39 patients (32.5%) into the pCR group and 81 patients (67.5%) into the non-pCR group. Baseline clinical and pathological characteristics of the full cohort and the two subgroups are shown in **Table 1**. Most participants were aged ≤50 years (67.5%), were premenopausal (69.2%), and had primary tumor sizes ≤50 mm (75.8%). A substantial proportion of patients were diagnosed with stage IIB–IIIC disease (72.5%), consistent with locally advanced breast cancer. Nearly all patients demonstrated a Ki-67 proliferation index ≥14% (96.7%). Comparative analyses revealed no significant differences between the pCR and non-pCR groups with respect to age, menopausal status, tumor size, lymph node involvement, Ki-67 index, or surgical approach to the breast ($P > .05$). In contrast, the pCR group exhibited a significantly lower rate of hormone receptor (HR) positivity (84.0% vs 59.0%, $P = .003$) and a significantly higher prevalence of HER2-positive tumors (27.2% vs 76.9%, $P < .001$) compared with the non-pCR group.

Table 1. Comparison of clinical characteristics between patients with and without pCR.

Patient Characteristic	Did Not Achieve pCR (n=81)	Achieved pCR (n=39)	All Patients (n=120)	P Value
Age (years)				
≤50	54 (66.7%)	27 (69.2%)	81 (67.5%)	.78
>50	27 (33.3%)	12 (30.8%)	39 (32.5%)	
Menopausal Status				
Premenopausal	54 (66.7%)	29 (74.4%)	83 (69.2%)	.39
Postmenopausal	27 (33.3%)	10 (25.6%)	37 (30.8%)	
Clinical Tumor Size				
cT1-2	59 (72.8%)	32 (82.1%)	91 (75.8%)	.27
cT3-4	22 (27.2%)	7 (17.9%)	29 (24.2%)	
Clinical Lymph Node Status				
cN0-1	67 (82.7%)	34 (87.2%)	101 (84.2%)	.53
cN2-3	14 (17.3%)	5 (12.8%)	19 (15.8%)	
Clinical Stage				
IIA	24 (29.6%)	9 (23.1%)	33 (27.5%)	.75
IIB	32 (39.5%)	19 (48.7%)	51 (42.5%)	
IIIA	18 (22.2%)	7 (17.9%)	25 (20.8%)	
IIIB	1 (1.2%)	0 (0%)	1 (0.8%)	
IIIC	6 (7.4%)	4 (10.3%)	10 (8.3%)	
Hormone Receptor (HR) Status				
Negative	13 (16.0%)	16 (41.0%)	29 (24.2%)	.003
Positive	68 (84.0%)	23 (59.0%)	91 (75.8%)	
HER2 Status				
Negative	59 (72.8%)	9 (23.1%)	68 (56.7%)	<.001
Positive	22 (27.2%)	30 (76.9%)	52 (43.3%)	
Breast Cancer Molecular Subtype				
HR+/HER2-	47 (58.0%)	4 (10.3%)	51 (42.5%)	<.001
HR+/HER2+	21 (25.9%)	19 (48.7%)	40 (33.3%)	
HR-/HER2+	4 (4.9%)	11 (28.2%)	15 (12.5%)	
Triple-Negative (TNBC)	9 (11.1%)	5 (12.8%)	14 (11.7%)	
Ki-67 Proliferation Index (%)				
<14	3 (3.7%)	1 (2.6%)	4 (3.3%)	.74
≥14	78 (96.3%)	38 (97.4%)	116 (96.7%)	
Surgical Procedure Performed				
Mastectomy	39 (48.1%)	15 (38.5%)	54 (45.0%)	.32
Breast-Conserving Surgery	42 (51.9%)	24 (61.5%)	66 (55.0%)	

Abbreviations: CTCs, circulating tumor cells; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; pCR, pathological complete response.

Link between circulating tumor cells and pathological complete response

CTCs were assessed in all 120 patients at baseline (T0), after two chemotherapy cycles (T1), and prior to surgery (T2). CTCs were identified in 105 patients (87.5%) at T0, 117 (97.5%) at T1, and 116 (96.7%) at T2. To determine optimal thresholds for predicting pCR based on CTC counts at T0, receiver operating characteristic (ROC) curves were generated for each CTC subtype, with cutoff points selected according to the maximum Youden index. Certain CTC subtypes at T0 showed an association with pCR achievement, whereas no such associations were observed at T1 or T2. As presented in **Table 2 and Figure 2**, patients with ≥4 total CTCs at T0 exhibited a markedly reduced pCR rate compared with those having <4 total CTCs (25.4% vs 42.9%, P = .044). In contrast, the counts of epithelial CTCs (E-CTC), hybrid

epithelial/mesenchymal CTCs (E/M-CTC), and mesenchymal CTCs (M-CTC) at T0 did not demonstrate significant links to pCR. Further analysis revealed that the presence of ≥1 F-CTC or ≥1 F-CTCE/M at T0 was independently linked to lower pCR rates (17.1% vs 59.1%, P < .001 for F-CTC; 18.0% vs 47.5%, P = .001 for F-CTCE/M). No similar associations emerged for F-CTCE or F-CTCM. Likewise, CTC subtype distributions at T1 and T2 showed no significant relation to pCR **(Table 2).**

Table 2. Counts of different CTC subtypes in patients achieving versus not achieving pCR at T0, T1, and T2.

