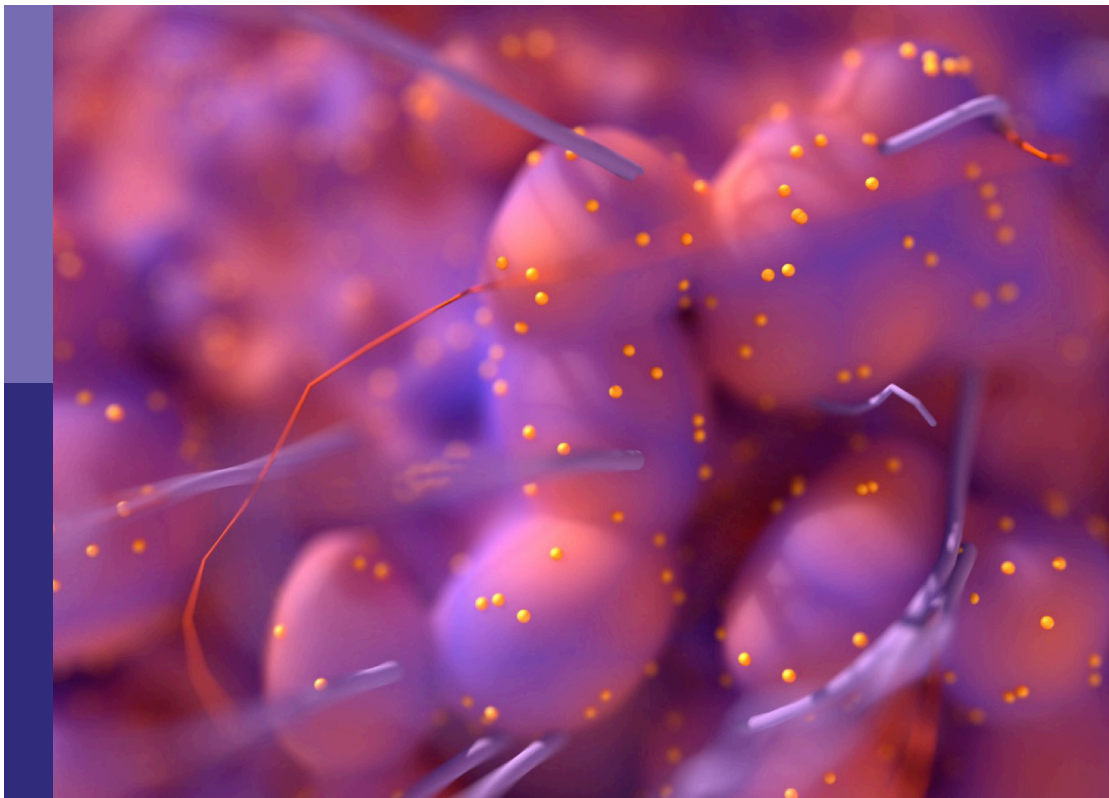
frontiers | Research Topics

Locoregional management of breast cancer: Multidisciplinary and individualized treatment

Edited by
Xiaosong Chen, Kunwei Shen, Walter Paul Weber, Jia-Yi Chen, Youlia M. Kirova and Yiding Chen

Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-83251-929-5
DOI 10.3389/978-2-83251-929-5

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Locoregional management of breast cancer: Multidisciplinary and individualized treatment

Topic editors

Xiaosong Chen — Shanghai Jiao Tong University, China
Kunwei Shen — Shanghai Jiao Tong University, China
Walter Paul Weber — University Hospital of Basel, Switzerland
Jia-Yi Chen — Shanghai Jiao Tong University, China
Youlia M. Kirova — Institut Curie, France
Yiding Chen — Zhejiang University, China

Citation

Chen, X., Shen, K., Weber, W. P., Chen, J.-Y., Kirova, Y. M., Chen, Y., eds. (2023). *Locoregional management of breast cancer: Multidisciplinary and individualized treatment*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-929-5

Table of contents

frontiers in Oncology

ORIGINAL RESEARCH
published: 13 October 2021
doi: 10.3389/fonc.2021.763119

Prognostic Factors and Surgery for Breast Cancer Patients With Locoregional Recurrence: An Analysis of 5,202 Consecutive Patients

Jiahui Huang, Yiwei Tong, Xiaosong Chen and Kunwei Shen**

Department of General Surgery, Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Purpose: With the application of "less extensive surgery" in breast cancer treatment, the pattern of locoregional recurrence (LRR) has significantly changed. This study aims to evaluate the risk and prognostic factors of LRR in a recent large breast cancer cohort.

Methods: Consecutive early breast cancer patients who received surgery from January 2009 to March 2018 in Shanghai Ruijin Hospital were retrospectively analyzed. LRR was defined as recurrence at the ipsilateral breast (IBTR), chest wall, or regional lymph nodes and without concurrent distant metastasis (DM). Patients' characteristics and survival were compared among these groups.

Results: Among 5,202 patients included, 87 (1.7%) and 265 (5.1%) experienced LRR and DM as first event after a median 47.0 (3.0–122.5) months' follow-up. LRR was significantly associated with large tumor size and positive lymph node status ($p < 0.05$). Forty (46.0%) patients received further salvage surgery after LRR and had a significantly better 3-year post-recurrence overall survival than those who did not (94.7% *vs.* 60.7%, $p = 0.012$). Multivariate analysis showed that salvage surgery for LRR was independently associated with better survival (HR = 0.12, 95% CI 0.02–0.93, $p = 0.043$) along with estrogen receptor (ER) positivity (HR = 0.33, 95% CI 0.12–0.91, $p = 0.033$).

Conclusion: LRR rate was relatively low in recent era of breast cancer treatment. Tumor size and lymph node status were associated with risk of LRR, and salvage surgery for selected LRR patients achieved an excellent outcome.

Keywords: breast cancer, risk factors, surgery, survival, locoregional recurrence

OPEN ACCESS

Edited by:
Tadahiko Shien, Okayama University, Japan

Reviewed by:
Shinsuke Sasada, Hiroshima University, Japan
Sota Asaga, Kyorin University Hospital, Japan

****Correspondence:***
Xiaosong Chen
chenxiaosong0156@hotmail.com
Kunwei Shen
kwshen@medmail.com.cn

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *23 August 2021*
Accepted: *22 September 2021*
Published: *13 October 2021*

Citation:
Huang J, Tong Y, Chen X and Shen K (2021) Prognostic Factors and Surgery for Breast Cancer Patients With Locoregional Recurrence: An Analysis of 5,202 Consecutive Patients. Front. Oncol. 11:763119. doi: 10.3389/fonc.2021.763119

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer mortality in females worldwide (1). With a better understanding of tumor biologic behavior, innovations in screening techniques, and the development of comprehensive multidisciplinary treatment strategies, more breast cancers can be diagnosed at early stages. Less extensive surgery, for instance, breast-conserving surgery (BCS) followed by radiotherapy and sentinel lymph node

biopsy (SLNB) in selected patients demonstrated equivalence with mastectomy and axillary lymph node dissection (ALND) in terms of survival but with less comorbidities (2, 3).

Locoregional recurrence (LRR) is a clinically relevant, predominant pattern of treatment failure in breast cancer. LRR patterns vary across initial surgical approach and mainly involve recurrence in chest wall post-mastectomy, residual breast after BCS, or regional lymph nodes (LNs). According to previous evidence, factors associated with increased risk of LRR include young age at diagnosis, greater tumor size, involvement of regional LN, high histological grade, triple negative (TN) or human epidermal growth factor receptor 2 (HER2)-positive subtype, lack of endocrine therapy, and omitting indicated adjuvant radiotherapy (4–6). Different from the palliative management of distant metastasis (DM), salvage surgery plays an important role in the comprehensive management of LRR. Patients who received salvage surgery for LRR reported relatively satisfactory 5-year overall survival (OS) ranging from 40.8% to 90.9% (7, 8), suggesting that selective LRR patients would benefit from salvage surgery and quite a number of LRR patients could be cured. However, retrospective series showed that between 15% and 37% patients with LRR had concurrent DM at the time of presentation (9–18). Disease outcomes and treatment strategies of these populations can be very different from those with LRR alone. The management of LRR should be based on systemic evaluation and be discussed in a multidisciplinary setting.

However, studies of LRR were mostly conducted in the late 1990s to early 2000s and in western populations. Following the change of initial surgical procedures from "maximal tolerable treatment" to "minimal effective treatment," the pattern of LRR has also significantly changed. With an increasing rate of BCS and SLNB, now we meet more patients with ipsilateral breast tumor recurrence (IBTR) and regional LN recurrence in clinical practice. To this end, the objective of this study was to analyze the risk and prognostic factors of LRR in the current "less extensive surgery" era.

PATIENTS AND METHODS

Patients

We retrospectively included consecutive female patients diagnosed with primary invasive breast cancer and received radical surgical treatment from January 2009 to March 2018 in Comprehensive Breast Health Center, Shanghai Ruijin Hospital. Patients with complete clinicopathological information, with at least 3 months of follow-up, were included in this study. Patient with *de novo* stage IV disease, with bilateral breast cancer, receiving neoadjuvant therapy for breast cancer, or with previous malignancy history were excluded from this study (**Supplementary Figure 1**). Patient baseline clinical characteristics were extracted from Shanghai Jiaotong University Breast Cancer Database (SJTU-BCDB).

Pathological Assessment

Histopathological assessment and immunohistochemical (IHC) evaluation were conducted in the Department of Pathology, Ruijin Hospital, by at least two independent experienced pathologists. Estrogen receptor (ER) positivity and progesterone receptor (PR) positivity were defined as 1% or more positive invasive tumor cells with nuclear staining (19). HER2 status was first determined by IHC staining and scored as 0, 1+, 2+, and 3+ according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline (20). Samples with HER2 IHC 2+ were further examined by fluorescence *in situ* hybridization (FISH). HER2 positivity was defined as HER2 IHC 3+ or FISH positive. Five breast cancer molecular subtypes were classified according to the 2013 St. Gallen breast cancer consensus (21): Luminal A (ER+/HER2–, Ki67 < 14%, and PR ≥ 20%), Luminal B HER2– (ER+/HER2–, Ki67 ≥ 14%, or ER+/HER2–, PR < 20%, or ER–/PR+/HER2–), Luminal B HER2+ (ER or PR+/HER2+), TN (ER–/PR–/HER2–), and HER2 enriched (ER–/PR–/HER2+).

Follow-Up and Disease Outcomes

Follow-up was accomplished annually by specialized breast cancer nurses in our center through outpatient medical history and/or phone calls. Recurrences in ipsilateral breast, chest wall, or regional LN (ipsilateral axillary, infra- and/or supraclavicular, or internal mammary LN) were considered LRR. DM included metastases to distant LN, bone, brain, liver, lung (including pleura and lymphangitic carcinomatosis), or others (including peritoneal, other organs not elsewhere classified, and skin not in the breast and chest wall). Patients with concurrent LRR and DM were categorized as DM as first recurrence event.

Recurrence-free interval (RFI) was defined as time from the date of breast cancer surgery to the date of first recurrence event. OS was defined as time from the date of breast cancer surgery to the date of death from any cause. Post-recurrence OS (PR-OS) was defined as the time from the date of first recurrence diagnosis to the time of death from any cause.

Statistical Analysis

Patients were categorized into three groups according to their recurrence status, i.e., recurrence-free, LRR, and DM groups. Descriptive characteristics of categorical variables were tested using chi-squared test or Fisher's exact test. Binary or multinomial logistic regression analysis was conducted to compare baseline clinicopathological features and adjuvant therapy among groups. Survival curves were plotted using the Kaplan–Meier method and compared between groups by log-rank test. Multivariate Cox proportional-hazards regression analyses were performed to calculate hazard ratios (HRs) and 95% confidence interval (CI) for recurrence and survival. All analyses were performed using IBM SPSS 22.0 (IBM Inc., Armonk, USA). All reported p-values were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Patient Baseline Characteristics

A total of 5,202 women were included in this study. The median age was 55 (range: 22–93) years. Patients' baseline clinicopathological

characteristics at initial diagnosis and treatment for primary breast cancer were summarized in **Table 1**. Four thousand four hundred fifty-four (85.6%) patients had invasive ductal carcinoma, and 1,723 (33.1%) had node-positive disease. ER positivity were identified in 3,769 (72.5%) patients, and 1,181 (22.7%) had HER2-positive disease. With regard to local and systemic treatment, BCS was performed in 1,597 (30.7%) patients, while others received mastectomy as initial surgery for breast cancer. Two thousand five hundred sixty-three (49.3%) patients received SLNB, 2,598 (49.9%) patients received ALND, and the remaining 41 (0.8%) patients did not receive surgery for the axilla. Adjuvant radiotherapy was performed in 2,539 patients, including 86.4% of patients who underwent BCS and in 32.1% of patients who received mastectomy.

Patient Characteristics Associated With First Recurrence Event

After a median follow-up of 47.0 (range: 3.0–122.5) months, 352 (6.8%) patients experienced breast cancer recurrence, including 87 (1.7%) LRR and 265 (5.1%) DM as first recurrence event. The 5-year estimated LRR rate was 2.2% in the whole population: 3.3% in patients receiving BCS and 1.7% in patients receiving mastectomy. Tumor size, pathological type, histological grade, LN status, ER status, PR status, Ki67 level, molecular subtype, surgery of the breast, surgery of the axilla, adjuvant chemotherapy, adjuvant radiotherapy, and adjuvant endocrine therapy were differently distributed among patients with no recurrence, LRR, and DM in the univariate model (all $p < 0.05$; **Table 1**), while no difference was observed in age, menopausal status, HER2 status, or adjuvant targeted therapy among three groups ($p > 0.05$).

Multivariate analysis demonstrated that tumor size ($p < 0.001$; **Table 2**), histological grade ($p < 0.001$), lymph node status ($p < 0.001$), molecular subtype ($p = 0.005$), surgery of the breast ($p < 0.001$), surgery of the axilla ($p < 0.001$), and adjuvant chemotherapy ($p = 0.013$) were independently associated with first recurrence events. Comparison between patients with LRR and recurrence-free showed that tumor size >2.0 cm (OR = 2.13, 95% CI 1.31–3.48, $p = 0.002$), positive LNs (OR = 3.24, 95% CI 1.75–6.02, $p < 0.001$), primary BCS (OR = 3.04, 95% CI 1.73–5.33, $p < 0.001$), not receiving adjuvant chemotherapy (OR = 2.48, 95% CI 1.37–4.50, $p = 0.003$), and not receiving adjuvant radiotherapy (OR = 1.91, 95% CI 1.07–3.42, $p = 0.030$) were independent risk factors for LRR. Regarding patients with DM as first recurrence event, LRR patients had higher rates of BCS (OR = 3.86, 95% CI 1.96–7.58, $p < 0.001$), SLNB (OR = 2.80, 95% CI 1.37–5.75, $p = 0.005$), not receiving adjuvant chemotherapy (OR = 2.81, 95% CI 1.37–5.75, $p = 0.013$), and not receiving adjuvant radiotherapy (OR = 2.52, 95% CI 1.21–5.20, $p = 0.042$).

Factors Influencing Salvage Surgery for Locoregional Recurrence Patients

Forty out of 87 (46.0%) LRR patients received further salvage surgery. **Table 3** summarizes the clinicopathological features associated with the reception of salvage surgery in LRR patients. Age at recurrence, primary tumor size, primary lymph node status, primary surgery of the breast and axilla, and LRR type significantly influenced the choice of surgery for LRR ($p < 0.05$; **Table 3**). Patients with IBTR received more salvage surgery as compared with LRR patients with chest wall recurrence or reginal LN recurrence ($p < 0.001$). Twenty-one out of 26 (80.8%) patients with IBTR received salvage surgery, all of whom received mastectomy with or without ALND. Only five patients with isolated IBTR did not receive surgery for LRR, including two patients refusing further treatment, two treated with endocrine therapy but not surgery due to advanced age, and one participating in a clinical trial of a new drug. Twelve out of 27 (44.4%) patients with chest wall recurrence received extended tumor excision, while seven out of 34 (20.6%) patients with regional LN recurrence received LN dissection surgery. Among 27 patients who did not receive surgery for regional LN recurrence, nine, 17, and one patients were with ALN recurrence, supraclavicular/infraclavicular LN recurrence, and internal mammary LN recurrence.

Multivariate analysis showed that primary tumor size ($p = 0.039$), primary surgery of the axilla ($p = 0.006$), and LRR type ($p < 0.001$) were factors that independently influenced the choice of surgery for LRR (**Table 4**). Patients with smaller primary tumor size, primary SLNB, and IBTR had significantly higher probability to receive surgical treatment for LRR. Patients with regional LN recurrence were less likely to receive surgery for LRR than were patients with IBTR only (OR = 0.07, 95% CI 0.02–0.30, $p < 0.001$), while the probability of surgery for LRR was comparable between patients with chest wall recurrence and IBTR (OR = 0.36, 95% CI 0.09–1.47, $p = 0.155$).

Survival Outcome With Different Recurrence Events

The estimated 5-year OS was 80.7%, 50.3%, and 98.8% for patients with LRR, patients with DM, and recurrence-free patients, respectively ($p < 0.001$, **Figure 1**). Among the 87 patients with LRR, 26, 27, and 34 patients had IBTR, chest wall recurrence, and LN recurrence, respectively. During a median post-recurrence follow-up time of 21.3 (range: 1.0–77.5) months, 30 deaths were recorded. PR-OS curve is shown in **Figure 2A**. Patients with LRR as first event had a significantly better PR-OS than those with DM (3-year PR-OS 75.0% *vs.* 37.1%; $p < 0.001$, **Figure 2A**).

Univariate analysis showed that primary tumor size ($p = 0.033$; **Supplementary Table 1**), primary ER status ($p = 0.033$), primary surgery of the axilla ($p = 0.034$), LRR type (regional LN *vs.* IBTR only, $p = 0.045$), and surgery of LRR ($p = 0.012$) were factors associated with PR-OS. The estimated 3-year PR-OS was 90.9%, 77.3%, and 60.3% in patients with recurrence type of IBTR, chest wall, and regional LN, respectively ($p = 0.132$, **Figure 2B**). The estimated 3-year PR-OS was 94.7% in patients receiving surgery after LRR, which was significantly higher than that not receiving surgery (60.7%, $p = 0.012$, **Figure 2C**). In multivariate analysis, ER positivity (HR = 0.33, 95% CI 0.12–0.91, $p = 0.033$) and salvage surgery of LRR (HR = 0.11, 95% CI 0.02–0.93, $p = 0.043$) were independently associated with better PR-OS for LRR patients (**Table 5**).

TABLE 1 | Clinicopathological characteristics at initial diagnosis and treatment for primary breast cancer by different first recurrence events.

	Total n	Recurrence-free n (%)	LRR n (%)	DM n (%)	p^a
Age					0.066
<50 years	1,835	1,701 (92.7)	41 (2.2)	93 (5.1)	
≥50 years	3,367	3,149 (93.5)	46 (1.4)	172 (5.1)	
Menopausal status					0.526
Pre-menopausal	2,101	1,961 (93.3)	39 (1.9)	101 (4.8)	
Post-menopausal	3,101	2,889 (93.2)	48 (1.5)	164 (5.3)	
Tumor size					<0.001
≤2 cm	3,067	2,936 (95.7)	40 (1.3)	91 (3.0)	
>2 cm	2,020	1,804 (89.3)	47 (2.3)	169 (8.4)	
NA*	115	110 (95.7)	0 (0.0)	5 (4.3)	
Pathological type					0.015
IDC	4,454	4,135 (92.8)	76 (1.7)	243 (5.5)	
ILC	149	138 (92.6)	2 (1.3)	9 (6.0)	
Other invasive cancer	599	577 (96.3)	9 (1.5)	13 (2.2)	
Histological grade					<0.001
I–II	2,528	2,402 (95.0)	29 (1.1)	97 (3.8)	
III	1,896	1,711 (90.2)	43 (2.3)	142 (7.5)	
NA*	778	737 (94.7)	15 (1.9)	26 (3.3)	
Lymph node status					<0.001
Negative	3,440	3,307 (96.1)	41 (1.2)	92 (2.7)	
Positive	1,723	1,512 (87.8)	43 (2.5)	168 (9.8)	
NA*	39	31 (79.5)	3 (7.7)	5 (12.8)	
ER					<0.001
Positive	3,769	3,553 (94.2)	51 (1.4)	165 (4.4)	
Negative	1,424	1,288 (90.4)	36 (2.5)	100 (7.0)	
NA*	9	9 (100.0)	0 (0.0)	0 (0.0)	
PR					<0.001
Positive	3,099	2,951 (95.2)	34 (1.1)	114 (3.7)	
Negative	2,091	1,887 (90.3)	53 (2.5)	151 (7.2)	
NA*	9	9 (100.0)	0 (0.0)	0 (0.0)	
HER2					0.403
Negative	3,797	3,553 (93.5)	56 (1.5)	188 (5.0)	
Positive	1,181	1,097 (92.9)	24 (2.0)	60 (5.1)	
NA*	215	191 (88.9)	7 (3.3)	17 (7.9)	
Ki67					<0.001
≤20%	2,734	2,587 (94.6)	37 (1.4)	110 (4.0)	
>20%	2,428	2,224 (91.6)	50 (2.1)	154 (6.3)	
NA*	40	39 (97.5)	0 (0.0)	1 (2.5)	
Molecular subtype					<0.001
Luminal A	922	897 (97.3)	6 (0.7)	19 (2.0)	
Luminal B HER2–	2,082	1,940 (93.2)	35 (1.7)	107 (5.1)	
Luminal B HER2+	567	542 (95.6)	5 (0.9)	20 (3.5)	
HER2 enriched	614	555 (90.4)	19 (3.1)	40 (6.5)	
TN	725	652 (90.0)	15 (2.1)	58 (8.0)	
NA*	292	264 (90.4)	7 (2.4)	21 (7.2)	
Surgery of the breast					<0.001
BCS	1,597	1,513 (94.7)	36 (2.3)	48 (3.0)	
Mastectomy	3,605	3,337 (92.6)	51 (1.4)	217 (6.0)	
Surgery of the axilla					<0.001
SLNB	2,563	2,491 (97.2)	33 (1.3)	39 (1.5)	
ALND	2,598	2,326 (89.5)	51 (2.0)	221 (8.5)	
No surgery	41	33 (80.5)	3 (7.3)	5 (12.2)	
Adjuvant chemotherapy					<0.001
No	1,636	1,561 (95.4)	33 (2.0)	42 (2.6)	
Yes	3,550	3,279 (92.4)	54 (1.5)	217 (6.1)	
NA*	16	10 (62.5)	0 (0.0)	6 (37.5)	
Adjuvant radiotherapy					0.001
No	2,647	2,500 (94.4)	58 (2.2)	89 (3.4)	
Yes	2,539	2,340 (92.2)	57 (2.2)	142 (5.6)	
NA*	16	10 (62.5)	0 (0.0)	6 (37.5)	
Adjuvant targeted therapy					0.277
No	4,319	4,021 (93.1)	73 (1.7)	225 (5.2)	

(Continued)

TABLE 1 | Continued

	Total n	Recurrence-free n (%)	LRR n (%)	DM n (%)	p^a
Yes	867	819 (94.5)	14 (1.6)	34 (3.9)	
NA*	16	10 (62.5)	0 (0.0)	6 (37.5)	
Adjuvant endocrine therapy					<0.001
No	1,570	1,418 (90.3)	44 (2.8)	108 (6.9)	
Yes	3,616	3,422 (94.6)	43 (1.2)	151 (4.2)	
NA*	16	10 (62.5)	0 (0.0)	6 (37.5)	

LRR, locoregional recurrence; DM, distant metastasis; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; NA, not available; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TN, triple negative; BCS, breast-conserving surgery; SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection.
[a]Compared between groups by chi-square test.
**Variable NA was not included in the analysis.*

TABLE 2 | Multivariate logistic regression of predictors for disease recurrence type*.

	Recurrence-free		Distant metastasis		*p*
	OR (95% CI)	*p*	OR (95% CI)	*p*	
Tumor size					<0.001
>2 cm	1.0		1.0		
≤2 cm	2.13 (1.31–3.48)	0.002	1.12 (0.64–1.97)	0.686	
Pathological type					0.182
IDC	1.0		1.0		
ILC	1.61 (0.28–9.40)	0.593	2.40 (0.32–18.02)	0.394	
Other invasive cancer	2.12 (0.63–7.05)	0.222	1.15 (0.27–4.93)	0.846	
Histological grade					<0.001
I-II	1.0		1.0		
III	0.66 (0.38–1.15)	0.145	0.98 (0.53–1.84)	0.961	
NA	0.41 (0.13–1.25)	0.116	0.53 (0.14–2.07)	0.361	
Lymph node status					0.048
Negative	3.24 (1.75–6.02)	<0.001	1.82 (0.89–3.73)	0.103	
Positive	1.0		1.0		
Molecular subtype					0.005
Luminal A	1.0		1.0		
Luminal B HER2–	0.41 (0.16–1.01)	0.052	0.78 (0.27–2.25)	0.642	
Luminal B HER2+	0.89 (0.23–3.40)	0.862	0.96 (0.21–4.32)	0.959	
HER2 enriched	0.34 (0.10–1.20)	0.094	0.55 (0.12–2.48)	0.440	
TN	0.52 (0.14–1.88)	0.316	1.30 (0.29–5.87)	0.730	
Surgery of the breast					<0.001
BCS	1.0		1.0		
Mastectomy	3.04 (1.73–5.33)	<0.001	3.86 (1.96–7.58)	<0.001	
Surgery of the axilla					<0.001
SLNB	1.0		1.0		
ALND	0.84 (0.46–1.51)	0.552	2.80 (1.37–5.75)	0.005	
Adjuvant chemotherapy					0.013
No	1.0		1.0		
Yes	2.48 (1.37–4.50)	0.003	2.81 (1.37–5.75)	0.013	
Adjuvant radiotherapy					0.090
No	1.0		1.0		
Yes	1.91 (1.07–3.42)	0.030	2.52 (1.21–5.20)	0.042	
Adjuvant endocrine therapy					
No	1.0		1.0		
Yes	2.14 (0.94–4.83)	0.069	1.64 (0.62–4.35)	0.320	

OR, odds ratio; CI, confidence interval; LRR, locoregional recurrence; DM, distant metastasis; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; NA, not available; HER2, human epidermal growth factor receptor 2; TN, triple negative; BCS, breast-conserving surgery; SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection.
**Reference category was LRR group.*

DISCUSSION

In this cohort of 5,202 consecutive breast cancer patients, we showed that LRR after radical surgery in the modern era is relatively low. Clinicopathological factors, including large tumor size, positive lymph node status, and molecular subtype, were significantly associated with increased risk of LRR. Primary surgical treatment for breast or adjuvant chemotherapy or

TABLE 3 | Univariate analysis for clinicopathological features related to salvage surgery decision for LRR patients.

	No surgery n (%)	Surgery n (%)	p[a]
Age at primary diagnosis			0.074
<50 years	18 (43.9)	23 (56.1)	
≥50 years	29 (63.0)	17 (37.0)	
Age at recurrence			0.028
<70 years	37 (49.3)	38 (50.7)	
≥70 years	10 (83.3)	2 (16.7)	
Menopausal status at primary diagnosis			0.078
Pre-menopausal	17 (43.6)	22 (56.4)	
Post-menopausal	30 (62.5)	18 (37.5)	
Tumor size*			0.004
≤2 cm	15 (37.5)	25 (62.5)	
>2 cm	32 (68.1)	15 (31.9)	
Pathological type*			0.970
IDC	41 (53.9)	35 (46.1)	
Other invasive cancer	6 (54.5)	5 (45.5)	
Histological grade*			0.078
I–II	14 (48.3)	15 (51.7)	
III	28 (65.1)	15 (34.9)	
NA	5 (33.3)	10 (66.7)	
Lymph node status*			<0.001
Negative	14 (34.1)	27 (65.9)	
Positive	33 (76.7)	10 (23.3)	
NA[†]	0 (0.0)	3 (100.0)	
ER*			0.498
Positive	21 (58.3)	15 (41.7)	
Negative	26 (51.0)	25 (49.0)	
PR*			0.871
Positive	29 (54.7)	24 (45.3)	
Negative	18 (52.9)	16 (47.1)	
HER2*			0.461
Negative	33 (58.9)	23 (41.1)	
Positive	12 (50.0)	12 (50.0)	
NA[†]	2 (28.6)	5 (71.4)	
Ki67*			0.387
≤20%	18 (48.6)	19 (51.4)	
>20%	29 (58.0)	21 (42.0)	
Molecular subtype*			0.447
Luminal A	2 (33.3)	4 (66.7)	
Luminal B HER2–	20 (57.1)	15 (42.9)	
Luminal B HER2+	2 (40.0)	3 (60.0)	
HER2 enriched	10 (52.6)	9 (47.4)	
TN	11 (73.3)	4 (26.7)	
NA[†]	2 (28.6)	5 (71.4)	
Primary surgery of the breast			0.001
BCS	12 (33.3)	24 (66.7)	
Mastectomy	35 (68,6)	16 (31.4)	
Primary surgery of the axilla			0.001
SLNB	11 (33.3)	22 (66.7)	
ALND	36 (70.6)	15 (29.4)	
No surgery	0 (0.0)	3 (100.0)	
LRR type			<0.001
IBTR	5 (19.2)	21 (80.8)	
Chest wall	15 (55.6)	12 (44.4)	
LNR	27 (79.4)	7 (20.6)	
RFI			0.246
≤24 months	21 (61.8)	13 (38.2)	
>24 months	26 (49.1)	27 (50.9)	

LRR, locoregional recurrence; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; NA, not available; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TN, triple negative; BCS, breast-conserving surgery; SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection; IBTR, ipsilateral breast tumor recurrence; LNR, lymph node recurrence; DM, distant metastasis; RFI, recurrence-free interval.

[a]*Compared between groups by chi-square test.*

**Tumor characteristics were from primary breast cancer.*

[†]*Variable NA was not included in the analysis.*

TABLE 4 | Multivariate analysis for clinicopathological features related to salvage surgery decision for LRR patients.

	Multivariate analysis	
	OR (95% CI)	***p***
Age at recurrence (<70 *vs.* ≥ 70 years)	5.37 (0.58–50.14)	0.140
Tumor size* (<2 *vs.* ≥2 cm)	3.29 (1.06–10.17)	0.039
Lymph node status* (negative *vs.* positive)	2.03 (0.52–8.00)	0.312
Primary surgery of the breast (BCS *vs.* mastectomy)	0.48 (0.06–3.75)	0.484
Primary surgery of the axilla (SLNB *vs.* ALND)	5.01 (1.60–15.68)	0.006
LRR type		
Chest wall only *vs.* IBTR only	0.36 (0.09–1.47)	0.155
Regional LNR only *vs.* IBTR only	0.07 (0.02–0.30)	<0.001

LRR, locoregional recurrence; OR, odds ratio; CI, confidence interval; BCS, breast-conserving surgery; SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection; IBTR, ipsilateral breast tumor recurrence; LNR, lymph node recurrence.

**Tumor characteristics were from primary breast cancer. Variable NA was not included in multivariate analysis.*

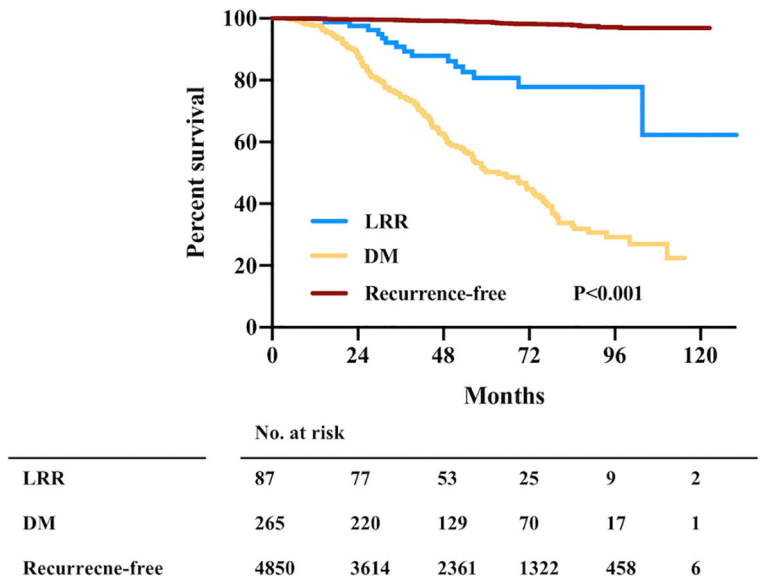


FIGURE 1 | Overall survival by first recurrence event in the whole population. LRR, locoregional recurrence; DM, distant metastasis; No., number.

radiotherapy also influenced the risk of LRR. Moreover, LRR patients had higher rates of receiving BCS or SLNB and not receiving adjuvant chemotherapy or radiotherapy compared with DM patients. Furthermore, we found that LRR types were related with salvage surgery choice after LRR. For patients receiving surgery after LRR, they could achieve an excellent outcome after recurrence.

According to the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) overview, which included trials up to year 2000 evaluating the effects of radiotherapy, the 5-year LRR rate was 7% in patients after BCS and radiotherapy and 6% in patients after mastectomy (22). A reduction of LRR has been seen in the recent years with the improvement in imaging, earlier diagnosis, surgical planning, and adjuvant therapy for breast cancer patients (5). In our study, the 5-year LRR rate was 2.8% in the whole population: 3.8% in patients receiving BCS and 2.5% in patients receiving mastectomy, which were quite low compared with the established evidence. The low LRR rate highlights the effect of multiple changes in breast cancer management over the past two decades.

Several clinicopathological factors as well as treatment patterns were associated with LRR after surgery in early breast cancer patients. Not surprisingly, in our study, we found that large tumor size, positive LN status, and primary BCS were identified as independent risk factors for LRR, which was consistent with previous studies (23, 24). Meanwhile, adjuvant chemotherapy and radiotherapy can effectively reduce the risk of LRR. Neoadjuvant chemotherapy was one of risk factors for local recurrence as reported by the EBCTCG meta-analysis (25), but neoadjuvant population was not included in our study. There was controversy in grouping patients when analyzing

the two populations together, since there is discordance of molecular biomarkers before and after neoadjuvant therapy, and the staging of patients will change after neoadjuvant therapy. Also, in neoadjuvant study, we usually use event-free survival to evaluate patients' outcome, which includes more information than recurrence-free interval that we evaluated in adjuvant studies. By reason of the foregoing, we excluded patients who received neoadjuvant therapy in this study, to make the evaluation standardized in the whole study population.

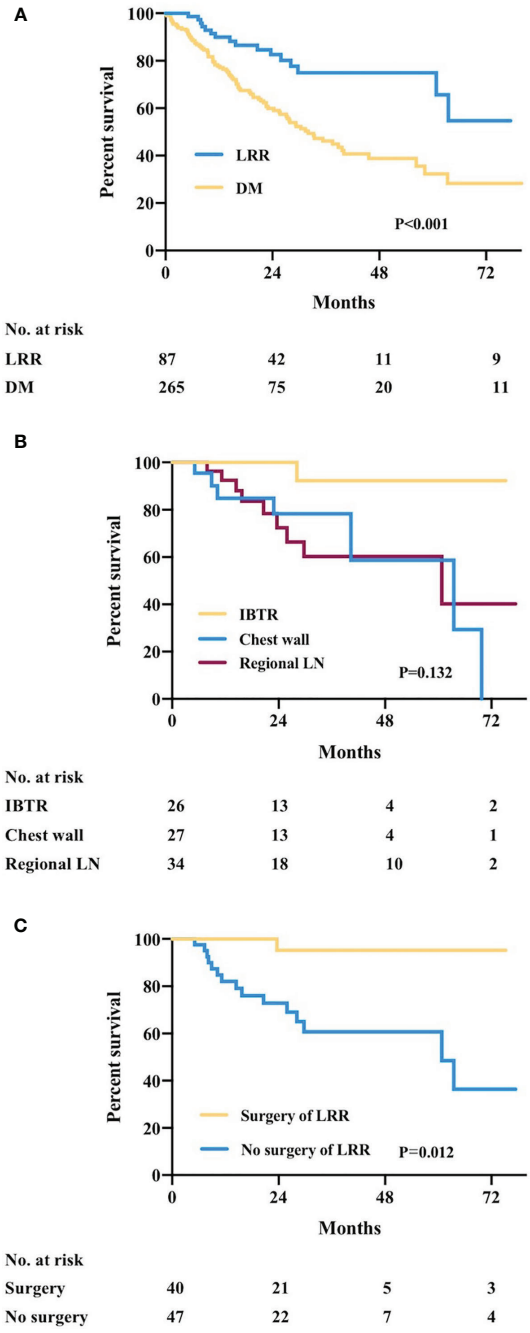


FIGURE 2 | Post-recurrence overall survival (PR-OS) by recurrence type. **(A)** PR-OS in recurrent patients by first recurrence event. **(B)** PR-OS in LRR patients by LRR type. **(C)** PR-OS in LRR patients receiving or not salvage surgery for LRR. LRR, locoregional recurrence; DM, distant metastasis; No., number; IBTR, ipsilateral breast tumor recurrence; LN, lymph node.

We also found that LRR was a less common recurrence event, as either first recurrence event or subsequent recurrence event comparing with DM. Few studies directly compared the difference between patients with different first recurrence events. Our study demonstrated that LRR patients had higher rate of receiving primary BCS, primary SLNB, and lower rate of receiving adjuvant chemotherapy or radiotherapy, indicating that more effective systemic and local treatment should be evaluated to further reduce the rate of LRR.

In the modern era of breast cancer treatment, management of LRR breast cancer patients remained a big challenge due to lower LRR events, fewer high quality clinical evidence, and relatively hard to follow-up patients. For patients who developed IBTR after BCS, the current standard of care is further salvage surgery, including salvage mastectomy or repeat BCS (26), which can achieve 59% to 90.9% 5-year OS after salvage surgery (11, 27–30). There is also another special consideration for patients with IBTR that whether it is "true recurrence" or "new primary," since new primaries should theoretically have a prognosis independent of the primary breast cancer. The rate of new primary breast cancer in patients with IBTR was 18%–58.9% in published studies (31–34), also strengthening the reason for surgery of IBTR. For patients with isolated chest wall recurrence, full-thickness chest wall resection can be performed with excellent survival and low morbidity. In a recent systematic meta-analysis of 48 studies accounting for 1,305 patients who received full-thickness resection for chest wall recurrence, the mortality was consistently low (<1%), and 5-year OS was 40.8% (8). Axillary recurrence rates are rare, ranging of 1% to 3% after adequate management of primary disease (35, 36). Salvage ALND was the first choice for selected patients and can be performed in 45.5% to 69.5% patients (37, 38). Surgery of LRR might be encouraged in patients who can achieve R0 resection. In our study, salvage surgery was performed in 46.0% of LRR patients: 80.8% for IBTR, 44.4% for chest wall recurrence, and 20.6% for regional LN recurrence. Patients with smaller primary tumor, receiving primary SLNB, and LRR type were related with the choice of surgery after LRR. Although the post-LRR follow-up period is short, and there was selective bias in patients receiving salvage surgery, we do observe that patients receiving surgery for LRR achieved a better PR-OS, which emphasized the importance of surgery as part of multidisciplinary management of LRR patients.

Some limitations of this study exist. The data were collected retrospectively, which may have led to selection bias. The follow-up time is relatively short, and only a small number of LRR events were recorded, given that LRR was less common in clinical practice. The actual site of recurrence may influence the possibility of surgery for LRR lesions and were not analyzed in this study. Details of the recurrence including site and pathologic features of the recurrent lesion are not completely collected, and we cannot distinguish whether there is true recurrence or new primary breast cancer in patients with

TABLE 5 | Multivariate analysis of factors associated with post-recurrence overall survival in patients with locoregional recurrence.

	Multivariate analysis	
	HR (95% CI)	***p***
Tumor size* (>2 *vs*. ≤2 cm)	1.06 (0.25–4.63)	0.934
ER* (positive *vs*. negative)	0.33 (0.12–0.91)	0.033
Primary surgery of the axilla (ALND *vs*. SLNB)	6.44 (0.83–49.63)	0.074
LRR type		0.496
Chest wall *vs*. IBTR only	3.52 (0.38–32.72)	0.262
LNR *vs*. IBTR only	2.40 (0.26–21.85)	0.337
Surgery of LRR (Yes *vs*. No)	0.12 (0.02–0.93)	0.043

HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; ALND, axillary lymph node dissection; SLNB, sentinel lymph node biopsy; LRR, locoregional recurrence; IBTR, ipsilateral breast tumor recurrence; LNR, lymph node recurrence.

**Tumor characteristics were from primary breast cancer. Patients with unknown lymph node status were not included in multivariate analysis.*

IBTR. Treatments of LRR out of surgery such as systemic therapy or radiotherapy and their impact on survival were not recorded or analyzed in this study. More comprehensive treatment data as well as longer follow-up are warranted to find the best management for LRR patients.

CONCLUSION

LRR rate was relatively low in the modern era of breast cancer treatment cohort. Large tumor size, positive lymph node status, and treatment strategies were associated with LRR. Moreover, LRR patients had a higher rate of receiving primary BCS or SLNB, and not receiving adjuvant chemotherapy or radiotherapy compared with DM patients. LRR patients treated with salvage surgery experienced excellent survival, indicating salvage surgery should play an important role in multidisciplinary treatment of LRR patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committees of Ruijin Hospital, Shanghai Jiaotong University School of Medicine. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This study was funded by the National Natural Science Foundation of China (81772797, 82072937); Shanghai Municipal Education Commission—Gaofeng Clinical Medicine Grant Support (20172007); Shanghai Jiao Tong University Yi Gong Jiao Cha Funding (YG2019QNA30); and Ruijin Hospital, Shanghai Jiao Tong University School of Medicine—"Guangci Excellent Youth Training Program" (GCQN-2019-B07). All these financial sponsors had no role in the study design, data collection, analysis, or interpretation.