CTC Category	Time Point T2 (after surgery)	Time Point T1 (after chemotherapy, before surgery)	Time Point T0 (before treatment)
	All patients (n = 120) pCR (n = 39) Non-pCR (n = 81) P-value	All patients (n = 120) pCR (n = 39) Non-pCR (n = 81) P-value	All patients (n = 120) pCR (n = 39) Non-pCR (n = 81) P-value
Total CTCs			
< 4	28 (23.3%) 9 (32.1%) 19 (67.9%) .96	18 (15.0%) 9 (50.0%) 9 (50.0%) .09	49 (40.8%) 21 (42.9%) 28 (57.1%) .044
≥ 4	92 (76.7%) 30 (32.6%) 62 (67.4%)	102 (85.0%) 30 (29.4%) 72 (70.6%)	71 (59.2%) 18 (25.4%) 53 (74.6%)
E-CTC (epithelial CTCs)			
< 2	42 (35.0%) 13 (31.0%) 29 (69.0%) .79	38 (31.7%) 15 (39.5%) 23 (60.5%) .27	53 (44.2%) 20 (37.7%) 33 (62.3%) .28
≥ 2	78 (65.0%) 26 (33.3%) 52 (66.7%)	82 (68.3%) 24 (29.3%) 58 (70.7%)	67 (55.8%) 19 (28.4%) 48 (71.6%)
E/M-CTC (hybrid epithelial/mesenchymal CTCs)			
< 3	54 (45.0%) 21 (38.9%) 33 (61.1%) .18	40 (33.3%) 16 (40.0%) 24 (60.0%) .22	63 (52.5%) 24 (38.1%) 39 (61.9%) .17
≥ 3	66 (55.0%) 18 (27.3%) 48 (72.7%)	80 (66.7%) 23 (28.8%) 57 (71.3%)	57 (47.5%) 15 (26.3%) 42 (73.7%)
M-CTC (mesenchymal CTCs)			
< 1	74 (61.7%) 27 (36.5%) 47 (63.5%) .24	66 (55.0%) 19 (28.8%) 47 (71.2%) .34	89 (74.2%) 27 (30.3%) 62 (69.7%) .39
≥ 1	46 (38.3%) 12 (26.1%) 34 (73.9%)	54 (45.0%) 20 (37.0%) 34 (63.0%)	31 (25.8%) 12 (38.7%) 19 (61.3%)
F-CTC (fibroblast-associated CTCs)			
< 1	67 (55.8%) 25 (37.3%) 42 (62.7%) .21	26 (21.7%) 9 (34.6%) 17 (65.4%) .80	44 (36.7%) 26 (59.1%) 18 (40.9%) <.001
≥ 1	53 (44.2%) 14 (26.4%) 39 (73.6%)	94 (78.3%) 30 (31.9%) 64 (68.1%)	76 (63.3%) 13 (17.1%) 63 (82.9%)
F-CTCE			
< 1	88 (73.3%) 33 (37.5%) 55 (62.5%) .052	71 (86.7%) 28 (39.4%) 43 (60.6%) .051	78 (65.0%) 28 (35.9%) 50 (64.1%) .28
≥ 1	32 (26.7%) 6 (18.8%) 26 (81.3%)	49 (13.3%) 11 (22.4%) 38 (77.6%)	42 (35.0%) 11 (26.2%) 31 (73.8%)
F-CTCE/M (hybrid fibroblast-associated)			

< 1	74 (61.7%) 27 (36.5%) 47 (63.5%) .24	36 (30.0%) 11 (30.6%) 25 (69.4%) .77	59 (49.2%) 28 (47.5%) 31 (52.5%) .001
≥ 1	46 (38.3%) 12 (26.1%) 34 (73.9%)	84 (70.0%) 28 (33.3%) 56 (66.7%)	61 (50.8%) 11 (18.0%) 50 (82.0%)
F-CTCM			
< 1	110 (91.7%) 36 (32.7%) 74 (67.3%) 1.0	85 (70.8%) 26 (30.6%) 59 (69.4%) .49	108 (90.0%) 37 (34.3%) 71 (65.7%) .33
≥ 1	10 (8.3%) 3 (30.0%) 7 (70.0%)	35 (29.2%) 13 (37.1%) 22 (62.9%)	12 (10.0%) 2 (16.7%) 10 (83.3%)

Abbreviations:
E-CTC: epithelial circulating tumor cells;
CTCs: circulating tumor cells;
E/M-CTC: biphenotypic epithelial/mesenchymal circulating tumor cells;
F-CTC: FTH1 gene-associated circulating tumor cells;
M-CTC: mesenchymal circulating tumor cells;
F-CTCM: FTH1 gene-associated mesenchymal circulating tumor cells.
F-CTCE/M: FTH1 gene-associated biphenotypic epithelial/mesenchymal circulating tumor cells;
F-CTCE: FTH1 gene-associated epithelial circulating tumor cells;

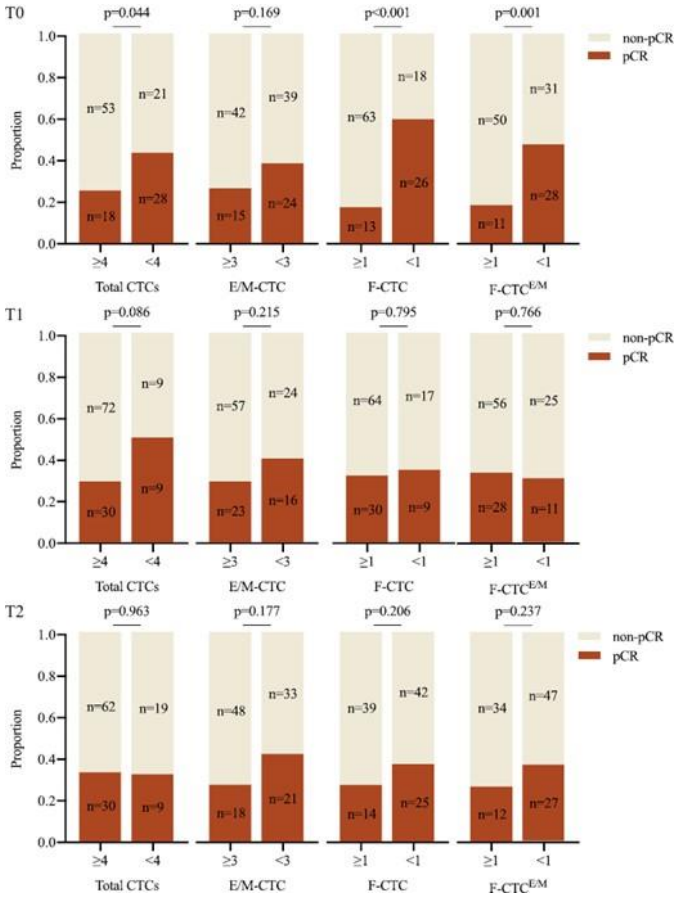
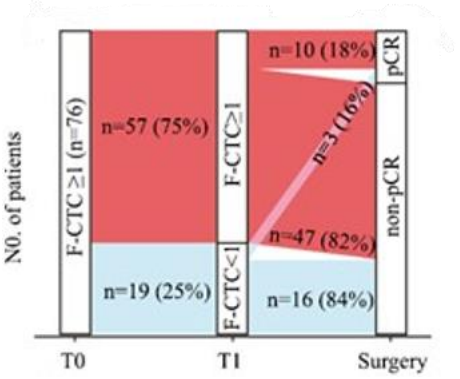
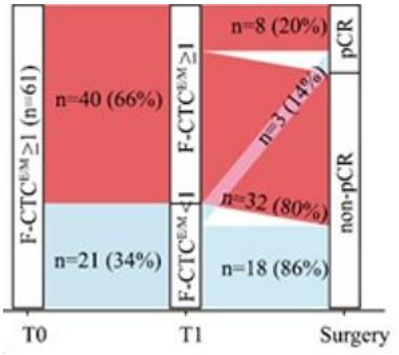