ACKNOWLEDGMENTS

The authors would like to thank Ms. Yidong Du for her assistance in inputting SJTU-BCDB.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.763119/full#supplementary-material

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2018) 68 (6):394–424. doi: 10.3322/caac.21492
2. Fisher B, Anderson S, Bryant J, Margolese RG, Deutsch M, Fisher ER, et al. Twenty-Year Follow-Up of a Randomized Trial Comparing Total Mastectomy, Lumpectomy, and Lumpectomy Plus Irradiation for the Treatment of Invasive Breast Cancer. *N Engl J Med* (2002) 347(16):1233–41. doi: 10.1056/NEJMoa022152
3. Rao R, Euhus D, Mayo HG, Balch C. Axillary Node Interventions in Breast Cancer: A Systematic Review. *JAMA* (2013) 310(13):1385–94. doi: 10.1001/jama.2013.277804
4. Wallgren A, Bonetti M, Gelber RD, Goldhirsch A, Castiglione-Gertsch M, Holmberg SB, et al. Risk Factors for Locoregional Recurrence Among Breast

Cancer Patients: Results From International Breast Cancer Study Group Trials I Through VII. *J Clin Oncol* (2003) 21(7):1205–13. doi: 10.1200/JCO.2003.03.130
5. McGuire A, Lowery AJ, Kell MR, Kerin MJ, Sweeney KJ. Locoregional Recurrence Following Breast Cancer Surgery in the Trastuzumab Era: A Systematic Review by Subtype. *Ann Surg Oncol* (2017) 24(11):3124–32. doi: 10.1245/s10434-017-6021-1
6. Holleczek B, Stegmaier C, Radosa JC, Solomayer EF, Brenner H. Risk of Loco-Regional Recurrence and Distant Metastases of Patients With Invasive Breast Cancer Up to Ten Years After Diagnosis - Results From a Registry-Based Study From Germany. *BMC Cancer* (2019) 19(1):520. doi: 10.1186/s12885-019-5710-5
7. Wadasadawala T, Vadgaonkar R, Bajpai J. Management of Isolated Locoregional Recurrences in Breast Cancer: A Review of Local and Systemic Modalities. *Clin Breast Cancer* (2017) 17(7):493–502. doi: 10.1016/j.clbc.2017.03.008
8. Wakeam E, Acuna SA, Keshavjee S. Chest Wall Resection for Recurrent Breast Cancer in the Modern Era: A Systematic Review and Meta-Analysis. *Ann Surg* (2018) 267(4):646–55. doi: 10.1097/SLA.0000000000002310
9. Schmoor C, Sauerbrei W, Bastert G, Schumacher M. Role of Isolated Locoregional Recurrence of Breast Cancer: Results of Four Prospective Studies. *J Clin Oncol* (2000) 18(8):1696–708. doi: 10.1200/JCO.2000.18.8.1696
10. Buchanan CL, Dorn PL, Fey J, Giron G, Naik A, Mendez J, et al. Locoregional Recurrence After Mastectomy: Incidence and Outcomes. *J Am Coll Surg* (2006) 203(4):469–74. doi: 10.1016/j.jamcollsurg.2006.06.015
11. Haffty BG, Fischer D, Beinfield M, McKhann C. Prognosis Following Local Recurrence in the Conservatively Treated Breast Cancer Patient. *Int J Radiat Oncol Biol Phys* (1991) 21(2):293–8. doi: 10.1016/0360-3016(91)90774-X
12. Harris EE, Hwang WT, Seyednejad F, Solin LJ. Prognosis After Regional Lymph Node Recurrence in Patients With Stage I-II Breast Carcinoma Treated With Breast Conservation Therapy. *Cancer* (2003) 98(10):2144–51. doi: 10.1002/cncr.11767
13. Tennant S, Evans A, Macmillan D, Lee A, Cornford E, James J, et al. CT Staging of Loco-Regional Breast Cancer Recurrence. A Worthwhile Practice? *Clin Radiol* (2009) 64(9):885–90. doi: 10.1016/j.crad.2009.05.006
14. Touboul E, Buffat L, Belkacemi Y, Lefranc JP, Uzan S, Lhuillier P, et al. Local Recurrences and Distant Metastases After Breast-Conserving Surgery and Radiation Therapy for Early Breast Cancer. *Int J Radiat Oncol Biol Phys* (1999) 43(1):25–38. doi: 10.1016/s0360-3016(98)00365-4
15. van Oost FJ, van der Hoeven JJ, Hoekstra OS, Voogd AC, Coebergh JW, van de Poll-Franse LV. Staging in Patients With Locoregionally Recurrent Breast Cancer: Current Practice and Prospects for Positron Emission Tomography. *Eur J Cancer* (2004) 40(10):1545–53. doi: 10.1016/j.ejca.2004.03.012
16. Yi M, Kronowitz SJ, Meric-Bernstam F, Feig BW, Symmans WF, Lucci A, et al. Local, Regional, and Systemic Recurrence Rates in Patients Undergoing Skin-Sparing Mastectomy Compared With Conventional Mastectomy. *Cancer* (2011) 117(5):916–24. doi: 10.1002/cncr.25505
17. Neuman HB, Schumacher JR, Francescatti AB, Adesoye T, Edge SB, Vanness DJ, et al. Risk of Synchronous Distant Recurrence at Time of Locoregional Recurrence in Patients With Stage II and III Breast Cancer (AFT-01). *J Clin Oncol* (2018) 36(10):975–80. doi: 10.1200/JCO.2017.75.5389
18. Elfgen C, Schmid SM, Tausch CJ, Montagna G, Guth U. Radiological Staging for Distant Metastases in Breast Cancer Patients With Confirmed Local and/or Locoregional Recurrence: How Useful are Current Guideline Recommendations? *Ann Surg Oncol* (2019) 26(11):3455–61. doi: 10.1245/s10434-019-07629-9
19. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College Of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. *J Clin Oncol* (2010) 28 (16):2784–95. doi: 10.1200/JCO.2009.25.6529
20. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *J Clin Oncol* (2013) 31(31):3997–4013. doi: 10.1200/JCO.2013.50.9984
21. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B, et al. Personalizing the Treatment of Women With Early Breast Cancer: Highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol Off J Eur Soc Med Oncol* (2013) 24(9):2206–23. doi: 10.1093/annonc/mdt303
22. Clarke M, Collins R, Darby S, Davies C, Elphinstone P, Evans V, et al. Effects of Radiotherapy and of Differences in the Extent of Surgery for Early Breast Cancer on Local Recurrence and 15-Year Survival: An Overview of the Randomised Trials. *Lancet* (2005) 366(9503):2087–106. doi: 10.1016/S0140-6736(05)67887-7
23. Harahap WA, Nindrea RD. Prognostic Factors of Local-Regional Recurrence in Patients With Operable Breast Cancer in Asia: A Meta-Analysis. *Open Access Maced J Med Sci* (2019) 7(4):690–5. doi: 10.3889/oamjms.2019.151
24. Houvenaeghel G, de Nonneville A, Cohen M, Classe JM, Reyal F, Mazouni C, et al. Isolated Ipsilateral Local Recurrence of Breast Cancer: Predictive Factors and Prognostic Impact. *Breast Cancer Res Treat* (2019) 173(1):111–22. doi: 10.1007/s10549-018-4944-2
25. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Long-Term Outcomes for Neoadjuvant *Versus* Adjuvant Chemotherapy in Early Breast Cancer: Meta-Analysis of Individual Patient Data From Ten Randomised Trials. *Lancet Oncol* (2018) 19(1):27–39. doi: 10.1016/S1470-2045(17)30777-5
26. Mahvi DA, Liu R, Grinstaff MW, Colson YL, Raut CP. Local Cancer Recurrence: The Realities, Challenges, and Opportunities for New Therapies. *CA Cancer J Clin* (2018) 68(6):488–505. doi: 10.3322/caac.21498
27. Doyle T, Schultz DJ, Peters C, Harris E, Solin LJ. Long-Term Results of Local Recurrence After Breast Conservation Treatment for Invasive Breast Cancer. *Int J Radiat Oncol Biol Phys* (2001) 51(1):74–80. doi: 10.1016/S0360-3016(01)01625-X
28. Galper S, Blood E, Gelman R, Abner A, Recht A, Kohli A, et al. Prognosis After Local Recurrence After Conservative Surgery and Radiation for Early-Stage Breast Cancer. *Int J Radiat Oncol Biol Phys* (2005) 61(2):348–57. doi: 10.1016/j.ijrobp.2004.06.011
29. Voogd AC, van Oost FJ, Rutgers EJ, Elkhuizen PH, van Geel AN, Scheijmans LJ, et al. Long-Term Prognosis of Patients With Local Recurrence After Conservative Surgery and Radiotherapy for Early Breast Cancer. *Eur J Cancer* (2005) 41(17):2637–44. doi: 10.1016/j.ejca.2005.04.040
30. Abner AL, Recht A, Eberlein T, Come S, Shulman L, Hayes D, et al. Prognosis Following Salvage Mastectomy for Recurrence in the Breast After Conservative Surgery and Radiation Therapy for Early-Stage Breast Cancer. *J Clin Oncol* (1993) 11(1):44–8. doi: 10.1200/JCO.1993.11.1.44
31. Veronesi U, Marubini E, Del Vecchio M, Manzari A, Andreola S, Greco M, et al. Local Recurrences and Distant Metastases After Conservative Breast Cancer Treatments: Partly Independent Events. *J Natl Cancer Inst* (1995) 87 (1):19–27. doi: 10.1093/jnci/87.1.19
32. Smith TE, Lee D, Turner BC, Carter D, Haffty BG. True Recurrence *vs.* New Primary Ipsilateral Breast Tumor Relapse: An Analysis of Clinical and Pathologic Differences and Their Implications in Natural History, Prognoses, and Therapeutic Management. *Int J Radiat Oncol Biol Phys* (2000) 48(5):1281–9. doi: 10.1016/s0360-3016(00)01378-x
33. Alexandrova E, Sergieva S, Kostova P, Mihaylova I, Katzarov D, Milev A. Ipsilateral in-Breast Tumor Recurrence After Breast Conserving Therapy: True Recurrence *Versus* New Primary Tumor. *J BUON* (2015) 20(4):1001–8.
34. Gujral DM, Sumo G, Owen JR, Ashton A, Bliss JM, Haviland J, et al. Ipsilateral Breast Tumor Relapse: Local Recurrence *Versus* New Primary Tumor and the Effect of Whole-Breast Radiotherapy on the Rate of New Primaries. *Int J Radiat Oncol Biol Phys* (2011) 79(1):19–25. doi: 10.1016/j.ijrobp.2009.10.074
35. Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Costantino JP, et al. Sentinel-Lymph-Node Resection Compared With Conventional Axillary-Lymph-Node Dissection in Clinically Node-Negative Patients With Breast Cancer: Overall Survival Findings From the NSABP B-32 Randomised Phase 3 Trial. *Lancet Oncol* (2010) 11(10):927–33. doi: 10.1016/S1470-2045(10)70207-2
36. Giuliano AE, Ballman KV, McCall L, Beitsch PD, Brennan MB, Kelemen PR, et al. Effect of Axillary Dissection *vs* No Axillary Dissection on 10-Year Overall Survival Among Women With Invasive Breast Cancer and Sentinel Node Metastasis: The ACOSOG Z0011 (Alliance) Randomized Clinical Trial. *JAMA* (2017) 318(10):918–26. doi: 10.1001/jama.2017.11470
37. Newman LA, Hunt KK, Buchholz T, Kuerer HM, Vlastos G, Mirza N, et al. Presentation, Management and Outcome of Axillary Recurrence From Breast Cancer. *Am J Surg* (2000) 180(4):252–6. doi: 10.1016/S0002-9610(00)00456-6
38. de Boer R, Hillen HF, Roumen RM, Rutten HJ, van der Sangen MJ, Voogd AC. Detection, Treatment and Outcome of Axillary Recurrence After Axillary

Clearance for Invasive Breast Cancer. *Br J Surg* (2001) 88(1):118–22. doi: 10.1046/j.1365-2168.2001.01637.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

ORIGINAL RESEARCH
published: 11 January 2022
doi: 10.3389/fonc.2021.760655

Efficacy and Safety of Albumin-Bound Paclitaxel Compared to Docetaxel as Neoadjuvant Chemotherapy for HER2-Negative Breast Cancer

Zhi-Dong Lv[1], Hong-Ming Song[1], Zhao-He Niu[1], Gang Nie[1], Shuai Zheng[2], Ying-Ying Xu[3], Wei Gong[4] and Hai-Bo Wang[1]**

[1] Breast Center, The Affiliated Hospital of Qingdao University, Qingdao, China, [2] Department of Breast and Thyroid Surgery, Heze Municipal Hospital, Heze, China, [3] Department of Breast Surgery, The First Affiliated Hospital of China Medical University, Shenyang, China, [4] Department of Thyroid and Breast Surgery, The Second People's Hospital of Kunshan, Kunshan, China

OPEN ACCESS

Edited by:
Yiding Chen,
Zhejiang University, China

Reviewed by:
Richard De Boer,
Peter MacCallum Cancer Centre, Australia
Liu Yiqiang,
Beijing Cancer Hospital, China

***Correspondence:**
Zhi-Dong Lv
zhidonglv@126.com
Hai-Bo Wang
qingyiwhb@126.com

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *18 August 2021*
Accepted: *13 December 2021*
Published: *11 January 2022*

Citation:
Lv Z-D, Song H-M, Niu Z-H, Nie G, Zheng S, Xu Y-Y, Gong W and Wang H-B (2022) Efficacy and Safety of Albumin-Bound Paclitaxel Compared to Docetaxel as Neoadjuvant Chemotherapy for HER2-Negative Breast Cancer. Front. Oncol. 11:760655. doi: 10.3389/fonc.2021.760655

Background: Nanoparticle albumin-bound paclitaxel (nab-paclitaxel) as neoadjuvant chemotherapy (NAC) for breast cancer remains controversial. We conducted a retrospective study to compare the efficacy and safety of nab-paclitaxel with those of docetaxel as neoadjuvant regimens for HER2-negative breast cancer.

Methods: In this retrospective analysis, a total of 159 HER2-negative breast cancer patients who had undergone operation after NAC were consecutively analyzed from May 2016 to April 2018. Patients were classified into the nab-paclitaxel group (n = 79, nab-paclitaxel 260 mg/m^2, epirubicin 75 mg/m^2, and cyclophosphamide 500 mg/m^2) and the docetaxel group (n = 80, docetaxel 75 mg/m^2, epirubicin 75 mg/m^2, and cyclophosphamide 500 mg/m^2) according to the drug they received for neoadjuvant treatment. The efficacy and adverse events were evaluated in the two groups.

Results: The pathological complete response (pCR)(ypT0/isN0) rate was significantly higher in the nab-paclitaxel group than in the docetaxel group (36.71% vs 20.00%; $P = 0.031$). The multivariate analysis revealed that therapeutic drugs, lymph node status, and tumor subtype were the most significant factor influencing treatment outcome. At a median follow-up of 47 months, disease-free survival (DFS) was not significantly different in those assigned to nab-paclitaxel compared with docetaxel (82.28% vs 76.25%; $P = 0.331$). The incidence of peripheral sensory neuropathy in the nab-paclitaxel group was higher than that in the docetaxel group (60.76% vs 36.25%; $P = 0.008$), while the incidence of arthralgia was observed more frequently in the docetaxel group (57.50% vs 39.97%; $P = 0.047$).

Conclusions: Compared with docetaxel, nab-paclitaxel achieved a higher pCR rate, especially those patients with triple-negative breast cancer or lymph node negative breast cancer. However, there was no significant difference in DFS between the two groups. This study provides a valuable reference for the management of patients with HER2-negative breast cancer.

Keywords: Her-2-negative breast cancer, pathological complete response, neoadjuvant chemotherapy, albumin-bound paclitaxel, docetaxel

INTRODUCTION

Neoadjuvant chemotherapy (NAC) has become a treatment option for patients with operable breast cancer (1). NAC performs as a platform to allow time for genetic testing, allow rapid assessment of drug efficacy, and provide important prognostic information (2). After receiving NAC, patients who attained superior pathological complete response (pCR) have been found to be associated with an extremely favorable survival benefit and proposed as a surrogate endpoint for predicting survival outcomes (3).

Taxane-based regimens are widely used in the NAC of human epidermal growth factor receptor 2 (HER2)-negative breast cancer (4). The conventional taxanes include docetaxel and paclitaxel. The NSABP B27 study found that the addition of docetaxel notably increased the pCR rate from 13.7% to 26.1% (5). Docetaxel is extremely hydrophobic and therefore requires a solvent to allow for parenteral administration. Docetaxel is formulated in polysorbate 80 and an ethanol diluent. These solvents are pharmacokinetically active and can cause a number of adverse reactions, such as hypersensitivity reactions and peripheral neuropathy (6). Nanoparticle albumin-bound paclitaxel (nab-paclitaxel) is a unique non-solvent-containing protein formulation. It can obviate the need for prophylactic anti-histamine and steroid treatment because of its much lower risk of hypersensitivity compared with conventional paclitaxel, although it is prone to causing peripheral neuropathy (7). Recently, nab-paclitaxel has been developed and administered to patients with breast cancer (8). Because nab-paclitaxel facilitates the accumulation of a higher paclitaxel dose into cancer cells, it has been expected to exert more feasible effects. Clinical trials of patients with metastatic breast cancer found that nab-paclitaxel achieves longer survival than docetaxel (9). However, evidence is insufficient to judge whether nab-paclitaxel is superior to docetaxel in a neoadjuvant setting. For example, when nab-paclitaxel is administered on days 1, 8, and 15, every 4 weeks, or docetaxel is administered on day 1, every 3 weeks, there is no difference between treatment groups (10). However, another study reported that higher PCR rates were achieved by the nab-paclitaxel group compared with the docetaxel regimen, particularly for the TNBC subpopulation and patients with a high Ki67 level (11). Real-world evidence of nab-paclitaxel as a NAC option for patients with HER2-negative breast cancer is limited (12). The response and adverse event assessments vary in different studies. Thus, to assess the clinical utility of nab-paclitaxel in NAC, we conducted this retrospective study to compare the efficacy and toxicity of nab-paclitaxel-based with those of docetaxel-based regimens used in patients with HER2-negative breast cancer.

Abbreviations: nab-paclitaxel, nanoparticle albumin-bound paclitaxel; pCR, pathological complete response; DFS, disease-free survival; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; PR, partial response; PD, progressive disease; SD, stable disease; TNBC, triple-negative breast cancer; IHC, immunohistochemistry; HR, hazard ratio; TEC, taxane–epirubicin–cyclophosphamide; CIs, confidence intervals.

PATIENTS AND METHODS

Patients

This is a retrospective study of HER2-negative breast cancer patients who received NAC and underwent surgery at the Affiliated Hospital of Qingdao University. The principle inclusion criterion was (1) age from 18 to 70 years; (2) pathologically confirmed invasive breast cancer; (3) HER2-negative; (4) clinical stage II–III disease; (5) received radical operation for breast cancer; (6) and received taxane–epirubicin–cyclophosphamide (TEC) chemotherapy before surgery. The exclusion criteria for the patients were as follows: (1) received any type of treatment prior to NAC treatment, including chemotherapy, targeted therapy, radiotherapy, or endocrine therapy; (2) with previous or synchronous invasive or *in situ* breast cancer, male breast cancer, bilateral breast cancer, or inflammatory breast cancer; and (3) with acute and chronic inflammatory disease, autoimmune disease, mental disease, severe liver, kidney insufficiency, or serious complications.

We included all consecutive patients meeting the inclusion/exclusion criteria from May 2016 to April 2018. A total of 184 patients were initially identified. Women who did not undergo radical operation after NAC (n = 3), had treatment records unavailable (n = 6), had previous breast cancer history (n = 1), had incomplete six cycles of chemotherapy (n = 12), or were lost to follow-up (n = 3) were excluded. Finally, 159 breast cancer patients enrolled in the study. The process of screening and grouping is shown in **Figure 1**. Patients were classified into the nab-paclitaxel group (n = 79) and the docetaxel group (n = 80) according to the drug they received for treatment. Patients in the nab-paclitaxel group received six cycles of every 3 weeks (q3w) nab-paclitaxel 260 mg/m^2, epirubicin 75 mg/m^2, and cyclophosphamide 500 mg/m^2. In the docetaxel group, the patients received six cycles of q3w docetaxel 75 mg/m^2, epirubicin 75 mg/m^2, and cyclophosphamide 500 mg/m^2. NAC

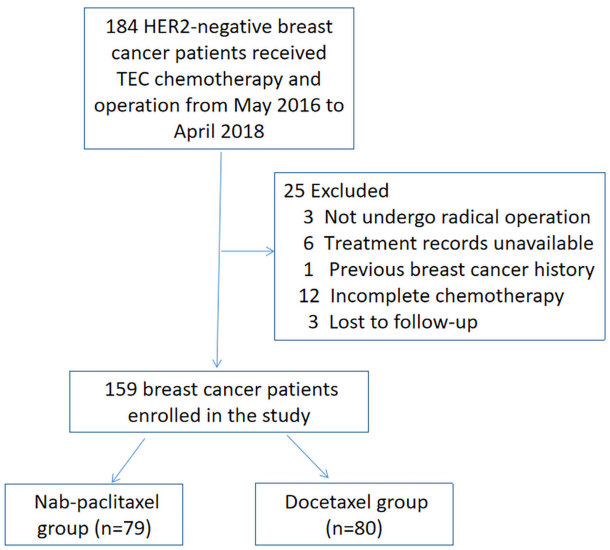


FIGURE 1 | The procedure of screening and grouping patients.

was delivered according to patients' specific disease features following the guidelines of the National Comprehensive Cancer Network. The physicians modified the regimen doses and schedules according to the tumor response and side effects. The docetaxel group received intravenous injection of dexamethasone before chemotherapy. A prophylactic injection of granulocyte colony stimulating factor was administered, and all patients underwent surgery 2–4 weeks after NAC.

Pathological diagnosis was obtained *via* core needle biopsy before initiating NAC. Immunohistochemistry (IHC) was used to assess estrogen receptor (ER), progesterone receptor (PR), HER2 status, and Ki-67 level. ER- and PR-positive were defined as ≥1% positively stained tumor cells. HER2 status was evaluated by IHC and fluorescence *in situ* hybridization (FISH). HER2-negative was defined as IHC scoring 1+ or 2+ with FISH non-amplified based on the American Society of Clinical Oncology Guidelines. The cells with Ki-67 were counted and expressed as the percentage of cells with positive nuclear staining among the total tumor cells. The molecular subtypes of breast cancer were classified according to the St. Gallen Consensus. All of the patients were Eastern Cooperative Oncology Group performance status 0–1. This study was approved by the Medical Ethics Committee of the Affiliated Hospital of Qingdao University (QYFY WZLL 26545). Considering the retrospective nature of this work, the requirement for informed consent was waived for individual participants as per the committee standards. To protect the patient's privacy, we have de-identified all patient details in this paper.

Response and Toxicity Assessments

Response assessments of NAC by ultrasonography or magnetic resonance imaging were performed within 1 week before NAC and before surgery. The pathology reports were reviewed by two pathologists to determine the pathological response category. The clinical tumor response to NAC was measured using RECIST 1.1. pCR was defined as no pathologic evidence of a residual invasive carcinoma in the breast or axillary lymph nodes (ypT0/isN0 status). Residual ductal carcinoma *in situ* was included under pCR. Partial response (PR) was defined as a decline of at least 30% in tumor maximum diameter, and progressive disease (PD) was defined as an increase of at least 20% from the baseline in the sum of all tumor diameter measurements. The disease was categorized as a stable disease (SD) when CR, PR, or PD was not noted. Patients were considered responders if they achieved CR or PR.

The treatment-related adverse events were calculated. Toxicities were graded using the National Cancer Institute Common Toxicity Criteria version 5.0. According to the literature, those effects that were reported to be associated with nab-paclitaxel or docetaxel were examined: leukopenia, thrombocytopenia, peripheral sensory neuropathy, nausea, oral mucositis, cardiotoxicity, rash, and arthralgia. All patients were followed up every 3 months by telephone or outpatient interview for at least 3 years. The disease-free survival (DFS) was calculated as the period from the date of surgery to the first observation of the tumor recurrence (local relapse and/or metastatic recurrence) or the last follow-up. The reporting of this study conforms to STROBE guidelines (13).

Statistical Analysis

Patient and tumor characteristics and pCR rates were compared between groups by Pearson's χ^2 test or Fisher's exact test. The sample size calculation was performed using the Stata software system (version 14.0, Stata Corp., College Station, TX, USA). Hazard ratios (HRs) and 95% confidence intervals (CIs) were obtained using the stratified Cox proportional hazards model. The Kaplan–Meier method was used to estimate the distributions of survival outcomes. Comparisons in survival rates between the treatment groups were assessed by the logistic regression analysis. The Cox model was used to control intergroup confounding prognostic variables. Statistical Package for Social Sciences (SPSS) (version 19.0, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis, and $P \leq 0.05$ was considered statistically significant.

RESULTS

Clinicopathological Characteristics

From May 2016 to April 2018, 79 patients who underwent nab-paclitaxel-based treatment and the other 80 patients who were administered the docetaxel-based regimens enrolled into the study and were available for analysis. All patients were diagnosed with invasive breast carcinoma using core needle and met the study criteria. The basic clinicopathological characteristics of all the subjects with breast cancer are shown in **Table 1**. The median age was 45 (25–67) years and 47 (27–69) years in the nab-paclitaxel group and docetaxel group, respectively. Baseline characteristics were comparable between the two groups, including age ($P = 0.940$), tumor size ($P = 0.474$),

TABLE 1 | Baseline characteristics of patients.

Subgroup	Nab-paclitaxel (n = 79)	Docetaxel (n = 80)	χ^2	*P*
Age (years)				
<50	41	43	0.006	0.940
≥50	38	37		
Tumor size			0.512	0.474
T1–2	36	42		
T3–4	43	38		
Grade			0.000	1.000
I–II	39	40		
III	40	40		
Lymph node status			2.976	0.084
Negative	25	37		
Positive	54	43		
Hormone receptor			0.051	0.822
Negative	24	22		
Positive	55	58		
Ki-67			0.276	0.599
≤20%	16	20		
>20%	63	60		
Clinical stage			0.307	0.580
II	38	43		
III	41	37		

grade ($P = 1.000$), lymph node status ($P = 0.084$), tumor subtype ($P = 0.822$), Ki-67 level ($P = 0.599$), and clinical stage ($P = 0.580$).

Efficacy

The overall response rates of the nab-paclitaxel and docetaxel groups were 89.87% (71/79) and 85.00% (68/80); the difference was not statistically significant ($P = 0.492$). After NAC, the pCR rate of the nab-paclitaxel regimens was 36.71% (29/79), which was higher than the rate of 20.00% (16/80) for the docetaxel regimens; the difference was statistically significant ($P = 0.031$) (**Table 2**). After subgroup analysis, patients with triple-negative breast cancer (TNBC) the in nab-paclitaxel group achieved a higher pCR rate than in the docetaxel group (62.50% vs 22.30%; $P = 0.015$). Furthermore, nearly half of the patients with T1–2 in the nab-paclitaxel group achieved pCR, which was significantly greater than in the docetaxel group (43.59% vs 19.05%; $P = 0.023$). The pCR rate of patients with lymph node negative in the nab-paclitaxel group was 56.00%, which was significantly higher than that in the docetaxel group ($P = 0.012$) (**Figure 2**). All of the 159 patients received radical surgery after NAC, 12 patients (15.19%) in the nab-paclitaxel group and 7 patients (8.75%) in the docetaxel group received breast-conserving surgery, and the other patients received mastectomy.

TABLE 2 | Pathological response.

Pathological response	Nab-paclitaxel (n = 79)	Docetaxel (n = 80)	χ^2	*P*
			4.676	0.031
pCR	29	16		
Non-pCR	50	64		
			0.473	0.492
CR+PR	71	68		
SD+PD	8	12		

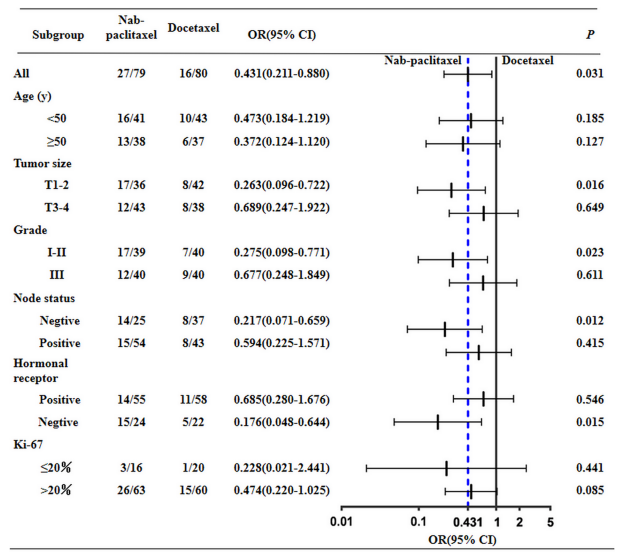


FIGURE 2 | Subgroup analysis of pathological complete response rate.

The results of multivariate logistic regression analysis for the pCR are shown in **Table 3**. In the combination analysis, nab-paclitaxel-based regimens displayed a significantly better pCR compared with the docetaxel-based regimens (OR, 2.777; 95% CI, 1.292–5.969; $P = 0.009$). Among the other four parameters, lymph node status (negative vs positive) and tumor subtype (TNBC vs HR+/HER2-) were the significant factor influencing treatment outcome favoring HER2-negative tumors.

Disease-Free Survival

The DFS analysis between the nab-paclitaxel group and docetaxel group was examined by using the Kaplan–Meier method with the log-rank test. After a median follow-up of 47 months, 33 of 159 patients (20.75%) experienced DFS events (14 in the nab-paclitaxel group and 19 in the docetaxel group). DFS was not significantly different in those assigned to the nab-paclitaxel group compared with the docetaxel group (82.28% vs 76.25%; $P = 0.331$). At the same time, we found that the DFS of nab-paclitaxel was 83.30% higher than that of 72.70% of docetaxel in TNBC patients, but the difference was not statistically significant ($P = 0.484$). In addition, we found that the DFS of nab-paclitaxel (81.80%) and docetaxel (77.6%) was similar in HR+/HER2- patients ($P = 0.473$). The Kaplan–Meier curves for DFS are depicted in **Figure 3**.

TABLE 3 | Multivariate analysis of pCR.

Variable	Effect	OR (95% CI)	*P*
Treatment	Nab-paclitaxel vs docetaxel	2.777 (1.292–5.969)	0.009
Tumor size	T1–2 vs T3–4	1.399 (0.658–2.973)	0.382
Grade	I–II vs III	1.068 (0.506–2.254)	0.864
Lymph node status	Positive vs negative	0.459 (0.215–0.979)	0.044
Tumor subtype	HR+/HER2- vs HR-/HER2-	0.375 (0.174–0.808)	0.012

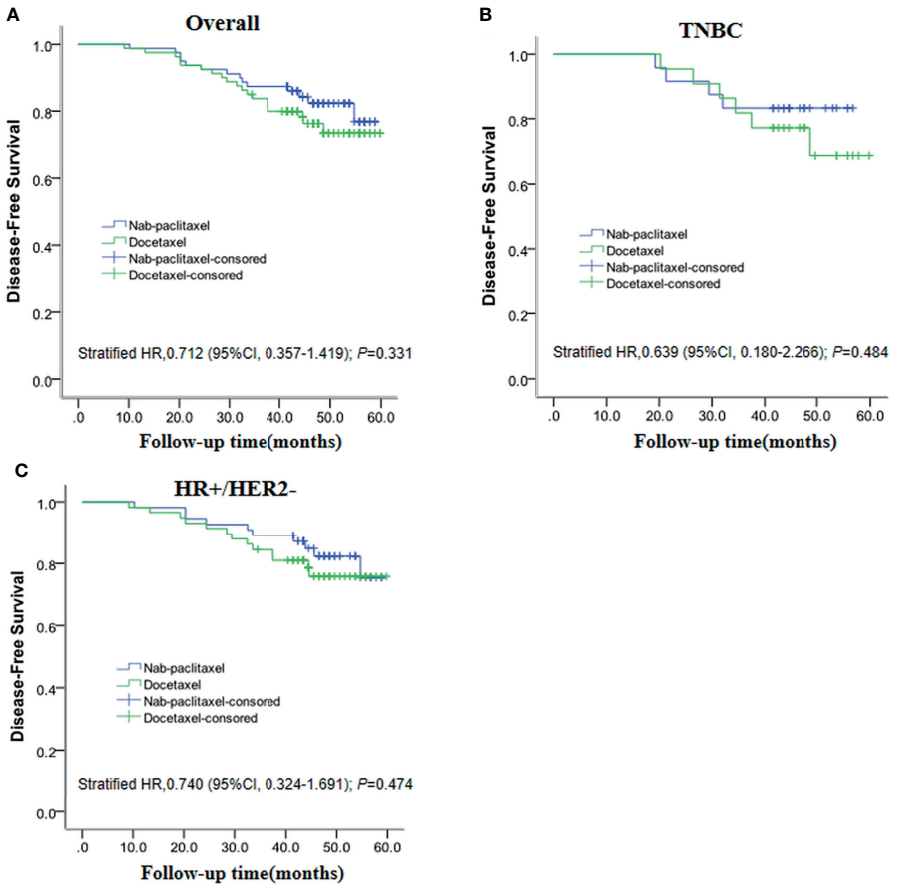


FIGURE 3 | Kaplan–Meier plots show disease-free survival for the entire **(A)**, TNBC **(B)**, and HR+/HER2- **(C)** populations.

Safety

Of the 159 enrolled patients in the two groups, all completed six cycles of NAC. During NAC, 94.94% patients in the nab-paclitaxel group had at least one drug-related adverse event compared with 95.00% of those treated with docetaxel. Most of the drug-related adverse events were mild and are listed in **Table 4**. Neutropenia is the most common adverse reaction during chemotherapy; the incidence was 86.08% in the nab-paclitaxel group and 90.00% in the docetaxel group, and there was no statistical significance between the two groups ($P = 0.420$). Peripheral sensory neuropathy (at any grade) occurred more often in patients allocated to the nab-paclitaxel group (60.76% vs 36.25%; $P = 0.008$), whereas the incidence of arthralgia (at any grade) in the nab-paclitaxel group was lower than in the docetaxel group (37.97% vs 57.50%; $P = 0.047$). The other adverse reactions, such as thrombocytopenia, nausea, oral mucositis, cardiotoxicity, and rash, were similar between the two groups, and there was no significant difference ($P > 0.05$).

DISCUSSION

Breast cancer has become the first malignant tumor in the world with a high incidence rate (14). NAC, as a platform allowing rapid reduction in tumor size and acquiring of drug sensitivity and prognosis information, has been increasingly employed in breast cancer (15). The gold standard for evaluating the efficacy of NAC is pathological response based on surgical specimens. Patients with pCR to NAC have been found to be associated with an extremely favorable survival benefit and proposed as a surrogate endpoint for predicting survival outcomes (16). Previous studies have revealed an enhanced delivery of nab-paclitaxel to tumors and less toxicity compared with docetaxel (17). As for NAC in early breast cancer, the difference in efficacy between nab-paclitaxel and docetaxel remains controversial. Therefore, we carried out this real-world study to retrospectively evaluate the efficacy and toxicity of the nab-paclitaxel-based and docetaxel-based regimens as NAC for HER2-negative breast cancer.

For nab-paclitaxel, it has been hypothesized that albumin-mediated delivery may result in enhanced transport of nab-paclitaxel to tumors (17) and improved tolerability profile of nab-paclitaxel compared with that of docetaxel at equimolar doses, with shorter infusion schedules and no premedication (18). In a trial of patients with metastatic breast cancer, nab-paclitaxel has been shown to achieve higher response rates and a longer time to progression compared to paclitaxel (9). The safety profiles of nab-paclitaxel were acceptable in most trials (19), but the data of head-to-head

TABLE 4 | Treatment-related adverse events.

Toxicity	Nab-paclitaxel (n = 79)	Docetaxel (n = 80)	χ^2	*P*
Neutropenia			1.734	0.420
0	11	8		
1–2	42	38		
3–4	26	34		
Thrombocytopenia			2.484	0.289
0	33	43		
1–2	39	30		
3–4	7	7		
Nausea			2.941	0.230
0	15	8		
1–2	37	38		
3–4	27	34		
Oral mucositis			0.802	0.669
0	44	46		
1–2	27	29		
3–4	8	5		
Cardiotoxicity			1.097	0.578
0	62	65		
1–2	16	15		
3–4	1	0		
Peripheral sensory neuropathy			9.700	0.008
0	31	51		
1–2	38	24		
3–4	10	5		
Rash			1.574	0.455
0	65	71		
1–2	12	7		
3–4	2	2		
Arthralgia			6.123	0.047
0	49	34		
1–2	26	39		
3–4	4	7		

comparison between nab-paclitaxel and docetaxel are still lacking. The present study highlights the real-world clinical benefits and adverse event profile of nab-paclitaxel administered as a NAC to patients with HER2-negative breast cancer. In this current study, among the 159 breast cancer patients, the pCR in patients treated with nab-paclitaxel was 36.71%, and pCR in those treated with docetaxel was 20.00%. The GeparSepto trial reported pCR rates in their nab-paclitaxel group of 42.7% for ypT0/isN0, which was much higher than our results (20). One possible reason is that our patients had a greater tumor burden, which may have reduced the pCR rates. In the GeparSepto trial, the primary tumor size was about 30 mm and about 45% patients were clinically assessed axillary node stage-positive; in comparison, the tumor size in our study was >40 mm and the proportion of patients categorized as clinically assessed axillary node stage-positive was much higher (68.35%). Furthermore, patients with more aggressive tumors seemed to benefit from nab-paclitaxel (21). Of note, in the GeparSepto trial, the pCR rate almost doubled in the TNBC cohort treated with nab-paclitaxel compared to that for paclitaxel. The present results showed that TNBC patients achieved significantly better pCR rates with nab-paclitaxel than with docetaxel. The pCR rate for patients with TNBC in the nab-paclitaxel group was 62.50%, which is higher than what the ETNA trial reported. In the present study, simultaneous application of taxane–epirubicin–cyclophosphamide may kill tumor cells more quickly and effectively reduce tumor load, while in the ETNA trial, it was the sequential application of those drugs. Another explanation could be that fewer patients enrolled and fewer prognostic events occurred in our analysis, which may affect the results of this study.

pCR is a strong predictor for favorable long-term prognosis in breast cancer. Nevertheless, it remains unclear how large a difference in pCR between nab-paclitaxel and docetaxel can translate into a difference in long-term clinical outcomes. The GeparSepto trial demonstrated that the absolute difference between the two treatment groups needed 20% to result in an improved iDFS (10). After a median follow-up of 47 months, 33 of 159 patients experienced DFS events (14 in the nab-paclitaxel group and 19 in the docetaxel group). Our results also showed that no statistically significant difference was observed for DFS between the nab-paclitaxel group and docetaxel group, though a trend of improved DFS was noted for nab-paclitaxel (82.28% vs 76.25%; $P = 0.331$). TNBC patients achieved a better pCR rate; we further analyzed the prognosis of those patients. Results of TNBC patients in DFS still showed a trend to favor nab-paclitaxel (83.30% vs 72.70%), but no statistical significance was found. This is consistent with the finding from the GeparSepto trial (10). Although in general, individual patients with a pCR also have an improved DFS, on a study level a pCR increase does not always translate into a significantly better long-term outcome. With the development of precision medicine, individual patient data with more molecular information might help dig deeper into the benefit population of nab-paclitaxel in the future.

Previous studies have demonstrated that nab-paclitaxel has almost identical toxicities as conventional taxanes except peripheral sensory neuropathy (22). In contrast to docetaxel, nab-paclitaxel does not utilize non-ionic surfactants to solubilize paclitaxel, which are known to contribute to toxicity and entrap paclitaxel within solvent-based micelles (23). Perhaps because nab-paclitaxel delivery is not complicated by solvents, a higher dose can be administered relative to docetaxel. TEC was a chemotherapy regimen with serious side effects, and leukopenia was the most common adverse reaction. In addition, allergy and vomiting were also common adverse effects. The toxicity profile in the present study was similar to that reported by the GeparSepto (20) and ETNA trials (21). Peripheral sensory neuropathy was more common in the nab-paclitaxel group, while neutropenia was common in the docetaxel group. In the GeparSepto trial, after dose amendment of nab-paclitaxel from 150 to 125 mg/m^2 continuous weekly for 12 weeks, the frequency of grade 3–4 peripheral sensory neuropathy in the nab-paclitaxel group decreased from 15% to 8% (24). In addition, nausea, arthralgia, and rash were comparable between the two groups, which was consistent with the results of previous findings (12). Long-term follow-up would be necessary to identify symptom relief patterns and their impact on quality of life.

This study had some potential limitations. First, this is a retrospective study without randomization and it was conducted in a single institution. As a result, there may be the potential selective bias and statistical error. Second, it is a small cohort study, which may affect the effectiveness of the results. In the future, a larger sample to verify the results is necessary. Finally, the follow-up of this study is relatively short, so more studies with long-time follow-up are needed to get a more accurate result.

In summary, our real-world study demonstrated that nab-paclitaxel was an effective cytotoxic drug in NAC for HER2-negative breast cancer, especially for patients with TNBC or lymph node negative diseases. However, there was no significant difference in DFS between the two groups. This study provides a valuable reference for the management of patients with HER2-negative breast cancer.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics and research committee of the Affiliated Hospital of Qingdao University. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

Z-DL, H-MS, and H-BW conceptualized and designed the study. Z-HN, GN, and SZ collected and analyzed the data and drafted the paper. SZ, Y-YX, and WG carried out the data analysis. All authors contributed to the article and approved the submitted version.

FUNDING

The present study was financially supported by Postdoctoral Science Foundation of China (No.2020M672001), Natural Science Foundation of Shandong Province (ZR2020MH274) and Qilu Health Outstanding Young Talents Project of Shandong Province.

REFERENCES

1. Heil J, Kuerer HM, Pfob A, Rauch G, Sinn HP, Golatta M, et al. Eliminating the Breast Cancer Surgery Paradigm After Neoadjuvant Systemic Therapy: Current Evidence and Future Challenges. *Ann Oncol* (2020) 31(1):61–71. doi: 10.1016/j.annonc.2019.10.012
2. Adachi Y, Okumura S, Sawaki M, Hattori M, Yoshimura A, Gondo N, et al. Effects of Neoadjuvant Chemotherapy on Operative Adverse Events and Chemotherapy and Radiotherapy in Patients Undergoing Immediate Breast Reconstruction. *Breast Cancer* (2020) 27(4):716–23. doi: 10.1007/s12282-020-01065-4
3. Makanjuola DI, Alkushi A, Al Anazi K. Defining Radiologic Complete Response Using a Correlation of Presurgical Ultrasound and Mammographic Localization Findings With Pathological Complete Response Following Neoadjuvant Chemotherapy in Breast Cancer. *Eur J Radiol* (2020) 130:109146. doi: 10.1016/j.ejrad.2020.109146
4. Sharma P, López-Tarruella S, García-Saenz JA, Khan QJ, Gómez HL, Prat A, et al. Pathological Response and Survival in Triple-Negative Breast Cancer Following Neoadjuvant Carboplatin Plus Docetaxel. *Clin Cancer Res* (2018) 24(23):5820–9. doi: 10.1158/1078-0432.CCR-18-0585
5. Mamounas EP, Anderson SJ, Dignam JJ, Bear HD, Julian TB, Geyer CE Jr, et al. Predictors of Locoregional Recurrence After Neoadjuvant Chemotherapy: Results From Combined Analysis of National Surgical Adjuvant Breast and Bowel Project B-18 and B-27. *J Clin Oncol* (2012) 30 (32):3960–6. doi: 10.1200/JCO.2011.40.8369
6. Kojima Y, Kawamoto H, Nishikawa T, Hayami R, Shimo A, Haku E, et al. Feasibility Study of Weekly Nanoparticle Albumin-Bound Paclitaxel (150 Mg/M^2) Followed by Fluorouracil, Epirubicin, and Cyclophosphamide Therapy as Neoadjuvant Chemotherapy for HER2-Negative Breast Cancer. *Clin Breast Cancer* (2018) 18(5):374–9. doi: 10.1016/j.clbc.2018.01.002
7. Mittendorf EA, Zhang H, Barrios CH, Saji S, Jung KH, Hegg R, et al. Neoadjuvant Atezolizumab in Combination With Sequential Nab-Paclitaxel and Anthracycline-Based Chemotherapy Versus Placebo and Chemotherapy in Patients With Early-Stage Triple-Negative Breast Cancer (IMpassion031): A Randomised, Double-Blind, Phase 3 Trial. *Lancet* (2020) 396(10257):1090–100. doi: 10.1016/S0140-6736(20)31953-X
8. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. IMpassion130 Trial Investigators. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *N Engl J Med* (2018) 379 (22):2108–21. doi: 10.1056/nejmoa1809615
9. Gennari A, Sun Z, Hasler-Strub U, Colleoni Z, Kennedy Z, Von Moos Z, et al. A Randomized Phase II Study Evaluating Different Maintenance Schedules of Nab-Paclitaxel in the First-Line Treatment of Metastatic Breast Cancer: Final Results of the IBCSG 42-12/BIG 2-12 SNAP Trial. *Ann Oncol* (2018) 29 (3):661–8. doi: 10.1093/annonc/mdx772
10. Kuwayama T, Nakamura S, Hayashi N, Takano T, Tsugawa K, Sato T, et al. Randomized Multicenter Phase II Trial of Neoadjuvant Therapy Comparing Weekly Nab-Paclitaxel Followed by FEC With Docetaxel Followed by FEC in HER2- Early-Stage Breast Cancer. *Clin Breast Cancer* (2018) 18(6):474–80. doi: 10.1016/j.clbc.2018.06.012
11. Li Y, Chen X, Zhu Q, Chen R, Xu L, Li S, et al. Retrospective Comparisons of Nanoparticle Albumin-Bound Paclitaxel and Docetaxel Neoadjuvant Regimens for Breast Cancer. *Nanomed (Lond)* (2021) 16(5):391–400. doi: 10.2217/nnm-2020-0458
12. Choi M, Park YH, Ahn JS, Im YH, Nam SJ, Cho SY, et al. Assessment of Pathologic Response and Long-Term Outcome in Locally Advanced Breast Cancers After Neoadjuvant Chemotherapy: Comparison of Pathologic Classification Systems. *Breast Cancer Res Treat* (2016) 160(3):475–89. doi: 10.1007/s10549-016-4008-4
13. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. *Ann Intern Med* (2007) 147:573–7. doi: 10.7326/0003-4819-147-8-200710160-00010
14. Takada M, Toi M. Neoadjuvant Treatment for HER2-Positive Breast Cancer. *Chin Clin Oncol* (2020) 9(3):32. doi: 10.21037/cco-20-123
15. Ersoy YE, Kadioglu H. Review of Novel Sentinel Lymph Node Biopsy Techniques in Breast Cancer Patients Treated With Neoadjuvant Chemotherapy. *Clin Breast Cancer* (2018) 18(4):e555–9. doi: 10.1016/j.clbc.2018.01.004
16. Hwang HW, Jung H, Hyeon J, Park YH, Ahn JS, Im YH, et al. A Nomogram to Predict Pathologic Complete Response (pCR) and the Value of Tumor-Infiltrating Lymphocytes (TILs) for Prediction of Response to Neoadjuvant Chemotherapy (NAC) in Breast Cancer Patients. *Breast Cancer Res Treat* (2019) 173(2):255–66. doi: 10.1007/s10549-018-4981-x
17. Yardley DA. Nab-Paclitaxel Mechanisms of Action and Delivery. *J Control Release* (2013) 170(3):365–72. doi: 10.1016/j.jconrel.2013.05.041
18. Patel TA, Ensor JE, Creamer SL, Boone T, Rodriguez AA, Niravath PA, et al. A Randomized, Controlled Phase II Trial of Neoadjuvant Ado-Trastuzumab Emtansine, Lapatinib, and Nab-Paclitaxel Versus Trastuzumab, Pertuzumab,

and Paclitaxel in HER2-Positive Breast Cancer (TEAL Study). *Breast Cancer Res* (2019) 21(1):100. doi: 10.1186/s13058-019-1186-0

19. Yardley DA, Coleman R, Conte P, Cortes J, Brufsky A, Shtivelband M, et al. Nab-Paclitaxel Plus Carboplatin or Gemcitabine Versus Gemcitabine Plus Carboplatin as First-Line Treatment of Patients With Triple-Negative Metastatic Breast Cancer: Results From the Tnacity Trial. *Ann Oncol* (2018) 29(8):1763–70. doi: 10.1093/annonc/mdy201
20. Untch M, Jackisch C, Schneeweiss A, Conrad B, Aktas B, Denkert C, et al. Nab-Paclitaxel Versus Solvent-Based Paclitaxel in Neoadjuvant Chemotherapy for Early Breast Cancer (GeparSepto-GBG 69): A Randomised, Phase 3 Trial. *Lancet Oncol* (2016) 17(3):345–56. doi: 10.1016/S1470-2045(15)00542-2
21. Gianni L, Mansutti M, Anton A, Calvo L, Bisagni G, Bermejo B, et al. Comparing Neoadjuvant Nab-Paclitaxel vs Paclitaxel Both Followed by Anthracycline Regimens in Women With ERBB2/HER2-Negative Breast Cancer-The Evaluating Treatment With Neoadjuvant Abraxane (ETNA) Trial: A Randomized Phase 3 Clinical Trial. *JAMA Oncol* (2018) 4(3):302–8. doi: 10.1001/jamaoncol.2017.4612
22. Nebot-Villacampa MJ, Zafra-Morales R, Alfaro-Olea A, Marín-Gorricho R, Casajús-Navasal A, Uriarte-Pinto M. Effectiveness and Safety of Nab-Paclitaxel/Gemcitabine in Locally Advanced or Metastatic Pancreatic Adenocarcinoma. *J Oncol Pharm Pract* (2020) 26(3):603–11. doi: 10.1177/1078155219862035
23. Zong Y, Peng Z, Wang X, Lu M, Shen L, Zhou J. Efficacy and Safety of Nab-Paclitaxel Plus S-1 Versus Nab-Paclitaxel Plus Gemcitabine for First-Line Chemotherapy in Advanced Pancreatic Ductal Adenocarcinoma. *Cancer Manag Res* (2020) 12:12657–66. doi: 10.2147/CMAR.S263773
24. Untch M, Jackisch C, Schneeweiss A, Schmatloch S, Aktas B, Denkert C, et al. NAB-Paclitaxel Improves Disease-Free Survival in Early Breast Cancer: GBG 69-GeparSepto. *J Clin Oncol* (2019) 37(25):2226–34. doi: 10.1200/JCO.18.01842

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

ORIGINAL RESEARCH
published: 07 February 2022
doi: 10.3389/fonc.2021.791995

Prior Local or Systemic Treatment: A Predictive Model Could Guide Clinical Decision-Making for Locoregional Recurrent Breast Cancer

Huai-liang Wu[1,2‡], Yu-jie Lu[3‡], Jian-wei Li[1,2], Si-yu Wu[1,2], Xiao-song Chen[3†] and Guang-yu Liu[1,2*†]*

[1] Department of Breast Surgery, Key Laboratory of Breast Cancer in Shanghai, Shanghai Cancer Center, Fudan University, Shanghai, China, [2] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China, [3] Department of General Surgery, Comprehensive Breast Health Center, Ruijin Hospital Shanghai Jiao Tong University School of Medicine, Shanghai, China

Introduction: Locoregional recurrent breast cancer indicates poor prognosis. No solid prediction model is available to predict prognosis and guide clinical management. Prior local treatment or systemic treatment remains controversial.

Methods: Locoregional recurrent breast cancer patients operated in Fudan University Shanghai Cancer Center were enrolled as a training cohort. An external validation cohort included breast cancer patients after locoregional recurrence from Ruijin Hospital, Shanghai Jiaotong University. A nomogram predicting overall survival after locoregional recurrence was established using multivariable Cox regression analysis while internal and external validation were performed to evaluate its calibration and discrimination.

Results: Overall, 346 and 96 breast cancer patients were included in the training cohort and the validation cohort separately. A nomogram was developed, including age, neoadjuvant chemotherapy, breast surgery, pathology type, tumor size, lymph node status, hormonal receptor and Her-2 status, disease-free interval, and sites of locoregional recurrence. It had modest calibration and discrimination in the training cohort, internal validation and external validation (concordance index: 0.751, 0.734 and 0.722, respectively). The nomogram classified 266 and 80 patients into low and high-risk subgroups with distinctive prognosis. Local treatment after locoregional recurrence was associated with improved overall survival in low-risk group ($P = 0.011$), while systemic therapies correlated with better outcomes only in high-risk group ($P < 0.001$).

Conclusion: A nomogram based on clinicopathological factors can predict prognosis and identify low and high-risk patients. Local treatment is a prior choice for low-risk patients whereas systemic treatment needs to be considered for high-risk patients, warranting further validation and exploration.

Keywords: locoregional recurrence, breast cancer, nomogram, local treatment, systemic treatment

OPEN ACCESS

Edited by:
Nicola Fusco,
University of Milan, Italy

Reviewed by:
Takahiro Kogawa,
Cancer Institute Hospital of Japanese Foundation for Cancer Research, Japan
Xiaowei Qi,
Army Medical University, China

****Correspondence:***
Guang-yu Liu
liugy688@163.com
Xiao-song Chen
chenxiaosong0156@hotmail.com

†ORCID:
Guang-yu Liu
orcid.org/0000-0002-1752-026X
Xiao-song Chen
orcid.org/0000-0002-3286-0035

‡These authors have contributed equally to this work

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *09 October 2021*
Accepted: *29 December 2021*
Published: *07 February 2022*

Citation:
Wu H-l, Lu Y-j, Li J-w, Wu S-y, Chen X-s and Liu G-y (2022) Prior Local or Systemic Treatment: A Predictive Model Could Guide Clinical Decision-Making for Locoregional Recurrent Breast Cancer. Front. Oncol. 11:791995. doi: 10.3389/fonc.2021.791995

INTRODUCTION

Breast cancer, the most common malignant tumor in women, was estimated to have 18.1 million newly diagnosed cases and to cause 9.6 million deaths in 2018 (1). Despite the development and regulation of standard comprehensive treatment, incidence rates of locoregional recurrent breast cancer after initial operation and systemic treatments remain 7%-15% (2–5). Locoregional recurrence (LRR) from early breast cancer after mastectomy or breast-conserving treatment (BCT) plus radiotherapy indicated poor prognosis, whereas locoregional recurrent breast cancers were more likely to precede local progression and/or distant metastasis (6, 7). Many previous studies have investigated predictive factors for the LRR from early breast cancer (8–11). According to a previous review on the multidisciplinary management of LRR from breast cancer, it summarized prognostic factors of LRR from breast cancer into three parts, including patient factors (age and family history), disease features [disease-free interval (DFI), biological features, initial disease stage, and sites of LRR], and previous treatment (initial surgery, systemic treatment, radiotherapy and resectable surgery after LRR) (12).

Once LRR occurs in patients with breast cancer, whether to perform chemotherapy and the priority between local treatment and systemic therapies remain unclear and controversial (13, 14). There are few prospective clinical cohorts of local treatment or systemic treatments after LRR to guide clinicians in making preferable decisions. Unavoidable case-by-case bias in the treatment choice and efficacy estimation due to the heterogeneity of recurrent disease and previous treatment is a major obstacle for starting prospective trials on post-LRR management. To date, clinicians have usually developed treatment strategies by multidisciplinary approaches for recurrent diseases (12, 13, 15). However, it is nearly impossible for physicians to treat each recurrent case through a multidisciplinary approach team. Considering these conundrums, a comprehensive clinical tool such as predictive models for post-LRR management is critically needed.

Predictive models for post-LRR patients contributed to therapeutic implications and socioeconomic considerations. Specifically, patients whose prognosis is poor may be considered for aggressive treatments, while those with an expected long-term survival might be saved from overtreatment and its related financial burden (16, 17). However, to the best of our knowledge, no previous study has included comprehensively significant prognostic factors to develop and externally validate predictive models for post-LRR breast cancer patients. Therefore, this study aims to derive and validate a predictive model using significant clinicopathological factors to guide clinical decision-making.

MATERIALS AND METHODS

Study Design and Participants

A retrospective two-cohort study was performed to investigate the prognosis of patients with breast cancer and the significance of local treatment and systemic treatment after LRR were evaluated. Patients with locoregional recurrent breast cancer treated between December 2007 and August 2020 in Fudan University Shanghai Cancer Center (FUSCC), Shanghai, China, were retrospectively included as a training cohort. An internal validation cohort was created by 500 bootstrap resamples of the training cohort. In addition, 96 patients with recurrent breast cancer were enrolled from the Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiaotong University School of Medicine (RJCBHC), between January 2009 and December 2018 as an external validation cohort. The inclusion criteria were as follows: 1) patients with primary or recurrent breast cancer who were admitted to FUSCC; 2) the presence of pathologically confirmed breast cancer; 3) locoregional recurrence of breast cancer; and 4) completed breast operation (mastectomy or BCT). The exclusion criteria were described in the **Supplementary Figure S1** and included: 1) phylodes tumors; 2) without completed clinical or pathological data; 3) with distant metastasis before LRR or with the first LRR; 4) male breast cancer; 5) highly suspected second primary lesions.

Baseline Characteristics, Follow-Up and Outcome

Patients' characteristics in the training cohort and external validation cohort, including age at diagnosis of breast cancer (≤ 35, 35-70 or ≥ 70 years old), body mass index, menopausal status, neoadjuvant chemotherapy (NACT) received or not, initial breast operation (mastectomy or BCT), initial axillary operation (axillary lymph node dissection or sentinel lymph node biopsy), histology grade (I, II, III), pathology [ductal carcinoma *in situ*, invasive ductal carcinoma (IDC), invasive lobular carcinoma and other types], tumor size (≤ 2.0 cm or > 2.0 cm), numbers of metastatic lymph nodes (LNs) after initial operation (0, 1-3, 4-10, or >10), estrogen receptor (ER) status, progesterone receptor (PR) status, hormonal receptor (HR) status and human epidermal growth factor receptor-2 (Her-2) status, DFI (≤ 2 years or > 2 years), and sites of LRR (chest wall, breast, nodal recurrence, and multiple sites), are displayed in **Table 1**. Treatment therapies after initial operation and after LRR were showed in the **Supplementary Table S1**.

ER and PR positivity were defined according to our previous studies (18). HR positivity was defined as the positivity of either ER or PR. The DFI was calculated from the time interval from the initial operation to the occurrence of the first LRR. LRR referred to breast cancer recurrence in the ipsilateral chest wall or breast or regional lymph nodes (axillary lymph node, clavicular lymph node and internal mammary lymph node) after excluding highly suspected second primary lesions. Besides, highly suspected second primary lesions were defined as that the recurrent tumors were found in the different quadrant or far from the primary tumor scar in isolated ipsilateral local recurrent patients (breast and chest wall), and patients with inconsistent immunohistochemistry status in reginal nodal recurrent patients (12). Multiple sites indicated that recurrent sites occurred in more than one region mentioned above. Distant metastasis (DM) referred to tumor recurrence outside the locoregional areas

TABLE 1 | Baseline characteristics of breast cancer patients with LRR.

Variable	Training cohort (%) *N* = 346	External validation cohort (%) *N* = 96	*P* value
Age at the diagnosis of breast cancer, year			0.671
≤35	35 (10.1%)	11 (11.5%)	
35-70	277 (80.1%)	73 (76.0%)	
≥70	34 (9.8%)	12 (12.5%)	
BMI			0.838
≤25	263 (76.0%)	72 (75.0%)	
>25	83 (24.0%)	24 (25.0%)	
Menopausal status			0.400
Premenopausal	142 (41.0%)	44 (45.8%)	
Postmenopausal	204 (59.0%)	52 (54.2%)	
Received NACT before surgery			0.310
No	272 (78.6%)	80 (83.3%)	
Yes	74 (21.4%)	16 (16.7%)	
Initial breast operation			0.093
BCT	89 (25.7%)	33 (34.4%)	
Mastectomy	257 (74.3%)	63 (65.6%)	
Initial axillary operation			0.115
Only SLNB	100 (28.9%)	38 (39.6%)	
ALND ± SLNB	238 (68.8%)	55 (57.3%)	
No axillary operation	8 (2.3%)	3 (3.1%)	
Histology grade			0.643
I	1 (0.3%)	1 (1.0%)	
II	100 (28.9%)	29 (30.2%)	
III	168 (48.6%)	49 (51.0%)	
Unknown	77 (22.3%)	17 (17.7%)	
Pathology			<0.001***
IDC	258 (74.6%)	87 (90.6%)	
ILC	5 (1.4%)	4 (4.2%)	
Others	83 (24.0%)	5 (5.2%)	
Tumor size, cm			0.567
≤2.0	170 (49.1%)	44 (45.8%)	
>2.0	176 (50.9%)	52 (54.2%)	
Positive LN			0.317
0	164 (47.4%)	46 (47.9%)	
1-3	82 (23.7%)	24 (25.0%)	
4-9	59 (17.1%)	10 (10.4%)	
≥10	41 (11.8%)	16 (16.7%)	
ER status			0.920
Negative	171 (49.4%)	48 (50.0%)	
Positive	175 (50.6%)	48 (50.0%)	
PR status			0.011*
Negative	195 (56.4%)	68 (70.8%)	
Positive	151 (43.6%)	28 (29.2%)	
HR status			0.726
Negative	166 (48.0%)	48 (50.0%)	
Positive	180 (52.0%)	48 (50.0%)	
HER-2 status			0.979
Negative	241 (69.7%)	67 (69.8%)	
Positive	105 (30.3%)	29 (30.2%)	
DFI to LRR			0.999
≤2 year	191 (55.2%)	53 (55.2%)	
>2 year	155 (44.8%)	43 (44.8%)	
Sites of LRR			0.441
Chest wall	129 (37.3%)	25 (26.0%)	
Breast	64 (18.5%)	33 (34.4%)	
Nodal recurrence	123 (35.5%)	31 (32.3%)	
Multiple sites	30 (8.7%)	7 (7.3%)	

**indicates P < 0.05; ***indicates P < 0.001.*

ALND, Axillary lymph node dissection; BCT, Breast-conserving treatment; BMI, Body mass index; DCIS, Ductal carcinoma in situ; DFI, Disease-free Interval; ER, Estrogen receptor; Her-2, Human epidermal growth factor receptor-2; HR, Hormonal receptor; IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; LN, Lymph node; LRR, Locoregional recurrence; NACT, Neoadjuvant chemotherapy treatment; PR, Progesterone receptor; SLNB, Sentinel lymph node biopsy.

mentioned above. Patients were censored when DM or death occurred or were lost to follow-up. Distant disease-free survival (DDFS) and overall survival (OS) after LRR were calculated from the time of the first LRR.

Statistical Analysis

Comparison of clinicopathological characteristics was evaluated using the χ^2 test or Fisher's exact test for categorical variables between the training cohort and validation cohort. DDFS and OS were evaluated using the Kaplan-Meier method, and the differences were compared using the unstratified log-rank test. Univariate and multivariate Cox regression were used to screen the risk factors. LASSO Cox regression analysis was implemented to further confirm the candidate prognostic factors and complete the construction of the predictive model.

A nomogram predicting OS was formulated based on the results of multivariate analysis and expert consensus. Model performance was assessed in the training cohort, internal validation cohort and external validation cohort through discrimination and calibration. Discrimination ability was assessed by receiver operating characteristic (ROC) analysis, and predictive accuracy was measured using the concordance index (C-index). The C-index ranges from 0.5 to 1.0 (random to perfect prediction) (19). Calibration analysis was performed through the comparison between predicted probabilities and actual probabilities. For predictive factors included in the nomogram, each value represented a score on the point axis. A total score is calculated by adding the scores for each item and locating this sum on the total point scale axis. The three vertical lines can be used to predict the probability of OS within 1 year, 2 years and 3 years (**Figure 1**). We used X-tile, a type of bioinformatics tool, to determine the appropriate cutoff points of predictive scores to stratify patients into low-risk and high-risk subgroups (20). All hypothesis tests were two-sided, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS statistical software (v22.0) and R statistical software (v3.5.2). The R packages used in this study are as follows: 'survival', survminer', 'rms', 'riskRegression', 'maxstat', 'pROC', 'plotROC', 'ggplot2', and 'nomogramFormula'.

RESULTS

Baseline Characteristics

A total of 346 patients in the training cohort and 96 patients in the external validation cohort who underwent mastectomy or BCT were included in the final analysis. The median follow-up after the initial operation was 57.1 months (range 5.3-152.3 months) in the FUSCC cohort and 49.8 months (range 12.77-113.57 months) in the RJCBHC cohort. Clinicopathological characteristics were similar between the training cohort and external validation cohort except for pathology and PR status (**Table 1**). Most of the patients had IDC in the external validation cohort, while the proportion of IDC in the training cohort was obviously lower (90.6% *vs* 74.6%, $P < 0.001$). In addition, therapeutic choices seemed to be significantly different between these two cohorts (**Supplementary Table S1**). The median

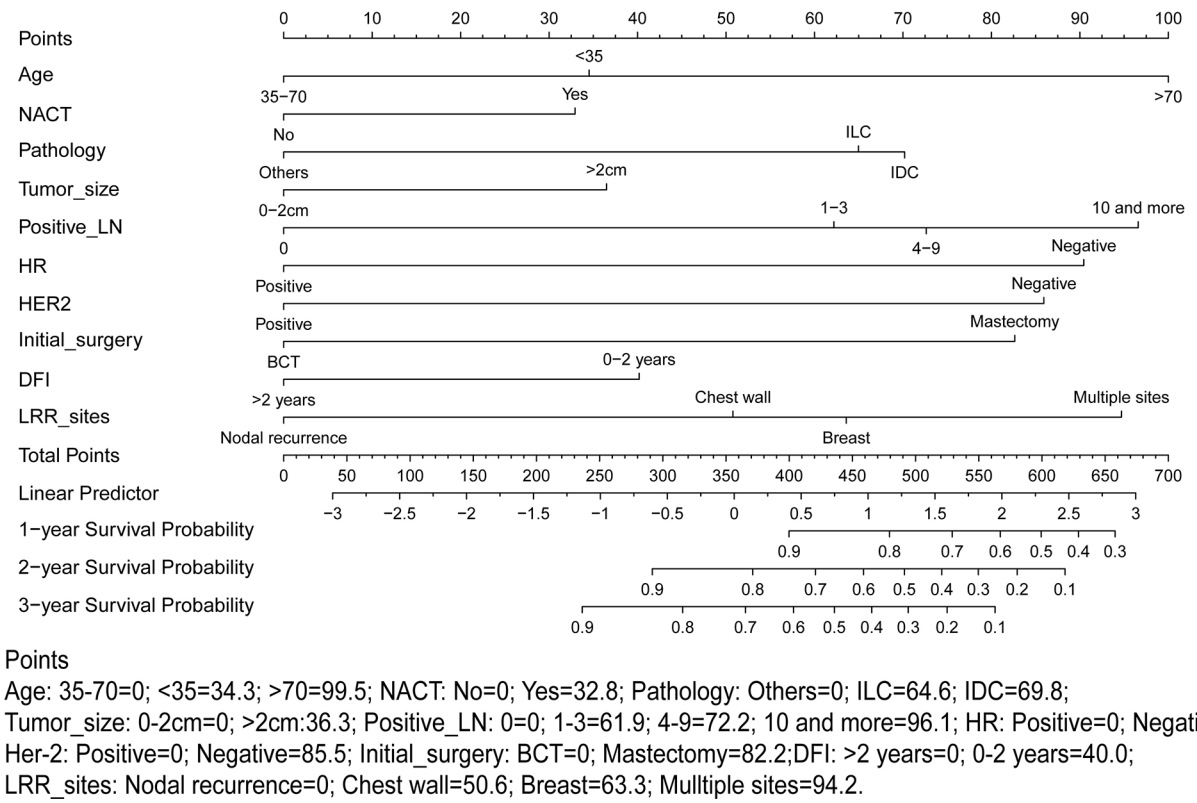


FIGURE 1 | Proposed nomogram using the training cohort to predict the probability of overall survival (OS) after locoregional recurrence (LRR). NACT, Neoadjuvant chemotherapy treatment; LN, Lymph node; HR, Hormonal receptor; HER-2, human epidermal growth factor receptor-2; LRR, Locoregional recurrence; DFI, Time interval from initial surgery to the LRR.

follow-up period after LRR was 29.1 months (range 0.1-134.4 months), while it was 25.1 months (range 3.4-85.8 months) in the external validation cohort. There were obviously higher death and DM rates in the training cohort (death rate: 33.5% *vs* 14.6%; DM rate 30.1% *vs* 6.3%).

Identified Prognostic Factors for Post-LRR Outcomes

The results of the univariate analysis and potential high-risk factors are listed in **Supplementary Table S2**. Multivariate analyses demonstrated that age group, NACT, pathology type, larger tumor size, metastatic lymph nodes, HR status, and Her-2 status were significantly associated with poor prognosis for post-LRR patients (**Table 2**).

Performance of the Predictive Nomogram

According to the high-risk factors identified in previous studies (12, 21), a nomogram predicting the probability of OS based on multivariate Cox regression analysis and expert opinions was constructed (**Figure 1**). A total score was calculated using age at the diagnosis of breast cancer, received NACT or not, pathology types, tumor size, number of positive lymph nodes, HR status, HER-2 status, type of initial breast operation, DFI, and location of LRR. Discrimination assessment showed good performance of the model (C-index: 0.751 and AUC: 0.775 [0.705-0.845]) and stable agreement (C-index: 0.734 and AUC: 0.774 [0.710-0.838]) in the original training and internal validation cohorts, respectively. However, this nomogram seemed to underestimate the OS probability (C-index: 0.722 and AUC: 0.679 [0.536-0.823]) in the external validation cohort (**Supplementary Figure S2**).

Clinical Implications of the Predictive Nomogram

Stratified by the nomogram model, we divided post-LRR patients into a low-risk group and a high-risk group (the cutoff point was 441.4). Stratification into low-risk and high-risk subgroups allowed significant distinction between the Kaplan-Meier curves for survival outcomes in both the training cohort and the external validation cohort (**Figure 2**). Clinicopathological characteristics were significantly different between the low-risk group and the high-risk group in the training cohort (**Supplementary Table S3**). Most high-risk patients presented with negative HR and Her-2 status (80.0% and 83.8%, respectively), while more than half (61.7%) were luminal breast cancer patients in the low-risk group.

To evaluate the priority of different treatment therapies after LRR, we examined the association of different therapies and prognosis in low-risk and high-risk patients. **Figure 3** indicates

TABLE 2 | Multivariate cox regression analysis of prognostic factors in the training cohort.

Variable	Distant-disease free survival (DDFS)			Overall survival (OS)		
	Hazard Ratio	95% Confidence Interval	*P* value	Hazard Ratio	95% Confidence Interval	*P* value
Age of diagnosis of breast cancer, yrs						
35-70	1.000 (reference)	—	—	1.000 (reference)	—	—
≤35	1.358	0.834-2.210	0.218	1.234	0.685-2.223	0.484
≥70	1.830	1.066-3.144	0.029*	2.219	1.205-4.087	0.011*
Receive NACT						
No	1.000 (reference)	—	—	1.000 (reference)	—	—
Yes	1.793	1.216-2.643	0.003**	1.519	0.950-2.430	0.081
Initial breast operation						
BCT	1.000 (reference)	—	—	1.000 (reference)	—	—
Mastectomy	1.096	0.572-2.100	0.783	2.102	0.756-5.844	0.154
Pathology						
IDC	1.000 (reference)	—	—	1.000 (reference)	—	—
ILC	1.014	0.243-4.237	0.984	1.000	0.134-7.443	1.000
Others	0.562	0.372-0.851	0.006**	0.485	0.289-0.813	0.006**
Tumor size						
≤2.0	1.000 (reference)	—	—	1.000 (reference)	—	—
>2.0	1.479	1.042-2.101	0.029*	1.438	0.945-2.187	0.090
Number of positive LNs						
0	1.000 (reference)	—	—	1.000 (reference)	—	—
1-3	1.648	1.088-2.494	0.018*	1.924	1.155-3.207	0.012*
4-9	2.058	1.276-3.320	0.003**	2.189	1.232-3.890	0.008**
≥10	2.527	1.543-4.141	<0.001***	2.601	1.453-4.656	0.001**
HR status						
Negative	1.000 (reference)	—	—	1.000 (reference)	—	—
Positive	0.540	0.324-0.899	0.018*	0.273	0.142-0.523	<0.001***
Her-2 status						
Negative	1.000 (reference)	—	—	1.000 (reference)	—	—
Positive	0.495	0.341-0.720	<0.001***	0.415	0.263-0.653	<0.001**
Adjuvant radiotherapy						
No	1.000 (reference)	—	—	1.000 (reference)	—	—
Yes	0.686	0.480-0.981	0.039*	0.676	0.444-1.029	0.068
Hormonal therapy						
No	1.000 (reference)	—	—	1.000 (reference)	—	—
Yes	1.597	0.955-2.672	0.074	1.678	0.889-3.169	0.110
DFI to LRR						
≤2, yrs	1.000 (reference)	—	—	1.000 (reference)	—	—
>2, yrs	0.770	0.546-1.087	0.138	0.662	0.443-1.012	0.057
Locoregional recurrence sites						
Breast	1.000 (reference)	—	—	1.000 (reference)	—	—
Chest wall	1.230	0.553-2.737	0.611	0.894	0.280-2.850	0.849
Nodal recurrence	1.188	0.567-2.490	0.648	0.563	0.184-1.720	0.313
Multiple sites	1.689	0.715-3.993	0.232	1.280	0.372-4.403	0.696

**indicates P < 0.05; **indicates P < 0.01; ***indicates P < 0.001.*

NACT, Neoadjuvant chemotherapy treatment; BCT, Breast-conserving treatment; IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; LN, Lymph node; HR, Hormonal receptor; Her-2, human epidermal growth factor receptor-2; DFI, Disease-free Interval; LRR, Locoregional recurrence.

the significance of local treatment in the low-risk group and systemic treatment in the high-risk group (HR: 0.513 [0.303-0.856] and 0.182 [0.085-0.387]). Specifically, resectable surgery seemed to be associated with longer survival for low-risk patients (HR: 0.548, $P = 0.029$) (**Supplementary Table S4**). Interestingly, chemotherapy and hormonal therapy in the high-risk group improved OS (HR: 0.386 and 0.200, $P = 0.011$ and 0.001, respectively). In contrast, chemotherapy seemed to not be associated with improved OS in the low-risk group, while resectable surgery did not correlate with better prognosis for high-risk patients. No significant benefits in OS were observed for radiotherapy in either the low-risk or high-risk group. However, radiotherapy following LRR was correlated with increased DDFS in the low-risk group but not in the high-risk group ($P = 0.024$ and 0.623, respectively) (**Supplementary Figure S3**).

To conclude that a schematic diagram of clinical management of post-LRR breast cancer patients based on the nomogram is illustrated (**Figure 4A**). Once locoregional recurrent breast cancer patients come to clinic, the nomogram could stratify these patients into low-risk or high-risk groups and provide prior treatment strategies for them. Furthermore, it is displayed that there are several examples of low-risk or high-risk patients in real-world practice (**Figure 4B**). For these two high-risk cases, multiple recurrent sites, negativity of Her-2, and short DFI were observed. However, it is inconsistent with our previous

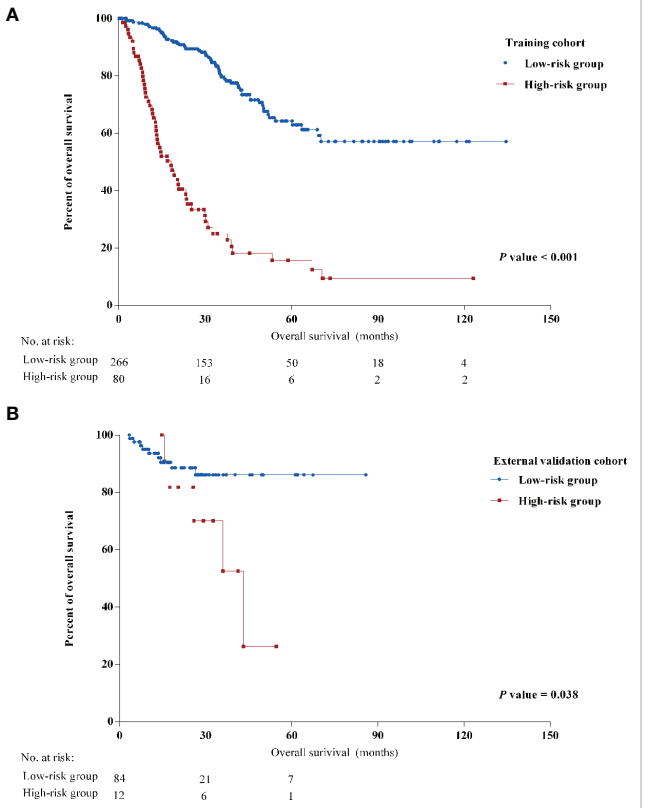


FIGURE 2 | Kaplan-Meier curves of overall survival (OS) of different risk groups in **(A)** the training cohort and **(B)** the external validation cohort.

perception, post-LRR luminal breast cancer patient (Case 3) with only one positive lymph node is regarded as a high-risk patient. Case 3 was still alive after receiving chemotherapy and hormonal therapy while case 4 only receiving radiotherapy was died within 4 months after LRR. This indicated the huge significance of systemic therapies for high-risk patients.

DISCUSSION

Our study demonstrated the relative importance of a breadth of high-risk prognostic variables for locoregional recurrent breast cancer patients, such as age at diagnosis of breast cancer, NACT, pathology type, tumor size, number of metastatic LNs, HR status, and Her-2 status. Furthermore, we constructed a nomogram to target and stratify patients into low-risk and high-risk subgroups. Local treatment was found to be correlated with improved survival in the low-risk group but not in the high-risk group. In contrast, systemic treatment is associated significantly improved OS only for high-risk patients. We expect that this predictive nomogram will prove helpful in further clinical practice on post-LRR management.

The nomogram reported and validated here was constructed to quantify the risks of several clinical profiles mentioned above. Through the nomogram, we found that the occurrence of LRR in multiple sites, 10 or more metastatic lymph nodes and age greater than 70 years were prognostic factors for poor OS. Similar findings were observed in previous studies (21–23). Interestingly, mastectomy was found to be a high-risk factor for post-LRR outcomes in the nomogram. The Danish 82b/c trials and another postmastectomy trial in British Columbia also found that the development of LRR and receipt of initial mastectomy were likely to precede metastatic disease (24, 25). Hence, prognosis of patients with LRR was significantly correlated with therapeutic choices before the occurrence of LRR. A consistent finding was noted in a recent clinical trial that intraoperative radiotherapy before LRR could achieve better prognosis compared to those receiving whole-breast external beam radiotherapy before (26). A previous risk stratification system using three robust risk factors, including positive LN status *vs* negative, DFI < 30 months *vs* ≥ 30 months and regional LN recurrence *vs* local recurrence, could guide patients in making a choice with estimated survival (21). However, in our nomogram, nodal recurrence seemed to be a protective factor for post-LRR patients. A possible explanation for this was the heterogeneity of different nodal recurrences. The survival data of the training cohort indicated that the OS of internal mammary LN and axillary LN recurrence was significantly better than that of chest wall recurrence, while clavicular LN recurrence showed an obviously adverse prognosis (**Supplementary Figure S4**). Furthermore, axillary recurrence was observed as a worse prognostic factor compared to those isolated breast recurrences. Jin et al. indicated a consistent finding that the 5-year OS of isolated breast recurrence was significantly higher than that of axillary nodal recurrence (100% *vs* 73.5%, p=0.021) (27).

Using another similar risk stratification system, Byoung Hyuck Kim et al. screened and targeted post-LRR patients with long-term survival through the initial pN stage, DFI interval and whether to perform resectable excision after LRR (15). Our study constructed a nomogram using more prognostic factors with internal and external validation to quantify the impacts of these prognostic factors in predicting OS. Moreover, it could estimate the probability of OS for patients and help them achieve a balance between potential benefits from treatments and socioeconomic factors such as financial cost and family considerations (28, 29). After further analysis on two different types of isolated local recurrence separately, similar findings were observed compared to the results reported above, which might demonstrate the stability of this predictive model (**Supplementary Table S5**).

The latest ESO-ESMO consensus for advanced breast cancer supported surgical excision if feasible for patients and if the recurrent sites are resectable (13). No surprisingly, resectable surgery was found to be associated with better OS in the low-risk group. However, some local recurrences involve extensive chest wall recurrence, and some regional recurrences occurring in surgically inaccessible sites cannot receive resectable surgery in the clinic. Anders N. Pedersen et al. found that the complete remission rate in resectable surgery was obviously higher than that in patients not given surgery (76% *vs* 43%, $P < 0.0001$) (30).

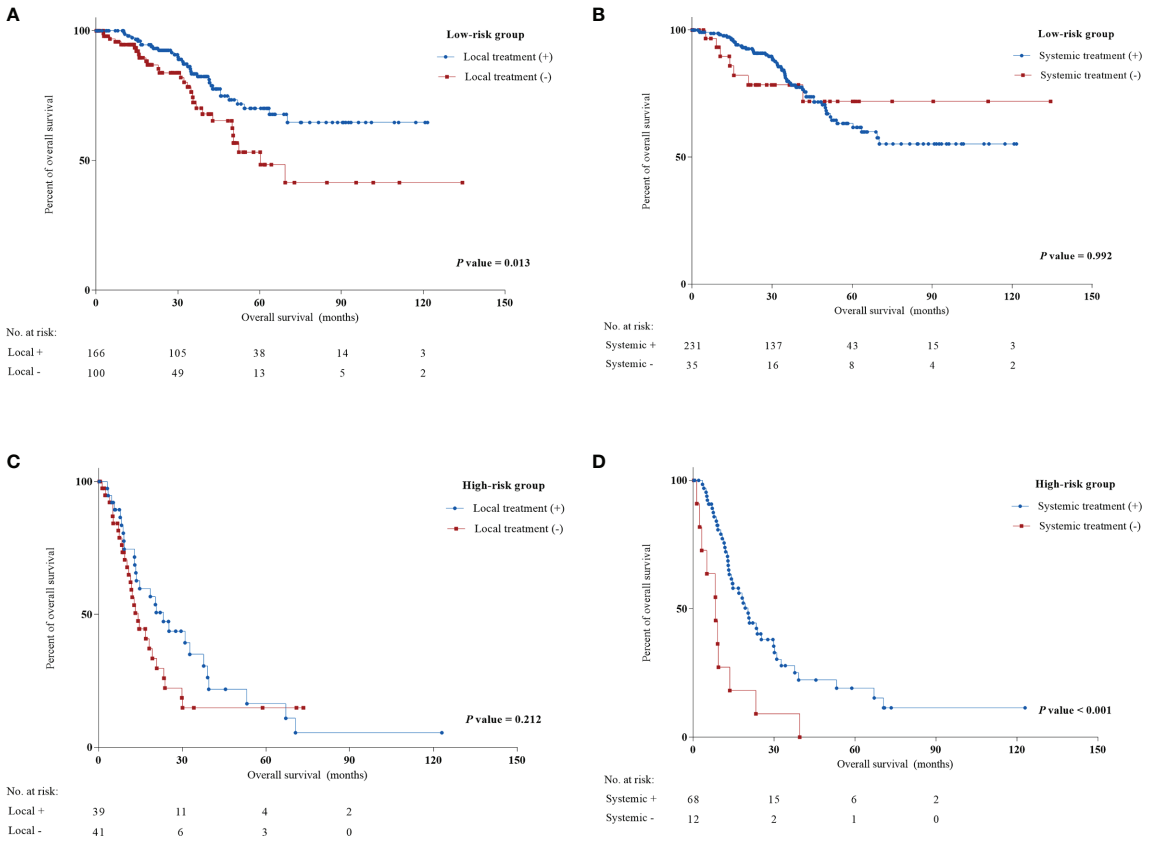


FIGURE 3 | Univariate Cox regression analysis of local treatment and systemic treatment in low and high-risk groups from the training cohort. **(A)** Local treatment in low-risk group; **(B)** systemic treatment in low-risk group; **(C)** local treatment in high-risk group; **(D)** systemic treatment in high-risk group.

In contrast, previous studies suggested that favorable prognosis correlated with the availability of resectable surgery (12, 31). Therefore, it is still unclear whether resectable surgery or resectable LRR originally contributed to a better prognosis. Radiotherapy was indicated for patients not previously irradiated, and the standard of reirradiation was still controversial (13). Through this nomogram, we could target patients with an expected poor prognosis (low-risk group). Further univariable analysis proved the significance of local treatment for low-risk patients. Although radiotherapy could not significantly improve OS, it was found to have a positive role in prolonging DDFS in the low-risk group (**Supplementary Figure S3**). Low-risk patients seemed to not obtain significant benefits from chemotherapy and other systemic treatments. Our findings were not only consistent with previous studies (26, 32) but also provided new therapeutic approaches for post-LRR patients.

In contrast, chemotherapy was only found to be correlated with longer survival for high-risk patients in our study. This finding was consistent with the risk stratification study mentioned above (15). Previously, the CALOR trial confirmed the positive role of chemotherapy in ER-negative patients but not in ER-positive patients (33). Owing to the limitations of the CALOR trial, it should not be concluded that all ER-positive patients cannot gain benefits from chemotherapy (33). Therefore, this study provides a new strategy to determine whether to use chemotherapy. Furthermore, except for chemotherapy, other systemic treatments should be considered based on their previous medical history and patient status (12, 13, 32). Tamoxifen improved the 5-year DFS of ER-positive LRR patients in the SAKK 23/82 trial (34). To date, no prospective trial has investigated the role of anti-Her-2 targeted therapy in Her-2-positive LRR patients. However, most experts still recommend the use of anti-Her-2 targeted therapy for post-LRR patients with Her-2 positivity as a standard treatment. Thus, with the combination of our findings and previous studies as well as expert consensus, physicians need to perform systemic therapies once patients meet the high-risk status in the clinic. This study also offers several real-world cases of both low and high-risk patients with therapeutic choices and survival after LRR to further support the clinical strategies mentioned above (**Figure 4B**).