Figure 2. Relationship between circulating tumor cells (CTCs) and pathological complete response (pCR). The associations of total CTCs, E/M-CTC, F-CTC, and F-CTCE/M status with pCR rates were evaluated at T0, T1, and T2.

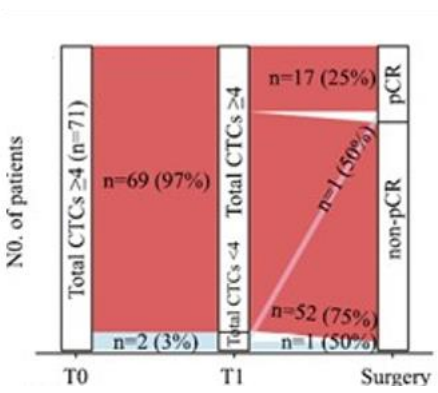
Alterations in the status of individual CTC subtypes showed no influence on pCR outcomes at either T1 or T2. For analyses of longitudinal variation, total CTCs, F-CTC, and F-CTCE/M were selected because their distributions at T0 differed according to pCR status. In addition, to determine whether temporal changes in CTCs identified solely by EMT markers had potential relevance, E/M-CTC was also incorporated into subsequent analyses, despite the lack of an association between E/M-CTC distribution at T0 and pCR rate. As illustrated in Figure 3, among 71 patients presenting with total CTCs ≥4 at T0, 69 did not experience a reduction at T1, and 17 of these patients ultimately achieved pCR. Of the 2 patients who exhibited a decline in total CTCs at T1, 1 attained pCR. No statistically significant difference in pCR rates was observed between these two groups, indicating that changes in total CTC levels at T1 were not associated with pCR. Likewise, variations in E/M-CTC, F-CTC, and F-CTCE/M at T1 demonstrated no relationship with pCR outcomes. Using the same analytical approach, changes in CTC counts at T2 were further examined; however, no correlation with pCR rate was identified **(Figure 3).**



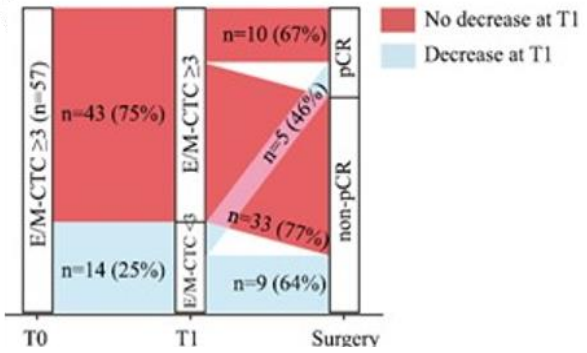
c)



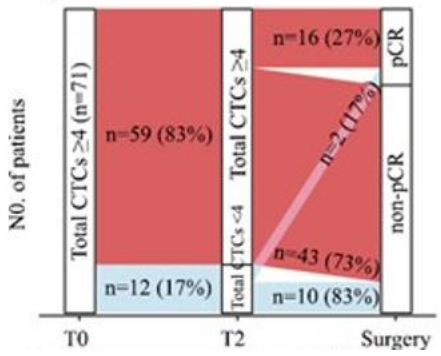
d)



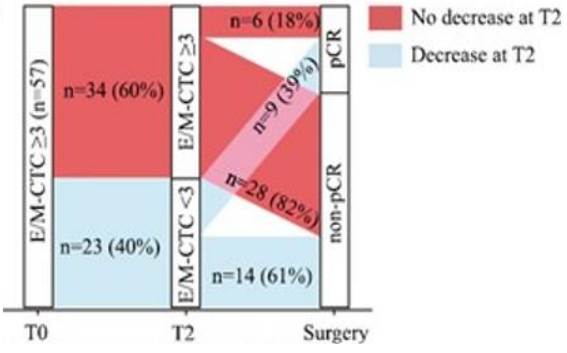
a)



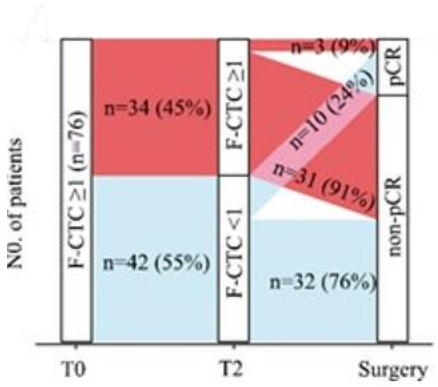
b)