To the best of our knowledge, this is the first study to establish a nomogram using many important prognostic factors from a relatively large training cohort accompanied by external validation to estimate the prognosis of post-LRR breast cancer

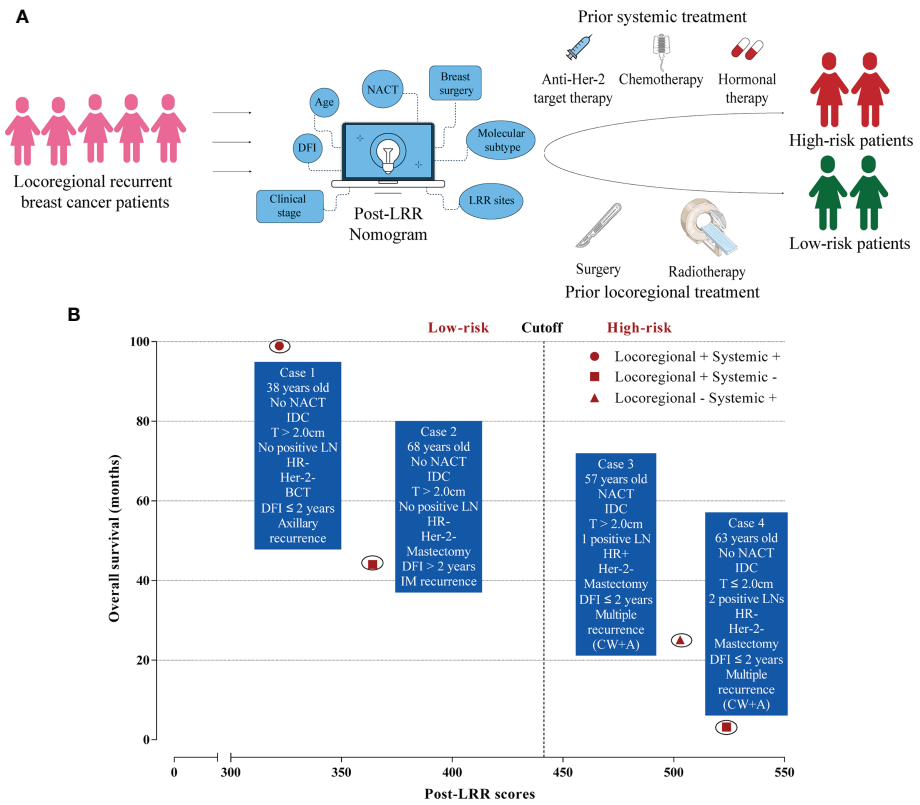


FIGURE 4 | Clinical management of locoregional recurrent breast cancer patients. **(A)** The schematic diagram of post-locoregional recurrent breast cancer patient management based on the nomogram; **(B)** Examples of low and high-risk patients in real-world practice. LRR, Locoregional recurrence; DFI, Disease-free interval; NACT, Neoadjuvant chemotherapy; IDC, Invasive ductal carcinoma; LN, Lymph node; HR, Hormonal receptor; BCT, Breast-conserving treatment; IM, Internal mammary; CW, Chest wall; A, Axillary.

patients. This nomogram showed modest performance and generalization in both internal and external validation. Moreover, based on the estimation of survival probability, patients could be divided into two different groups and had corresponding therapeutic strategies. Patients could clearly consider and decide their own treatment therapies according to the expected survival reported by the nomogram and their willingness.

Despite these important advantages, this study had several limitations. First, as a retrospective study, it would unavoidably have selection bias. It was also unable to investigate the correlation between clinicopathological factors and occurrence of LRR after initial surgery without the clinical data of all patients treated in the same period. Second, therapeutic choices and incidence rates of events were obviously different between the training and external validation cohorts, causing worse agreement in the external validation. Third, owing to the technical limitation, it's inevitable to exclude all second primary breast tumor patients apart in our study. Finally, our nomogram dealt only with clinicopathologic profiles and needed longer follow-up. This nomogram should be applied with caution until validated in a randomized clinical trial with different treatment strategies in the future.

CONCLUSION

The post-LRR predictive nomogram was developed and externally validated for patients with breast cancer and could guide oncologists in making prognosis-related clinical decisions. Local treatments following LRR could be initial choices rather than systemic treatment for low-risk patients, while systemic treatment might be considered once identified as high-risk patients.

DATA AVAILABILITY STATEMENT

The datasets that support the findings of this study are kept in institutional file storage on an internal server at the Fudan University Shanghai Cancer Centre Department of breast cancer. In order to protect patient privacy, these data were unavailable to public. The data will be made available on reasonable request from the corresponding author. For all data requests, please contact Dr Guang-yu Liu (Department of Breast Surgery, Fudan University Shanghai Cancer Centre, Shanghai, P.R. China. liugy688@163.com) or Dr Xiao-song Chen (Comprehensive Breast Health Center, Ruijin Hospital,

Shanghai Jiao Tong University School of Medicine, Shanghai, China. chenxiaosong0156@hotmail.com).

ETHICS STATEMENT

Ethical approval was not provided for this study on human participants because as a retrospective cohort study, all the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: X-sC and G-yL. Data curation and formal analysis: H-lW and Y-jL. Investigation: H-lW, Y-jL, J-wL, and S-yW. Methodology: H-lW, Y-jL, J-wL, and S-yW. Project administration and supervision: X-sC and G-yL. Validation: H-lW and Y-jL. Visualization: H-lW and Y-jL. Writing - original draft: H-lW and Y-jL. Writing - review and editing: H-lW, Y-jL, J-wL, S-yW, X-sC, and G-yL. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors thank all participating patients and clinicians for contributing data to this study. Some vectors in the graphical abstract were attributed from the Vecteezy.com.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.791995/full#supplementary-material

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2018) 68 (6):394–424. doi: 10.3322/caac.21492
2. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2020. *CA Cancer J Clin* (2020) 70(1):7–30. doi: 10.3322/caac.21590
3. Schmoor C, Sauerbrei W, Bastert G, Schumacher M. Role of Isolated Locoregional Recurrence of Breast Cancer: Results of Four Prospective Studies. *J Clin Oncol Off J Am Soc Clin Oncol* (2000) 18(8):1696–708. doi: 10.1200/JCO.2000.18.8.1696
4. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE Jr, et al. Four-Year Follow-Up of Trastuzumab Plus Adjuvant Chemotherapy for Operable Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: Joint Analysis of Data From NCCTG N9831 and NSABP B-31. *J Clin Oncol Off J Am Soc Clin Oncol* (2011) 29(25):3366–73. doi: 10.1200/JCO.2011.35.0868
5. Clarke M, Collins R, Darby S, Davies C, Elphinstone P, Evans V, et al. Effects of Radiotherapy and of Differences in the Extent of Surgery for Early Breast Cancer on Local Recurrence and 15-Year Survival: An Overview of the Randomised Trials. *Lancet (London England)* (2005) 366(9503):2087–106. doi: 10.1016/S0140-6736(05)67887-7
6. Clemons M, Danson S, Hamilton T, Goss P. Locoregionally Recurrent Breast Cancer: Incidence, Risk Factors and Survival. *Cancer Treat Rev* (2001) 27 (2):67–82. doi: 10.1053/ctrv.2000.0204
7. Aebi S, Gelber S, Anderson SJ, Lang I, Robidoux A, Martin M, et al. Chemotherapy for Isolated Locoregional Recurrence of Breast Cancer (CALOR): A Randomised Trial. *Lancet Oncol* (2014) 15(2):156–63. doi: 10.1016/S1470-2045(13)70589-8
8. Lu Y, Tong Y, Huang J, Lin L, Wu J, Fei X, et al. Primary 21-Gene Recurrence Score and Disease Outcome in Loco-Regional and Distant Recurrent Breast Cancer Patients. *Front Oncol* (2020) 10:1315. doi: 10.3389/fonc.2020.01315
9. Gillon P, Touati N, Breton-Callu C, Slaets L, Cameron D, Bonnefoi H. Factors Predictive of Locoregional Recurrence Following Neoadjuvant Chemotherapy in Patients With Large Operable or Locally Advanced Breast Cancer: An Analysis of the EORTC 10994/BIG 1-00 Study. *Eur J Cancer (Oxford Engl 1990)* (2017) 79:226–34. doi: 10.1016/j.ejca.2017.04.012
10. Millar EK, Graham PH, O'Toole SA, McNeil CM, Browne L, Morey AL, et al. Prediction of Local Recurrence, Distant Metastases, and Death After Breast-Conserving Therapy in Early-Stage Invasive Breast Cancer Using a Five-Biomarker Panel. *J Clin Oncol Off J Am Soc Clin Oncol* (2009) 27(28):4701–8. doi: 10.1200/JCO.2008.21.7075
11. Gentilini O, Botteri E, Veronesi P, Sangalli C, Del Castillo A, Ballardini B, et al. Repeating Conservative Surgery After Ipsilateral Breast Tumor Reappearance: Criteria for Selecting the Best Candidates. *Ann Surg Oncol* (2012) 19 (12):3771–6. doi: 10.1245/s10434-012-2404-5
12. Buchholz TA, Ali S, Hunt KK. Multidisciplinary Management of Locoregional Recurrent Breast Cancer. *J Clin Oncol Off J Am Soc Clin Oncol* (2020) 38 (20):2321–8. doi: 10.1200/JCO.19.02806
13. Cardoso F, Paluch-Shimon S, Senkus E, Curigliano G, Aapro MS, Andre F, et al. 5th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 5)(Dagger). *Ann Oncol Off J Eur Soc Med Oncol* (2020) 31(12):1623–49. doi: 10.1016/j.annonc.2020.09.010
14. Gradishar WJ, Anderson BO, Abraham J, Aft R, Agnese D, Allison KH, et al. Breast Cancer, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* (2020) 18(4):452–78. doi: 10.6004/jnccn.2020.0016
15. Kim BH, Shin KH, Chie EK, Kim JH, Kim K, Hwang KT, et al. Identifying Long-Term Survival Candidates Among Patients With Isolated Locoregionally Recurrent Breast Cancer: Implications of the Use of Systemic Chemotherapy. *J Breast Cancer* (2020) 23(3):279–90. doi: 10.4048/jbc.2020.23.e31
16. Clark GM. Do We Really Need Prognostic Factors for Breast Cancer? *Breast Cancer Res Treat* (1994) 30(2):117–26. doi: 10.1007/BF00666054
17. Altman DG. Prognostic Models: A Methodological Framework and Review of Models for Breast Cancer. *Cancer Invest* (2009) 27(3):235–43. doi: 10.1080/07357900802572110
18. Wu S, Wang Y, Li J, Zhang N, Mo M, Klimberg S, et al. Subtype-Guided (18) F-FDG PET/CT in Tailoring Axillary Surgery Among Patients With Node-Positive Breast Cancer Treated With Neoadjuvant Chemotherapy: A Feasibility Study. *Oncologist* (2020) 25(4):e626–33. doi: 10.1634/theoncologist.2019-0583
19. Hanley JA, McNeil BJ. A Method of Comparing the Areas Under Receiver Operating Characteristic Curves Derived From the Same Cases. *Radiology* (1983) 148(3):839–43. doi: 10.1148/radiology.148.3.6878708
20. Camp RL, Dolled-Filhart M, Rimm DL. X-Tile: A New Bio-Informatics Tool for Biomarker Assessment and Outcome-Based Cut-Point Optimization. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2004) 10(21):7252–9. doi: 10.1158/1078-0432.CCR-04-0713

21. Lee YJ, Park H, Kang CM, Gwark SC, Lee SB, Kim J, et al. Risk Stratification System for Groups With a Low, Intermediate, and High Risk of Subsequent Distant Metastasis and Death Following Isolated Locoregional Recurrence of Breast Cancer. *Breast Cancer Res Treat* (2020) 179(2):315–24. doi: 10.1007/s10549-019-05469-5
22. Anderson SJ, Wapnir I, Dignam JJ, Fisher B, Mamounas EP, Jeong JH, et al. Prognosis After Ipsilateral Breast Tumor Recurrence and Locoregional Recurrences in Patients Treated by Breast-Conserving Therapy in Five National Surgical Adjuvant Breast and Bowel Project Protocols of Node-Negative Breast Cancer. *J Clin Oncol Off J Am Soc Clin Oncol* (2009) 27(15):2466–73. doi: 10.1200/JCO.2008.19.8424
23. Shenouda MN, Sadek BT, Goldberg SI, Keruakous AR, Croft BJ, Abi Raad RF, et al. Clinical Outcome of Isolated Locoregional Recurrence in Patients With Breast Cancer According to Their Primary Local Treatment. *Clin Breast Cancer* (2014) 14(3):198–204. doi: 10.1016/j.clbc.2013.12.007
24. Danish Breast Cancer Cooperative G, Nielsen HM, Overgaard M, Grau C, Jensen AR, Overgaard J. Study of Failure Pattern Among High-Risk Breast Cancer Patients With or Without Postmastectomy Radiotherapy in Addition to Adjuvant Systemic Therapy: Long-Term Results From the Danish Breast Cancer Cooperative Group DBCG 82 B and C Randomized Studies. *J Clin Oncol Off J Am Soc Clin Oncol* (2006) 24(15):2268–75. doi: 10.1200/JCO.2005.02.8738
25. Ragaz J, Olivotto IA, Spinelli JJ, Phillips N, Jackson SM, Wilson KS, et al. Locoregional Radiation Therapy in Patients With High-Risk Breast Cancer Receiving Adjuvant Chemotherapy: 20-Year Results of the British Columbia Randomized Trial. *J Natl Cancer Inst* (2005) 97(2):116–26. doi: 10.1093/jnci/djh297
26. Arthur DW, Winter KA, Kuerer HM, Haffty BG, Cuttino LW, Todor DA, et al. NRG Oncology-Radiation Therapy Oncology Group Study 1014: 1-Year Toxicity Report From a Phase 2 Study of Repeat Breast-Preserving Surgery and 3-Dimensional Conformal Partial-Breast Reirradiation for In-Breast Recurrence. *Int J Radiat Oncol Biol Phys* (2017) 98(5):1028–35. doi: 10.1016/j.ijrobp.2017.03.016
27. Jin ZY, Lu YJ, Chen WG, Li YF, Chen XS, Shen KW. Factor Analysis of Diagnosis and Surgical Treatment of Local Regional Recurrence in Breast Cancer Patients. *Zhonghua Wai Ke Za Zhi* (2019) 57(5):366–72. doi: 10.3760/cma.j.issn.0529-5815.2019.05.009
28. He J, Zhang JX, Chen CT, Ma Y, De Guzman R, Meng J, et al. The Relative Importance of Clinical and Socio-Demographic Variables in Prognostic Prediction in Non-Small Cell Lung Cancer: A Variable Importance Approach. *Med Care* (2020) 58(5):461–7. doi: 10.1097/MLR.0000000000001288
29. van Giessen A, Peters J, Wilcher B, Hyde C, Moons C, de Wit A, et al. Systematic Review of Health Economic Impact Evaluations of Risk Prediction Models: Stop Developing, Start Evaluating. *Value Health* (2017) 20(4):718–26. doi: 10.1016/j.jval.2017.01.001
30. Pedersen AN, Moller S, Steffensen KD, Haahr V, Jensen M, Kempel MM, et al. Supraclavicular Recurrence After Early Breast Cancer: A Curable Condition? *Breast Cancer Res Treat* (2011) 125(3):815–22. doi: 10.1007/s10549-010-0918-8
31. Kuo SH, Huang CS, Kuo WH, Cheng AL, Chang KJ, Chia-Hsien Cheng J. Comprehensive Locoregional Treatment and Systemic Therapy for Postmastectomy Isolated Locoregional Recurrence. *Int J Radiat Oncol Biol Phys* (2008) 72(5):1456–64. doi: 10.1016/j.ijrobp.2008.03.042
32. Wadasadawala T, Vadgaonkar R, Bajpai J. Management of Isolated Locoregional Recurrences in Breast Cancer: A Review of Local and Systemic Modalities. *Clin Breast Cancer* (2017) 17(7):493–502. doi: 10.1016/j.clbc.2017.03.008
33. Wapnir IL, Price KN, Anderson SJ, Robidoux A, Martin M, Nortier JWR, et al. Efficacy of Chemotherapy for ER-Negative and ER-Positive Isolated Locoregional Recurrence of Breast Cancer: Final Analysis of the CALOR Trial. *J Clin Oncol Off J Am Soc Clin Oncol* (2018) 36(11):1073–9. doi: 10.1200/JCO.2017.76.5719
34. Waeber M, Castiglione-Gertsch M, Dietrich D, Thurlimann B, Goldhirsch A, Brunner KW, et al. Adjuvant Therapy After Excision and Radiation of Isolated Postmastectomy Locoregional Breast Cancer Recurrence: Definitive Results of a Phase III Randomized Trial (SAKK 23/82) Comparing Tamoxifen With Observation. *Ann Oncol Off J Eur Soc Med Oncol* (2003) 14(8):1215–21. doi: 10.1093/annonc/mdg347

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 09 February 2022
doi: 10.3389/fonc.2022.803804

A Prospective Self-Controlled Study of Indocyanine Green, Radioisotope, and Methylene Blue for Combined Imaging of Axillary Sentinel Lymph Nodes in Breast Cancer

Yuting Jin[†], *Long Yuan*[†], *Yi Zhang*, *Peng Tang*, *Ying Yang*, *Linjun Fan*, *Li Chen*, *Xiaowei Qi*[*] *and Jun Jiang*[*]

Department of Breast Surgery, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China

OPEN ACCESS

Edited by:
Yiding Chen,
Zhejiang University, China

Reviewed by:
Tomoharu Sugie,
Kansai Medical University Hospital, Japan
Zhenggui Du,
Sichuan University, China

***Correspondence:**
Jun Jiang
jcbd@medmail.com.cn
Xiaowei Qi
qxw9908@foxmail.com

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *28 October 2021*
Accepted: *10 January 2022*
Published: *09 February 2022*

Citation:
Jin Y, Yuan L, Zhang Y, Tang P, Yang Y, Fan L, Chen L, Qi X and Jiang J (2022) A Prospective Self-Controlled Study of Indocyanine Green, Radioisotope, and Methylene Blue for Combined Imaging of Axillary Sentinel Lymph Nodes in Breast Cancer. Front. Oncol. 12:803804. doi: 10.3389/fonc.2022.803804

Purpose: This self-controlled study aimed to clarify whether indocyanine green (ICG) could be an alternative tracer in the absence of radioisotope (RI) for combined imaging of axillary sentinel lymph node (SLN) in breast cancer.

Methods: Primary breast cancer, clinically axillary node-negative patients (n = 182) were prospectively enrolled from March 2015 to November 2020. ICG, methylene blue (MB), and RI were used to perform axillary sentinel lymph node biopsy (SLNB). The main observation index was the positivity of ICG + MB vs. RI + MB in axillary SLNB; the secondary observation indicators were the axillary SLN detection rate, mean number of axillary SLNs detected, mean number of metastatic axillary SLNs detected, and safety.

Results: All 182 patients had axillary SLNs; a total of 925 axillary SLNs were detected. Pathological examination confirmed metastatic axillary SLN in 42 patients (total of 79 metastatic SLNs). Positivity, detection rate of SLNs, detection rate of metastatic SLNs, and the number of metastatic SLNs detected were comparable with RI+MB and ICG+MB ($p > 0.05$). The mean number of axillary SLNs detected was significantly higher with ICG +MB than with RI+MB (4.99 ± 2.42 vs. 4.02 ± 2.33, $p < 0.001$). No tracer-related adverse events occurred.

Conclusions: ICG appears to be a safe and effective axillary SLN tracer, and a feasible alternative to RI in combined imaging for axillary SLN of breast cancer.

Keywords: breast cancer, indocyanine green, radioisotope, combined imaging, sentinel lymph node biopsy

INTRODUCTION

Sentinel lymph node biopsy (SLNB) for breast cancer is a minimally invasive technique that can provide accurate axillary staging (1, 2). When SLNB is negative, axillary lymph node dissection (ALND) (1, 3, 4) can be avoided, and the patient is spared the suffering caused by complications such as upper limb lymph edema, nerve damage, local pain, numbness, and shoulder stiffness (1).

A key factor for the accuracy of axillary SLNB is the tracer that is used (5). Currently, the commonly used tracers are radioisotope (RI), blue dye, and RI plus blue dye (dual-tracer method). The American Society of Clinical Oncology (ASCO) recommends the dual tracer method for axillary SLNB because of its high detection rate (>90%), low false-negative rate (5%–10%), and the short learning curve (6). However, high cost, radiation exposure, relatively complicated surgical preparations, painful preoperative injections, and the need for nuclear medicine personnel are some of the disadvantages associated with RI use (7). In addition, the RI method cannot provide clear and intuitive visual guidance during the surgery and may therefore impair the surgeon's ability to locate the sentinel lymph node. With the increasing demand for day surgery, the relatively long time taken for the application of RI tracing cannot be ignored either (8). Therefore, there is much ongoing research to identify lymphatic tracers that could replace RI.

In recent years, indocyanine green (ICG), a common near-infrared fluorescent tracer, has been increasingly used in axillary SLNB; the advantages are lack of radiation exposure, ease of use, and a short learning curve. The passage of ICG through the lymphatic vessels can be visualized in real time, greatly facilitating surgery and shortening operation time. There is also no "blue tattooing" or blue dye pollution in the operation area (9, 10). The success rate with ICG has been found to be higher than that with blue dye and comparable to that with RI (11). In a previous prospective controlled study, we obtained comparable results with the combination of ICG plus methylene blue (MB) and RI plus MB (12). However, some authors have found that body mass index is significantly related to the detection of ICG fluorescence in skin lymphatic vessels, and age and obesity may reduce the probability of successful axillary SLNB (3, 13–16).

This prospective study aimed to clarify whether ICG could be an alternative tracer in the absence of RI for axillary SLN mapping in breast cancer. We compared the efficacies of ICG +MB with RI+MB for detection of axillary SLN in early breast cancer patients; to avoid the effects of BMI, age, and anatomical variations of the lymphatic system, we adopted a self-controlled protocol.

PATIENTS AND METHODS

Patients

Between March 2015 and November 2020, a total of 182 patients with primary breast cancer scheduled to undergo axillary SLNB were enrolled in this study. All patients had diagnosis confirmed by core needle biopsy and were clinically and radiologically lymph node negative. Patients with clinically or radiologically suspicious lymph nodes, inflammatory breast cancer, distant metastases, previous axillary surgery, or hypersensitivity to iodine or ICG were excluded from the study (**Figure 1**).

This study was approved by the Medical Ethics Committee of the Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China (clinical trial registration no. ChiCTR2000030729). All patients signed informed consent forms before the operation. This study strictly followed the Declaration of Helsinki and relevant clinical trial specifications, laws, and regulations.

Reagents and Equipment

The tracers used in this study were 1% MB solution (Jumpcan Pharmaceutical Group Co., Ltd., Taixing, China); 99Tcm-colloids (3.7×10^7 Bq, Shihong Pharmaceutical, Beijing, China); and 1.25% ICG solution (Dandong Pharmaceutical Co., Ltd., Liaoning, China). The fluorescent vascular imaging system (MDM-I, Mingde, Langfang, China) and Neo2000 Gamma Detection System (Neoprobe Corporation, OH, USA) were used to detect the signal of ICG-positive and RI-positive lymph nodes.

Procedure

This study was a self-controlled trial. Confounding factors such as BMI, age, and individual differences in lymphatic anatomy

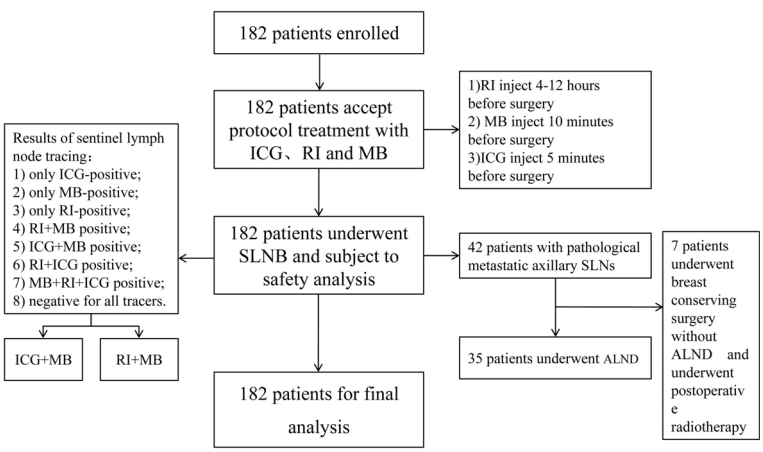


FIGURE 1 | Flow diagram.

were reduced by the use of three different tracers, indocyanine green, radioisotope, and methylene blue, in the same patient. The tracers were injected into the subareolar area; 1 ml of RI was injected subdermally 4–12 h before surgery, 1 ml of 1% MB was injected subdermally in the disinfected periareolar region 10 min before surgery, and 1 ml of 1.25% ICG was injected intradermally 5 min before surgery, followed by massage for another 5 min (12). Lymph nodes were detected by gamma detector, naked eyes and fluorescent detector, respectively (**Figure 2**). The lights of the operating area should be turned off when detecting lymphatic and lymph nodes by a fluorescent detector. Axillary SLNB was performed by experienced surgeons following standard operating procedures. Patients with negative axillary SLN on intraoperative frozen section examination did not undergo ALND. For patients with definite axillary SLN metastasis or indeterminate results, the decision on whether to proceed with ALND was according to the current guidelines and the patient's preoperatively expressed preference.

MB-positive lymph nodes were those that appeared blue to the naked eye or those with blue-stained lymph vessels entering them. RI-positive lymph nodes were those in which the gamma detector showed threshold count >10% of the maximum lymph node count (17). ICG-positive lymph nodes were those that showed fluorescent bright spots on the fluorescence imager. All tracer-positive lymph nodes were removed during the resection; other suspicious lymph nodes (large, hard) were also removed. The "tracer status" of the removed lymph nodes was recorded before they were sent for intraoperative frozen section examination. There were eight possible tracer combinations for each lymph node: 1) only ICG-positive; 2) only MB-positive; 3) only RI-positive; 4) RI+MB positive; 5) ICG+MB positive; 6) RI+ICG positive; 7) MB+RI+ICG positive; or 8) negative for all tracers.

For the purpose of this analysis, the total number of axillary SLNs detected was defined as the tracer-positive lymph nodes (18).

Pathological Examination and Postoperative Treatment

Isolated tumor cells (ITCs) refer to tumor lesions in lymph nodes with a diameter less than 0.2 mm or tumor cells in a single section with a diameter less than 0.2 mm. Micrometastasis was defined as the tumor metastasis with the largest diameter more than 0.2 mm but no more than 2 mm. Metastatic lymph nodes with a maximum diameter of tumor metastasis more than 2 mm were considered to be macrometastases. For patients who did not receive neoadjuvant chemotherapy, metastatic axillary SLNs were those with macrometastasis and micrometastasis; ITCs and no metastasis were defined as axillary SLN negative. For patients who received neoadjuvant chemotherapy, metastatic axillary SLN were those with axillary SLN macrometastasis, micrometastasis, and ITCs; no metastasis was defined as negative. Diagnosis and treatment were according to National Comprehensive Cancer Network (NCCN) guidelines and the Chinese Anti-Cancer Association guidelines for breast cancer.

Tracer-related complications occurring within 1 week of injection of the tracer were recorded; the complications included regional or systemic allergic reactions, infection at the injection site, and serious adverse events requiring clinical treatment or causing disability or death.

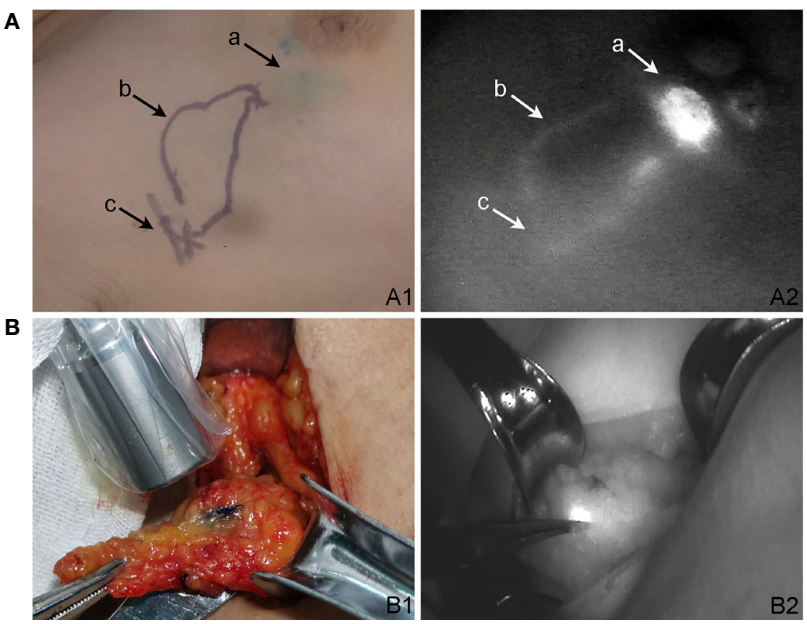


FIGURE 2 | Detection of tracer-positive lymph node. **(A)** ICG skin development. **(A1)** ICG percutaneous lymphography and sentinel lymph node localization. **(A2)** Lymphatic pathway under the fluorescent detector. (a) ICG injection location. (b) Lymphatic vessels highlighted by ICG. (c) Sentinel lymph node location. **(B)** The development of sentinel lymph nodes after subcutaneous incision. **(B1)** Blue-stained lymph node was detected by the γ detector. **(B2)** The same lymph node was ICG positive under the fluorescent detector."

Statistical Analysis

Positivity was defined as the number of patients with metastatic SLN detected by a tracer or combination divided by the total number of patients with metastatic SLN. Axillary SLN detection rate was defined as the number of patients with SLNs detected by one or more of the tracers divided by the total number of patients. Patients with negative axillary SLN did not undergo ALND surgery, and so the true positive rate, true negative rate, positive predictive value, and negative predictive value could not be calculated (8). The false-negative rate is difficult to calculate either. The main observation index was positivity. The secondary observation indicators were the axillary SLN detection rate, the mean number of axillary SLNs detected, the mean number of metastatic axillary SLNs detected, and safety.

The paired chi-square test was used to compare positivity, total axillary SLN detection rate, and metastatic axillary SLN detection rate. The paired t test was used to compare the number of axillary SLNs detected and the number of metastatic axillary SLNs detected. Average values are presented as mean ± SD. Because of the multiple comparisons involved, corrected statistical significance was set at $p < 0.002$ (α/number of comparisons, $\alpha = 0.05$). SPSS 25.0 (IBM Corp., Armonk, NY, USA) and Microsoft Office Excel 2007 were used for statistical analysis.

RESULTS

Demographics and Tumor Characteristics

A total of 182 patients (median age, 48 years; age range, 31–74 years) were enrolled in this study. The mean BMI was 23.5 kg/m^2 (range, 18.4–42.4 kg/m^2). The tumor was located in the upper outer quadrant in 38.6% of patients. Neoadjuvant chemotherapy was administered to 30 (16.5%) patients. 42 patients (24.2%) had metastatic axillary SLNs, ALND was performed for 35 (19.2%) patients, and 7 patients with metastatic axillary SLNs who underwent breast conserving surgery did not receive ALND according to the results of the Z0011 study (19). No patient underwent a second operation due to inconsistent pathological examinations. **Table 1** summarizes the clinicopathological characteristics of the patients.

TABLE 1 | Demographic and clinical characteristics of the 182 patients.

Characteristic		n	%
Age (years)	<50	104	57.1
	≥50	78	42.9
Menopausal status	Premenopausal	109	59.9
	Postmenopausal	73	40.1
BMI[a] (kg/m^2)	<24	106	58.2
	≥24	76	41.8
Tumor side	Left	100	54.9
	Right	82	45.1
Tumor location	Upper outer quadrant	66	36.3
	Lower outer quadrant	30	16.5
	Upper inner quadrant	48	26.4
	Lower inner quadrant	17	9.3
	Nipple–areolar area	21	11.5
Neoadjuvant chemotherapy	Yes	30	16.5
	No	152	83.5
Histological type	*In situ*	13	7.1
	Invasive non-specific cancer	153	84.1
	Invasive specific cancer	16	8.8
T stage	Tis[b]	13	7.1
	T1	90	49.5
	T2	78	42.9
	T3	1	0.5
Type	Luminal A	54	29.7
	Luminal B	47	25.8
	Her-2 positive	57	31.3
	TNBC[c]	24	13.2
Breast surgery	Mastectomy	130	71.4
	Lumpectomy	52	28.6
Axillary surgery	SLNB[d]	147	80.8
	SLNB+ALND[e]	35	19.2

[a]*Body mass index.*
[b]*Tumor in situ.*
[c]*Triple-negative breast cancer.*
[d]*Sentinel lymph node biopsy.*
[e]*Axillary lymph node dissection.*

Sentinel Lymph Node Tracer Status

All 182 patients had axillary SLNs. A total of 925 axillary SLNs were detected (mean, 5.1 per patient). In two patients, the detected SLNs were only ICG positive; in both cases, pathology showed lymph node metastases. In 178 patients, there was obvious percutaneous lymphography and the SLNs were ICG positive; thus, the detection rate with ICG alone was 97.8% (178/182). The detection rate with RI+MB was 98.8% (180/182), while the detection rate with ICG+MB was 100% (182/182; **Table 2**).

Pathological examination confirmed metastatic SLNs in 42 patients (a total of 79 metastatic axillary SLNs). In 32 patients, the metastatic nodes were successfully detected by ICG, MB, and RI. The positivity was 90.5% (38/42) for both ICG and RI, and 83.3% (35/42) for MB.

ICG+MB vs. RI+MB

Among 42 patients with metastatic axillary SLNs, 37 patients were detected by both ICG+MB and RI+MB, 3 patients were detected only by ICG+MB, and 2 patients were detected only by RI+MB. RI +MB detected 68 metastatic SLNs in 39 patients, and ICG+MB detected 74 metastatic SLNs in 40 patients; the proportion of patients with SLN metastases identified by the two methods was not significantly different (92.9% (39/42) vs. 95.2% (40/42), $p = 1.000$; **Table 3**), and the mean number of metastatic SLNs detected by the two methods was not significantly different (0.37 ± 0.88 vs. 0.41 ± 1.01, $p = 0.332$). The overall detection rate was not significantly different with the two methods: 100% (182/182) with ICG+MB vs. 98.9% (178/182) with RI+MB. The mean number of axillary SLNs detected was higher with ICG+MB than with RI+MB (4.99 ± 2.42 vs. 4.02 ± 2.34, $p < 0.001$).

The metastatic SLN detection rate based on SLNs with ICG+MB was no less than that of RI+MB (93.7% (74/79) vs. 86.1% (68/79), $p = 0.114$). ICG+MB detected a total of 909 axillary SLNs, and RI +MB detected a total of 732 axillary SLNs. Therefore, the SLN detection rate based on SLNs with ICG+MB was higher than that of RI+MB (98.3% (909/925) vs. 79.1% (732/925), $p = 0.000$).

In addition, we performed a subgroup analysis. Thirty neoadjuvant chemotherapy patients and 152 non-neoadjuvant

TABLE 2 | Axillary sentinel lymph node tracer status.

Category	Positivity[d]	SLN Detection Rate	SLN Number	Metastatic SLN Number
ICG[a]	90.5% (38/42)	97.8% (178/182)	4.63 ± 2.51	0.37 ± 0.98
MB[b]	83.3% (35/42)	89.6% (163/182)	3.12 ± 2.48	0.29 ± 0.76
RI[c]	90.5% (38/42)	94.5% (172/182)	3.30 ± 2.10	0.30 ± 0.72
RI+MB	92.9% (39/42)	98.9% (180/182)	4.02 ± 2.34	0.37 ± 0.88
ICG+MB	95.2% (40/42)	100% (182/182)	4.99 ± 2.42	0.41 ± 1.01
RI+ICG	100% (42/42)	100% (182/182)	4.93 ± 2.41	0.41 ± 1.01
ICG+MB+RI	100% (42/42)	100% (182/182)	5.08 ± 2.41	0.43 ± 1.04

[a]*Indocyanine green.*
[b]*Methylene blue.*
[c]*Radioisotope.*
[d]*Positivity was defined as the number of patients with metastatic SLNs detected by one or more of the tracers divided by the total number of patients with metastatic SLN.*

TABLE 3 | Comparison of efficacies of ICG+MB and RI+MB.

Efficacy Parameter	ICG[a]+MB[b]	RI[c]+MB	*p*
Positivity[d]	95.2% (40/42)	92.9% (39/42)	1.000
SLN detection rate	100% (182/182)	98.9% (180/182)	0.480
Metastatic SLN number	0.41 ± 1.01	0.37 ± 0.88	0.332
SLN number	4.99 ± 2.42	4.02 ± 2.34	0.000

[a]*Indocyanine green.*
[b]*Methylene blue.*
[c]*Radioisotope.*
[d]*Positivity was defined as the number of patients with metastatic SLNs detected by one or more of the tracers divided by the total number of patients with metastatic SLNs.*

chemotherapy patients were analyzed and compared in terms of positivity, SLN detection rate, SLN number, and metastatic SLN number. The results showed that the positivity and SLN detection rates of ICG+MB and RI+MB were equal (100%) in 30 patients after neoadjuvant chemotherapy. Similarly, the positivity and SLN detection rates of ICG+MB and RI+MB were 100% in 30 neoadjuvant chemotherapy patients compared with 152 non-neoadjuvant chemotherapy patients. The SLN number and metastatic SLN number in the neoadjuvant chemotherapy group were smaller than those in the non-neoadjuvant chemotherapy group (4.37 ± 2.47 vs. 5.22 ± 2.38, $p = 0.075$; 0.23 ± 0.57 vs. 0.47 ± 1.10, $p = 0.247$). SLN number with the RI+MB method in the neoadjuvant chemotherapy group was smaller than that in the non-neoadjuvant chemotherapy group (3.07 ± 1.96 vs. 4.21 ± 2.36, $p = 0.014$). However, SLN number with the ICG+MB method showed no significant difference between the neoadjuvant chemotherapy group and the non-neoadjuvant chemotherapy group (4.30 ± 2.45 vs. 5.13 ± 2.40, $p = 0.086$). For neoadjuvant chemotherapy patients, SLN number with the ICG+MB method was greater than that with the RI+MB method (4.30 ± 2.45 vs. 3.07 ± 1.96, $p = 0.036$).

Safety

No patient had tracer-related local or systemic allergic reactions, injection site infection, or any serious adverse events.

DISCUSSION

In patients with early breast cancer, axillary SLNB is currently the standard method to assess tumor spread to the axilla. In a survey conducted in China, among 110 hospitals performing >200 breast cancer surgeries per year, the majority (69/110, 62.73%) used dye (mainly MB) as the tracer for SLNB; the dual tracer of RI+MB was used only in 16/110 (14.55%) hospitals, probably because of the limited availability of RI tracers (20). Several authors have found ICG to be an excellent tracer in terms of safety, feasibility, and accuracy (21–26). The feasibility of replacing RI with ICG for axillary SLN tracing in breast cancer is currently a hot topic of research.

Recognizing that obesity, age, anatomy, and other factors may affect the success rate of ICG axillary SLN tracing, we adopted a self-controlled protocol to compare the efficacy of different tracers. No significant difference was found between ICG+MB and RI+MB in positivity, axillary SLN detection rate, metastatic axillary SLN detection rate, and number of metastatic axillary SLNs detected. In addition, we compared the effect of the two tracer methods based on the number of lymph nodes. The results showed that ICG+MB was no less than RI+MB, and even higher than RI+MB in terms of detection rate (based on lymph node calculation). These findings are consistent with most previous reports (12, 21, 22, 27, 28). The ICG+MB method detected a significantly larger number of axillary SLNs and may be able to reduce the false-negative rate (29). As far as we know, this is the largest self-controlled study to date comparing the ICG+MB method and the RI+MB method.

The advantages of the RI include longer concentration of radioactivity in the SLN and easier operation, and it allows the surgeon to find the "hot spot" without cutting through the skin, which is not possible with the biological dye method. However, due to radiological contamination of RI and inconvenience of use, it cannot be widely applied in hospitals in China. Near-infrared imaging provides γ-ray tissue penetration, without exposing the patient to radiation. Another advantage is that the cost of ICG is about 21.9% that of RI (8). In our hospital, no matter how many tracers are used, the operation fee is only charged once. In terms of patient costs, patients using the ICG tracer spent less than a third of the cost of RI. The vascular fluorescence imager can provide real-time guidance and greatly reduce the difficulty of surgery; ICG tracing is therefore excellent for training of young breast surgeons (30–32).

In 2020, Goonawardena et al. (23) conducted a systematic review of the application of ICG and RI for breast cancer axillary SLN tracing and reported a detection rate and sensitivity of

81.9%–100% and 65.2%–100%, respectively. They found no significant difference in the detection rate and sensitivity between ICG and RI. In the current study, the detection rate and positivity with ICG were 97.8% and 90.5%, respectively, which is consistent with the results of Goonawardena et al. Although the detection rate with ICG is high, there are disadvantages associated with the use of ICG alone. First, accidental cutting of lymphatic vessels could result in fluorescent pollution of the operative area and make it difficult to identify the truly metastatic axillary lymph nodes and, thereby, also increase procedure time (33). Second, ICG fluorescence penetrance is only about 1 cm, and so metastatic SLN in some deep axillary regions may be missed (5, 34). Using the combination of MB and ICG can solve these problems. Previous studies have shown that the detection time for each axillary SLN is significantly shorter, and the number of axillary SLNs detected significantly more, with ICG+MB than with ICG alone (28, 33).

In this study, the ICG+MB method detected 100% of axillary SLNs. Our result is consistent with a study from Japan, which reported a detection rate >99% (35). In our study, the positivity of ICG+MB was 95.2%, which is similar to the 94.4% reported from our previous study (12). Positivity and the metastatic SLN detection rate were also close to the results of a recent study in China (95.2% vs. 96.6%; 93.7% vs. 94.1%) (36). Interestingly, we found that the number of axillary SLNs detected was higher with ICG+MB than with RI+MB. This has not been reported by other authors. According to previous reports, the mean number of axillary SLNs detected is higher with ICG (1.3–5.4 per patient) than with MB or RI (11, 23, 29, 37–39). Our study also found that a higher number of SLNs were detected by ICG (mean, 4.6 by ICG vs. 3.3 by RI). The explanation may be that the ICG molecules are smaller and so more easily migrate through the lymphatic system; further, fluorescence imaging sensitivity is higher (37, 40). In recent years, surgeons have studied the risk factors for axillary non-sentinel lymph node (nSLN) metastases in patients with axillary SLN metastases. It was found that the number of SLN metastases is an independent risk factor for axillary nSLN metastases (41). Therefore, it is more important to remove multiple SLNs rather than single or two SLNs, especially for surgeons following the Z0011 trial. Direct comparison between ICG+MB method and RI+MB method showed that the efficacy of the ICG+MB method is no less than that of the RI +MB method; this finding is consistent with the results of our previous research (12).

Some scholars consider that neoadjuvant chemotherapy may affect the lymphatic vessels of patients. The neoadjuvant chemotherapy patients enrolled in our study were all patients who had clinically negative axillary lymph nodes examined before surgery and had no downstaging of axillary lymph nodes after neoadjuvant chemotherapy. Moreover, 30 patients with neoadjuvant chemotherapy not only met the requirements of neoadjuvant chemotherapy guidelines but also met the guidelines of sentinel lymph node biopsy (42, 43). Secondly, we further conducted subgroup analysis and compared the positive rate, SLN detection rate, number of SLNs detected, and number of SLN metastases. Our results showed that the introduction of ICG could reduce the impact of neoadjuvant chemotherapy on the number of SLNs detected. In addition, many studies on sentinel lymph node tracers did not include patients with neoadjuvant chemotherapy, which is also one of the characteristics of our study.

In two patients in the present study, the detected axillary SLNs were only ICG positive, but pathological examination confirmed metastases. This suggests that the use of ICG+MB +RI might reduce the false-negative rate with the RI+MB method. However, when using ICG as a tracer, great care must be taken to avoid intraoperative ICG leakage as it may negate the benefits of fluorescence imaging. Exploration should be carried out only after opening the axillary fascia; blind exploration in the fat tissue must be strictly avoided (5).

This study has limitations. First, there was no long-term follow-up, and so data on postoperative recurrence, metastasis, and survival were not available. Second, this was a single-center study with a relatively small sample size; thus, although the data suggest that ICG is a feasible alternative to RI, the findings need to be confirmed in large randomized trials.

CONCLUSION

ICG appears to be a reliable, safe, intuitive, and effective tracer for axillary SLNB in patients with breast cancer. Our previous study and the present study indicated that ICG+MB could offer comparable performance compared to RI+MB in SLN mapping. Therefore, ICG may be the preferred method when RI is not available or convenient to use. Multicenter clinical trials are warranted to further verify the current findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of the Southwest Hospital, Third Military Medical University (Army Medical University). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XQ and JJ had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: YJ, LY, YZ, XQ, JJ. Acquisition, analysis, or interpretation of data: YJ, LY, PT, YY, LF, LC. Drafting of the manuscript: YJ, LY. Critical revision of

the manuscript for important intellectual content: XQ, JJ. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by grants from the Chongqing Science and Health Joint Medical Research Project (No. 2019QNXM003, No. 2020FYYX027) Military Medical Staff Innovation Plan of Southwest Hospital (No. SWH2018BJLC-04), Army Medical University (No. XZ-2019-505-042), and Program of National Key Clinical Specialist Construction (No. 413F1Z113).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.803804/full#supplementary-material

REFERENCES

1. Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Costantino JP, et al. Sentinel-Lymph-Node Resection Compared With Conventional Axillary-Lymph-Node Dissection in Clinically Node-Negative Patients With Breast Cancer: Overall Survival Findings From the NSABP B-32 Randomised Phase 3 Trial. *Lancet Oncol* (2010) 11(10):927–33. doi: 10.1016/S1470-2045(10)70207-2
2. Park YH, Senkus-Konefka E, Im SA, Pentheroudakis G, Saji S, Gupta S, et al. Pan-Asian Adapted ESMO Clinical Practice Guidelines for the Management of Patients With Early Breast Cancer: A KSMO-ESMO Initiative Endorsed by CSCO, ISMPO, JSMO, MOS, SSO and TOS. *Ann Oncol* (2020) 4:451–69. doi: 10.1016/j.annonc.2020.01.008
3. Goyal A, Newcombe RG, Chhabra A, Mansel RE. Factors Affecting Failed Localisation and False-Negative Rates of Sentinel Node Biopsy in Breast Cancer–Results of the ALMANAC Validation Phase. *Breast Cancer Res Treat* (2006) 2:203–8. doi: 10.1007/s10549-006-9192-1
4. Qiu SQ, Zhang GJ, Jansen L, de Vries J, Schroder CP, de Vries E, et al. Evolution in Sentinel Lymph Node Biopsy in Breast Cancer. *Crit Rev Oncol Hematol* (2018) 123:83–94. doi: 10.1016/j.critrevonc.2017.09.010
5. Jiang J, Wang S. Guideline for Fluorescence Tracer Sentinel Lymph Node Biopsy of Breast Cancer. *Chin J Breast Dis (Electron Ed)* (2017) 04:193–7. doi: 10.3877/cma.j.issn.1674-0807.2017.04.001
6. Lyman GH, Giuliano AE, Somerfield MR, Benson AR, Bodurka DC, Burstein HJ, et al. American Society of Clinical Ncology Guideline Recommendations for Sentinel Lymph Node Biopsy in Early-Stage Breast Cancer. *J Clin Oncol* (2005) 23:7703–20. doi: 10.1200/JCO.2005.08.001
7. Krynyckyi BR, Miner M, Ragonese JM, Firestone M, Kim CK, Machac J. Technical Aspects of Performing Lymphoscintigraphy: Optimization of Methods Used to Obtain Images. *Clin Nucl Med* (2000) 12:978–85. doi: 10.1097/00003072-200012000-00003
8. Somashekhar SP, Kumar CR, Ashwin KR, Zaveri SS, Jampani A, Ramya Y, et al. Can Low-Cost Indo Cyanine Green Florescence Technique for Sentinel Lymph Node Biopsy Replace Dual Dye (Radio-Colloid and Blue Dye) Technique in Early Breast Cancer: A Prospective Two-Arm Comparative Study. *Clin Breast Cancer* (2020) 5:e576–83. doi: 10.1016/j.clbc.2020.03.013
9. Jung SY, Kim SK, Kim SW, Kwon Y, Lee ES, Kang HS, et al. Comparison of Sentinel Lymph Node Biopsy Guided by the Multimodal Method of Indocyanine Green Fluorescence, Radioisotope, and Blue Dye Versus the Radioisotope Method in Breast Cancer: A Randomized Controlled Trial. *Ann Surg Oncol* (2014) 4:1254–9. doi: 10.1245/s10434-013-3437-0
10. vanderVorst JR, Schaafsma BE, Verbeek FP, Hutteman M, Mieog JS, Lowik CW, et al. Randomized Comparison of Near-Infrared Fluorescence Imaging Using Indocyanine Green and 99(M) Technetium With Or Without Patent Blue for the Sentinel Lymph Node Procedure in Breast Cancer Patients. *Ann Surg Oncol* (2012) 13:4104–11. doi: 10.1245/s10434-012-2466-4
11. Ahmed M, Purushotham AD, Douek M. Novel Techniques for Sentinel Lymph Node Biopsy in Breast Cancer: A Systematic Review. *Lancet Oncol* (2014) 8:e351–62. doi: 10.1016/S1470-2045(13)70590-4
12. Yuan L, Qi X, Zhang Y, Yang X, Zhang F, Fan L, et al. Comparison of Sentinel Lymph Node Detection Performances Using Blue Dye in Conjunction With Indocyanine Green Or Radioisotope in Breast Cancer Patients: A Prospective Single-Center Randomized Study. *Cancer Biol Med* (2018) 4:452–60. doi: 10.20892/j.issn.2095-3941.2018.0270
13. Samorani D, Fogacci T, Panzini I, Frisoni G, Accardi FG, Ricci M, et al. The Use of Indocyanine Green to Detect Sentinel Nodes in Breast Cancer: A Prospective Study. *Eur J Surg Oncol* (2015) 1:64–70. doi: 10.1016/j.ejso.2014.10.047
14. Cao YM, Wang SH, Guo JJ, Yang DQ, Tong FZ, Zhou B, et al. Combination of ICG and Methylene Blue for Mapping Sentinel Lymph Nodes in Early Breast Cancer Patients. *Chin J Gen Surg* (2014) 02:119–22. doi: 10.3760/cma.j.issn.1007-631X.2014.02.013
15. Mazouni C, Koual M, De Leeuw F, Conversano A, Leymarie N, Rimareix F, et al. Prospective Evaluation of the Limitations of Near-Infrared Imaging in Detecting Axillary Sentinel Lymph Nodes in Primary Breast Cancer. *Breast J* (2018) 6:1006–9. doi: 10.1111/tbj.13123
16. Valente SA, Al-Hilli Z, Radford DM, Yanda C, Tu C, Grobmyer SR. Near Infrared Fluorescent Lymph Node Mapping With Indocyanine Green in Breast Cancer Patients: A Prospective Trial. *J Am Coll Surg* (2019) 4:672–8. doi: 10.1016/j.jamcollsurg.2018.12.001
17. Breast Cancer Professional Committee of Chinese Anti-Cancer Association. Guidelines and Norms for Diagnosis and Treatment of Breast Cancer by Chinese Anti-Cancer Association (2015 Edition). *Chn J Oncol* (2015) 09:692–754. doi: 10.3969/j.issn.1007-3969.2015.09.010
18. Wishart GC, Loh SW, Jones L, Benson JR. A Feasibility Study (ICG-10) of Indocyanine Green (ICG) Fluorescence Mapping for Sentinel Lymph Node Detection in Early Breast Cancer. *Eur J Surg Oncol* (2012) 8:651–6. doi: 10.1016/j.ejso.2012.05.007
19. Giuliano AE, Hunt KK, Ballman KV, Beitsch PD, Whitworth PW, Blumencranz PW, et al. Axillary Dissection Vs No Axillary Dissection in Women With Invasive Breast Cancer and Sentinel Node Metastasis: A Randomized Clinical Trial. *JAMA* (2011) 6:569–75. doi: 10.1001/jama.2011.90
20. Guo R, Li L, Zhang Q, Xiu BQ, Yang BL, Wang J, et al. Current Status of Sentinel Lymph Node Biopsy for Breast Cancer in China: A Cross-Sectional Study. *Chn J Oncol* (2020) 03:166–73. doi: 10.19401/j.cnki.1007-3639.2020.03.002
21. Kelley LM, Holmes DR. Tracer Agents for the Detection of Sentinel Lymph Nodes in Breast Cancer: Current Concerns and Directions for the Future. *J Surg Oncol* (2011) 1:91–6. doi: 10.1002/jso.21791
22. Yuan L, Zhou Y, Hu Y, Wei HY, Pan QW, Zhang KY, et al. Indocyanine Green Combined With Methylene Blue for Sentinel Lymph Node Biopsy in Breast Cancer Patients. *Chin J Breast Dis (Electron Ed)* (2016) 02:87–91. doi: 10.1016/j.ejso.2012.05.007
23. Goonawardena J, Yong C, Law M. Use of Indocyanine Green Fluorescence Compared to Radioisotope for Sentinel Lymph Node Biopsy in Early-Stage Breast Cancer: Systematic Review and Meta-Analysis. *Am J Surg* (2020) 3:665–76. doi: 10.1016/j.breast.2020.01.016
24. Ballardini B, Santoro L, Sangalli C, Gentilini O, Renne G, Lissidini G, et al. The Indocyanine Green Method is Equivalent to the (99m)Tc-Labeled Radiotracer Method for Identifying the Sentinel Node in Breast Cancer: A Concordance and Validation Study. *Eur J Surg Oncol* (2013) 12:1332–6. doi: 10.1016/j.ejso.2013.10.004
25. Vermersch C, Raia-Barjat T, Chapelle C, Lima S, Chauleur C. Randomized Comparison Between Indocyanine Green Fluorescence Plus (99M) Technetium and (99M)Technetium Alone Methods for Sentinel Lymph Node Biopsy in Breast Cancer. *Sci Rep* (2019) 1:6943. doi: 10.1038/s41598-019-43473-3
26. Sugie T, Kinoshita T, Masuda N, Sawada T, Yamauchi A, Kuroi K, et al. Evaluation of the Clinical Utility of the ICG Fluorescence Method Compared

With the Radioisotope Method for Sentinel Lymph Node Biopsy in Breast Cancer. *Ann Surg Oncol* (2016) 1:44–50. doi: 10.1245/s10434-015-4809-4
27. He K, Chi C, Kou D, Huang W, Wu J, Wang Y, et al. Comparison Between the Indocyanine Green Fluorescence and Blue Dye Methods for Sentinel Lymph Node Biopsy Using Novel Fluorescence Image-Guided Resection Equipment in Different Types of Hospitals. *Transl Res* (2016) 178:74–80. doi: 10.1016/j.trsl.2016.07.010
28. Guo J, Yang H, Wang S, Cao Y, Liu M, Xie F, et al. Comparison of Sentinel Lymph Node Biopsy Guided by Indocyanine Green, Blue Dye, and Their Combination in Breast Cancer Patients: A Prospective Cohort Study. *World J Surg Oncol* (2017) 1:196. doi: 10.1186/s12957-017-1264-7
29. Ban EJ, Lee JS, Koo JS, Park S, Kim SI, Park BW. How Many Sentinel Lymph Nodes are Enough for Accurate Axillary Staging in T1-2 Breast Cancer? *J Breast Cancer* (2011) 4:296–300. doi: 10.4048/jbc.2011.14.4.296
30. Toh U, Iwakuma N, Mishima M, Okabe M, Nakagawa S, Akagi Y. Navigation Surgery for Intraoperative Sentinel Lymph Node Detection Using Indocyanine Green (ICG) Fluorescence Real-Time Imaging in Breast Cancer. *Breast Cancer Res Treat* (2015) 2:337–44. doi: 10.1007/s10549-015-3542-9
31. Shen S, Xu Q, Zhou Y, Mao F, Guan J, Sun Q. Comparison of Sentinel Lymph Node Biopsy Guided by Blue Dye With Or Without Indocyanine Green in Early Breast Cancer. *J Surg Oncol* (2018) 8:1841–7. doi: 10.1002/jso.25058
32. Verbeek FP, Troyan SL, Mieog JS, Liefers GJ, Moffitt LA, Rosenberg M, et al. Near-Infrared Fluorescence Sentinel Lymph Node Mapping in Breast Cancer: A Multicenter Experience. *Breast Cancer Res Treat* (2014) 2:333–42. doi: 10.1007/s10549-013-2802-9
33. Ji Y, Luo N, Jiang Y, Li Q, Wei W, Yang H, et al. Clinical Utility of the Additional Use of Blue Dye for Indocyanine Green for Sentinel Node Biopsy in Breast Cancer. *J Surg Res* (2017) 215:88–92. doi: 10.1016/j.jss.2017.03.038
34. Mok CW, Tan SM, Zheng Q, Shi L. Network Meta-Analysis of Novel and Conventional Sentinel Lymph Node Biopsy Techniques in Breast Cancer. *BJS Open* (2019) 4:445–52. doi: 10.1002/bjs5.50157
35. Sugie T, Kassim KA, Takeuchi M, Hashimoto T, Yamagami K, Masai Y, et al. A Novel Method for Sentinel Lymph Node Biopsy by Indocyanine Green Fluorescence Technique in Breast Cancer. *Cancers (Basel)* (2010) 2:713–20. doi: 10.3390/cancers2020713
36. Wang C, Tong F, Cao Y, Liu P, Zhou B, Liu H, et al. Long-Term Follow-Up Results of Fluorescence and Blue Dye Guided Sentinel Lymph Node Biopsy in Early Breast Cancer. *Breast Cancer Res Treat* (2021) 188:361–8. doi: 10.21203/rs.3.rs-229024/v1
37. Sugie T, Sawada T, Tagaya N, Kinoshita T, Yamagami K, Suwa H, et al. Comparison of the Indocyanine Green Fluorescence and Blue Dye Methods in Detection of Sentinel Lymph Nodes in Early-Stage Breast Cancer. *Ann Surg Oncol* (2013) 7:2213–8. doi: 10.1245/s10434-013-2890-0
38. Sugie T, Ikeda T, Kawaguchi A, Shimizu A, Toi M. Sentinel Lymph Node Biopsy Using Indocyanine Green Fluorescence in Early-Stage Breast Cancer: A Meta-Analysis. *Int J Clin Oncol* (2017) 1:11–7. doi: 10.1007/s10147-016-1064-z
39. Zada A, Peek MC, Ahmed M, Anninga B, Baker R, Kusakabe M, et al. Meta-Analysis of Sentinel Lymph Node Biopsy in Breast Cancer Using the Magnetic Technique. *Br J Surg* (2016) 11:1409–19. doi: 10.1002/bjs.10283
40. Inoue T, Nishi T, Nakano Y, Nishimae A, Sawai Y, Yamasaki M, et al. Axillary Lymph Node Recurrence After Sentinel Lymph Node Biopsy Performed Using a Combination of Indocyanine Green Fluorescence and the Blue Dye Method in Early Breast Cancer. *Breast Cancer Tokyo* (2016) 2:295–300. doi: 10.1007/s12282-014-0573-8
41. Maimaitiaili A, Wu D, Liu Z, Liu H, Muyiduli X, Fan Z. Analysis of Factors Related to Non-Sentinel Lymph Node Metastasis in 296 Sentinel Lymph Node-Positive Chinese Breast Cancer Patients. *Cancer Biol Med* (2018) 15 (3):282–9. doi: 10.20892/j.issn.2095-3941.2018.0023
42. Breast Cancer Professional Committee of Chinese Anti-Cancer Society. Chinese Association of Cancer Diagnosis and Treatment Guidelines for Breast Cancer (2013 Edition). *China Cancer J* (2013) 23(08):637–93. doi: 10.19401/j.cnki.1007-3639.2021.03.004
43. Gradishar WJ, Anderson BO, Blair SL, Burstein HJ, Cyr A, Elias AD, et al. Breast Cancer Version 3.2014. *J Natl Compr Canc Netw* (2014) 12(4):542–90. doi: 10.6004/jnccn.2014.0058

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

CLINICAL TRIAL
published: 10 February 2022
doi: 10.3389/fonc.2022.833742

The Effectiveness of Traditional Chinese Medicine Combined With Surgery to Treat Granulomatous Mastitis: A Propensity-Matched Analysis

Reziya Sawuer[1†], *Chunyu Wu*[1†], *Zhenping Sun*[1*] *and Sheng Liu*[2*]

[1] Department of Breast Surgery (Integrated Traditional and Western Medicine), Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [2] Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

OPEN ACCESS

Edited by:
Xiaosong Chen,
Shanghai Jiao Tong University, China

Reviewed by:
Chang Yao,
Nanjing University of Chinese Medicine, China
Jiayi Wu,
Shanghai Jiao Tong University, China

***Correspondence:**
Sheng Liu
sliu_tcm@163.com
Zhenping Sun
scptcm@126.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: 12 December 2021
Accepted: 17 January 2022
Published: 10 February 2022

Citation:
Sawuer R, Wu C, Sun Z and Liu S (2022) The Effectiveness of Traditional Chinese Medicine Combined With Surgery to Treat Granulomatous Mastitis: A Propensity-Matched Analysis. Front. Oncol. 12:833742. doi: 10.3389/fonc.2022.833742

Purpose: The etiology and pathology of granulomatous mastitis (GLM) are still unknown. Expert consensus on the treatment of GLM has not been developed. The objective of this study is to study the effectiveness of traditional Chinese medicine (TCM) combined with surgery in treating GLM.

Materials and Methods: A retrospective cohort study was implemented at Longhua Hospital of Shanghai University of Traditional Chinese Medicine in China between September 2019 and August 2021. Female patients were included according to the propensity-score matching (PSM) method and balanced according to age and BMI. Patients with GLM diagnosed by pathology and a course of disease ≥ 6 months were included in this trial. Patients were divided into the TCM alone group or TCM + surgery group.

Results: In total, 168 female patients were assessed and 102 patients were included in the study after PSM (51 in the TCM group and 51 in the TCM + surgery group). The average age of the patients was 32 years (21-47 years). There was no significant baseline characteristics difference between two groups after PSM. The suppuration rate in the TCM + surgery group was less than that in the TCM group (64.7% *vs.* 83.35%, $P < 0.05$), and the TCM + surgery group had a higher 9-month cure rate than the TCM group (86.3% *vs.* 52.9%, $P < 0.05$). The full course of disease in the TCM + surgery group was shorter than that in the TCM group (253.9 ± 117.3 days *vs.* 332.5 ± 111.6 days, $P < 0.05$).

Conclusions: TCM combined with surgery can improve the cure rate and shorten the full course of GLM treatment, indicating surgery should be integrated in the clinical management of GLM.

Keywords: granulomatous mastitis, surgery, traditional Chinese medicine, clinical outcome, integrated Chinese and Western surgery

Abbreviations: GLM, granulomatous mastitis; TCM, traditional Chinese medicine.

INTRODUCTION

Granulomatous mastitis (GLM), also known as idiopathic granulomatous mastitis and granulomatous mastitis, is an inflammation in which macrophages and neutrophils infiltrate the breast lobules to form necrotic granulomatous lesions, which was first described by Kessler et al. (1). The typical clinical manifestations of this disease include breast pain, breast mass, nipple depression, nipple overflow, axillary lymph node enlargement, nonlactating breast abscess, fistula, etc. (2, 3). There was no obvious specificity in the clinical manifestations of GLM. Thus, GLM is easily confused with inflammatory breast cancer, and the diagnosis of GLM mainly requires histopathological examination (4–6).

Because the etiology and pathology of GLM are unclear, there is no unified treatment plan to treat GLM. At present, the reported treatment includes surgical treatment and conservative treatments. Although the healing time of surgical treatment is short, the wound complications and recurrence rate are relatively high, which makes surgeons carefully choose this treatment (7, 8). Conservative treatments mainly include antibiotic treatment, steroid treatment, and traditional Chinese medicine (TCM) treatment. The effect of the antibiotic treatment is poor (9). Steroid therapy can cause complications, and serious side effects limit its long-term use (10). TCM treatment, used as a supplementary treatment for evidence-based diseases, has a significant therapeutic effect on inflammatory diseases (11, 12). Additionally, the therapeutic effect of TCM on GLM was recognized (13). A previous study suggested that the 9-month cure rate of TCM was 63%, which was equivalent to that of the TCM surgical treatment (68%) (14). Although the curative effect of TCM was apparent, the treatment time was relatively long. At present, although GLM is a benign lesion, there is no ideal treatment. This disease is difficult to cure, has a high recurrence rate, and causes a tremendous psychological burden to patients (15). Therefore, the present trial intends to study whether TCM combined with surgical treatment can improve the outcome of GLM using the propensity score matching method.

MATERIALS AND METHODS

Patients

A retrospective cohort study was performed at Longhua Hospital of Shanghai University of Traditional Chinese Medicine in China between September 2019 and August 2021. Female patients were included through strict inclusion and exclusion criteria screening.

Inclusion criteria: (I) GLM was diagnosed by pathology (based on the pathological diagnosis of Shanghai Longhua Hospital); (2) Course of disease ≥ 6 months; (3) Non pregnant and nonlactating female patients aged 18-45 years (including 18 and 45 years); (4) There was no severe heart, lung, liver and kidney dysfunction; (5) The patients voluntarily participated in this clinical study, did not participate in other clinical trials and signed informed consent.

Exclusion criteria: (I) patients with serious diseases (such as malignant tumors) or mental diseases that affect their survival; (2) diagnosis of systemic lupus erythematosus, rheumatism, or other known autoimmune diseases; (3) abnormal liver and kidney function (ALT, AST, BUN, etc.) exceeds 20% of the upper limit of normal value and other laboratory indices with clinical significance.

The nonexposure group was treated with oral and external applications of traditional Chinese medicine. The exposure group was treated with TCM combined with surgery. The small incision lesion resection and suture was employed for the surgery operation. Golden ointment was used for external application. The drug use was adjusted appropriately according to the patient's condition. Oral and external application of traditional Chinese medicine continued until recovery. For the exposure group, oral and external application of TCM was used until no obvious redness, swelling and pain were found in the mass. The patient was then admitted to the hospital for small incision resection and suture. After the operation, the above TCM was taken until the patient recovered.

The formula of traditional Chinese medicine is (addition and subtraction of homemade formula): Radix Bupleuri 6 g, Scutellariae Radix 9 g, Curcumae Radix 9 g, Atractylodes Macrocephala Koidz. 15 g, Poria Cocos (Schw.) Wolf. 15 g, Radix Salviae 15 g, raw Crataegi Folium 15 g, and Herba Taraxaci 30 g. The external application treatment was JinHuang ointment made by our hospital, which is provided by the herbal medicine room of our hospital.

Serum liver and kidney function tests were performed every 3 months during the oral administration of traditional Chinese medicine in the two groups to exclude adverse drug reactions.

Comparison of Clinical Outcomes Using PSM

The clinical data between the two groups were collected, including general information such as age, height, and weight, marriage and childbearing, time of onset, time interval from the last postpartum to onset, time interval from stopping lactation to onset, course of the disease, lactation, history of previous drug use, history of breast trauma, and history of other diseases. The propensity scores were calculated through logistic regression analysis, including age and BMI. The nearest neighbor matching method to match patients was used through the propensity score matching method. The caliper width was equal to 0.2 times the logit standard deviation of the propensity score. After matching, the statistical significance and standardized differences in the covariate balance were reviewed.

Evaluation and Follow-Up

The cure rate was calculated at 6 months and 9 months of treatment to evaluate the curative effect. Three and 6 months after the cure, the patient was asked to return to the hospital for an ultrasound review to assess the recurrence rate.

Standard of Cure

The evaluation criteria for acne mastoid carbuncle ("Diagnosis and Efficacy Criteria for TCM Diseases" version 2017) and the "Consensus of Traditional Chinese Medicine Experts on the Diagnosis and Treatment of Granulomatous Mastitis" issued by the Breast Disease Branch of the Chinese Society of Chinese Medicine in 2017 were used to evaluate the cure criteria. (I)

Healed: the mass disappeared, the fistula healed, and the systemic symptoms subsided; ultrasound showed no remaining lesions. (II) Clinical recovery: the systemic symptoms disappeared, the original inflammatory lesions were clinically untouchable, and the ulcers or sores were healed. (III) Improvement: the redness and heat pain disappeared, the mass shrank, and the fistula was nearly healed; ultrasound showed that the heterogeneous hypoechoic area or mixed gyrus area was significantly smaller than before. (IV) Unhealed: the mass did not disappear, the fistula did not heal, and the lesion area was even enlarged; ultrasound showed that the heterogeneous hypoechoic area or mixed back area was identical to the previous observation or enlarged, or the liquid dark area was visible.

The cure (includes healed and clinical recovery mentioned above I and II) rate is the ratio of the number of cured cases in each group to the total number of cases in the group at the sixth and ninth months from the beginning of the enrollment.

The total course of treatment is the first time that B-ultrasound shows no lesions, serum recovery period or postoperative changes since the self-hospital treatment.

The enrollment and analysis process of GLM patients are shown in **Figure 1**.

Statistical Methods

A patient follow-up information database was established using the Excel software, and the SPSS 24.0 statistical software was used for statistical data analysis. For the descriptive statistical analysis, the measurement data are expressed as the mean ± standard deviation ($x \pm s$). The count data, i.e., classified data, are represented by the number of cases and the composition ratio.

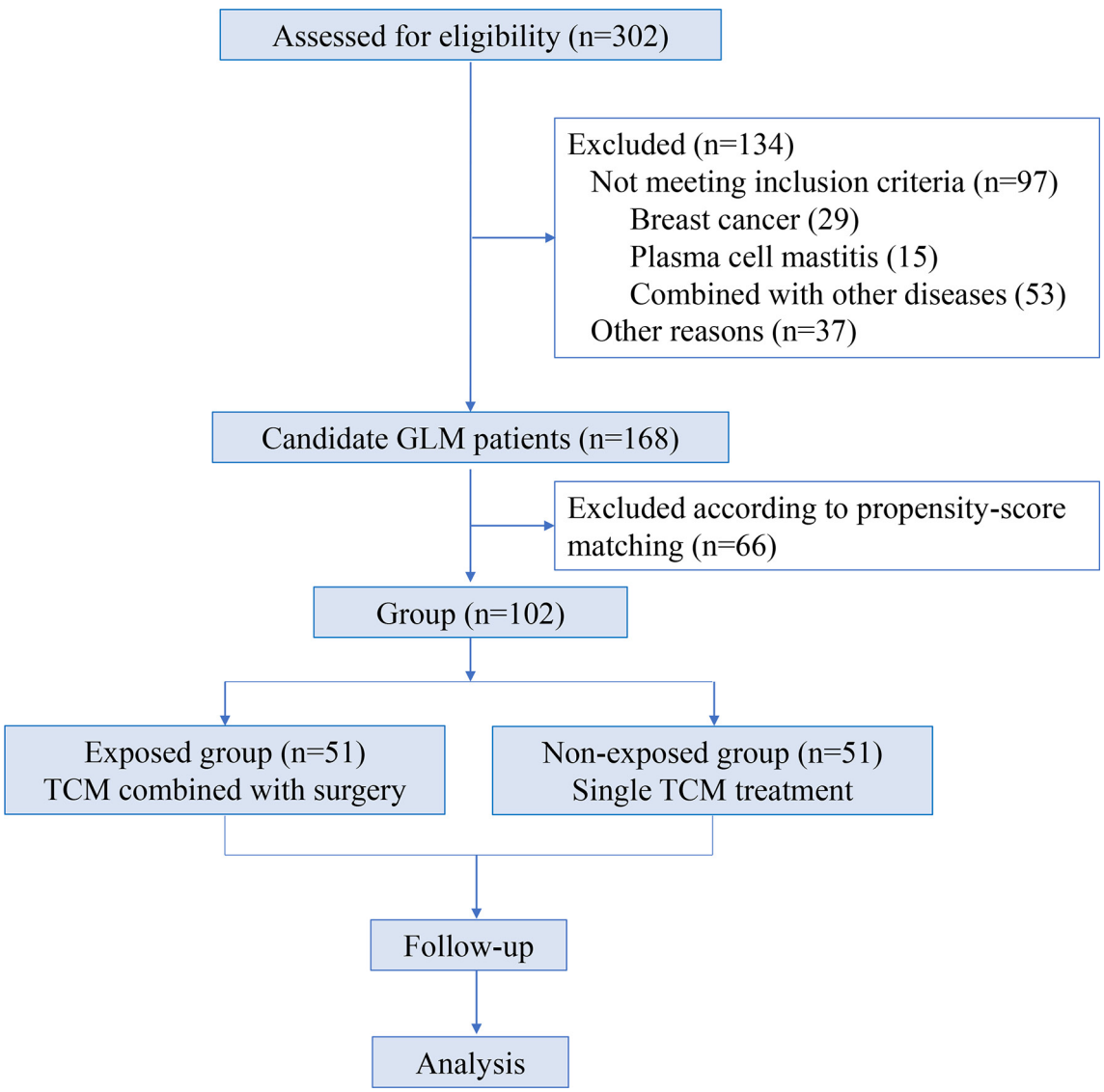


FIGURE 1 | Flow diagram of the trial.

For the comparative analysis of the two groups, the measurement data were measured by t-test or Wilcoxon rank-sum test, and the count data or rank data were compared by chi-square test or rank-sum test. The results were considered statistically significant at P<0.05.

RESULTS

Baseline Characteristics Between Two Groups Were Well Balanced Following PSM

In total, 302 female GLM patients were employed at Longhua Hospital of Shanghai University of Traditional Chinese Medicine in China between September 2019 and August 2021. Among them, 168 patients met the inclusion and exclusion criteria. Finally, 102 cases were included and divided into two groups according to the matching propensity score in this study (**Figure 1**).

The clinical data between the two groups before and after matching the propensity score were analyzed, including general conditions such as age, body mass index (BMI), marriage and childbirth, onset time, time interval after last childbirth to onset, time interval between stopping breastfeeding, onset, course of the disease, breastfeeding, history of past drug use, history of breast trauma, and history of other diseases (**Table 1**). Compared with the integrated traditional Chinese and surgery group, the body mass index (BMI) of the patients in the TCM group before the tendency matching was greater (24.9 ± 3.7 *vs.* 24.4 ± 3.5, p=0.019), the proportion of these cases with a history of acute mastitis during lactation was greater (26.5% *vs.* 9.8%, p=0.015), the proportion of these cases with fever symptoms after the onset was higher (25.6% *vs.* 11.8%, p=0.044), and the time from onset to our hospital treatment was shorter (32.1 ± 42.0 *vs.* 62.3 ± 58.7, p=0.002, **Table 1**). After the propensity score matching, the primary conditions of patients between the two groups were balanced, and the difference was not significant.

TCM Combined With Surgery Reduce the Suppuration Rate

During the treatment period, there were 33 cases and 42 cases of purulent TCM combined with surgery and TCM. The proportion of purulent patients in the TCM-surgery group was significantly less than that in the TCM group (64.7% *vs.* 83.4%, P=0.043) as shown in **Figure 2A**.

TCM Plus Surgery Significantly Reduced Total Duration of Treatment

The total treatment durations of the exposed group and the nonexposed group were 254 ± 117 days and 333 ± 112 days, respectively (**Figure 2B**). The median days of the treatments between TCM plus surgery and TCM were 260 and 330 days. Compared with the TCM group, the TCM-surgery group had a significantly shorter total course time with a p value 0.001 (**Figure 2B**).

TCM Combined With Surgery Increased Cure Rate

According to the evaluation criteria for acne mastoid carbuncle approved by the Breast Disease Branch of the Chinese Society of Chinese Medicine in 2017, only 9 (17.6%) patients in the non-exposed group were healed after 9 months of treatment. Interesting, in the exposed group, one (2.0%) patient was healed within 3 months of treatment, and 14 (27.5%) patients were healed at 9 months. In addition, the clinical recovery cases of the non-exposure group at 3, 6 and 9 months of treatment were 1 (2.0%), 7 (13.7%) and 18 (35.3%), respectively. While, the clinical recovery cases of the exposure group at 3, 6 and 9 months of treatment were 14 (27.5%), 20 (39.2%) and 30 (58.8%), which were significantly higher than the non-exposed group (P<0.001). In summary, the number of cured cases at 3 months, 6 months and 9 months of TCM combined with surgery was 15 cases (29.4%), 26 cases (50.1%), and 44 cases (86.3%), respectively. Correspondingly, there were 1 (1.96%), 7 (13.7%), and 27 (52.9%) cured cases in the TCM group at 3 months, 6 months and 9 months, respectively (**Table 2**). During the three different follow-up times, the TCM-surgery group had a significantly higher cure rate than the TCM group (P<0.01, **Table 2** and **Figure 3**).

No Significant Adverse Reactions Between the Two Groups

During the treatment period in the exposure group, 3 patients developed wound fluid accumulation, no obvious adverse reactions were found in the non-exposed group. However, there was no significant difference in adverse reactions between the two groups during treatment (P=0.24).

DISCUSSION

GLM, which is a chronic inflammatory benign disease, has a higher incidence among Han Chinese women, and its incidence accounts for approximately 3.5% of benign breast diseases in women (16). GLM tends to occur in women of childbearing age, and most patients have a history of pregnancy and breastfeeding (17). In this study, all patients were married women of childbearing age, had an average age of 32 years (21-47 years), and had a history of pregnancy. Moreover, approximately 88% of the patients had a history of breastfeeding. These results are consistent with previous reports. In addition, the average time between the onset of the patient and the last postpartum period was 42 months, and 11.8% of the patients had a history of acute mastitis.

The etiology and pathogenesis of granulomatous lobular mastitis is not yet fully understood. Some researchers believe that the pathogenesis of GLM is due to ductal epithelial damage, which transfers luminal secretions to lobular connective tissue, causing local inflammation of the connective tissue, which in turn allows macrophages and lymphocytes to migrate to this area, causing local granulomatous inflammation (18). The factors that ultimately induce ductal epithelial injury include pregnancy, breastfeeding, history of trauma, hyperprolactinemia,

TABLE 1 | Baseline characteristics and propensity score matching.

Types	Full queue			Probability score matching		
	Exposed group (n=51)	Non-Exposed group (n=117)	p	Exposed group (n=51)	Non-Exposed group (n=51)	p
Age*	31.94 ± 4.23	32.06 ± 4.37	0.45	31.94 ± 4.23	33.33 ± 5.41	0.078
Hyperprolactinemia			0.86			0.63
Yes	12 (23.5%)	29 (24.8%)		12 (23.5%)	10 (19.6%)	
No	39 (76.5%)	88 (75.2%)		39 (76.5%)	41 (80.4%)	
History of breast trauma			0.084			0.28
Yes	13 (25.5%)	46 (39.3%)		13 (25.5%)	18 (35.3%)	
No	38 (74.5%)	71 (60.7%)		38 (74.5%)	33 (64.7%)	
History of oral contraceptives			0.52			0.51
Yes	4 (7.8%)	13 (11.1%)		4 (7.8%)	6 (11.8%)	
No	47 (92.2%)	104 (88.9%)		47 (92.2%)	45 (88.2%)	
History of inverted nipples			0.86			0.69
Yes	23 (45.1%)	51 (43.6%)		23 (45.1%)	21 (41.2%)	
No	28 (54.9%)	66 (56.4%)		28 (54.9%)	30 (58.8%)	
History of nipple discharge			0.043			0.135
Yes	13 (25.5%)	15 (12.8%)		13 (25.5%)	7 (13.7%)	
No	38 (74.5%)	102 (87.2%)		38 (74.5%)	44 (86.3%)	
Breastfeeding history						0.22
Yes	43 (84.3%)	104 (88.9%)	0.41	43 (84.3%)	47 (92.2%)	
No	8 (15.7%)	13 (11.1%)		8 (15.7%)	4 (7.8%)	
Acute mastitis			0.044			1.0
Yes	6 (11.76%)	30 (25.6%)		6 (11.76%)	6 (11.76%)	
No	45 (88.24%)	87 (74.4%)		45 (88.24%)	45 (88.24%)	
BMI*	24.35 ± 3.5	24.88 ± 3.7	0.019	24.35 ± 3.5	23.94 ± 2.97	0.53
Postpartum and onset interval (months)	46.63 ± 25.31	43.18 ± 23.62	0.24	46.63 ± 25.31	37.67 ± 28.8	0.15
Interval between weaning and onset (months)	41.58 ± 22.28	34.08 ± 23.28	0.053	41.58 ± 22.28	33.18 ± 22.75	0.19
Accompanying fever after the onset			0.015			0.16
Yes	5 (9.8%)	31 (26.5%)		5 (9.8%)	10 (19.61%)	
No	46 (90.2%)	86 (73.5%)		46 (90.2%)	41 (80.39%)	
Accompanying erythema nodules after the onset			0.63			0.56
Yes	6 (11.76%)	17 (14.5%)		6 (11.76%)	8 (15.69%)	
No	45 (88.24%)	100 (85.5%)		45 (88.24%)	43 (84.31%)	
Lump staging at visit			0.23			0.12
Lump stage	31 (60.78%)	51 (43.6%)		31 (60.78%)	22 (43.14%)	
Abscess	10 (19.61%)	34 (29.1%)		10 (19.61%)	8 (15.69%)	
Rupture period	7 (13.73%)	21 (17.9%)		7 (13.73%)	15 (29.41%)	
Post-collapse	3 (5.88%)	11 (9.4%)		3 (5.88%)	6 (11.76%)	
Interval between onset and treatment in our hospital (days)	62.31 ± 58.69	32.14 ± 42.02	0.002	62.31 ± 58.69	64.98 ± 40.76	0.4

**The matching factor.*

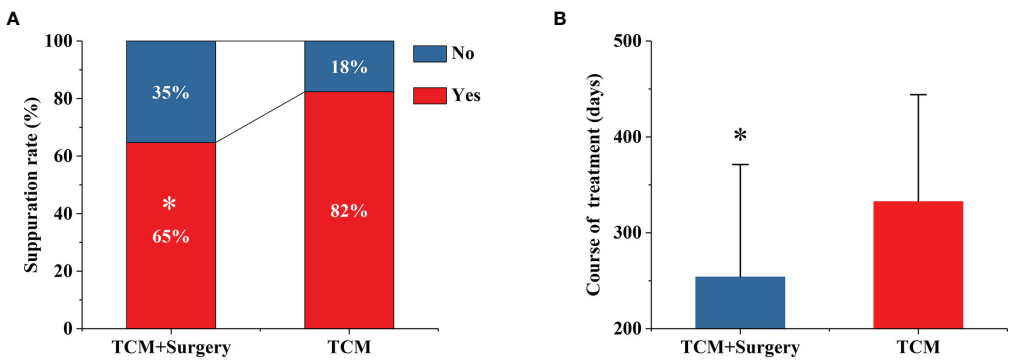


FIGURE 2 | TCM combined with surgery reduce the suppuration rate **(A)** and total duration of treatment **(B)**. *P < 0.05 *vs.* TCM group.

TABLE 2 | Cure cases (rate) between the two groups.

Groups	Types	3 months	6 months	9 months
Exposed group	Healed	1 (2.0%)	6 (11.8%)*	14 (27.5%)
	Clinical Recovery	14 (27.5%)***	20 (39.2%)**	30 (58.8%)*
	Improvement	34 (66.7%)***	25 (49.0%)***	6 (11.8%)***
	Unhealed	2 (3.9%)	0 (0%)	1 (2.0%)
	Total cure	15 (29.4%)***	26 (50.1%)***	44 (86.3%)***
Non-Exposed group	Healed	0 (0%)	0 (0%)	9 (17.6%)
	Clinical Recovery	1 (2.0%)	7 (13.7%)	18 (35.3%)
	Improvement	48 (94.1%)	42 (82.4%)	24 (47.1%)
	Unhealed	2 (3.9%)	2 (3.9%)	0 (0%)
	Total cure	1 (2.0%)	7 (13.7%)	27 (52.9%)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. Non-Exposed group.*

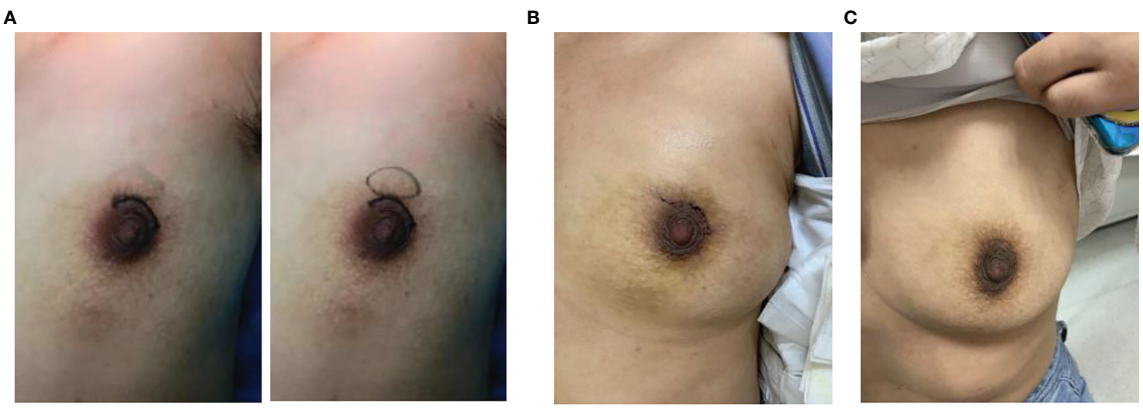


FIGURE 3 | Typical representative photos of GLM before surgery **(A)**, one week after surgery **(B)**, and one month after surgery **(C)**.

and obesity (18). In this study, 21.6% of patients had high prolactin, 30.1% of patients had breast trauma before the onset, 8.3% of patients had oral contraceptives, 43.1% of patients had inverted nipples, and the average BMI index of these cases was ≥23.9. The results of this study consistent with that of previous studies, suggest that pregnancy, breastfeeding, trauma history, obesity and other factors are risk factors for GLM (18).

There is no clear consensus on the treatment of GLM. At present, the most reported treatments are drug treatment and surgical treatment. Grover et al. believed that steroids or immunomodulatory drugs are used as conventional treatments, and surgical resection is widely adopted as the last treatment for refractory cases (19). Zhou et al. also suggested that drug treatment was recommended as a priority (20). If the patient has a relapse or the drug is not effective, surgery will be used for treatment. However, other researchers believe that the therapeutic effect of immunomodulatory (steroid) therapy is usually poor (21). Therefore, surgical treatment is still one of the main ways to treat GLM abroad. For surgical treatment, Zhang et al. believed that appropriate acute inflammation control methods should be used before surgery, and appropriate surgical methods should be selected after the inflammation has been controlled (13). This strategy has a better therapeutic effect than the traditional extensive resection; i.e., its cosmetic effect is good, the recovery time is short, and the prognosis is better. Wang et al. found that surgery after steroid treatment has a faster curative effect and a lower recurrence rate than steroid treatment alone (22). Zhang et al. found that TCM YangHe decoction combined with surgical treatment had a higher cure rate and a lower recurrence rate than surgical treatment alone (23). Zuo et al. also believed that the treatment of GLM with integrated traditional Chinese and Western medicine could improve the aesthetics of both breasts, thus it is worthy of clinical recommendation (24). A recent review reveals that the effectiveness of TCM plus surgery in the treatment of GLM is similar to that of surgical operation, but TCM plus surgery can significantly improve the satisfaction of patients with breast shape (25). Therefore, the consensus of experts on diagnosis and treatment of granulomatous lobular mastitis in traditional Chinese Medicine was initiated by the China Association of Chinese Medicine (CACM) (26). Although Chinese medicine has a clear curative effect, the treatment time of traditional Chinese medicine is long, and most patients suffer from long-term dressing changes. Therefore, traditional Chinese medicine combined with surgery was used to treat GLM. In the early stage of the disease, TCM was used to reduce the mass and limit the inflammation, and finally the appropriate operation was performed to reduce the damage to the breast shape. Finally, the

purpose of shortening the course of treatment and reducing the pain caused by dressing changes was achieved.