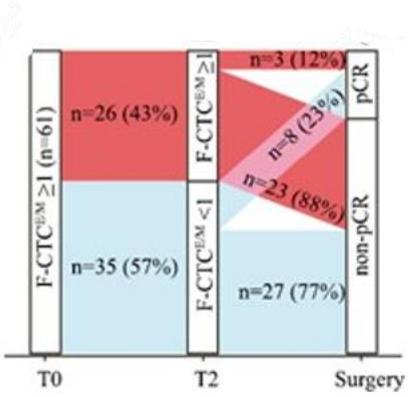
e)



f)



g)



h)

Figure 3. Impact of temporal variations in CTC counts on pathological complete response (pCR) at T1 and T2. Panels a–d: pCR rates according to numerical changes in total CTCs, E/M-CTC, F-CTC, and F-CTCE/M at T1. Panels e–h: pCR rates according to numerical changes in total CTCs, E/M-CTC, F-CTC, and F-CTCE/M at T2.

Among all evaluated factors, F-CTC status at baseline (T0) was identified as an independent determinant of pCR.

From the results summarized in **Table 3,** patients exhibiting HR positivity (odds ratio [OR] = 0.28, 95% confidence interval [CI], 0.12–0.66, P = .004), total CTC count ≥4 (OR = 0.45, 95% CI, 0.21–0.99, P = .046), F-CTC ≥1 (OR = 0.14, 95% CI, 0.06–0.33, P < .001), or F-CTCE/M ≥1 (OR = 0.24, 95% CI, 0.11–0.56, P = .001) were significantly less likely to achieve pCR. In contrast, HER2-positive status strongly favored achieving pCR (OR = 8.94, 95% CI, 3.67–21.80, P < .001). Other clinicopathological characteristics, as well as modifications in CTC levels at T1 or T2, did not show significant associations with pCR outcomes.

After adjusting for HR and HER2, baseline F-CTC remained a statistically significant independent predictor of pCR (OR = 0.16, 95% CI = 0.03–0.95, P = .043). Considering the known influence of HER2 on treatment response, we conducted a stratified multivariate logistic regression based on HER2 status. Similarly, in the HER2-positive subgroup, baseline F-CTC independently predicted decreased likelihood of achieving pCR (HR = 0.08, 95% CI, 0.01–0.98, P = .048).

Table 3. Results of univariate and multivariate logistic regression analyses for factors influencing pCR rates.

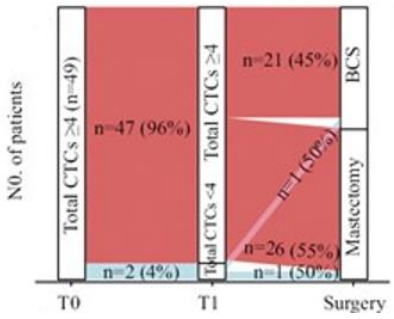
Variables	Multivariable analysis		Univariable analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age: ≤50 vs >50	/	/	0.89 (0.39-2.02)	.78
Menstrual status: premenopausal vs postmenopausal	/	/	0.69 (0.29-1.62)	.39
Tumor size: cT1-2 vs cT3-4	/	/	0.59 (0.23-1.52)	.27
Lymph nodes: cN0-1 vs cN2-3	/	/	0.70 (0.23-2.12)	.53
HR status: negative vs positive	0.23 (0.08-0.68)	.008	0.28 (0.12-0.66)	.004
HER2 status: negative vs positive	7.53 (2.75-20.61)	<.001	8.94 (3.67-21.80)	<.001
Ki-67 level: <14% vs ≥14%	/	/	1.46 (0.15-14.52)	.75
Total CTCs at T0: <4 vs ≥4	0.75 (0.24-2.35)	.75	0.45 (0.21-0.99)	.046
E/M-CTC at T0: <3 vs ≥3	0.16 (0.03-0.95)	.043	0.14 (0.06-0.33)	<.001
F-CTC at T0: <1 vs ≥1	/	/	0.58 (0.27-1.26)	.17
F-CTCE/M at T0: <1 vs ≥1	1.45 (0.23-9.14)	.69	0.24 (0.11-0.56)	.001

Total CTCs at T1: <4 vs ≥4	/	/	0.42 (0.15-1.15)	.09
E/M-CTC at T1: <3 vs ≥3	/	/	0.89 (0.35-2.22)	.80
F-CTC at T1: <1 vs ≥1	/	/	0.61 (0.27-1.34)	.22
F-CTCE/M at T1: <1 vs ≥1	/	/	1.14 (0.49-2.64)	.77
Total CTCs at T2: <4 vs ≥4	/	/	1.02 (0.41-2.53)	.96
E/M-CTC at T2: <3 vs ≥3	/	/	0.60 (0.28-1.32)	.21
F-CTC at T2: <1 vs ≥1	/	/	0.61 (0.24-1.52)	.29
F-CTCE/M at T2: <1 vs ≥1	/	/	0.61 (0.27-1.38)	.24
Change in total CTCs at T1: no decrease vs decrease	/	/	3.06 (0.18-51.59)	.44
Change in E/M-CTC at T1: no decrease vs decrease	/	/	0.88 (0.22-3.61)	.86
Change in F-CTC at T1: no decrease vs decrease	/	/	1.83 (0.50-6.74)	.36
Change in F-CTCE/M at T1: no decrease vs decrease	/	/	0.94 (0.28-3.19)	.93
Change in total CTCs at T2: no decrease vs decrease	/	/	0.54 (0.11-2.73)	.45
Change in E/M-CTC at T2: no decrease vs decrease	/	/	3.23 (0.81-12.86)	.10
Change in F-CTC at T2: no decrease vs decrease	/	/	3.00 (0.89-10.12)	.08
Change in F-CTCE/M at T2: no decrease vs decrease	/	/	2.27 (0.54-9.58)	.26