Traditional Chinese medicine doctors believes that granulomatous lobular mastitis belongs to the category of "acne breast carbuncle". There is no clear name of GLM in ancient books. According to its special clinical manifestations and pathogenesis, Professors Gu Bohua and Lu Deming of Shanghai School of Gu's Surgery proposed the name "acne breast carbuncle" in 1980 (27). They believe that GLM first has nipple deformity or duct dilation and subsequently stagnation of liver qi, failure to follow blood, stagnation of qi and blood stasis, agglomeration into masses, long-term depression, and heat stagnation because of emotional discomfort, which result in meat rot and abscess. Eventually, it becomes a fistula after ulceration. In this study, TCM combined with surgery was used to treat GLM, and Chinese medicines such as Radix Bupleuri, Curcumae Radix, Radix Salviae, and Herba Taraxaci were given to soothe the liver, clear heat, promote blood circulation and remove blood stasis. The local lesions were excised and sutured approximately 4.7 months after the oral administration of TCM. The results of the study suggest that the TCM-surgery group had a significantly lower purulent lesion rate than the TCM group. This result indicates that the use of surgical treatment at the appropriate time can significantly reduce local suppuration, which shortens the course of treatment and reduces pain. The cure rates at 3 months, 6 months and 9 months in the TCM-surgery group were significantly better than those in the TCM group. Moreover, the total treatment course of TCM with surgery was significantly shorter than that of TCM. During the treatment period, there were 3 patients with wound effusion in the traditional Chinese medicine combined with the operation group after surgery. After symptomatic extraction of the effusion and Chinese medicine treatment, the wound healed after an average delay of 2 weeks. There were no obvious adverse reactions in the traditional Chinese medicine group. Therefore, Chinese medicine combined with surgery to treat GLM is worth recommending in clinical practice.

The present study has some limitations. First, this investigation was a retrospective cohort study, and there may be research bias. Second, the sample size in this study was relatively small, and the conclusions of this study must be verified by further multi-center clinical trials with larger sample sizes. Third, the follow-up time of this study was not sufficiently long, and longer clinical follow-up is required for these patients.

CONCLUSIONS

In summary, Chinese medicine combined with surgical treatment can increase the cure rate of GLM, shorten the course of treatment, and reduce the rate of suppuration of local lesions. Therefore, this treatment strategy is worthy of clinical recommendation and is expected to become a comprehensive treatment for GLM.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the clinical trial ethics committee in Longhua Hospital of Shanghai University of Traditional Chinese Medicine. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SL and ZS designed the research. CW and RS performed the research. CW and RS contributed analytic tools. CW and RS wrote the paper. SL and ZS reviewed the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by grants from the Shanghai Municipal Health Commission (Grant Number ZY3-LCPT-2-1002), and Shanghai Municipal Science and Technology Commission (Grant Number 20Z21900300), as well as Shanghai Hospital Development Center (No. SHDC2020CR1050B).

REFERENCES

1. Kessler E, Wolloch Y. Granulomatous Mastitis: A Lesion Clinically Simulating Carcinoma. *Am J Clin Pathol* (1972) 58(6):642–6. doi: 10.1093/ajcp/58.6.642
2. Martinez-Ramos D, Simon-Monterde L, Suelves-Piqueres C, Queralt-Martin R, Granel-Villach L, Laguna-Sastre JM, et al. Idiopathic Granulomatous Mastitis: A Systematic Review of 3060 Patients. *Breast J* (2019) 25(6):1245–50. doi: 10.1111/tbj.13446
3. Pluguez-Turull CW, Nanyes JE, Quintero CJ, Alizai H, Mais DD, Kist KA, et al. Idiopathic Granulomatous Mastitis: Manifestations at Multimodality Imaging and Pitfalls. *Radiographics* (2018) 38(2):330–56. doi: 10.1148/rg.2018170095
4. Ichinose Y, Kosaka Y, Saeki T, Fujimoto A, Nukui A, Asano A, et al. Granuloma After Breast Conserving Surgery-a Report of Three Cases. *J Surg Case Rep* (2021) 2021(6):rjab199. doi: 10.1093/jscr/rjab199
5. Oddó D, Domínguez F, Gómez N, Méndez GP, Navarro ME. Granulomatous Lobular Mastitis Associated With Ductal Carcinoma *In Situ* of the Breast. *SAGE Open Med Case Rep* (2019) 7:2050313X19836583. doi: 10.1177/2050313X19836583
6. Chandanwale S, Naragude P, Shetty A, Sawadkar M, Raj A, Bhide A, et al. Cytomorphological Spectrum of Granulomatous Mastitis: A Study of 33 Cases. *Eur J Breast Health* (2020) 16(2):146–51. doi: 10.5152/ejbh.2020.5185
7. Chirappapha P, Thaweepworadej P, Supsamutchai C, Biadul N, Lertsithichai P. Idiopathic Granulomatous Mastitis: A Retrospective Cohort Study Between 44 Patients With Different Treatment Modalities. *Ann Med Surg (Lond)* (2018) 36:162–7. doi: 10.1016/j.amsu.2018.11.001
8. Azlina AF, Ariza Z, Arni T, Hisham AN. Chronic Granulomatous Mastitis: Diagnostic and Therapeutic Considerations. *World J Surg* (2003) 27(5):515–8. doi: 10.1007/s00268-003-6806-1
9. Aghajanzadeh M, Hassanzadeh R, Alizadeh Sefat S, Alavi A, Hemmati H, Esmaeili Delshad MS, et al. Granulomatous Mastitis: Presentations,

Diagnosis, Treatment and Outcome in 206 Patients From the North of Iran. *Breast* (2015) 24(4):456–60. doi: 10.1016/j.breast.2015.04.003
10. Mathew Thomas V, Alexander SA, Bindal P, Vredenburgh J. Idiopathic Granulomatous Mastitis-a Mystery Yet to be Unraveled: A Case Series and Review of Literature. *Cureus* (2020) 12(2):e6895. doi: 10.7759/cureus.6895
11. Lü JM, Yao Q, Chen C. Ginseng Compounds: An Update on Theirmolecular Mechanisms and Medical Applications. *Curr Vasc Pharmaco* (2009) l7:293–302. doi: 10.2174/157016109788340767
12. Zhang C, Lei S, Kong C, Tan Y, Dai X, He J, et al. Clinical Study on Surgical Treatment of Granulomatous Lobular Mastitis. *Gland Surg* (2019) 8(6):712–22. doi: 10.21037/gs.2019.11.12
13. Chu A, Fan Y, Wang C, Lai B, Pei X. Current Status and Analysis of Traditional Chinese and Western Medicine Treatment for Granulomatous Mastitis. *Chin J Tradit Chin Medi Pharm* (2020) 35(12):5971–4.
14. Wang R, Zhou X, Wang Y, Song X, Liu S. Clinical Study on the Treatment of 118 Cases of Granulomatous. Mastitis by "Clearing and Resolving Therapy". *J Liaoning Univ Tradit Chin Med* (2019) 21(11):115–8.
15. Uysal E, Soran A, Sezgin E, Turkan H, Girgin S, Karanlik H, et al. Factors Related to Recurrence of Idiopathic Granulomatous Mastitis: What do We Learn From a Multicentre Study? *ANZ J Surg* (2018) 88:635–9. doi: 10.1111/ans.14115
16. Jiang L, Li X, Sun B, Ma T, Kong X, Yang Q. Clinicopathological Features of Granulomatous Lobular Mastitis and Mammary Duct Ectasia. *Oncol Lett* (2020) 19(1):840–8. doi: 10.3892/ol.2019.11156
17. Azizi A, Prasath V, Canner J, Gharib M, Sadat Fattahi A, Naser Forghani M, et al. Idiopathic Granulomatous Mastitis: Management and Predictors of Recurrence in 474 Patients. *Breast J* (2020) 26(7):1358–62. doi: 10.1111/tbj.13822
18. Halim NA, Uthman I, Rammal R, Assi HI. Idiopathic Granulomatous Mastitis Presenting as a Breast Pseudotumor: Case Reports With Review of the Literature. *Case Rep Rheumatol* (2018) 2018:4264012. doi: 10.1155/2018/4264012
19. Grover H, Grover SB, Goyal P, Hegde R, Gupta S, Malhotra S, et al. Clinical and Imaging Features of Idiopathic Granulomatous Mastitis - the Diagnostic Challenges and a Brief Review. *Clin Imaging* (2021) 69:126–32. doi: 10.1016/j.clinimag.2020.06.022
20. Zhou F, Liu L, Liu L, Yu L, Wang F, Xiang Y, et al. Comparison of Conservative Versus Surgical Treatment Protocols in Treating Idiopathic Granulomatous Mastitis: A Meta-Analysis. *Breast Care (Basel)* (2020) 15 (4):415–20. doi: 10.1159/000503602
21. Zhu Q, Wang L, Wang P. The Identification of Gene Expression Profiles Associated With Granulomatous Mastitis. *Breast Care (Basel)* (2021) 16 (4):319–27. doi: 10.1159/000507474
22. Wang J, Zhang Y, Lu X, Xi C, Yu K, Gao R, et al. Idiopathic Granulomatous Mastitis With Skin Rupture: A Retrospective Cohort Study of 200 Patients Who Underwent Surgical and Nonsurgical Treatment. *J Invest Surg* (2021) 34 (7):810–5. doi: 10.1080/08941939.2019.1696905
23. Zhang X, Li J, Hu XJ. Postoperative Yanghe Decoction Regimen Improves Outcomes for Idiopathic Granulomatous Mastitis: A Retrospective Cohort Study. *Med (Baltimore)* (2020) 99(45):e23136. doi: 10.1097/MD.0000000000023136
24. Zuo X, Shi X, Gao X, Lai R, Liu P, Zhao Z. A Retrospective Study on 221 Patients With Granulomatous Lobular Mastitis Treated by a Combination of Traditional Chinese Medicine and Western Medicine. *Ann Ital Chir* (2021) 92:135–41.
25. Xu B, Wang L, Liu X, Lai M, Zhang X, Kang M. Investigation on Present Status of Diagnosis and Treatment of Granulomatous Lobular Mastitis in China. *J N Chin Med* (2019) 51(2):279–83. doi: CNKI:SUN:REND.0.2019-02-087
26. China Association of Chinese Medicine (CACM). *Consensus of Experts on Diagnosis and Treatment of Granulomatous Lobular Mastitis in Traditional Chinese Medicine*. Beijing: China traditional Chinese Medicine Press (2020).
27. Gu BH. *Practical Chinese Surgery*. Shanghai: Shanghai Science and Technology Press (1985).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

ORIGINAL RESEARCH
published: 24 February 2022
doi: 10.3389/fonc.2022.789198

Practical Model to Optimize the Strategy of Adjuvant Postmastectomy Radiotherapy in T1-2N1 Breast Cancer With Modern Systemic Therapy

Fei-Fei Xu, Lu Cao, Cheng Xu, Gang Cai, Shu-Bei Wang, Wei-Xiang Qi and Jia-Yi Chen**

Department of Radiation Oncology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

OPEN ACCESS

Edited by:
Yiding Chen,
Zhejiang University, China

Reviewed by:
Fabiana Gregucci,
Ospedale Generale Regionale Francesco Miulli, Italy
Jun Wang,
Sichuan University, China

***Correspondence:**
Jia-Yi Chen
chenjiayi0188@aliyun.com
Wei-Xiang Qi
qiweixiang1113@163.com

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *04 October 2021*
Accepted: *28 January 2022*
Published: *24 February 2022*

Citation:
Xu F-F, Cao L, Xu C, Cai G, Wang S-B, Qi W-X and Chen J-Y (2022) Practical Model to Optimize the Strategy of Adjuvant Postmastectomy Radiotherapy in T1-2N1 Breast Cancer With Modern Systemic Therapy. Front. Oncol. 12:789198. doi: 10.3389/fonc.2022.789198

Purpose: The effect of adjuvant irradiation after mastectomy in early-stage breast cancer patients remains controversial. The present study aims to explore the clinical benefit obtained from adjuvant radiotherapy among post-mastectomy pT1-2N1 breast cancer patients who received adjuvant modern systemic therapy.

Methods: Medical records of consecutive patients with pT1-2N1 breast cancer who received mastectomy in our institution between January 2009 and December 2016 were retrospectively reviewed. High-risk features consist of patient age, number of positive lymph nodes, T stage, and Ki67 index, which were developed previously at our institution using early-stage breast cancer patients after mastectomy without adjuvant radiotherapy. Differences of survival and local recurrence were compared between no-postmastectomy radiotherapy (PMRT) and PMRT group according to number of risk factors. The time-to-event curves were calculated by the Kaplan–Meier methods and compared by the log-rank test. Propensity score matching (PSM) was performed to reduce the imbalances in patient characteristics.

Results: A total of 548 patients were enrolled (no-PMRT: 259 and PMRT: 289). After a median follow-up of 69 months, the 5-year rate of DFS, BCSS, and LRR in the overall cohort was 90.2%, 97.4%, and 3.6%, respectively. PMRT did not significantly improve DFS, BCSS, and LRRFS in the whole cohort. Patients were divided into low-risk (with no or one risk factor) and high-risk (with two or more risk factors) groups. According to the univariable and multivariable analysis, high-risk group (HR = 1.81, 95% CI 1.11–2.98, $p = 0.02$) was demonstrated as an independent risk factor for DFS. For the high-risk group, PMRT significantly improved DFS from 81.4% to 91.9% and BCSS from 95.5% to 98.6% and decreased the 5-year rate of LRR from 5.6% to 1.4%, respectively ($p < 0.01$, $p = 0.05$, and $p = 0.06$). However, no survival benefit from PMRT was observed in the low-risk group in terms of DFS, BCSS, and LRR ($p = 0.45$, $p = 0.51$, and $p = 0.99$, respectively). In multivariate analysis, PMRT remained an independent prognostic factor

for DFS (HR = 0.50, 95% CI 0.24–1.00, p = 0.05) in the high-risk group. After PSM analysis, the survival benefit of PMRT was sustained in high-risk patients.

Conclusion: PMRT significantly improved DFS in high-risk pT1-2N1 breast cancer patients, but not in low-risk patients. Independent validation of our scoring system is recommended.

Keywords: breast cancer, T1-2N1, postmastectomy radiotherapy, risk factors, survival prognosis

INTRODUCTION

Globally, breast cancer is the most commonly diagnosed cancer in women with a growing trend in both incidence and mortality (1). On the molecular level, breast cancer is a heterogeneous disease, which could be categorized into subtypes mainly based on the presence or absence of molecular markers for human epidermal growth factor receptor 2 (HER2) and hormone receptors [HR, including estrogen receptor (ER) and progesterone receptor] and/or BRCA mutations (2). As a result, a multidisciplinary team is recommended to determine the optimal locoregional (surgery and radiation therapy) and systemic management strategies for breast cancer. Modern systemic therapies, including endocrine therapy for HR-positive disease, anti-HER2 therapy for HER2-positive disease, chemotherapy based on anthracycline and taxane, bone-stabilizing agents, poly (ADP-ribose) polymerase inhibitors for BRCA mutation carriers, and immunotherapy, have been demonstrated to significantly improve the survival outcomes of breast cancer patients (3). Post-mastectomy radiotherapy (PMRT) is an important local treatment for breast cancer with microscopic residual disease. In general, the indications for PMRT were strongly recommended for breast cancer involving a tumor size of >5 cm, presence of more than three positive lymph nodes, or positive surgical margins (4).

However, the role of PMRT in pT1-2N1 breast cancer patients remains debated in daily clinic (5). An updated report from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) in 2014 confirmed that adjuvant PMRT significantly reduced both recurrence and breast cancer mortality in the women with one to three positive lymph nodes (6, 7). However, this meta-analysis has been criticized for its limitations, mainly less intensive systemic therapy, limited axillary dissection in some trials, and the sub-optimal radiation techniques. The high locoregional recurrence (LRR) of 20.3% at 10 years in EBCTCG meta-analysis is also quite far from the LRR rated reported in later trials (8). In addition, the clinical benefit obtained from PMRT significantly varies with primary tumor size and number of positive lymph node. A recent study from University of Chicago showed that PMRT improved the survival prognosis among patients with 3 positive lymph nodes and tumors 2–5 cm in size, but no beneficial effect for patients with 1–2 positive nodes and tumors 2 cm in size or smaller (9). Thus, investigating risk factors is critically important to identify early-stage breast cancer patients who might benefit from PMRT after mastectomy (4, 10).

A number of risk factors for survival in early breast cancer patients have been reported, but the results are controversial (11). Prior to the present study, we have established a nomogram for predicting the prognosis of patients with pN0-1 breast cancer who were treated with mastectomy and without adjuvant radiotherapy (12). The model was externally validated in an independent cohort of 1,356 patients from one phase III trial (NCT00041119). Finally, pathological T stage, number of positive lymph nodes, age, and Ki67 index were found to be significant predictors for breast cancer specific survival (BCSS) in post-mastectomy breast cancer with pN0-1. In the present study, we aim to validate whether the practical prognostic scoring system based on these four risk factors in our previous study can identify high-risk pT1-2N1 breast cancer patients who could benefit from PMRT.

MATERIALS AND METHODS

Patients' Selection

From January 2009 to December 2016, a total of 642 consecutive newly diagnosed invasive breast cancer patients undergoing mastectomy and sentinel lymph node biopsy or axillary lymph node dissection with pathological T1-2N1 were identified at our institution. Ninety-four patients were excluded from the present analysis for the following reasons: (1) neoadjuvant chemotherapy; (2) lack of information about tumor size, pathological type, Ki67 index, and radiotherapy; (3) pathologically diagnosed as ductal carcinoma i*n situ*, lobular carcinoma *in situ*, or Paget's disease. Finally, 548 patients were enrolled for analysis in the present study.

Adjuvant Radiotherapy

For patients treated with adjuvant PMRT, dose prescription to the chest wall (CW) and regional nodes (supraclavicular, infraclavicular with or without internal mammary lymph nodes) was 50 Gy in 25 fractions. CW irradiation was given using field-in-field forward-planned intensity-modulated radiotherapy using photons and regional nodes were treated using an anterior mixed photon and electron beam. The volume delineation and definition were determined according to the Radiation Therapy Oncology Group (RTOG) guidelines (13).

Outcome's Definitions

Disease-free survival (DFS) was defined as the time from surgery to the time of the first recurrence in the ipsilateral chest wall or in regional nodal or distant sites or death from any cause. BCSS was

defined as the time from surgery till death of breast cancer. LRR was defined as the time from surgery to the time of a first recurrence in the ipsilateral chest wall or in the ipsilateral regional nodal (including axillary, supraclavicular, infraclavicular, and internal mammary lymph nodes). Follow-up time was calculated from the date of surgery to the first event or last confirmed date of breast cancer disease-free status.

Statistical Analysis

For categorical variables, differences between the no-PMRT and PMRT groups were evaluated by using Pearson's chi square statistics. The time-to-event curves were calculated by the Kaplan–Meier methods and compared by the log-rank test. Hazard ratios (HRs) and corresponding 95% CIs were estimated using the Cox proportional hazards regression model. Given the difference between patients with and without PMRT, PSM was applied to balance measurable confounders. Patients were matched based on their estimated propensity using 1:1 matching *via* nearest method without replacement with a caliper of 0.05. All statistical tests were two-sided and $p < 0.05$ was considered significant. The software package SPSS 24.0 (IBM corporation, USA) was used for analysis.

RESULTS

Baseline Characteristics

In total, 548 patients who received mastectomy and were diagnosed as pT1-2N1 breast cancer were enrolled. A total of 289 patients were treated with adjuvant PMRT, and all completed scheduled radiotherapy. The baseline characteristics of these patients are listed in **Table 1**. The median age at diagnosis was 56 years (range, 28–91). The median tumor size was 2.5 cm (range, 0.3–5.0) in the whole cohort. Among 455 patients who received adjuvant chemotherapy, 86.6% received anthracycline and taxane-based chemotherapy. In ER-positive patients, 89.6% received the endocrine therapy. Anti-HER2 therapy was given to 62.9% of HER2-positive patients.

As shown in **Table 1**, patients in the PMRT group had more risk factors including younger age, larger tumor, more axillary lymph nodes involved, and unfavorable biomarkers. Accordingly, higher portion of patients received chemotherapy in the PMRT group ($p < 0.01$).

Survival Outcomes in Overall Cohort and Different Subgroups

After a median follow-up of 69 months (range, 2–128), 7 patients developed LRR only, 37 patients had distant metastasis only, and 13 patients developed LRR and distant metastasis. A total of 32 patients died in the entire cohort, with 23 attributed to breast cancer. The 5-year rate of DFS and BCSS was 90.2% and 97.4%, respectively. The 5-year rate of LRR was 3.6%.

Four risk parameters, established and validated by our previous study to be independent risk factors for predicting BCSS in pN0-1 breast cancer patients receiving mastectomy, namely, age (≤40 versus >40 years old), number of positive lymph nodes (1–2 versus 3 positive lymph nodes), T stage (T1 versus T2), and Ki67 index (≤20% versus >20%), were utilized to divide patients into a low-risk group, which was defined as patients with no or one risk factor, and a high-risk group, which was defined as patients with two or more risk factors. There were 286 and 262 patients in the low-risk group and high-risk group, respectively, in which 127 and 162 patients received PMRT, respectively. Five-year rates of DFS, BCSS, and LRR were 92.6% versus 87.5% ($p = 0.05$), 97.5% versus 97.2% ($p = 0.49$), and 4.1% versus 3.2% ($p = 0.55$) between the low- and high-risk subgroups, respectively (shown in **Figure 1**).

Univariate and Multivariate Analysis for Survival Outcomes

In the whole cohort, chemotherapy (Yes vs. No) and risk group (high risk vs. low risk) were found to be significant prognostic factors for DFS ($p < 0.01$ and $p = 0.04$, respectively) by univariate analysis. By multivariate analysis, no chemotherapy (HR = 2.69, 95% CI 1.51–4.79, $p < 0.01$) and high-risk group (HR = 1.81, 95% CI 1.11–2.98, $p = 0.02$) remained independent risk factors for DFS. The detailed univariable and multivariable analysis for DFS is shown in **Table 2**.

Survival Benefits From PMRT in Different Risk Groups

After a median follow-up of 69 months (range 2–128), 8 and 15 breast cancer deaths occurred in the PMRT and no-PMRT group, respectively. No significant difference was found between PMRT and no-PMRT groups in terms of 5-year rate of DFS (91.7% vs. 88.8%, $p = 0.13$, **Figure 2A**), BCSS (98.5% vs. 96.4%, $p = 0.37$, **Figure 2B**), and LRR (2.7% vs. 4.5%, $p = 0.19$, **Figure 2C**).

For the high-risk group, the Kaplan–Meier survival analysis indicated that PMRT significantly improved 5-year rate of DFS from 81.4% to 91.9% ($p < 0.01$, **Figure 2D**), BCSS from 95.5% to 98.6% ($p = 0.05$, **Figure 2E**), and LRR from 5.6% to 1.4% with marginal significance ($p = 0.06$, **Figure 2F**). For the low-risk group, there was no significant difference in DFS, BCSS, and LRR between PMRT and no-PMRT patients (**Figures 2G–I**). By multivariate analysis, chemotherapy and PMRT remained independent prognostic factors for DFS (HR = 0.27, 95% CI 0.12–0.58, $p < 0.01$ and HR = 0.50, 95% CI 0.24–1.00, $p = 0.05$, respectively) in the high-risk group. The results of univariate and multivariate survival analysis in the high-risk and low-risk groups separately are detailed in **Supplementary Table 1**.

Since the baseline characteristic significantly varied between the PMRT group and no-PMRT group, we performed PSM to reduce the potentially selection bias. After PSM analysis, a total of 392 matched patients were finally included for analysis. No significant difference was observed between PMRT and no-PMRT groups in the overall cohort (shown in **Supplementary Table 2**). Similarly, the 5-year rate of DFS in patients treated with PMRT was comparable to those who did not receive PMRT (90.6% vs. 88.5%, $p = 0.36$) in overall matched cohort. Consistent with previous results, patients with more than two high-risk

TABLE 1 | Patient and treatment characteristics.

Characteristics	Whole cohort (*N* = 548)	No-PMRT (*N* = 259)	PMRT (*N* = 289)	*p*-value
Age (years)				<0.01
Median (range)	56 (28–91)	58 (29–91)	54 (28–78)	
≤40	50 (9.1)	14 (5.4)	36 (12.5)	
>40	498 (90.9)	245 (94.6)	253 (87.5)	
Menopausal status				0.09
Premenopausal	198 (36.1)	84 (32.4)	119 (39.4)	
Postmenopausal	350 (63.9)	175 (67.6)	179 (60.6)	
Tumor size (cm)				0.09
Median (range)	2.5 (0.3–5.0)	2.0 (0.5–5.0)	2.5 (0.3–5.0)	
≤2.0	254 (46.4)	130 (50.2)	124 (42.9)	
2.0-5.0	294 (53.6)	129 (49.8)	165 (57.1)	
Nuclear grade				0.05
Low-Intermediate	282 (57.1)	146 (61.6)	136 (52.9)	
High	212 (42.9)	91 (38.4)	121 (47.1)	
Unknown	58	23	35	
Axillary surgery				0.01
SLNB alone	12 (2.2)	10 (3.9)	2 (0.7)	
ALND	536 (97.8)	249 (96.1)	287 (99.3)	
Number of resected LN	16 (2–35)	16 (2–35)	15 (4–34)	0.86
Number of positive LN				<0.01
1–2	450 (82.1)	226 (87.3)	224 (77.5)	
3	98 (17.9)	33 (12.7)	65 (22.5)	
ER status				<0.01
Positive	416 (76.6)	217 (84.1)	199 (69.8)	
Negative	127 (23.4)	41 (15.9)	86 (30.2)	
Unknown	5	1	4	
Ki67 index				<0.01
≤20%	203 (37.0)	112 (43.2)	91 (31.5)	
>20%	345 (63.0)	147 (56.8)	198 (68.5)	
HER2 status				<0.01
Positive	129 (23.7)	48 (18.6)	81 (28.3)	
Negative	415 (76.3)	210 (81.4)	205 (71.7)	
Unknown	4	1	3	
Molecular subtype				<0.01
Luminal	416 (76.6)	217 (83.8)	199 (69.8)	
HER2 positive	62 (11.4)	18 (7.0)	44 (15.4)	
Triple negative	65 (12.0)	23 (8.9)	42 (14.7)	
Unknown	5	1	4	
Chemotherapy				<0.01
Yes	455 (86.2)	193 (77.2)	262 (94.2)	
No	73 (13.8)	57 (22.8)	16 (5.8)	
Unknown	20	9	11	
Target therapy in HER2 positive (*n* = 129)				0.46
Yes	78 (62.9)	27 (58.7)	51 (65.4)	
No	46 (37.1)	19 (41.3)	27 (34.6)	
Unknown	5	2	3	
Endocrine therapy in ER positive (*n* = 416)				0.10
Yes	353 (89.6)	184 (87.2)	169 (92.3)	
No	41 (10.4)	27 (12.8)	14 (7.7)	
Unknown	22	6	16	

factors remained a poor independent risk factor for DFS in multivariate analysis (HR = 1.81, 95% CI 1.05–3.11, $p = 0.03$). In addition, survival benefit obtained from PMRT remained significant among breast cancer with more than two high-risk factors after PSM (5-year rate of DFS: 92.3% vs. 82.1%, $p = 0.03$), while no significant survival benefit from PMRT was observed among patients presented with less than one low-risk factor after PSM (5-year rate of DFS: 89.1% versus 93.6%, $p = 0.29$).

In conclusion, our results were consistent before or after PSM, which further confirmed that the high-risk group, defined as patients with two or more risk factors, was an independent risk factor for DFS, and PMRT should be recommended for patients in that population.

DISCUSSION

In this study, we investigated the impact of PMRT on survival outcomes among patients with T1-2N1 breast cancer treated with modern systematic therapies by using a scoring system

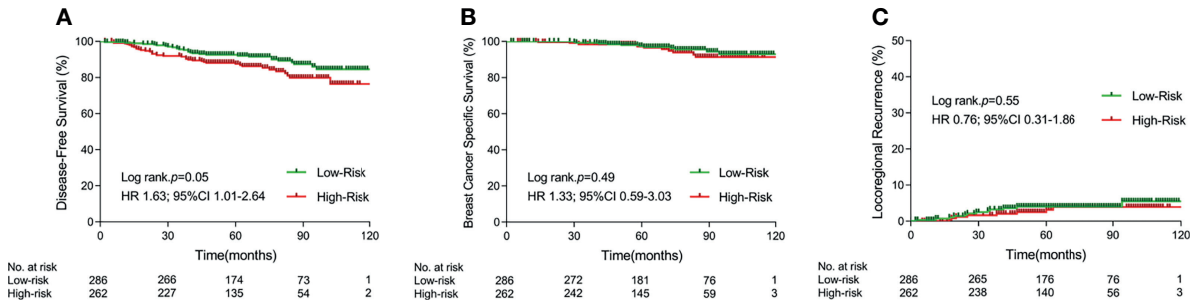


FIGURE 1 | Kaplan–Meier curves for 5-year disease-free survival **(A)**, 5-year breast cancer specific survival **(B)**, and 5-year locoregional recurrence **(C)** in different risk cohorts. (PMRT, postmastectomy radiotherapy).

composed of 4 clinical–pathological risk factors. Based on the number of risk factors, we divided patients into two risk groups [low risk (0–1 risk factor) vs. high-risk (≥2 risk factors)]. In the multivariate analysis, we found that the high-risk group and chemotherapy were two independent risk factors for DFS and survival benefit of PMRT was limited to the high-risk group only.

The trend in the receipt of PMRT in patients with T1-2N1 breast cancer has varied significantly over years and utilization of PMRT has increased from 14.1% to 23.5% in Asia in recent years (14). Nevertheless, significant controversy remains regarding the benefit of PMRT or regional nodal irradiation (RNI) in this population. Prior to the present study, the DBCG 82 b&c randomized trials demonstrated lower risk of LRR and better survival outcomes with the addition of PMRT (15). However, the higher risk of LRR and suboptimal BCSS have been attributed to the less than standard systemic therapy and axillary surgery in the DBCG trials. Subsequently, McBride et al. investigated the clinical benefit of PMRT among patients with T1-2N1 breast cancer and treated with mastectomy and modern systemic treatment, but no significant difference in 5-year LRR was observed (16). Another study performed by Muhsen et al., which recruited 1,087 patients with pT1-2N1 breast cancer, found no survival benefit from PMRT (17). A large sample analysis from the surveillance, epidemiology, and end results program (SEER) data also found that the survival outcomes were comparable between PMRT and no-PMRT patients in the modern era (14). Consistent with previous results, we also found that in the general population of T1-2N1 breast cancer patients, the clinical benefit from PMRT in the era of modern systemic therapy was not significant. In our cohort, adjuvant chemotherapy was prescribed to 86.2% of included patients and up to 88.5% of patients had ≥10 axillary lymph nodes removed. As a result, in the era of modern systemic therapy with adjuvant chemotherapy typically containing anthracycline and taxanes, higher proportion of HER2-positive patients receiving anti-HER2 therapy, and standard adjuvant hormonal therapy in HR-positive patients, the risk of tumor recurrence has been significantly decreased. Therefore, establishing a risk scoring system is critically important to identify early-stage breast cancer patients who might benefit from PMRT after mastectomy.

A practical reference risk stratifying system appears to be essential to identify patients who would benefit most from PMRT at the present time. A number of risk factors that have been identified by nomograms combining different risk factors have been developed as well (18, 19). The most representative risk factors identified were patient age, number of positive lymph nodes, histological grade, and lympho-vascular invasion (20). However, the absolute risk of LRR and survival benefit from PMRT with regard to risk stratifying system in T1–2N1 patients after mastectomy remain heterogeneous. Data from SEER population claimed that the benefit of PMRT was observed in patients with high-risk (2 or 3 positive nodes with tumors 2–5 cm in size) but not in patients with low-risk disease (1 or 2 positive nodes with tumors <2 cm in size) (9). A more recent retrospective study by Park et al. found that close resection margin was the only independent factor for worse prognosis among post-mastectomy patients undergoing modern systematic therapies (21). Molecular subtypes play a critical part in the decision-making of systemic therapy, but its role in tailoring local–regional radiotherapy remains undefined, even though ongoing studies aim to explore this. The TAILOR RT trial sponsored by the Canadian Cancer Trials Group is investigating the role of PMRT in favorable patients with one to three positive axillary nodes who have ER-positive tumors with low-risk Oncotype DX recurrence scores (NCT03488693). Our analysis failed to recognize the molecular subtypes as a significant prognostic factor of DFS by univariate analysis and multivariate analysis. One possible reason might be the improvement of patients' survival with the application of comprehensive systemic therapy with almost 86.2% of patients receiving 4–8 cycles of chemotherapy of anthracycline or taxane, 62.9% of patients receiving anti-HER2 therapy in HER2-positive subtypes, and 89.6% patients receiving endocrine therapy in ER-positive subtypes in our study. Consistent with our results, a large sample study, which enrolled 1,474 postmastectomy patients staged pT1-2N1 between 2006 and 2012, showed that molecular subtypes also failed to significantly influence the survival and local prognosis with application of optimal systemic therapy (98.1% with anthracycline or taxane chemotherapy, 95.1% with hormonal therapy in HR-positive

TABLE 2 | The univariate and multivariable analyses for outcomes.

Characteristics	DFS					
	Univariate analyses			Multivariable analyses		
	N of event	5-year rate	*p*-value	HR	95% CI	*p*-value
Age (years)			0.59			
≤40	7	89.2				
>40	61	90.3				
Menopausal status			0.16			
Premenopausal	19	93.6				
Postmenopausal	49	88.7				
Tumor size (cm)			0.06			
≤2.0	25	94.2				
2.0–5.0	43	88.2				
Nuclear grade			0.37			
Low-Intermediate	33	91.7				
High	30	87.6				
Axillary surgery			0.85			
SLNB alone	1	90.9				
ALND	67	90.5				
Number of positive LN			0.38			
1–2	53	90.5				
3	15	89.1				
ER status			0.51			
Positive	17	86.8				
Negative	51	91.1				
Ki67 index			0.38			
≤20%	20	92.9				
>20%	48	88.9				
HER2 status			0.36			
Positive	19	90.6				
Negative	49	88.6				
Molecular subtype			0.39			
Luminal	51	91.1				
HER2 positive	10	84.6				
Triple negative	7	88.7				
Chemotherapy			<0.01			
Yes	51	91.0		1		
No	16	84.5		2.69	1.51–4.79	<0.01
Target therapy			0.18			
Yes	13	86.9				
No	54	90.6				
Endocrine therapy			0.28			
Yes	45	91.8				
No	22	85.7				
PMRT			0.13			
Yes	26	91.7				
No	42	88.8				
Subgroups			0.04			
Low risk	29	92.6		1		
High risk	39	87.5		1.81	1.11–2.98	0.02

patients, and 47.4% with anti-HER2 therapy in HER2-positive patients) (22). As yet, the molecular subtype is not included in National Comprehensive Cancer Center (NCCN) guidelines to guide the decision-making of PMRT in this population (23). Results of prospective trials are still of great importance to define the role of molecular subtypes and other more elaborate biomarkers in the decision-making of radiotherapy.

In our previous study, patients with positive or close surgical margin were excluded while age, number of positive lymph nodes, tumor size, and Ki67 index remained as independent risk factors for BCSS in T1-2N0-1 breast cancer (12). Our results showed that benefits from PMRT were disparate between different risk groups. PMRT significantly improved DFS in the high-risk group with 2–4 risk factors while it did not improve in the low-risk group with 0–1 risk factor. Although it was a single-center experience, it provided a basis to conduct a multiple institutional study in a second phase. The randomized SUPREMO trial was prospectively designed to evaluate the role of PMRT in 1,688 women with intermediate-risk breast cancer defined as T1-2N1, T3N0, or T2N0 with lympho-vascular invasion and high grade who underwent mastectomy

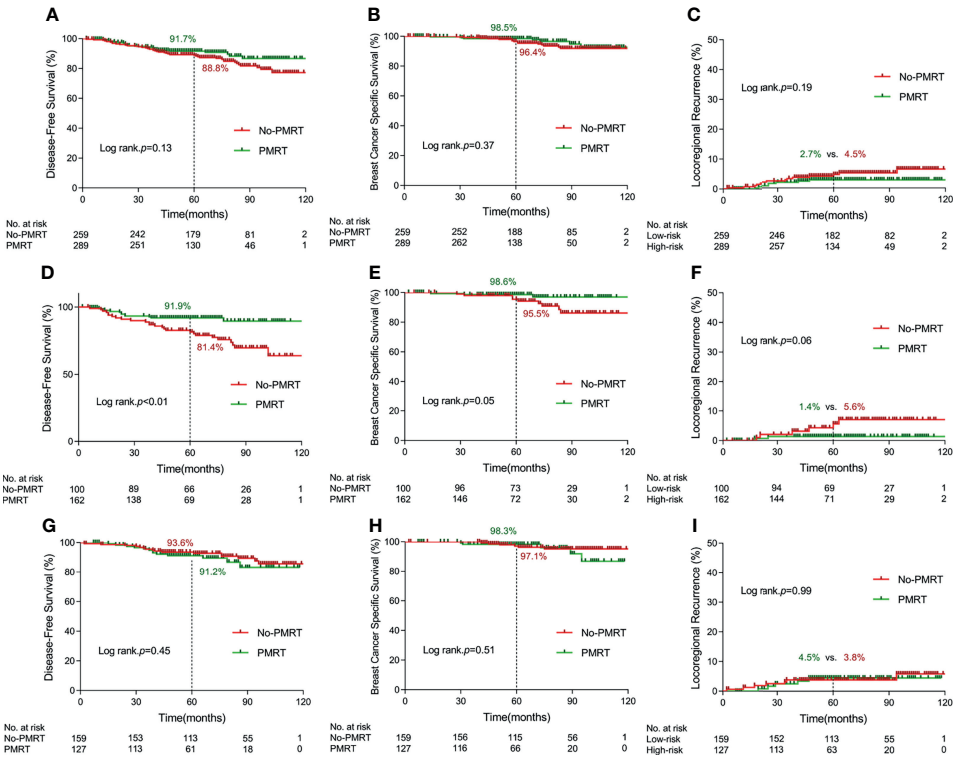


FIGURE 2 | Kaplan–Meier curves for 5-year disease-free survival, 5-year breast cancer specific survival, and 5-year locoregional recurrence according to delivery of postmastectomy radiotherapy in terms of different cohorts. [**(A–C)** in the whole cohort; **(D–F)** in the high-risk subgroup; **(G–I)** in the low-risk group] (PMRT, postmastectomy radiotherapy).

between 2006 and 2013 (24, 25). The results of this study are expected by the end of 2023.

Recent studies incorporating information on the molecular profile of breast cancer aim to further tailor radiotherapeutic decisions based on risk stratification and potentially intrinsic radiosensitivity of different subtypes. Shao et al. conducted a retrospective cohort-based study and demonstrated that among patients with high-risk factors (T2 stage and 3 positive lymph nodes disease), PMRT prolonged over-survival only in the Luminal A subtype, but not for the triple-negative and HER2-positive subgroups (26). In addition to those known prognostic biomarkers, genomic profile will provide additional prognostic information to risk stratification. However, most of these studies were retrospective; thus, the evidence was relatively low. An observational cohort study using data from the American National Cancer Database (NCDB) and SEER found that the improved survival associated with PMRT was limited to patients with a low Oncotype DX recurrence score (RS) (27). Others had reported that RS could not define the patients who will benefit from PMRT or not (28). Mamounas et al. though found that a high RS predicted a higher risk of LRR in general, while such association was not established when N1 patients receiving mastectomy were further analyzed (29). At the present time, majority of the panel of 2021 SG-BCC agree that commercially available multigene signatures (e.g., MammaPrint and Recurrence Score) should not provide a solid recommendation for deciding RNI (92%) or PMRT (89%) when prospective trials such as TAILOR RT are still ongoing (30). Most of the ongoing trials integrating genomic profile are focused on ER-positive, HER2-negative tumors with one to three positive axillary nodes (31). To acknowledge the advantage of molecular and genomic profile in individualizing risk in a defined population, the inconvenience that other molecular subtypes are not covered by most of the trials should also be noticed. While awaiting these results, our present analysis provides a practical model of available clinical–pathological information and biomarkers in consideration of an individualized PMRT.

There are limitations of this study that need to be mentioned. First, this is a retrospective study of our institute; thus, potential selection bias could not be excluded. Second, the median follow-up of 69 months is relatively limited, which might underestimate the actual survival outcomes of this patient population.

In summary, our retrospective study provided a practical model to optimize the triage of PMRT in a highly debatable population, T1-2N1 breast cancer patients. The risk scoring system composed of four clinical–pathological risk factors can be applied to identify the high-risk patients who might benefit from PMRT undergoing modern systemic adjuvant therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Ruijin Hospital Affiliated Medicine School of Shanghai Jiao Tong University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Concept, design, analysis and interpretation of data, and manuscript writing: F-FX, W-XQ, and J-YC. Collection of data and final approval of manuscript: F-FX, LC, CX, GC, S-BW, W-XQ, and J-YC. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported in part by the National Key Research and Development Program of China (grant numbers 2016YFC0105409), Clinical Research Plan of SHDC (grant numbers SHDC2020CR2052B and SHDC2020CR4070), Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant Support (grant numbers 20171904), National Natural Science Foundation of China (grant numbers 81673102, 81602791, 81702601, 81803164, and 81972963), Shanghai Jiaotong University Translational Medicine Fund Support (grant numbers ZH2018QNA54), Special Construction of Integrated Chinese and Western Medicine in General Hospital (grant numbers ZHYY-ZXYJHZ X-2-201704 and ZHYY-ZXYJHZ X-2-201913), and Scientific and Technological Innovation Action Plan of Shanghai Science and Technology Committee (grant numbers 19411950900 and 19411950901).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.789198/full#supplementary-material

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: Cancer J Clin* (2018) 68 (6):394–424. doi: 10.3322/caac.21492
2. Waks AG, Winer EP. Breast Cancer Treatment: A Review. *JAMA* (2019) 321 (3):288–300. doi: 10.1001/jama.2018.19323
3. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, et al. Breast Cancer. *Nat Rev Dis Primers* (2019) 5(1):66. doi: 10.1038/s41572-019-0111-2
4. Recht A, Comen EA, Fine RE, Fleming GF, Hardenbergh PH, Ho AY, et al. Postmastectomy Radiotherapy: An American Society of Clinical Oncology, American Society for Radiation Oncology, and Society of Surgical Oncology Focused Guideline Update. *Pract Radiat Oncol* (2016) 6(6):e219–e34. doi: 10.1016/j.prro.2016.08.009
5. Chagpar AB. Debate: Postmastectomy Radiation Therapy in T1/2N1 Disease. *Ann Surg Oncol* (2021) 28(10):5456–60. doi: 10.1245/s10434-021-10500-5
6. McGale P, Taylor C, Correa C, Cutter D, Duane F, Ewertz M, et al. Effect of Radiotherapy After Mastectomy and Axillary Surgery on 10-Year Recurrence and 20-Year Breast Cancer Mortality: Meta-Analysis of Individual Patient Data for 8135 Women in 22 Randomised Trials. *Lancet (London England)* (2014) 383(9935):2127–35. doi: 10.1016/s0140-6736(14)60488-8
7. Kayali M, Abi Jaoude J, Tfayli A, El Saghir N, Poortmans P, Zeidan YH. Post-Mastectomy Radiation Therapy in Breast Cancer Patients With 1-3 Positive Lymph Nodes: No One Size Fits All. *Crit Rev Oncol Hematol* (2020) 147:102880. doi: 10.1016/j.critrevonc.2020.102880
8. Moo TA, McMillan R, Lee M, Stempel M, Patil S, Ho A, et al. Selection Criteria for Postmastectomy Radiotherapy in T1-T2 Tumors With 1 to 3 Positive Lymph Nodes. *Ann Surg Oncol* (2013) 20(10):3169–74. doi: 10.1245/s10434-013-3117-0
9. Huo D, Hou N, Jaskowiak N, Winchester DJ, Winchester DP, Yao K. Use of Postmastectomy Radiotherapy and Survival Rates for Breast Cancer Patients With T1-T2 and One to Three Positive Lymph Nodes. *Ann Surg Oncol* (2015) 22(13):4295–304. doi: 10.1245/s10434-015-4528-x
10. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. Early Breast Cancer: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up. *Ann Oncol* (2019) 30(10):1674. doi: 10.1093/annonc/mdz189
11. Bazan JG, Majithia L, Quick AM, Wobb JL, Terando AM, Agnese DM, et al. Heterogeneity in Outcomes of Pathologic T1-2n1 Breast Cancer After Mastectomy: Looking Beyond Locoregional Failure Rates. *Ann Surg Oncol* (2018) 25(8):2288–95. doi: 10.1245/s10434-018-6565-8
12. Qi WX, Cao L, Xu C, Zhao S, Chen J. Established and Validated Novel Nomogram for Predicting Prognosis of Post-Mastectomy Pn0-1 Breast Cancer Without Adjuvant Radiotherapy. *Cancer Manage Res* (2021) 13:3517–27. doi: 10.2147/cmar.s292233
13. White J, Tai A, Arthur D, Buchholz T, MacDonald S, Marks L, et al. *Breast Cancer Atlas for Radiation Therapy Planning: Consensus Definitions. Book Breast Cancer Atlas for Radiation Therapy Planning*. (2009).
14. Li FY, Lian CL, Lei J, Wang J, Hua L, He ZY, et al. Real-World Impact of Postmastectomy Radiotherapy in T1-2 Breast Cancer With One to Three Positive Lymph Nodes. *Ann Transl Med* (2020) 8(7):489. doi: 10.21037/atm.2020.03.49
15. Danish Breast Cancer Cooperative G, Nielsen HM, Overgaard M, Grau C, Jensen AR, Overgaard J. Study of Failure Pattern Among High-Risk Breast Cancer Patients With or Without Postmastectomy Radiotherapy in Addition to Adjuvant Systemic Therapy: Long-Term Results From the Danish Breast Cancer Cooperative Group DBCG 82 B and C Randomized Studies. *J Clin Oncol* (2006) 24(15):2268–75. doi: 10.1200/JCO.2005.02.8738
16. McBride A, Allen P, Woodward W, Kim M, Kuerer HM, Drinka EK, et al. Locoregional Recurrence Risk for Patients With T1,2 Breast Cancer With 1-3 Positive Lymph Nodes Treated With Mastectomy and Systemic Treatment. *Int J Radiat Oncol Biol Phys* (2014) 89(2):392–8. doi: 10.1016/j.ijrobp.2014.02.013
17. Muhsen S, Moo TA, Patil S, Stempel M, Powell S, Morrow M, et al. Most Breast Cancer Patients With T1-2 Tumors and One to Three Positive Lymph Nodes Do Not Need Postmastectomy Radiotherapy. *Ann Surg Oncol* (2018) 25(7):1912–20. doi: 10.1245/s10434-018-6422-9
18. Montero A, Ciérvide R, García-Aranda M, Rubio C. Postmastectomy Radiation Therapy in Early Breast Cancer: Utility or Futility? *Crit Rev Oncol Hematol* (2020) 147:102887. doi: 10.1016/j.critrevonc.2020.102887
19. Tang Y, Zhang YJ, Zhang N, Shi M, Wen G, Cheng J, et al. Nomogram Predicting Survival as a Selection Criterion for Postmastectomy Radiotherapy

in Patients With T1 to T2 Breast Cancer With 1 to 3 Positive Lymph Nodes. *Cancer* (2020) 126 Suppl 16:3857–66. doi: 10.1002/cncr.32963
20. Kassak F, Rossier C, Picardi C, Bernier J. Postmastectomy Radiotherapy in T1-2 Patients With One to Three Positive Lymph Nodes - Past, Present and Future. *Breast* (2019) 48:73–81. doi: 10.1016/j.breast.2019.09.008
21. Park HJ, Shin KH, Kim JH, Ahn SD, Kim JY, Park W, et al. Incorporating Risk Factors to Identify the Indication of Post-Mastectomy Radiotherapy in N1 Breast Cancer Treated With Optimal Systemic Therapy: A Multicenter Analysis in Korea (KROG 14-23). *Cancer Res Treat Off J Korean Cancer Assoc* (2017) 49(3):739–47. doi: 10.4143/crt.2016.405
22. Wang X, Zhang L, Zhang X, Luo J, Wang X, Chen X, et al. Impact of Clinical-Pathological Factors on Locoregional Recurrence in Mastectomy Patients With T1-2N1 Breast Cancer: Who Can Omit Adjuvant Radiotherapy? *Breast Cancer Res Treat* (2021) 190(2):277–86. doi: 10.1007/s10549-021-06378-2
23. Gradishar WJ, Moran MS, Abraham J, Aft R, Agnese D, Allison KH, et al. NCCN Guidelines® Insights: Breast Cancer, Version 4.2021. *J Natl Compr Cancer Netw JNCCN* (2021) 19(5):484–93. doi: 10.6004/jnccn.2021.0023
24. Velikova G, Williams LJ, Willis S, Dixon JM, Loncaster J, Hatton M, et al. Quality of Life After Postmastectomy Radiotherapy in Patients With Intermediate-Risk Breast Cancer (SUPREMO): 2-Year Follow-Up Results of a Randomised Controlled Trial. *Lancet Oncol* (2018) 19(11):1516–29. doi: 10.1016/s1470-2045(18)30515-1
25. Kunkler IH, Canney P, van Tienhoven G, Russell NSGroup MESTM. Elucidating the Role of Chest Wall Irradiation in 'Intermediate-Risk' Breast Cancer: The MRC/EORTC SUPREMO Trial. *Clin Oncol (R Coll Radiol)* (2008) 20(1):31–4. doi: 10.1016/j.clon.2007.10.004
26. Wei J, Jiang Y, Shao Z. The Survival Benefit of Postmastectomy Radiotherapy for Breast Cancer Patients With T1-2N1 Disease According to Molecular Subtype. *Breast* (2020) 51:40–9. doi: 10.1016/j.breast.2020.03.003
27. Goodman CR, Seagle BL, Kocherginsky M, Donnelly ED, Shahabi S, Strauss JB. 21-Gene Recurrence Score Assay Predicts Benefit of Post-Mastectomy Radiotherapy in T1-2 N1 Breast Cancer. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2018) 24(16):3878–87. doi: 10.1158/1078-0432.ccr-17-3169
28. Zhang WW, Tong Q, Sun JY, Hua X, Long ZQ, Deng JP, et al. 21-Gene Recurrence Score Assay Could Not Predict Benefit of Post-Mastectomy Radiotherapy in T1-2 N1mic ER-Positive HER2-Negative Breast Cancer. *Front Oncol* (2019) 9:270. doi: 10.3389/fonc.2019.00270
29. Mamounas EP, Liu Q, Paik S, Baehner FL, Tang G, Jeong JH, et al. 21-Gene Recurrence Score and Locoregional Recurrence in Node-Positive/ER-Positive Breast Cancer Treated With Chemo-Endocrine Therapy. *J Natl Cancer Inst* (2017) 109(4):1–8. doi: 10.1093/jnci/djw259
30. Thomssen C, Balic M, Harbeck N, Gnant M. St. Gallen/Vienna 2021: A Brief Summary of the Consensus Discussion on Customizing Therapies for Women With Early Breast Cancer. *Breast Care (Basel)* (2021) 16(2):135–43. doi: 10.1159/000516114
31. Ohri N, Haffty BG. Is There a Role for Postmastectomy Radiation (PMRT) in Patients With T1-2 Tumors and One to Three Positive Lymph Nodes Treated in the Modern Era? *Ann Surg Oncol* (2018) 25(7):1788–90. doi: 10.1245/s10434-018-6493-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor YC is currently organizing a Research Topic with the author JC.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

ORIGINAL RESEARCH
published: 03 March 2022
doi: 10.3389/fonc.2022.784839

A Clinical Assessment of a Magnetic Resonance Computer-Aided Diagnosis System in the Detection of Pathological Complete Response After Neoadjuvant Chemotherapy in Breast Cancer

Haiyong Peng[1†], Shaolei Yan[1†], Xiaodan Chen[2], Jiahang Hu[3], Kaige Chen[1], Ping Wang[1], Hongxia Zhang[1], Xiushi Zhang[1†] and Wei Meng[1*†]*

[1] Radiology Department, Harbin Medical University, Harbin Medical University Cancer Hospital, Harbin, China, [2] Department of Computer Technology, Harbin Institute of Technology University, Harbin, China, [3] Department of Radiology, Hongqi Hospital Affiliated to Mudanjiang Medical University, Mudanjiang, China

OPEN ACCESS

Edited by:
Kunwei Shen,
Shanghai Jiao Tong University, China

Reviewed by:
Vinay Ayyappan,
University of Pennsylvania,
United States
Xu Ling,
Peking University First Hospital, China

***Correspondence:**
Xiushi Zhang
xiushiz@126.com
Wei Meng
articlemengwei@163.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: 28 September 2021
Accepted: 07 February 2022
Published: 03 March 2022

Citation:
Peng H, Yan S, Chen X, Hu J, Chen K, Wang P, Zhang H, Zhang X and Meng W (2022) A Clinical Assessment of a Magnetic Resonance Computer-Aided Diagnosis System in the Detection of Pathological Complete Response After Neoadjuvant Chemotherapy in Breast Cancer. Front. Oncol. 12:784839. doi: 10.3389/fonc.2022.784839

Purpose: This study aimed to assess the diagnostic performance and the added value to radiologists of different levels of a computer-aided diagnosis (CAD) system for the detection of pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) in patients with breast cancer. Besides, to investigate whether tumor molecular typing is associated with the efficiency of diagnosis of the CAD systems.

Methods: 470 patients were identified with breast cancers who underwent NAC and post MR imaging between January 2016 and March 2019. The diagnostic performance of radiologists of different levels and the CAD system were compared. The added value of the CAD system was assessed and subgroup analyses were performed according to the tumor molecular typing.

Results: Among 470 patients, 123 (26%) underwent pCR. The CAD system showed a comparable specificity as the senior radiologist (83.29% vs. 84.15%, p=0.488) and comparable area under the curve (AUC) (0.839 vs. 0.835, p =0.452). The performance of all radiologists significantly improved when aided by the CAD system (P<0.05), And there were no statistical differences in terms of sensitivity, specificity and accuracy between the two groups with CAD assistance(p>0.05).The AUC values for identifying pCR in TN patients were significant (0.883, 95%CI: 0.801-0.964, p < 0.001).

Conclusion: The CAD system assessed in this study improves the performance of all radiologists, regardless of experience. The molecular typing of breast cancer is potential influencer of CAD diagnostic performance.

Keywords: breast cancer, MRI, computer-aided diagnosis, pathological complete response, neoadjuvant chemotherapy (NAC)

INTRODUCTION

With the wide application of neoadjuvant chemotherapy in the treatment of breast cancer patients, it has become an essential part of the treatment of breast cancer, especially stage II and III breast cancer (1, 2). Its curative effect directly affects the follow-up treatment and prognosis of patients. Effective NAC can reduce tumor stage, make breast conserving surgery possible, and even achieve preoperative pathological complete remission (pCR) in up to 30% of patients (3, 4). The efficacy of chemotherapy varies and depends on the subtypes of breast cancers (5). HER2- positive and triple-negative patients are more likely to achieve pCR, and surgery is expected to be avoided (6). As a consequence, accurate recognition of treatment response is crucial to optimize patient management and treatment adjustment.

Conventional imaging modalities, such as mammography and ultrasound, show limited accuracy in predicting treatment response after NAC (7, 8), Magnetic Resonance Imaging (MRI) is currently used in clinical practice to assess the response at the end of NAC. Several studies have investigated the value of breast MRI for assessing or predicting treatment response to NAC (9–11). However, MRI has limitations when used clinically because image interpretation is based on the radiologist's visual assessment.

Computer-aided diagnosis (CAD) has attracted significant attention from researchers as a newly developed technique that can enhance radiologists' interpretation and overcome subjective limitations (12–15). The CAD detection and diagnosis methods are based on machine learning approaches that extract features based on shape, texture, and statistical values, assessing or predicting treatment response to NAC. Several studies have shown that the CAD system has superior capability and performance (16, 17). However, few studies have evaluated the changes in diagnosis performance when the CAD system combined with radiologists with various levels of experience in assessing response to chemotherapy after treatment.

Therefore, this retrospective study aimed to validate the clinical role of the CAD systems in the assessment of pCR and to evaluate its value in improving doctors' diagnosis performance. Besides, the association between the efficiency of diagnosis of the CAD systems and tumor subtypes was discussed.

MATERIALS AND METHODS

The institutional review board approved this retrospective study. Informed consent was obtained from all the patients. All patients in whom invasive breast cancer were diagnosed between January 2016 and March 2019, treated with neoadjuvant chemotherapy, and who underwent breast MR imaging before neoadjuvant chemotherapy were eligible. The chemotherapy regimens were drawn up according to the neoadjuvant therapy regimens of NCCN guidelines breast cancer version 1.2016 including (regimen I): AC-T(doxorubicin 60mg/m2 plus cyclophosphamide 600mg/m2 IV day 1 every 21 days for 4 cycles followed by docetaxel 100mg/m2 IV day 1 every 21 days for 4 cycles); (regimen II):TAC(docetaxel 75mg/m2 plus doxorubicin 50mg/m2 plus cyclophosphamide 500mg/m2 every 21 days for 6 cycles). Trastuzumab or Partuzumab would employ in HER2/neu positive patients (Trastuzumab:the dose was 4mg/kg for the first use; the followed dose was 2mg/kg, i.e., every 21 days for 1 year; Partuzumab: the dose was 840mg/kg for the frst use; the followed dose was 420mg/kg, i.e., every 21 days for 1 year). A total of 493 patients (mean age: 49.6 ± 10.09 years; range: 24-70 years) and 470 masses (mean size before chemotherapy: 19.03 ± 7.1mm; range: 6-55mm) underwent core needle biopsy or surgery. Twenty-three patients were excluded from the study group, because the patient had unilateral multifocal cancers and the correlation between the tumor in MRI and postoperative pathological examination was uncertain. A flowchart of the study population is presented in **Figure 1**.

MR images were obtained using a 3.0T MR scanner (Philips Achieva 3.0T). The patients adopted a prone position and put their breasts into the dedicated phased-array breast coil. Imaging parameters for DCE-MRI were are as follows:

Axial T1-weighted imaging (repetition time (TR) = 495 ms; echo time (TE) = 10 ms; slice thickness/gap = 3 mm/0 mm; matrix = 512; number of signal averaged (NSA) = 1; field of view (FOV) = 340 mm × 340 mm); axial T2-weighted imaging (TR = 4213 ms, TE = 120 ms, slice thickness/gap = 3 mm/0 mm, matrix = 512, NSA = 1, FOV = 340 mm × 340 mm); T2-weighted fat-saturated imaging using a spectral selection attenuated inversion recovery (SPAIR) (TR = 4216 ms, TE = 60 ms, inversion delay (IR) = 120 ms, slice thickness/gap = 3 mm/0 mm, matrix = 352, NSA = 1, FOV = 340 mm × 340 mm); and T1-weighted high-resolution isotropic volume examination (THRIVE) (TR = 4.4 ms, TE = 2.2 ms, flip angle = 12°; matrix = 352; FOV = 340 mm × 340 mm; number of sections = 110; acquisition time: 256 seconds). MR imaging data sets were acquired once before gadolinium (Gd)- diethylenetriamine penta-acetic acid (DTPA) (Bayer scheming pharma AG, Berlin, Germany) injection and at 90-second intervals

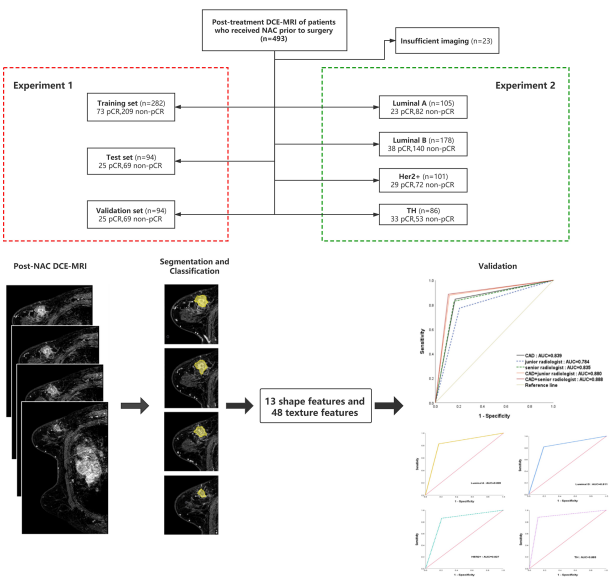


FIGURE 1 | Patients selection flowchart and the composition of the training, test, and verification sets. pCR, pathologic complete response; NAC, neoadjuvant chemotherapy.

upon injection of 0.1 mmol/kg Gd-DTPA(followed by an intravenous saline flush of 20 ml), for a total imaging duration of 5–8 minutes.

Segmentation and Classification

We first used an encoder-decoder network called Unet to segment the tumor region in the MRI, shown in **Figure 2**. The encoder network in Unet extracts the deep semantic features in MRI, and the decoder network upsamples the features to the size of the original image. The backbone of the encoder is resnet18, and the strategy of the decoder is upsampling step by step. The learning rate of training is 1e-5, and epochs are 500. The weight decay is 5e-4 and the training optimizer is Adam. The loss function is Cross Entropy. Thus, the Unet model segment the tumor region from the background. And then we extracted shape features and texture features of tumor. The 13 shape features describe the appearance of tumor, which include roundness, aspect ratio, average normalized radial length, the normalized standard deviation of radial length, average normalized entropy radial length, area ratio, aspect ratio, number of leaflets, needle shape, boundary roughness, direction angle, normalized ellipse circumference and normalized ellipse contour. The 48 texture features show the details inside tumors obtained using gray level co-occurrence matrix (GLCM). Moreover, we extracted energy, correlation, contrast and entropy under three steps with four directions. The 13 shape features and 48 texture features were input into the support vector machine to execute pCR or non-pCR classification. The goal of Support Vector Machine (SVM) is to find a hyperplane to separate the two classes of data and maximize the margin in the meantime. The data which is closest to the margin is called a support vector and the distance between the hyperplane and any support vector is 1.

Observer Study

The MR images were assessed by a senior radiologist of more than ten years' experience and then assessed by a junior radiologist of three years' experience. The two groups of radiologists analyzed the integrated computer workstation images without access to the final histological results. The diagnosis of the pCR was based on whether the tumor volume disappeared or marked and constantly homogeneous enhancement fibrous tissue on DCE-MRI. Only the largest mass was used for evaluation if a patient had multiple residual masses after NAC. If there was disagreement between the two radiologists, they reviewed the images together, obtaining a consensus.

Pathological Diagnoses

All breast lesions were pathologically confirmed by surgery or biopsy. Pathological complete remission (pCR) was defined as no residual invasive tumor cells in primary breast lesions after therapy, but ductal carcinoma in situ(DCIS) can exist. Lesions were divided into pCR and non-pCR groups, and all the lesions were divided into molecular subtypes. All the assessments were performed by a pathologist who had more than ten years' experience. Tumor characteristics are presented in **Table 1**.

Statistical Analysis

The diagnostic performance of the radiologist assisted by the CAD system was defined as positive when the criteria met one of the two categories: the radiologist and the CAD system. The SPSS software (version 20.0, IBM Corp, Armonk, NY, United States) and MedCalc software (version 15.2, Mariakerke, Belgium) were used to analyze the data. Taking molecular subtypes as the standard, the separate diagnostic ROC curves of luminal a, luminal B, HER2 +, TN were constructed; The ROC curves for the separate diagnosis of junior radiologist, senior radiologist and CAD and the joint diagnosis of junior radiologist and CAD, senior radiologist and CAD were constructed by comparing the pathological results. and the area under the curve (AUC) and sensitivity, specificity and accuracy were calculated. Chi square

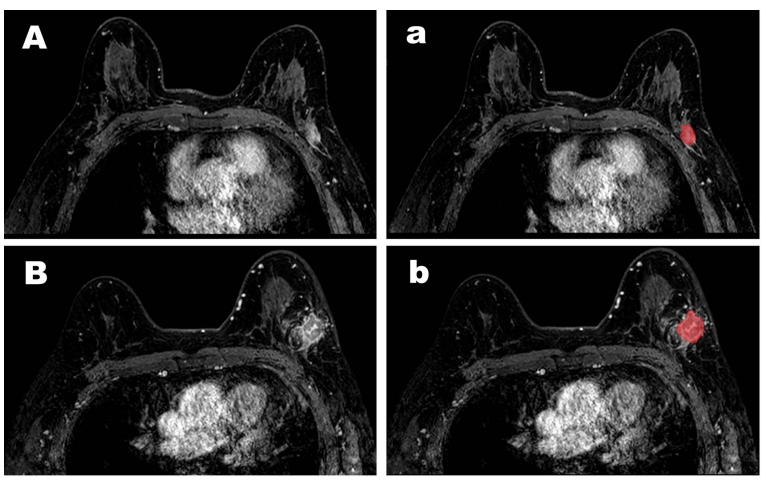


FIGURE 2 | Representative cases of pCR **(A)** and non-pCR **(B)**. For the case **(A)**, both the CAD system and the senior radiologists diagnosed it as a pCR but the junior radiologists diagnosed it as a non-pCR. For the case **(B)**, both the CAD system and the senior and the junior radiologists diagnosed it as a non-pCR. The images (a, b) for the segmentation results were obtained by computer-aided diagnosis system.

TABLE 1 | Breakdown of dataset by pathological complete response status.

	pCR	Non-pCR	All patients
Number of patients	123 (32–66)	347 (24–70)	470 (24–70)
Mean Age (y)*	54	48	50
Tumor diameter (mm)*			
Mean	22.1	32.2	29.0
SD	12.5	13.9	14.3
Receptor status			
Luminal A	23	82	105
Luminal B	38	140	178
HER-2+	29	72	101
TN	33	53	86
Surgery type			
Breast conservation	114	218	332
Mastectomy	9	129	138

**Data are means, with ranges in parentheses.*

test was used to compare the sensitivity, specificity and accuracy of different diagnostic methods. Inspection level α=0.5.

RESULTS

There were pCR and non-pCR in the 493 patients (mean age: 49.6 ± 10.09 years; range: 24-70 years). The experimental data were 470 MRI masses (average size before NAC: 19.03 ± 7.1 mm, range:6-55mm), of which 347(74%) were non-pCR, and 123(26%)were pCR. The non-pCR images and pCR images were divided into 5 parts respectively. Each time, 3 parts were taken as the training set, 1 part as the verification set and 1 part as the test set.

The diagnostic performances of the CAD system, radiologists in the different groups, and CAD-assisted radiologists for detecting pCR were summarized in **Table 2**.

The CAD system exhibited no statistically significant difference in terms of specificity compared with the senior radiologist(83.29% versus 84.15%,p=0.488),and CAD has higher sensitivity while the accuracy were lower in the CAD system than those in the senior radiologist(84.55% vs. 82.93%,p=0.005;83.61% vs. 83.83%,p=0.037, respectively). When compared with the junior radiologist, the CAD system resulted in markedly increased sensitivity and accuracy and higher specificity in the classification of pCR (84.55% vs.77.24%, p <0.001; 83.83% vs.78.94%, p<0.001; 83.29% vs.79.54%, p = 0.007, respectively). When the CAD system was used to assist the senior and junior radiologists, the sensitivity, specificity and accuracy of diagnosis were significantly improved, no matter junior radiologist or senior radiologist($p \leq 0.001$).And there was no statistical difference terms of sensitivity, specificity and accuracy between the two groups with CAD assistance(87.80% vs.88.62%, p =0.525; 88.18% vs.89.04%, p=0.713; 88.94% vs.88.09%, p = 0.525, respectively). ROC analysis comparing the diagnostic performance of CAD systems, radiologists, and CAD-assisted radiologists is shown in **Table 2** and **Figure 3**. The AUCs were 0.784 for the junior radiologist,0.835 for the senior radiologist,0.839 for the CAD system, 0.880 for the CAD-assisted junior radiologist,0.888 for the CAD-assisted senior radiologist.

Results of performance of CAD in different molecular subtypes are listed in **Table 3** and **Figure 4**. Out of the 123 patients who achieved pCR, twenty-three breast cancers were luminal A, thirty-eight were luminal B, twenty-nine were HER2-enriched, and thirty-three were triple-negative. The AUC values for identifying pCR in TN patients were significant (0.883, 95% CI: 0.801-0.964, p < 0.001), and the specificity, sensitivity and accuracy achieved 88.68%, 87.88% and 88.37%, respectively.

DISCUSSION

MR plays a crucial role in the assessment of response to chemotherapy during treatment. However, the usefulness of MR may be limited for the diagnostic performance of it varies

TABLE 2 | Diagnostic performance of CAD system, radiologists and CAD-assisted radiologists.

Method	AUC	95%CI	Sensitivity	Specificity	Accuracy
Junior radiologist	0.784	0.734-0.833	77.24	79.54	78.94
Senior radiologist	0.835	0.791-0.880	82.93	84.15	83.83
CAD	0.839	0.796-0.883	84.55	83.29	83.61
Junior radiologist+CAD	0.880	0.841-0.919	87.80	88.18	88.09
Senior radiologist+CAD	0.888	0.851-0.926	88.62	89.04	88.94
P^{a1}	0.049		<0.001	0.007	<0.001
P^{a2}	0.452		0.005	0.488	0.037
P^{b1}	0.001		<0.001	<0.001	<0.001
P^{b2}	0.037		<0.001	0.001	<0.001
P*	0.380		0.525	0.713	0.525

P^{a1} is CAD vs. Junior radiologist; P^{a2} is CAD vs. Senior radiologist; P^{b1} is Junior radiologist vs. Junior radiologist+CAD.
P^{b2} is Senior radiologist vs. Senior radiologist+CAD; P is Junior radiologist+CAD vs. Senior radiologist+CAD.*

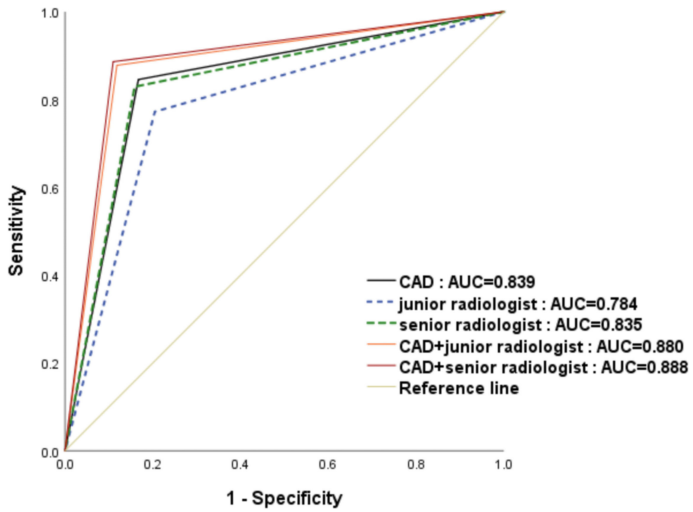


FIGURE 3 | The receiver operating characteristic (ROC) curves for the performance of the computer-aided diagnosis (CAD) system, the senior radiologist, the junior radiologist, and CAD-assisted radiologists. The area under the ROC curve for the combination of senior radiologists and CAD was significantly highest.

TABLE 3 | Diagnostic efficacy of the diagnosis of CAD among subtype.

All patients	AUC	95%CI	P	Sensitivity	Specificity	Accuracy
Luminal A	0.828	0.726-0.929	<0.001	82.61	82.93	82.86
Luminal B	0.811	0.731-0.892	<0.001	81.58	80.71	80.90
HER2+	0.827	0.736-0.918	<0.001	86.20	84.72	85.15
TN	0.883	0.801-0.964	<0.001	87.88	88.68	88.37

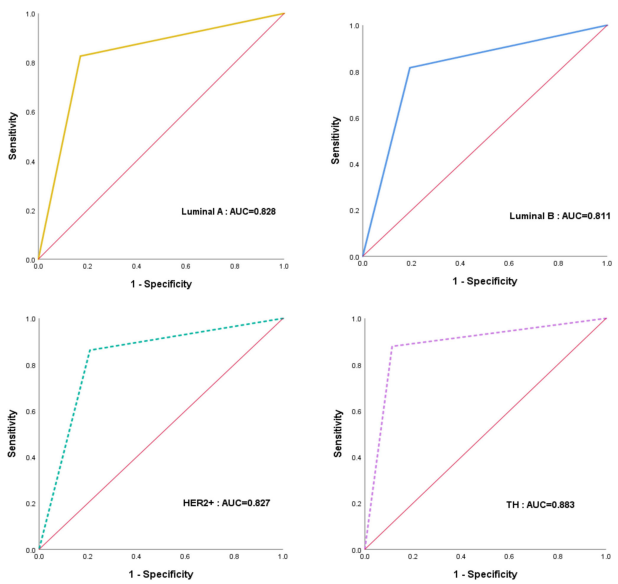


FIGURE 4 | The receiver operating characteristic (ROC) curves for the performance of CAD in different molecular subtypes.

from person to person, which depends on the experience of a radiologist to a large extent. The CAD system based on artificial intelligence has been developed to assist radiologists in analyzing images, shortening the time cost of the diagnostic process, and reducing interobserver variability.

In this study, a clinical assessment was performed to evaluate the value of the CAD system in the MRI diagnosis of pCR. This retrospective study showed that the CAD system generally performed comparably to qualitative assessments by the senior radiologist in terms of specificity but had a higher sensitivity and lower accuracy. In addition, the specificity, sensitivity and accuracy of the CAD system were remarkably higher than that of the junior radiologist.

The added value of the CAD system was also evaluated in this study. Our study showed that CAD assistance significantly improves all radiologists' performance, which was consistent with some studies (12, 18, 19). With the assistance of the CAD system, the junior radiologist showed a significant increase in AUC from 0.784 to 0.880 ($P<0.001$). The diagnostic performance of senior radiologists was also improved and statistically significant($P<0.05$). The improved AUC indicated that the CAD system might function as a supplementary opinion to avoid missed diagnoses, especially for less-experienced radiologists. As shown in the study, the CAD system improved radiologist specificity, which implied that the CAD system could play a constructive role in reducing unnecessary biopsies or follow-up imaging studies to assess response to chemotherapy.

The study contributes to several clinical implications. First, the CAD system in this study can automatically recognize and analyze MR images. Therefore, it is also possible to overcome the disadvantages caused by the visual assessment of radiologists, which demonstrates an opportunity for the combination between radiologists and machines in future clinical practice. Second, the CAD system exhibited no statistically significant difference in specificity compared with the senior radiologist. In addition, the sensitivity and accuracy were higher. This finding implied that the CAD system could reduce unnecessary biopsies and also help to lighten the load of radiologists. Besides, all individual radiologists significantly improved with CAD assistance, which could serve as a supplementary diagnosis for radiologists to minimize missed diagnoses, Especially for inexperienced radiologists. Lastly, the CAD system's diagnostic efficiency for assessing response to chemotherapy during treatment was evaluated, which further reflected the clinical value of the CAD system.

We further analyzed whether the efficiency of diagnosis of the CAD systems was affected by molecular typing. In previous studies, Cain developed a multivariate machine learning model using 288 pre-NAC MRIs. They found that this model was significantly associated with pCR in TN/HER2 + patients, reaching an AUC of 0.707 (20). Braman also identified that the TN/HER2 + combined tumor subtype could predict pCR more accurately than the HR and HER2 + tumor subtypes (AUC = 0. 93) by extracting intratumoral and peritumoral features (21). However, they grouped TN and HER2 + patients into a combined TN/HER2+ cohort because of insufficient sample sizes. Moreover, they used the pre-NAC MRI images, which is different from our study. One of our methodologies vital advantages was that our experiments utilize computers to process segmentation, classification, and subtyping of tumors simultaneously. Moreover, we extracted 13 shape features and 48 texture features of tumor to improve the classification. In summary, TN cancers seemed to carry distinct radiomic signatures that enable CAD to separate from breast cancers with other features. One possible explanation for the findings may be that the TN subtypes demonstrated more necrosis so the texture may be more features in the images.

This study also has some limitations. First of all, the sample capacity was relatively small, and selection bias was inevitable due to the retrospective study nature. Therefore, additional studies with a more significant number of NAC cases are required to establish the clinical value of CAD in predicting the pCR after NAC. Second, the MRI scans we used were only two-dimensional rather than three-dimensional. So, it may not have represented the entire tumor exactly. Finally, no formal training for the processed images was used in our study. Although the processed images' features were familiar to the radiologists, a training set to allow radiologists to become familiar with the CAD method might enhance their confidence to use it.

In conclusion, the CAD system assessed in this study improves the performance of all radiologists, regardless of experience, in classifying pCR on MRI. The molecular typing of breast cancer is a potential influencer of CAD diagnostic performance. Future work will address using a larger independent dataset for testing to improve its diagnostic performance and evaluate the clinical role of CAD diagnosis. CAD systems may improve the specificity of MRI and yield high clinical impact, especially among radiologists with limited experience in MRI.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HP and SY created the datasets, interpreted the data and defined the clinical labels. XC, XZ, HZ, PW, KC, and JH developed the network architecture and training and testing setup. HP and SY created the figures and performed statistical analysis. HP wrote the manuscript. WM provided the clinical expertise and guidance on the study design. WM supervised the project. All authors contributed to the article and approved the submitted version.

FUNDING

The work was supported by the National Natural Science Foundation of China (81901701), the Postdoctoral Science Foundation of the Ministry of Heilongjiang Province(LBH-Q20123)and Harbin Medical University Cancer Hospital Haiyan fund (JJMS2021-31, JJZD2021-15).

REFERENCES

1. Gradishar WJ, Anderson BO, Abraham J, Aft R, Agnese D, Allison KH, et al. Breast Cancer, Version 3.2020, Nccn Clinical Practice Guidelines in Oncology. *J Natl Compr Cancer Netw: JNCCN* (2020) 18:452–78. doi: 10.6004/jnccn.2020.0016
2. Kaufmann M. International Expert Panel on the Use of Primary (Preoperative) Systemic Treatment of Operable Breast Cancer: Review and Recommendations. *J Clin Oncol* (2003) 21:2600–8. doi: 10.1200/JCO.2003.01.136
3. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, et al. Effect of Preoperative Chemotherapy on the Outcome of Women With Operable Breast Cancer. *J Clin Oncol* (1998) 16:2672–85. doi: 10.1200/JCO.1998.16.8.2672
4. Spring LM, Fell G, Arfe A, Sharma C, Greenup R, Reynolds KL, et al. Pathologic Complete Response After Neoadjuvant Chemotherapy and Impact on Breast Cancer Recurrence and Survival: A Comprehensive Meta-Analysis. *Clin Cancer Res* (2020) 26:2838–48. doi: 10.1158/1078-0432.CCR-19-3492
5. Maur M. Primary Systemic Therapy in Operable Breast Cancer: Clinical Data and Biological Fall-Out. *Ann Oncol* (2006) 17(Suppl 5):v158–64. doi: 10.1093/annonc/mdj973
6. Rauch GM, Kuerer HM, Adrada B, Santiago L, Moseley T, Candelaria RP, et al. Biopsy Feasibility Trial for Breast Cancer Pathologic Complete Response Detection After Neoadjuvant Chemotherapy: Imaging Assessment and Correlation Endpoints. *Ann Surg Oncol* (2018) 25:1953–60. doi: 10.1245/s10434-018-6481-y
7. Chagpar AB, Middleton LP, Sahin AA, Dempsey P, Buzdar AU, Mirza AN, et al. Accuracy of Physical Examination, Ultrasonography, and Mammography in Predicting Residual Pathologic Tumor Size in Patients Treated With Neoadjuvant Chemotherapy. *Ann Surg* (2006) 243:257–64. doi: 10.1097/01.sla.0000197714.14318.6f
8. Keune JD, Jeffe DB, Schootman M, Hoffman A, Gillanders WE, Aft RL. Accuracy of Ultrasonography and Mammography in Predicting Pathologic Response After Neoadjuvant Chemotherapy for Breast Cancer. *Am J Surg* (2010) 199:477–84. doi: 10.1016/j.amjsurg.2009.03.012
9. Hylton NM, Blume JD, Bernreuter WK, Pisano ED, Rosen MA, Morris EA, et al. Locally Advanced Breast Cancer: Mr Imaging for Prediction of Response to Neoadjuvant Chemotherapy–Results From Acrin 6657/I-Spy Trial. *Radiology* (2012) 263:663–72. doi: 10.1148/radiol.12110748
10. Uematsu T, Kasami M, Yuen S. Neoadjuvant Chemotherapy for Breast Cancer: Correlation Between the Baseline MR Imaging Findings and Responses to Therapy. *Eur Radiol* (2010) 20:2315–22. doi: 10.1007/s00330-010-1813-8
11. Loo CE, Straver ME, Rodenhuis S, Muller SH, Wesseling J, Vrancken Peeters MJ, et al. Magnetic Resonance Imaging Response Monitoring of Breast Cancer During Neoadjuvant Chemotherapy: Relevance of Breast Cancer Subtype. *J Clin Oncol Off J Am Soc Clin Oncol* (2011) 29:660. doi: 10.1200/JCO.2010.31.1258
12. Meinel LA, Stolpen AH, Berbaum KS, Fajardo LL, Reinhardt JM. Breast Mri Lesion Classification: Improved Performance of Human Readers With a Backpropagation Neural Network Computer-Aided Diagnosis (Cad) System. *J Magn Reson Imaging* (2010) 25:89–95. doi: 10.1002/jmri.20794
13. Deurloo EE, Muller SH, Peterse JL, Besnard AP, Gilhuijs KG. Clinically and Mammographically Occult Breast Lesions on MR Images: Potential Effect of Computerized Assessment on Clinical Reading. *Radiology* (2005) 234:693–701. doi: 10.1148/radiol.2343031580
14. Meeuwis C, van de Ven SM, Stapper G, Fernandez Gallardo AM, van den Bosch MA, Mali WP, et al. Computer-Aided Detection (CAD) for Breast MRI: Evaluation of Efficacy at 3.0 T. *Eur Radiol* (2010) 20:522–8. doi: 10.1007/s00330-009-1573-5
15. Arazi-Kleinman T, Causer PA, Jong RA, Hill K, Warner E. Can Breast MRI Computer-Aided Detection (CAD) Improve Radiologist Accuracy for Lesions Detected at MRI Screening and Recommended for Biopsy in a High-Risk Population? *Clin Radiol* (2009) 64:1166–74. doi: 10.1016/j.crad.2009.08.003
16. Williams TC, DeMartini WB, Partridge SC, Peacock S, Lehman CD. Breast MR Imaging: Computer-Aided Evaluation Program for Discriminating Benign From Malignant Lesions. *Radiology* (2007) 244:94–103. doi: 10.1148/radiol.2441060634
17. Makkat S, Luypaert R, Stadnik T, Bourgain C, Sourbron S, Dujardin M, et al. Deconvolution-Based Dynamic Contrast-Enhanced MR Imaging of Breast Tumors: Correlation of Tumor Blood Flow With Human Epidermal Growth Factor Receptor 2 Status and Clinicopathologic Findings–Preliminary Results. *Radiology* (2008) 249:471–82. doi: 10.1148/radiol.2492071147
18. Lehman CD, Peacock S, DeMartini WB, Chen X. A New Automated Software System to Evaluate Breast MR Examinations: Improved Specificity Without Decreased Sensitivity. *AJR Am J Roentgenol* (2006) 187:51–6. doi: 10.2214/AJR.05.0269
19. Penn AI, Kumar N, Thompson SF, Schnall MD, Wang F, Gatsonis G, et al. Preliminary Performance Analysis of Breast MRI CAD System. *Med Imaging Int Soc Optics Photon* (2001) 4322:1944–53. doi: 10.1117/12.431088
20. Cain EH, Saha A, Harowicz MR, Marks JR, Marcom PK, Mazurowski MA. Multivariate Machine Learning Models for Prediction of Pathologic Response to Neoadjuvant Therapy in Breast Cancer Using MRI Features: A Study Using an Independent Validation Set. *Breast Cancer Res Treat* (2019) 173:455–63. doi: 10.1007/s10549-018-4990-9
21. Braman NM, Etesami M, Prasanna P, Dubchuk C, Gilmore H, Tiwari P, et al. Intratumoral and Peritumoral Radiomics for the Pretreatment Prediction of Pathological Complete Response to Neoadjuvant Chemotherapy Based on Breast DCE-MRI. *Breast Cancer Res* (2017) 19:57. doi: 10.1186/s13058-017-0846-1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

CASE REPORT
published: 29 March 2022
doi: 10.3389/fonc.2022.878645

Chylous Leakage After Breast-Conserving Surgery and Axillary Clearance: Case Report and Management Strategies

Liang Yin[1]*, *Peiqing Chen*[1], *Enock Adjei Agyekum*[2], *Xiudi Xiao*[1] and *Xiaoqin Qian*[2]

[1] Department of Breast Surgery, Jiangsu University Affiliated People's Hospital, Zhenjiang, China, [2] Department of Ultrasound, Jiangsu University Affiliated People's Hospital, Jiangsu University, Zhenjiang, China

Chylous leakage is a rare complication of breast and axillary surgery. We present a case of chylous leakage inside the breast following breast-conserving surgery and axillary lymph node dissection. The majority of chylous leakages in the breast are managed with conservative measures aimed at reducing lymphatic fluid production and outflow. Surgical intervention is required in cases of conservative treatment failure and high output chylous leakage. To the best of our knowledge, this is the first case report of chyles leaks inside the breast following breast-conserving surgery that was successfully treated surgically.