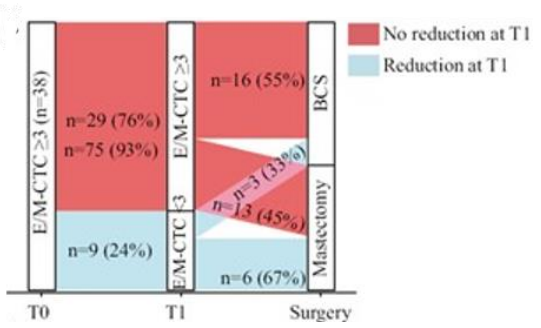
Abbreviations:
CI: confidence interval;
CTCs: circulating tumor cells;
E-CTC: epithelial circulating tumor cells;
E/M-CTC: biphenotypic epithelial/mesenchymal circulating tumor cells;
F-CTC: FTH1 gene-associated circulating tumor cells;
F-CTCE: FTH1 gene-associated epithelial circulating tumor cells;
F-CTCE/M: FTH1 gene-associated biphenotypic epithelial/mesenchymal circulating tumor cells;
F-CTCM: FTH1 gene-associated mesenchymal circulating tumor cells;
HER2: human epidermal growth factor receptor 2;
HR: hormone receptor;
M-CTC: mesenchymal circulating tumor cells;
OR: odds ratio;

At initial assessment, 87 out of 120 patients (72.5%) presented with stage IIB–IIIC breast cancer, making them unsuitable candidates for breast-conserving surgery (BCS) before systemic therapy. These patients were expected to undergo neoadjuvant chemotherapy (NAC) to potentially downstage their tumors. After completion of NAC, all 87 patients proceeded to surgery, with 43 (49.4%) ultimately receiving BCS.

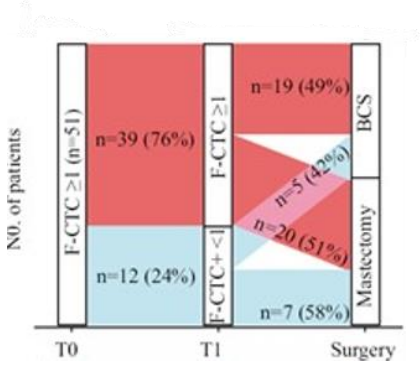
Figure 4 illustrates that changes in total CTCs, E/M-CTC, F-CTC, and F-CTCE/M at T1 did not show a significant effect on the likelihood of BCS. We further examined whether variations in CTC status at T2 influenced surgical outcomes. Among the 51 patients with F-CTC ≥1 at T0, 20 exhibited a reduction in F-CTC by T2, and 5 of these underwent BCS. Conversely, in 31 patients whose F-CTC did not decrease, 19 underwent BCS. This resulted in a significantly higher BCS rate in patients showing a decline in F-CTC at T2 compared with those with stable or increased levels (61% vs 25%, P = .011). Such an association was not observed for total CTCs, E/M-CTC, or F-CTCE/M at T2.



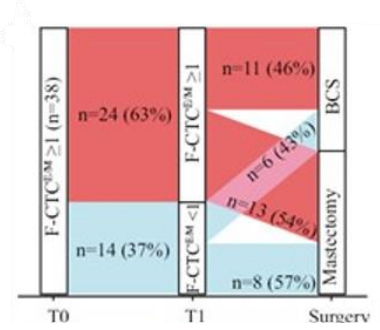
a)



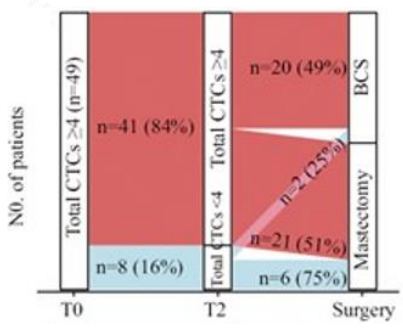
b)



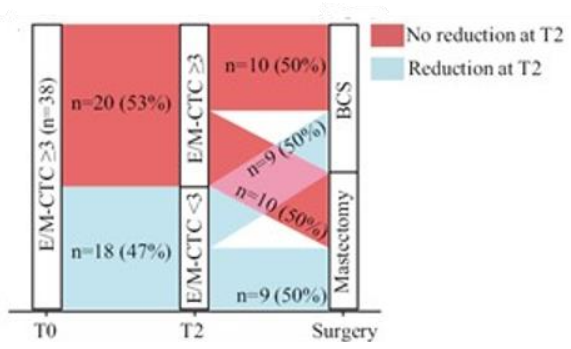
c)



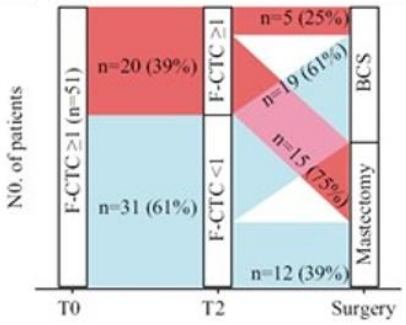
d)



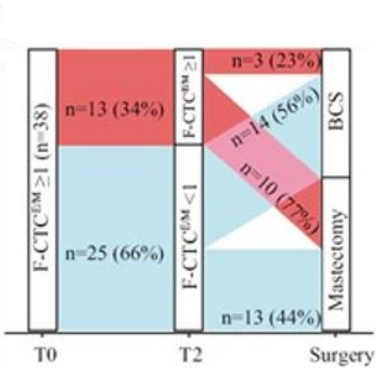
e)



f)



g)



h)

Figure 4. Impact of CTC count variations on breast-conserving surgery (BCS) rates at T1 and T2 in patients with locally advanced breast cancer.

Panels a–d: Comparison of BCS rates based on numerical changes in total CTCs, E/M-CTC, F-CTC, and F-CTCE/M at T1.

Panels e–h: Comparison of BCS rates based on numerical changes in total CTCs, E/M-CTC, F-CTC, and F-CTCE/M at T2.

In univariate analysis, certain clinical factors were linked to a lower likelihood of BCS. Specifically, postmenopausal patients had decreased odds of undergoing BCS (OR = 0.32, 95% CI, 0.12–0.82, P = .02), as did patients with tumors larger than 50 mm (OR = 0.15, 95% CI, 0.05–0.42, P < .001) and those with

clinical lymph node stages 2–3 (OR = 0.28, 95% CI, 0.09–0.87, P = .03). Conversely, a reduction in F-CTC at T2 was associated with higher chances of receiving BCS (OR = 4.75, 95% CI, 1.37–16.47, P = .01).

When these variables were simultaneously analyzed in a multivariate logistic regression, tumor size remained a significant negative predictor (OR = 0.18, 95% CI, 0.04–0.75, P = .02), while preoperative reduction in F-CTC continued to positively predict BCS (OR = 4.54, 95% CI, 1.14–18.08, P = .03) **(Table 4).**

Table 4. Logistic regression analyses (univariate and multivariate) identifying variables associated with BCS rates in patients with locally advanced breast cancer.

Variables	Multivariable analysis		Univariable analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age: ≤50 vs >50	/	/	0.63 (0.27-1.52)	.30
Menstrual status: premenopausal vs postmenopausal	0.60 (0.16-2.28)	.45	0.32 (0.12-0.82)	.02
Tumor size: cT1-2 vs cT3-4	0.18 (0.04-0.75)	.02	0.15 (0.05-0.42)	<.001
Lymph nodes: cN0-1 vs cN2-3	0.43 (0.08-2.23)	.31	0.28 (0.09-0.87)	.03
HR status: negative vs positive	/	/	0.42 (0.15-1.12)	.08
HER2 status: negative vs positive	/	/	0.79 (0.34-1.84)	.59
Total CTCs at T0: <4 vs ≥4	/	/	0.66 (0.28-1.55)	.34
E/M-CTC at T0: <3 vs ≥3	/	/	1.04 (0.45-2.43)	.93
F-CTC at T0: <1 vs ≥1	/	/	0.80 (0.34-1.87)	.60
F-CTCE/M at T0: <1 vs ≥1	/	/	0.72 (0.31-1.68)	.44
Total CTCs at T1: <4 vs ≥4	/	/	0.97 (0.33-2.88)	.96
E/M-CTC at T1: <3 vs ≥3	/	/	2.15 (0.88-5.25)	.09
F-CTC at T1: <1 vs ≥1	/	/	0.85 (0.31-2.35)	.75
F-CTCE/M at T1: <1 vs ≥1	/	/	1.66 (0.66-4.17)	.28
Total CTCs at T2: <4 vs ≥4	/	/	1.11 (0.40-3.08)	.84
E/M-CTC at T2: <3 vs ≥3	/	/	1.26 (0.54-2.92)	.59
F-CTC at T2: <1 vs ≥1	/	/	0.44 (0.18-1.05)	.06
F-CTCE/M at T2: <1 vs ≥1	/	/	0.69 (0.28-1.68)	.41
Change in total CTCs at T1: no decrease vs decrease	/	/	1.24 (0.07-21.00)	.88
Change in E/M-CTC at T1: no decrease vs decrease	/	/	0.58 (0.13-2.51)	.46
Change in F-CTC at T1: no decrease vs decrease	/	/	0.75 (0.20-2.78)	.67
Change in F-CTCE/M at T1: no decrease vs decrease	/	/	0.89 (0.24-3.35)	.86
Change in total CTCs at T2: no decrease vs decrease	/	/	0.35 (0.06-1.94)	.23
Change in E/M-CTC at T2: no decrease vs decrease	/	/	1.00 (0.28-3.57)	1.00
Change in F-CTC at T2: no decrease vs decrease	4.54 (1.14-18.08)	.03	4.75 (1.37-16.47)	.01
Change in F-CTCE/M at T2: no decrease vs decrease	/	/	4.24 (0.94-19.26)	.06

Abbreviations:
BCS: breast-conserving surgery;
CI: confidence interval;
CTCs: circulating tumor cells;
E-CTC: epithelial circulating tumor cells;
E/M-CTC: biphenotypic epithelial/mesenchymal circulating tumor cells;
F-CTC: FTH1 gene-associated circulating tumor cells;
F-CTCE: FTH1 gene-associated epithelial circulating tumor cells;
F-CTCE/M: FTH1 gene-associated biphenotypic epithelial/mesenchymal circulating tumor cells;
F-CTCM: FTH1 gene-associated mesenchymal circulating tumor cells;

HER2: human epidermal growth factor receptor 2;
HR: hormone receptor;
M-CTC: mesenchymal circulating tumor cells;
OR: odds ratio.

Association between CTCs and event-free survival (EFS)

During a median follow-up period of 13 months (interquartile range: 10–16), metastases were observed in a subset of patients: 3 developed chest wall metastases, 2 experienced bone metastases, and 1 had adrenal metastases. No definitive predictors of EFS were identified, likely due to the limited follow-up duration.

In this prospective, observational cohort, we investigated the association between FTH1 gene-associated CTCs (F-CTC) and the response to neoadjuvant chemotherapy (NAC) in non-metastatic breast cancer. We observed that F-CTC ≥1 at baseline independently predicted a lower likelihood of achieving pathological complete response (pCR), whereas a reduction in F-CTC following NAC independently correlated with higher breast-conserving surgery (BCS) rates. The prognostic implications of FTH1 expression will be clarified with longer follow-up.

Previous studies have reported that overall CTC count is a prognostic factor in breast cancer, though its correlation with pCR has not been clearly established [6]. The heterogeneity of minimal residual disease (MRD) can be explored by evaluating CTC subtypes using RNA in situ hybridization. In our cohort, baseline F-CTC was a significant independent predictor of pCR, likely reflecting elevated FTH1 expression in primary tumors. FTH1 overexpression enhances cellular iron sequestration and prevents accumulation of lipid reactive oxygen species (ROS), thereby inhibiting ferroptosis [26, 27], which may contribute to chemotherapy resistance. Consequently, patients with detectable F-CTC tend to show lower sensitivity to chemotherapy and anti-HER2 therapy, making pCR more difficult to achieve.

CTCs expressing mesenchymal markers have enhanced migratory and invasive capacity [28, 29], raising the question of whether E/M-CTC might influence NAC response. In this study, E/M-CTC levels at baseline did not significantly impact pCR, and further research is required to assess their role in metastatic disease.

Changes in CTC counts at T1 or T2 did not affect pCR in this cohort. Prior studies have noted fluctuations in CTC numbers during NAC in early-stage breast cancer, potentially due to chemotherapy-induced tumor cell release [30]. These variations suggest that dynamic changes may not reliably reflect tumor status, highlighting the importance of baseline CTC assessment for predicting NAC efficacy.

Compared with mastectomy, BCS offers better cosmetic outcomes and less psychological and quality-of-life impact [31]. In our study, in addition to tumor size, reduction in F-CTC post-NAC significantly influenced BCS rates. This may be explained by decreased FTH1-mediated iron sequestration in primary tumors, leading to ROS accumulation via the Fenton reaction and ferroptosis, thereby facilitating local tumor shrinkage and a higher likelihood of BCS.

No significant effect of CTC subtypes at T0 or their dynamic changes at T1/T2 on EFS was observed, possibly due to the short follow-up. Extended follow-up will be necessary to clarify the impact of F-CTC on survival outcomes.

FTH1 was used as a novel biomarker for CTC classification in this study, offering a non-invasive method to evaluate NAC response. Patients with detectable F-CTC before NAC may benefit from intensified chemotherapy regimens. For those without F-CTC reduction post-NAC, wider surgical margins during BCS and reconstruction planning may be warranted. Further studies on the mechanisms of these CTC subtypes could inform the development of targeted therapeutics.

This study has limitations. It was conducted at a single center, introducing potential selection bias. Follow-up duration remains limited, and extended survival analyses are needed. Unlike circulating tumor DNA (ctDNA), which reflects only DNA-level information, CTCs provide RNA, protein, and cellular-level data [32], making these assays complementary. CTC testing is cost-effective and well-tolerated in clinical practice. Ongoing ctDNA studies (ChiCTR2100048870) at our center aim to identify predictive molecular markers for breast cancer therapy.

Conclusion

F-CTC correlates with NAC efficacy and may serve as an early predictor of treatment response. Utilizing FTH1-based CTC monitoring could enable timely, personalized interventions in non-metastatic breast cancer, potentially improving long-term outcomes.

Acknowledgments: We are grateful to all patients and their families and all staff at the study center. We appreciate the technical support from SurExam Bio-Tech Co. Ltd Xiaoyu Pu. This article was approved for publication by the Academic Standardization Office of Sun Yat-sen Memorial Hospital, Sun Yat-sen University.

Conflict of Interest: None

Financial Support: This work was supported by the Natural Science Foundation of China (81872139, 82072907, 82003311, U1911204 and 51861125203); Project of The Beijing Xisike Clinical Oncology Research Foundation (Y-Roche2019/2-0078, Y-pierrefabre202102-0107); Project of The Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation (2020B1212060018); the Fundamental Research Funds for the Central Universities, Sun Yat-sen University (2022005); High-tech, Major and Characteristic Technology Projects in Guangzhou Area (2023-2025) (2023P-ZD14); National Key R&D Program of China (2021YFC3001000); Guangzhou Science and Technology Program (No. 202102010272).

Ethics Statement: The study was approved by the ethics committee of Sun Yat-sen Memorial Hospital (SYSEC-KY-KS-2021-103). Written informed consent was obtained from all participants in the study.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30. https://doi.org/10.3322/caac.21590.
2. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the glob- al cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-1953. https://doi. org/10.1002/ijc.31937.
3. Pantel K, Alix-Panabieres C. Liquid biopsy and minimal residual disease - latest advances and implications for cure. Nat Rev Clin Oncol. 2019;16(7):409-424. https://doi.org/10.1038/s41571-019- 0187-3.
4. Serrano MJ, Rovira PS, Martinez-Zubiaurre I, et al. Dynamics of circulating tumor cells in early breast cancer under neoadjuvant therapy. Exp Ther Med. 2012;4(1):43-48. https://doi.org/10.3892/etm.2012.540.
5. Lucci A, Hall CS, Lodhi AK, et al. Circulating tumour cells in non-metastatic breast cancer: a prospective study. Lancet Oncol. 2012;13(7):688-695. https://doi.org/10.1016/S1470-2045(12)70209-7.
6. Bidard FC, Michiels S, Riethdorf S, et al. Circulating tumor cells in breast cancer patients treated by neoadjuvant chemotherapy: a meta- analysis. J Natl Cancer Inst. 2018;110(6):560-567. https://doi. org/10.1093/jnci/djy018.
7. Joosse SA, Pantel K. Biologic challenges in the detection of cir- culating tumor cells. Cancer Res. 2013;73(1):8-11. https://doi. org/10.1158/0008-5472.CAN-12-3422.
8. Gorges TM, Tinhofer I, Drosch M, et al. Circulating tumour cells escape from EpCAM-based detection due to epithelial-to- mesenchymal transition. BMC Cancer. 2012;12:178. https://doi. org/10.1186/1471-2407-12-178.
9. Wu S, Liu S, Liu Z, et al. Classification of circulating tumor cells by epithelial-mesenchymal transition markers. PLoS One. 2015;10(4):e0123976. https://doi.org/10.1371/journal. pone.0123976.
10. Dong J, Zhu D, Tang X, et al. Circulating tumor cells in pulmo- nary vein and peripheral arterial provide a metric for PD-L1 diag- nosis and prognosis of patients with non-small cell lung cancer. PLoS One. 2019;14(7):e0220306. https://doi.org/10.1371/journal. pone.0220306.
11. Guan X, Ma F, Li C, et al. The prognostic and therapeutic impli- cations of circulating tumor cell phenotype detection based on epithelial-mesenchymal transition markers in the first-line che- motherapy of HER2-negative metastatic breast cancer. Cancer Commun (Lond). 2019;39(1):1. https://doi.org/10.1186/s40880- 018-0346-4.
12. Qi LN, Xiang BD, Wu FX, et al. Circulating tumor cells undergoing EMT provide a metric for diagnosis and prognosis of patients with hepatocellular carcinoma. Cancer Res. 2018;78(16):4731-4744. https://doi.org/10.1158/0008-5472.CAN-17-2459.
13. Lu B, Chen XB, Ying MD, et al. The role of ferroptosis in can- cer development and treatment response. Front Pharmacol. 2017;8:992. https://doi.org/10.3389/fphar.2017.00992.

14. Dixon SJ, Stockwell BR. The hallmarks of ferroptosis. Ann Rev Can- cer Biol. 2019;3(1):35-54. https://doi.org/10.1146/annurev-cancer- bio-030518-055844.
15. Kanojia D, Zhou W, Zhang J, et al. Proteomic profiling of can- cer stem cells derived from primary tumors of HER2/Neu trans- genic mice. Proteomics. 2012;12(22):3407-3415. https://doi.org/10.1002/pmic.201200103.
16. Liu NQ, De Marchi T, Timmermans AM, et al. Ferritin heavy chain in triple negative breast cancer: a favorable prognostic marker that relates to a cluster of differentiation 8 positive (CD8+) effec- tor T-cell response. Mol Cell Proteomics. 2014;13(7):1814-1827. https://doi.org/10.1074/mcp.M113.037176.
17. Galadari S, Rahman A, Pallichankandy S, Thayyullathil F. Reactive oxygen species and cancer paradox: To promote or to suppress?. Free Radic Biol Med. 2017;104:144-164. https://doi.org/10.1016/j. freeradbiomed.2017.01.004.
18. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mech- anisms and health implications. Cell Res. 2021;31(2):107-125. https://doi.org/10.1038/s41422-020-00441-1.
19. Higgy NA, Salicioni AM, Russo IH, Zhang PL, Russo J. Differential expression of human ferritin H chain gene in immortal human breast epithelial MCF-10F cells. Mol Carcinog. 1997;20(4):332-339. https://doi.org/10.1002/(sici)1098-2744(199712)20:4<332::aid- mc2>3.0.co;2-l.
20. Moloney JN, Cotter TG. ROS signalling in the biology of cancer. Semin Cell Dev Biol. 2018;80:50-64. https://doi.org/10.1016/j. semcdb.2017.05.023.
21. Epsztejn S, Glickstein H, Picard V, et al. H-ferritin subunit overex- pression in erythroid cells reduces the oxidative stress response and induces multidrug resistance properties. Blood. 1999;94(10):3593-3603.
22. Ebrahimi N, Adelian S, Shakerian S, et al. Crosstalk between ferro- ptosis and the epithelial-mesenchymal transition: implications for inflammation and cancer therapy. Cytokine Growth Factor Rev. 2022;64:33-45. https://doi.org/10.1016/j.cytogfr.2022.01.006.
23. Wang M, Li S, Wang Y, et al. Gambogenic acid induces ferropto- sis in melanoma cells undergoing epithelial-to-mesenchymal tran- sition. Toxicol Appl Pharmacol. 2020;401:115110. https://doi.org/10.1016/j.taap.2020.115110.
24. Chen K, Zeng Y, Jia H, et al. Clinical outcomes of breast- conserving surgery in patients using a modified method for cav- ity margin assessment. Ann Surg Oncol. 2012;19(11):3386-3394. https://doi.org/10.1245/s10434-012-2331-5.
25. Schmid P, Cortes J, Dent R, et al. Event-free survival with pem- brolizumab in early triple-negative breast cancer. N Engl J Med. 2022;386(6):556-567. https://doi.org/10.1056/NEJMoa2112651.
26. Li J, Cao F, Yin HL, et al. Ferroptosis: past, present and future. Cell Death Dis. 2020;11(2):88. https://doi.org/10.1038/s41419-020- 2298-2.
27. Wang Y, Chang S, Wu Q, et al. The protective role of mitochondrial fer- ritin on erastin-induced ferroptosis. Front Aging Neurosci. 2016;8:308.
28. Yao D, Dai C, Peng S. Mechanism of the mesenchymal-epithelial transition and its relationship with metastatic tumor formation. Mol Cancer Res. 2011;9(12):1608-1620. https://doi.org/10.1158/1541- 7786.MCR-10-0568.
29. Iwatsuki M, Mimori K, Yokobori T, et al. Epithelial-mesenchymal transition in cancer development and its clinical significance. Cancer Sci. 2010;101(2):293-299. https://doi.org/10.1111/j.1349- 7006.2009.01419.x.
30. Kaigorodova EV, Savelieva OE, Tashireva LA, et al. Heterogeneity of circulating tumor cells in neoadjuvant chemotherapy of breast cancer. Molecules. 2018;23(4):727. https://doi.org/10.3390/mole- cules23040727.
31. van Maaren MC, de Munck L, de Bock GH, et al. 10 year survival after breast-conserving surgery plus radiotherapy compared with mastectomy in early breast cancer in the Netherlands: a population- based study. Lancet Oncol. 2016;17(8):1158-1170. https://doi. org/10.1016/S1470-2045(16)30067-5.
32. Tellez-Gabriel M, Knutsen E, Perander M. Current status of circu- lating tumor cells, circulating tumor dna, and exosomes in breast cancer liquid biopsies. Int J Mol Sci. 2020;21(24):9457.