Keywords: chylous leakage, breast cancer, breast-conserving, axillary clearance, case report management strategies

OPEN ACCESS

Edited by:
Xiaosong Chen,
Shanghai Jiao Tong University, China

Reviewed by:
Gianni Lazzarin,
Abano Terme Hospital, Italy
Ali Coskun,
Izmir Bozyaka Training and Research Hospital, Turkey

***Correspondence:**
Liang Yin
justinfly2080@gmail.com

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *18 February 2022*
Accepted: *07 March 2022*
Published: *29 March 2022*

Citation:
Yin L, Chen P, Agyekum EA, Xiao X and Qian X (2022) Chylous Leakage After Breast-Conserving Surgery and Axillary Clearance: Case Report and Management Strategies. Front. Oncol. 12:878645. doi: 10.3389/fonc.2022.878645

INTRODUCTION

Chylous leakage is a well-known complication of the neck, thoracic, and upper gastrointestinal surgery. Its incidence ranges from 0.5% to 8.3% in neck dissection, with the majority of cases occurring on the left side due to thoracic duct injury (1, 2). However, its occurrence following breast and axillary surgery is a rare occurrence. In breast cancer surgery, the reported incidence ranges from 0.36% to 0.84% in the literature (3). Given the exceedingly rare occurrence, there is currently little guidance on the diagnosis and management of chylous leakage. We present a case of chylous leakage after breast-conserving surgery and axillary clearance in a patient with solid papillary carcinoma of the right breast.

CASE REPORT

A 67-year-old woman came to our hospital complaining of a lump in her right breast. An ultrasound of the breast revealed a 2.5*2-cm solid mass in the upper outer quadrant of the right breast, with no enlarged right axillary lymph node. Mammography revealed a high density, ill-defined lump in the upper outer quadrant of the right breast. The patient was diagnosed with stage IIA breast cancer. We decided to proceed with breast-conserving surgery, namely a lumpectomy and sentinel lymph node biopsy using methylene blue injection through a single incision, after discussing treatment options with the patient. During the operation, the rapid freezing pathology

revealed solid papillary carcinoma with a negative surgical margin. On frozen, one of three sentinel lymph nodes tested positive for malignancy, necessitating level II axillary lymph node dissection. In the axillary and breast cavity, a single drain was placed. Histopathological examination revealed a highly differentiated lumina A and 25mm solid papillary carcinoma in the right breast. A total of 16 lymph nodes were removed. One of them was found to have tumor metastasis. The patient was given endocrine therapy in the form of 1 mg of anastrozole orally every day.

Her postoperative recovery went smoothly. The drain was removed on postoperative day (POD) 5, and the patient was discharged on POD 7. On POD 10, she presented to our facility with a slight swelling of the axilla. By puncturing the axillary cavity, 20 milliliters of a milky fluid were aspirated. Initially, we tried conservative treatment such as closed suction drainage, a compressive bandage, and a low-fat diet. A biochemistry analysis of the drainage fluid revealed 1201 mg/dL of triglycerides, and the celiac test was positive. However, the daily output of the drain was kept at around 200ml for two months (**Figure 1**). Following the failure of conservative management, the patient refused radiotherapy. After consulting with the patient, we decided to perform exploration and mastectomy. The clear fluid was observed to be coming from a single duct located in the breast surgery bed rather than the axillary cavity. The duct was ligated, and the breast was removed. The incision was closed after one drain was placed. Drain output dropped to 80ml/d after the re-operation, and it remained slightly milky for the next 10 days. The drain was removed, and there has been no evidence on a regular diet.

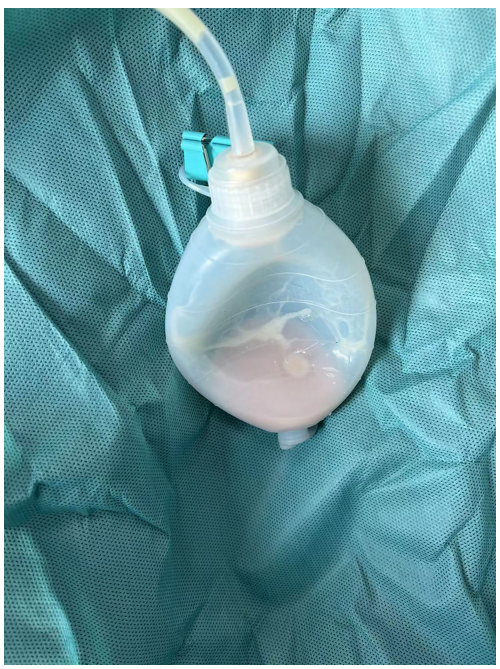


FIGURE 1 | After percutaneous drainage, chylous fluid was present in bulb.

DISCUSSION

Because of the anatomically more remote position of the thoracic duct, chylous leakages are uncommon after breast and axillary surgery. However, as the results show, chylous leaks are not limited to the left side. Thoracic duct anatomical variants are well documented in the literature (4). This is not surprising given that only 50% of people have the typical anatomy. The duct may empty on the right in 2-3% of cases, and bilateral emptying occurs in 1.5% (5). Furthermore, the type of axillary procedure used may play a role in determining which patients will experience chylous leakage. Because the duct collapses after injury, it is difficult to recognize lymphatic duct injuries intraoperatively. Because of the rarity of lymphatic trunk injury and the lack of well-known risk variables, it is also difficult to predict injury to the lymphatic trunks preoperatively.

To the best of our knowledge, this is the first and only case of celiac leakage in the breast following breast-conserving surgery and axillary lymph node dissection. David T Pointer Jr described a case of chyle leak after breast-conserving surgery and sentinel lymph node biopsy. The celiac leakage was discovered at the site of a sentinel lymph node biopsy rather than in the breast (6). The other cases occurred after mastectomy and axillary lymph node dissection, which had more extensive surgery than our presented case (7, 8).

Chylous leakage is typically diagnosed when a milky white fluid drains from the surgical drain. Biochemical testing of the fluid's electrolyte, protein, and lipid content, all of which are compatible with chyle in these cases, confirms the definitive diagnosis. Lymphoscintigraphy or computed tomography is a useful tool for locating chyle fistulas and confirming chyle collection.

The majority of chylous leakages respond to conservative management. To avoid the formation of a collection, a low-volume leak can be handled simply by draining and monitoring. Negatively pressured drainage and free drainage were described in the literature, and the use of pressure bandaging in conjunction with drainage was also mentioned (9). Local injection of hypertonic glucose or meglumine diatrizoate was thought to be an effective treatment for refractory chylous leakage, because drugs can cause aseptic inflammation, resulting in lymphatic vessel closure (10, 11). Dietary fats are known to increase chyle volume, a low-fat diet may help to reduce flow volumes and allow damaged lymphatic capillaries to repair. As a result, in primary conservative management, a diet rich in medium-chain triglycerides (MCT) or parenteral nutrition support is recommended (12). Some authors advocate the use of octreotide to reduce chylous output by inhibiting gastrointestinal motility and secretions (13).

Surgical intervention of chyle leak, on the other hand, has been discussed in a number of studies (6, 14, 15). Some authors believe that early surgical intervention may be beneficial in patients who have failed to respond to initial dietary and/or medical interventions. Because the risk of re-exploration of the axilla and breast is low, and earlier chylous fistula ligation can prevent subsequent oncologic treatments from being delayed, the damaged lymphatic channel is directly ligated during surgery.

Intraoperative orogastric or nasogastric boluses of "heavy cream," as demonstrated by Pointer and colleagues, can aid in the identification of the leaking vessel (6). As an alternative, plugging with gel foam, adhesive, local muscle rotation flaps, or other packing materials could be considered.

CONCLUSION

Chylous leakage following breast conserving surgery and axillary clearance is a rare but significant complication. The majority of chylous leakage occurs during axillary surgery; however, we should be aware of the possibility of chylous leakage during breast surgery as well. Individualized management of chylous leakage following breast and axillary dissection is required. Early surgical intervention is recommended for conservative treatment failure and high output fistulas.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Jiangsu University Affiliated People's Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

Research project of Jiangsu Maternal and Child Health Association (FYX202004). This study was financially supported by National Natural Science Foundation of China (Project No.:81971629) and Research Project of Jiangsu Maternal and Child Health Association (Grant No. FYX202004).

REFERENCES

1. Park I, Her N, Choe JH, Kim JS, Kim JH. Management of Chyle Leakage After Thyroidectomy, Cervical Lymph Node Dissection, in Patients With Thyroid Cancer. *Head Neck* (2018) 40(1):7–15. doi: 10.1002/hed.24852
2. Ahn D, Sohn JH, Jeong JY. Chyle Fistula After Neck Dissection: An 8-Year, Single-Center, Prospective Study of Incidence, Clinical Features, and Treatment. *Ann Surg Oncol* (2015) 22 Suppl 3:S1000–S6. doi: 10.1245/s10434-015-4822-7
3. Daggett JD, Watt AW, Smith PD. Chyle Leak Following Right Axillary Lymph Node Dissection: A Case Report and Review of Current Literature. *Int J Surg Case Rep* (2016) 20:68–73. doi: 10.1016/j.ijscr.2015.12.044
4. Chalret du Rieu M, Baulieux J, Rode A, Mabrut JY. Management of Postoperative Chylothorax. *J Visc Surg* (2011) 148(5):e346–e52. doi: 10.1016/j.jviscsurg.2011.09.006
5. Lee GM, Stowell JT, Pope K, Carter BW, Walker CM. Lymphatic Pathways of the Thorax: Predictable Patterns of Spread. *AJR Am J Roentgenol* (2021) 216 (3):649–58. doi: 10.2214/AJR.20.23523
6. Pointer DT, Durgan DM, Kis B, Khakpour N, Kiluk JV. High-Output Chyle Leak After Breast-Conserving Surgery and Sentinel Lymph Node Biopsy. *Breast J* (2020) 26(3):514–6. doi: 10.1111/tbj.13533
7. Tan B, Bloom R. Chylous Fistula After Sentinel Lymph Biopsy. *J Plast Reconstr Aesthet Surg* (2016) 69(6):873–4. doi: 10.1016/j.bjps.2016.03.009
8. Al-Ishaq Z, Gupta S, Collins MA, Sircar T. Chyle Leak Following an Axillary Sentinel Lymph Node Biopsy for Breast Cancer in a Patient With Superior Vena Caval Thrombosis - A Case Report and Review of the Literature. *Ann R Coll Surg Engl* (2018) 100(6):e147–e9. doi: 10.1308/rcsann.2018.0074
9. Lee YS, Nam K-H, Chung WY, Chang H-S, Park CS. Postoperative Complications of Thyroid Cancer in a Single Center Experience. *J Korean Med Sci* (2010) 25(4):541–5. doi: 10.3346/jkms.2010.25.4.541
10. Huo J, Ou D, Chen C, Zhao J. Local Injection of Hypertonic Glucose for Treating Refractory Chylous Leakage: The First Case Report. *Asian J Surg* (2021) 44(12):1568–9. doi: 10.1016/j.asjsur.2021.08.042
11. Bai Y, Tao X, Xu C, Zhu Y. Diagnosis and Treatment of Chylous Leakage After Modified Radical Mastectomy for Breast Cancer: A Case Report. *J Int Med Res* (2021) 49(6):3000605211021375. doi: 10.1177/03000605211021375
12. Casirati A, Mulazzani G, Luksch R, Morosi C, Biasoni D, Della Valle S. Nutritional Management of Chylous Leakage Following Surgery for High-Risk Neuroblastoma in a Toddler: A Case Report. *Eur J Clin Nutr* (2021) 6:1–3. doi: 10.1038/s41430-021-00988-1
13. Swanson MS, Hudson RL, Bhandari N, Sinha UK, Maceri DR, Kokot N. Use of Octreotide for the Management of Chyle Fistula Following Neck Dissection. *JAMA Otolaryngol Head Neck Surg* (2015) 141(8):723–7. doi: 10.1001/jamaoto.2015.1176
14. Baek JM, Lee JA, Nam YH, Sung GY, Lee DS, Won JM. Chylous Leakage: A Rare Complication After Axillary Lymph Node Dissection in Breast Cancer and Surgical Management. *J Breast Cancer* (2012) 15(1):133–4. doi: 10.4048/jbc.2012.15.1.133
15. Farkas N, Wong J, Monib S, Thomson S. A Systematic Review of Chyle Leaks and Their Management Following Axillary Surgery. *Eur J Surg Oncol* (2020) 46(6):931–42. doi: 10.1016/j.ejso.2020.01.029

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 13 April 2022
doi: 10.3389/fonc.2022.830138

Pan-Immune-Inflammation Value: A New Prognostic Index in Operative Breast Cancer

Fei Lin[1,2†], Li-Ping Zhang[4†], Shuang-Yan Xie[1,2†], Han-Ying Huang[1,2], Xiao-Yu Chen[1,2], Tong-Chao Jiang[1,2], Ling Guo[1,3‡] and Huan-Xin Lin[1,2*‡]*

[1] State Key Laboratory of Oncology in South China, Guangdong Key Laboratory of Nasopharyngeal Carcinoma Diagnosis and Therapy, Sun Yat-sen University Cancer Center, Collaborative Innovation Center for Cancer Medicine, Guangzhou, China, [2] Department of Radiotherapy, Sun Yat-sen University Cancer Center, Guangzhou, China, [3] Department of Nasopharyngeal Carcinoma, Sun Yat-sen University Cancer Center, Guangzhou, China, [4] Department of Oncology, Guangdong Province Hospital of Integrated of Traditional Chinese and Western Medicine, Foshan, China

OPEN ACCESS

Edited by:
Jia-Yi Chen,
Shanghai Jiao Tong University, China

Reviewed by:
Wei-Xiang Qi,
Shanghai Jiao Tong University, China
Ming Ye,
Shanghai JiaoTong University, China
Kazim Senol,
Uludağ University, Turkey

***Correspondence:**
Huan-Xin Lin
linhx@sysucc.org.cn
Ling Guo
guoling@sysucc.org.cn

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *06 December 2021*
Accepted: *15 March 2022*
Published: *13 April 2022*

Citation:
Lin F, Zhang L-P, Xie S-Y, Huang H-Y, Chen X-Y, Jiang T-C, Guo L and Lin H-X (2022) Pan-Immune-Inflammation Value: A New Prognostic Index in Operative Breast Cancer. Front. Oncol. 12:830138. doi: 10.3389/fonc.2022.830138

Background: To build a predictive scoring model based on simple immune and inflammatory parameters to predict postoperative survival in patients with breast cancer.

Methods: We used a brand-new immuno-inflammatory index—pan-immune-inflammation value (PIV)—to retrospectively evaluate the relationship between PIV and overall survival (OS), and based on the results of Cox regression analysis, we established a simple scoring prediction model based on several independent prognostic parameters. The predictive accuracy of the model was evaluated and independently validated.

Results: A total of 1,312 patients were included for analysis. PIV was calculated as follows: neutrophil count (10^9/L) × platelet count (10^9/L) × monocyte count (10^9/L)/ lymphocyte count (10^9/L). According to the best cutoff value of PIV, we divided the patients into two different subgroups, high PIV (PIV > 310.2) and low PIV (PIV ≤ 310.2), associated with significantly different survival outcomes (3-year OS, 80.26% vs. 86.29%, respectively; 5-year OS, 62.5% vs. 71.55%, respectively). Six independent prognostic factors were identified and used to build the scoring system, which performed well with a concordance index (C-index) of 0.759 (95% CI: 0.715–0.802); the calibration plot showed good calibration.

Conclusions: We have established and verified a simple scoring system for predicting prognosis, which can predict the survival of patients with operable breast cancer. This system can help clinicians implement targeted and individualized treatment strategies.

Keywords: breast cancer, nomogram, PIV, index, prognosis

INTRODUCTION

Breast cancer is the most common malignant tumor in women (1, 2) and has the highest incidence among all malignancies affecting women according to the WHO (3). Although the overall survival (OS) rate of breast cancer has improved owing to advancements in diagnosis and treatment over the past decades (4), there is still a non-negligible fraction of patients with poor outcomes, and the latest

study shows that some high-income countries report continuous substantial improvements exceeding 2% annual mortality reduction of breast cancer; however, many low- and middle-income countries have changeless or even increasing mortality rates (5, 6). Moreover, it is known that breast cancer patients with the same clinical stage and receiving the same treatment may have completely different outcomes. Because of such prognostic heterogeneity (7), carrying out individualized precision treatment is paramount to treatment success. Therefore, it is necessary to identify new and suitable alternative biomarkers for better prognostic stratification and prediction of treatment outcomes.

At present, many studies have proven that inflammatory factors such as IL-6 and TGF-β, inflammatory reactions, and the immune system are associated with the development and progression of two types of cancer attributable to chronic inflammatory disease: cholangiocarcinoma and colitis-associated colorectal cancer (8, 9). Research has shown that macrophages, essential components of the immune-inflammatory response, are implicated in inflammatory mechanisms and can therefore facilitate tumorigenesis in colorectal cancer (10). In addition, studies have demonstrated that hematologic parameters such as lymphocyte level are promising biomarkers of the body's immune and inflammation status (11). In recent years, research on immune-inflammatory biomarkers (IIBs), compared with traditional tumor-related biomarkers, that can affect the prognosis of breast cancer has shown significant progress. Several easy-to-obtain and blood-based IIBs have been proven as potential independent prognostic factors in breast cancer, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) (12–16).

Because of the complex interactions between the tumor and host immune-inflammatory responses (17), the abovementioned indicators based on simple calculations inevitably limit the prediction power of the prognosis. The pan-immune-inflammation value (PIV), a new comprehensive biomarker involving the neutrophil, platelet, monocyte, and lymphocyte counts, has been proven to be a strong predictor of survival outcomes with better performance than other well-known IIBs in patients with metastatic colorectal cancer (18). However, the prognostic value of PIV is rarely reported in breast cancer. Therefore, this study aimed to clarify the prognostic value of PIV in breast cancer.

METHODS

Patients

In all, 1,312 patients were included in this retrospective study who underwent surgery at the Sun Yat-sen University Cancer Center (SYSUCC; Guangzhou, China) between December 2010 and October 2012. The inclusion criteria were as follows: 1) pathologically confirmed breast cancer and 2) receipt of mastectomy or lumpectomy. The exclusion criteria were as follows: 1) relapse and *de novo* breast cancer; 2) complicated with another primary tumor; 3) ductal carcinoma *in situ* (DCIS); 4) male breast cancer; 5) receipt of any antitumor treatment before surgery; 6) concurrent hematological, autoimmune, or acute/chronic inflammatory disease; 7) incomplete laboratory data resulting in the non-calculation of the PIV indicator; and 8) follow-up loss. This study was approved by the Research Ethics Committee of SYSUCC. All patients' data were confidential.

Data Collection and Definitions

The list of patients who visited our hospital was obtained from the follow-up department. Then the patient's laboratory data were checked through the case system and recorded in Excel in detail. Laboratory data were collected 1 week before surgery (at first diagnosis, before any treatment), and clinicopathological data were collected from the patients' medical records. The calculation formula of each indicator was as follows: SII = platelet count (10^9/L) × neutrophil count/lymphocyte count (10^9/L); NLR = neutrophil count (10^9/L)/lymphocyte count (10^9/L); PLR = platelet count (10^9/L)/lymphocyte count (10^9/L) (19); and PIV = neutrophil count (10^9/L) × platelet count (10^9/L) × monocyte count (10^9/L)/lymphocyte count (10^9/L) (20). According to the calculation formula mentioned above, the PIV and other indicators were calculated in Excel, and the sorted data were analyzed for further statistical analysis using R. Patients were staged according to the eighth edition American Joint Committee on Cancer—Tumor, Node, and Metastases (AJCC-TNM) staging system (21). The expression of estrogen receptor (ER) and progesterone receptor (PR) were scored using the St. Gallen criteria (22). Human epidermal growth factor receptor-2 (HER-2) status was assessed according to the American Society of Clinical Oncology–College of American Pathologists guidelines (23, 24) by using immunohistochemistry or fluorescence *in situ* hybridization (FISH) test. HER-2-negative status was defined as immunohistochemistry showing HER-2+/++, or the FISH test results are negative, or the FISH test was not performed; HER-2-positive status was defined as immunohistochemical staining = 3+ or FISH positive/chromogenic *in situ* hybridization positive.

Follow-Up

Follow-up was performed telephonically or through a regular outpatient surveillance system to record the condition of patients or the cause and date of death if the patient had already died. In this study, the endpoint was OS—defined as the time between the date of diagnosis and death due to any reason. The date of the last follow-up was considered the study endpoint for all surviving patients. The date of the last follow-up was considered for patients who did not reach the study endpoint.

Statistical Analysis

Continuous variables were presented as the median and interquartile range (IQR). Categorical variables were presented as frequency and percentage. A chi-square test and the Mann–Whitney U test were used to analyze the association between PIV groups and other clinicopathological characteristics. In this research, a two-tailed p-value <0.05 was considered to indicate statistical significance. Maximally selected rank statistics were used to determine the optimal cutoff of continuous variables. Survival curves were plotted using the Kaplan–Meier method,

and significance was determined by the log-rank test. All factors with a p-value <0.05 detected in univariate analyses were entered into the multivariate model to identify independent prognostic factors. Before multivariate analyses, the proportional hazards assumption test was performed using the Schoenfeld residuals. The variables with p-value <0.05 in the multivariate analyses were finally selected to build a prognostic model, which was presented as a nomogram. Concordance index (C-index) was used to evaluate the predictive accuracy of the nomogram. The calibration curves were used to predict the ability of the calibration between the predicted and actual survival. To avoid overfitting, 1,000 bootstrap samples and 10-fold cross-validation were also applied. All analyses were performed with R software (http://www.R-project.org; version 4.0.2) and SPSS 25.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

The Optimal Cutoff Value of Pan-Immune-Inflammation Value

The optimal cutoff value for PIV was 310.2 in the whole cohort by using maximally selected rank statistics (**Supplementary Figure 1**).

Patient Characteristics and Relationship Between Pan-Immune-Inflammation Value and Clinicopathological Factors

A total of 1,312 breast cancer patients were enrolled in this study. The relationship between clinicopathological characteristics and PIV of the whole cohort is presented in **Table 1**. Briefly, the median age of the patients was 48 years (IQR, 41–57). From the perspective of the clinical stage, 317 (24.2%), 679 (51.7%), and 316 (24.1%) patients were diagnosed with stage I, II, and III cancer, respectively. Overall, 1,109 (84.5%) patients had invasive ductal carcinoma, and 203 (15.5%) had other pathological types. The median body mass index (BMI) of the patients was 23 (IQR, 20.8–25.2). The median follow-up time was 78.4 months (IQR, 53.1–88). The median PIV of the patients was 135.2 (IQR, 87.6–213.7). Further, 387 (29.5%) patients were HER-2 positive, and 925 (70.5%) were negative. The median values of the pretreatment platelet count, neutrophil count, monocyte count, and lymphocyte count were 225×10^9/L, 3.65×10^9/L, 0.32×10^9/L, and 1.9×10^9/L, respectively.

The analysis of the relationship between PIV and various clinicopathological factors showed that PIV was significantly associated with ER status ($p = 0.02$).

The whole cohort was randomly divided into a training set and a validation set (ratio: 7:3) (**Table 2**). With respect to the PIV group, 819 (89.0%) and 341 (87.0%) patients were assigned to the low-PIV group in the training set and validation set, respectively.

Survival Analysis of Pan-Immune-Inflammation Value Groups

According to the optimal cutoff value of PIV, the whole cohort was divided into two groups: the low-PIV group (PIV ≤ 310.2) and the high-PIV group (PIV > 310.2). **Figure 1** shows the significant survival differences between the two groups. The 3-year OS rates in the low-PIV group and the high-PIV group were 86.29% and 80.26%, respectively; the 5-year OS rates in the low-PIV group and the high-PIV group were 71.55% and 62.50%, respectively (hazard ratio (HR): 1.737, 95% CI: 1.096–2.755, log-rank test, $p = 0.016$).

Moreover, we performed univariate and multivariate Cox regression analyses for OS. Indicators that related to breast cancer clinically and common IIBs such as NLR, PLR, and SII were selected in the univariate analysis. The results have shown that T stage, N stage, histopathological type, ER status, PR status, HER-2 status, Ki-67, NLR, and PIV were potential factors associated with OS (**Table 3**). The global p-value was 0.231 of the PH-test, which means that the constructed multi-regression analysis model is successful. The abovementioned indicators were further analyzed in the multivariable Cox regression analysis. In the final model, we observed that T stage, N stage, histopathological type, PR status, Ki-67, and PIV were significant independent prognostic factors of breast cancer (**Table 3**), which is graphically presented as **Supplementary Figure 2**.

In the training set and validation set, we conducted survival analyses and univariate and multivariate Cox regression analyses (**Tables S1, S2**). The Kaplan–Meier survival curves of the training cohort and validation cohort are presented in **Supplementary Figure 3** (training cohort, HR: 1.831, 95% CI: 1.077–3.111, log-rank test, $p = 0.021$; validation cohort, HR: 1.687, 95% CI: 1.156–3.068, log-rank test, $p = 0.024$). The results of survival analysis were consistent with those of the whole set (all log-rank $p < 0.05$). The results of univariate analysis and multivariate Cox regression analyses were in line with the whole set as well.

Prognostic Analysis and Building the Model

Based on the abovementioned independent factors, a prognostic model for the prediction of the 1-, 3-, and 5-year OS was built and graphically presented as a nomogram (**Figure 2**). The prognostic model showed a good discriminating ability for OS prediction, with a C-index of 0.759 (95% CI: 0.715–0.802). The calibration curves of 1-, 3-, and 5-year OS illustrated good calibration between the predicted and actual survival probabilities in the whole cohort (**Figure 3**).

Subgroup Analysis of Common Clinical Variables

Subgroup analysis shows that there was no interaction between PIV and clinicopathological characteristics in the whole cohort (all $p > 0.05$, **Figure 4**).

DISCUSSION

The concept of tumor immunoediting includes the following three phases: elimination, equilibrium, and escape (25). The mechanism of immune escape is very complicated, which involves tumor-

TABLE 1 | The relationship between PIV and clinicopathological characteristics in the whole cohort.

Characteristic	Total (N = 1,312)	High-PIV group (N = 152)	Low-PIV group (N = 1,160)	*p*
Age (years), median (IQR)	48 (41–57)	46 (40–55)	48 (41–57)	0.127
Tumor stage				0.292
T1	467 (35.6)	52 (34.2)	415 (35.8)	
T2	719 (54.8)	79 (52.0)	640 (55.2)	
T3	65 (5.0)	10 (6.6)	55 (4.7)	
T4	61 (4.6)	11 (7.2)	50 (4.3)	
Node stage				0.178
N0	687 (52.4)	69 (45.4)	618 (53.3)	
N1	345 (26.3)	42 (27.6)	303 (26.1)	
N2	163 (12.4)	26 (17.1)	137 (11.8)	
N3	117 (8.9)	15 (9.9)	102 (8.8)	
Clinical stage				–
I	317 (24.2)	30 (19.7)	287 (24.7)	
II	679 (51.7)	73 (48.0)	606 (52.3)	
III	316 (24.1)	49 (32.3)	267 (23.0)	
BMI kg/m^2, median (IQR)	23 (20.8–25.2)	23.4 (21.0–25.7)	22.9 (20.8–25.1)	0.158
Histological type				0.902
Invasive ductal carcinoma	1,109 (84.5)	129 (84.9)	980 (84.5)	
Others	203 (15.5)	23 (15.1)	180 (15.5)	
ER status				0.020*
Positive	942 (71.8)	97 (63.8)	845 (72.8)	
Negative	370 (28.2)	55 (36.2)	315 (27.2)	
PR status				0.413
Positive	842 (64.2)	93 (61.2)	749 (64.6)	
Negative	470 (35.8)	59 (38.8)	411 (35.4)	
HER-2 status				0.975
Positive	387 (29.5)	45 (29.6)	342 (29.5)	
Negative	925 (70.5)	107 (70.4)	818 (70.5)	
Ki-67				0.349
>14%	575 (43.8)	72 (47.4)	503 (43.4)	
≤14%	737 (56.2)	80 (52.6)	657 (56.6)	
Adjuvant chemotherapy				0.285
Yes	1,066 (81.3)	111 (73.0)	955 (82.3)	
No	246 (18.7)	41 (27.0)	205 (17.7)	
Radiotherapy				0.761
Yes	350 (26.7)	43 (28.3)	307 (26.5)	
No	962 (73.3)	109 (71.7)	853 (73.5)	
Endocrine therapy				0.818
Yes	680 (51.8)	79 (52.0)	601 (51.8)	
No	632 (48.2)	73 (48.0)	559 (48.2)	
Target therapy				0.485
Yes	95 (7.2)	15 (9.9)	80 (6.9)	
No	1,217 (92.8)	137 (90.1)	1,080 (93.1)	
PLT (10^9/L), median (IQR)	225 (190.0–265.0)	272 (236.9–310.5)	220.5 (186.0–255.2)	–
NE (10^9/L), median (IQR)	3.7 (2.9–4.6)	5.3 (4.4–7.0)	3.5 (2.8–4.3)	–
MONO (10^9/L), median (IQR)	0.3 (0.2–0.4)	0.5 (0.4–0.6)	0.3 (0.2–0.4)	–
LY (10^9/L), median (IQR)	1.9 (1.6–2.3)	1.63 (1.4–2.2)	1.91 (1.6–2.3)	–

PIV low group (PIV ≤ 310.2) and PIV high group (PIV > 310.2).

PIV, pan-immune-inflammation value; IQR, interquartile range; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; PLT, platelet count; NE, neutrophil count; MONO, monocyte count; LY, lymphocyte count.

*$p < 0.05$.

associated antigens, tumor gene mutation, several types of immune cells, an inflammatory microenvironment, and a tumor microenvironment (TME) (26). The TME includes not only the tumor cells but also immune and inflammatory cells (27, 28). One study showed that tumor cells interact with platelets both inside the TME and in the bloodstream or ascitic fluid (29). Another study reported that neutrophils promote tumor cell growth and progression by secreting cytokines and chemokines so as to offer a proper microenvironment for tumor cells (30). Tumor-associated macrophages (TAMs) are derived from circulating monocytes and play a crucial role in the formation of TME by promoting tumor progression and metastasis (31). The characteristics of the TME are hypoxia, chronic inflammation, and immunosuppression, which make a more complex network mechanism to regulate the relationship between systemic inflammation, local immune response, cancer progression, and patient survival (32–34).

In the current study, we used real-world data to assess the prognostic value of the PIV in operable breast cancer. The results showed that PIV, a new immune-inflammation score, was an independent predictor for breast cancer. Another study on PIV in metastatic colorectal cancer arrived at a similar conclusion as

TABLE 2 | The baseline characteristics between the training and validation datasets.

Characteristics	Training set (N = 920)	Validation set (N = 392)
Age (years), median (IQR)	48 (42–57)	47 (44–55)
Tumor stage		
T1	330 (35.9%)	137 (35.0%)
T2	508 (55.2%)	211 (53.8%)
T3	39 (4.2%)	26 (6.6%)
T4	43 (4.7%)	18 (4.6%)
Node stage		
N0	470 (51.0%)	217 (55.4%)
N1	254 (27.6%)	91 (23.2%)
N2	123 (13.4%)	40 (10.2%)
N3	73 (8.0%)	44 (11.2%)
Clinical stage		
I	222 (24.2%)	95 (24.2%)
II	476 (51.7%)	203 (51.8%)
III	222 (24.1%)	94 (24.0%)
Histological type		
Invasive ductal carcinoma	781 (84.9%)	328 (83.7%)
Others	139 (15.1%)	64 (16.3%)
ER status		
Positive	661 (71.8%)	281 (71.7%)
Negative	259 (28.2%)	111 (28.3%)
PR status		
Positive	591 (64.2%)	251 (64.0%)
Negative	329 (35.8%)	141 (36.0%)
HER-2 status		
Positive	202 (22.0%)	82 (20.9%)
Negative	718 (78.0%)	310 (79.1%)
Ki-67		
>14%	403 (43.8%)	172 (43.9%)
≤14%	517 (56.2%)	220 (56.1%)
PIV		
>310.2	101 (11.0%)	51 (13.0%)
≤31.02	819 (89.0%)	341 (87.0%)

IQR, interquartile range; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2.

ours (18). Patients with low PIV have a better prognosis than those with high. What is more, we compared the effectiveness of PIV and the traditional TNM staging system in predicting prognosis by using time-dependent receiver operating characteristic (ROC) analysis; it revealed that PIV had higher accuracy in predicting OS than the traditional TNM staging system (**Supplementary Figure 4**), further highlighting the clinical application value of PIV.

In routine clinical work, for breast cancer, clinicians often determine the treatment according to molecular subtype, gene expression features, and clinical stage. In our study, subgroup analysis showed that there was no interaction between PIV and clinicopathological characteristics, which proves that the PIV has predictive consistency for each subgroup statistically. Of course, its real clinical application value needs to be confirmed by larger-scale data and prospective studies in the future.

The results of correlation analysis showed a relationship between PIV and ER status; the exact reason why PIV and ER status are significantly associated remains unclear. One of the possible reasons is selection bias. Although this association is statistically significant, it remains to be seen in future studies whether there is a true clinical relevance.

Several studies also have shown that patients with high levels of NLR, PLR, and SII have a poor prognosis in operable breast cancer (14, 35–37). The conclusion is as follows: NLR, PLR, and SII were independent prognostic factors in these studies. These findings were different from ours. As to NLR in our study, patients with low NLR showed a better prognosis than those with high NLR (**Supplementary Figure 5**), which is consistent with other studies (14) (38, 39). However, further multivariate Cox regression analysis showed that NLR is not an independent prognostic factor for breast cancer patients, while PIV is. The reason for this difference remains unclear. Perhaps, the small sample size of patients in this study did not allow us draw a conclusion between NLR and independent prognostic factors. As to PLR and SII, the univariate analysis, showed that neither PLR nor SII was a potential factor associated with OS for breast cancer in our study. The relationship between NLR, PLR, SII, and breast cancer prognosis is complex: many reports concluded that NLR (14, 40, 41), PLR (37, 42, 43), and SII (16, 44, 45) were independent prognostic factors for breast cancer, but there are also many studies that do not support the conclusion mentioned above (46–49). This may be related to the selection of the population and the included variables. In our current research, 1,312 patients were included in this retrospective study who underwent surgery at the Sun Yat-sen University Cancer Center between December 2010 and October 2012, and we included a new variable, PIV, a novel indicator of combined immuno-inflammation nutrition; time-dependent ROC curves show that PIV has better prognostic value than NLR, PLR, and SII (**Supplementary Figure 6**).

As PIV is a relatively novel biomarker, few studies on PIV have been reported thus far. A previous study (50) showed that a low PIV value predicts better chemotherapy response and survival in breast cancer patients treated with neoadjuvant chemotherapy. Another previous study (20) showed that PIV is a new and potent predictor of OS in HER-2-positive advanced BC patients treated with first-line trastuzumab–pertuzumab-containing biochemotherapy.

Our study has some limitations. First, there were inevitable flaws due to the nature of the retrospective observational design (51). Second, there were a relatively limited number of patients enrolled in this study. Third, patients included in this research were from a single cancer center. Therefore, potential selection bias could have led to data not being representative of the true distribution of PIV values in the whole cohort.

What is more, there was a very important point that the methods of obtaining the optimal cutoff value of PIV varied among studies. One study (52) used the median value of this parameter in the clinical cohort, while others (53–55) used the ROC curve to obtain the optimal value. In this study, we classified the candidate continuous index according to the cutoff point determined by the maximally selected rank statistics using the "maxstat" package of R software (56), a widely recognized and applied method in many studies (57–59). Thus, the cutoff value of PIV varies among studies, which limited the clinical use of this

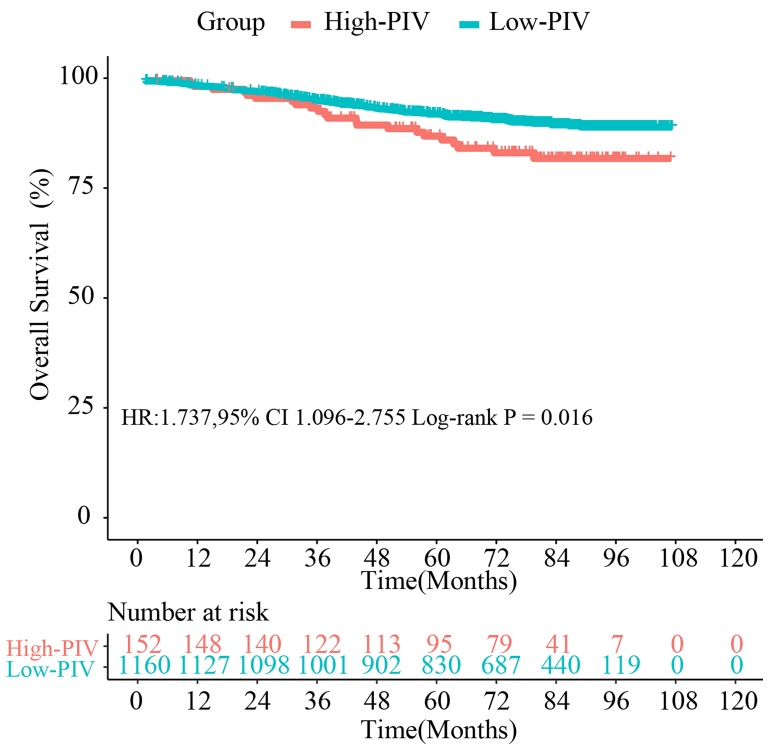


FIGURE 1 | Kaplan–Meier survival curves with breast cancer after surgery between the high-PIV group and low-PIV group in the whole cohort. PIV, pan-immune-inflammation value.

TABLE 3 | Univariate and multivariate analyses of overall survival.

Characteristic	Univariate analysisHazard ratio (95% CI)	*p*	Multivariate analysisHazard ratio (95% CI)	*p*
Age (years)	1.153 (0.811–1.640)	0.427	–	–
T stage#	2.415 (1.547–3.771)	<0.001*	1.633 (1.027–2.596)	0.038*
N stage#	5.572 (3.823–8.121)	<0.001*	4.719 (3.195–6.971)	<0.001*
Histopathological Type	2.674 (1.306–5.473)	0.007*	2.668 (1.302–5.468)	0.007*
ER status	0.572 (0.399–0.822)	0.002*	0.902 (0.521–1.563)	0.713
PR status	0.568 (0.399–0.808)	0.002*	0.695 (0.483–0.998)	0.049*
HER-2 status	1.691 (1.181–2.421)	0.004*	1.231 (0.845–1.793)	0.279
Ki-67	2.197 (1.526–3.162)	<0.001*	1.713 (1.175–2.497)	0.005*
NLR group	1.440 (1.0122.051)	0.043*	1.598 (0.574–2.365)	0.064
PLR group	1.488 (0.981–2.232)	0.062	–	–
SII group	1.356 (0.903–2.037)	0.142	–	–
PIV group	1.737 (1.096–2.755)	0.016*	1.720 (1.083–2.730)	0.021*

A Cox proportional hazards model was used to conduct multivariate analyses. All variables were transformed into categorical variables. HRs of variables were calculated as follows: Age (>48 vs. ≤48 years); T stage (T1 vs. T234); N stage (N012 vs. N3); histological Type (invasive ductal carcinoma vs. others); ER (negative vs. positive); PR (negative vs. positive); HER-2 (negative vs. positive); Ki-67 (≤14% vs. >14%); NLR group (≤1.99 vs. >1.99); PLR group (≤160.25 vs. >160.25); SII group (≤642.23 vs. >642.23); PIV group (≤310.20 vs. >310.20).

HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; PIV, pan-immune-inflammation value.

#According to the eighth edition of the Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) staging system.

**p < 0.05.*

biomarker. The cutoff value determined in this study needs more research for further verification.

In addition, it must be mentioned that though we have established the model by randomly dichotomizing into the training and testing groups (at 7:3) in our study, we established the model by using the training cohort and validated the model by using the testing cohort. Also, we have made multifaceted efforts to validate our results. We tried to use different cohorts from public databases to validate the findings outlined in this study. Public databases

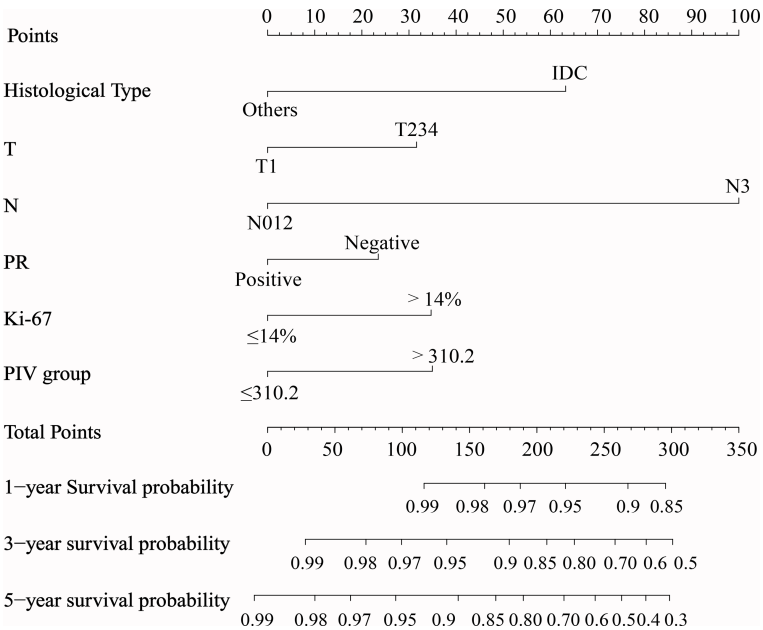


FIGURE 2 | Nomogram to predict 1-, 3-, and 5-year overall survival generated using the whole cohort. IDC, invasive ductal carcinoma.

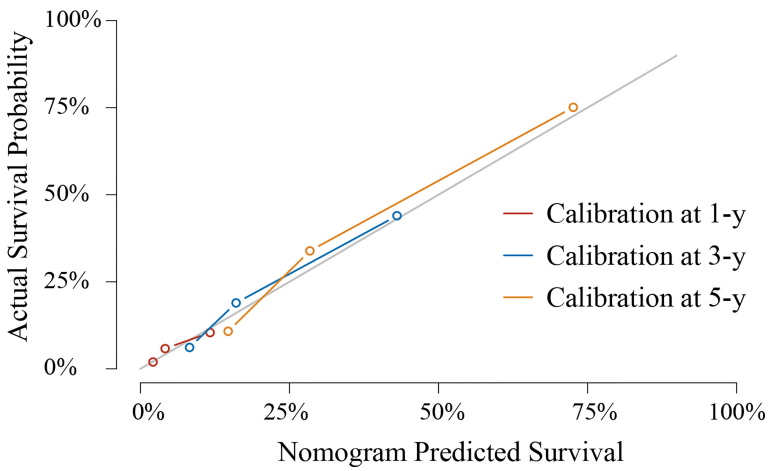


FIGURE 3 | Calibration curves to predict 1-, 3-, and 5-year overall survival in the whole cohort.

such as the Surveillance, Epidemiology, and End Results (SEER) database and The Cancer Genome Atlas (TCGA) database were tried for validation, but none of them provided laboratory data (platelet counts, etc.). To the best of our knowledge, there are no available published public databases containing routine preoperative laboratory data. Also, we have been seeking data help from colleagues at Sun Yat-sen Memorial Hospital and Guangdong Provincial People's Hospital in China, as well as from the organization Korean Breast Cancer Society in Korea. There are still some difficulties; regrettably, we have not obtained enough external validation data to confirm our findings so far. But we are actively seeking cooperation from other centers to verify the results. This is a limitation that should be considered.

The main strength of this study is that we believe we have supplemented the current knowledge of supporting evidence that PIV is independently related to survival outcomes in patients with breast cancer. We hope that future studies can further validate and confirm the application of the PIV indicator to other cancers as well.

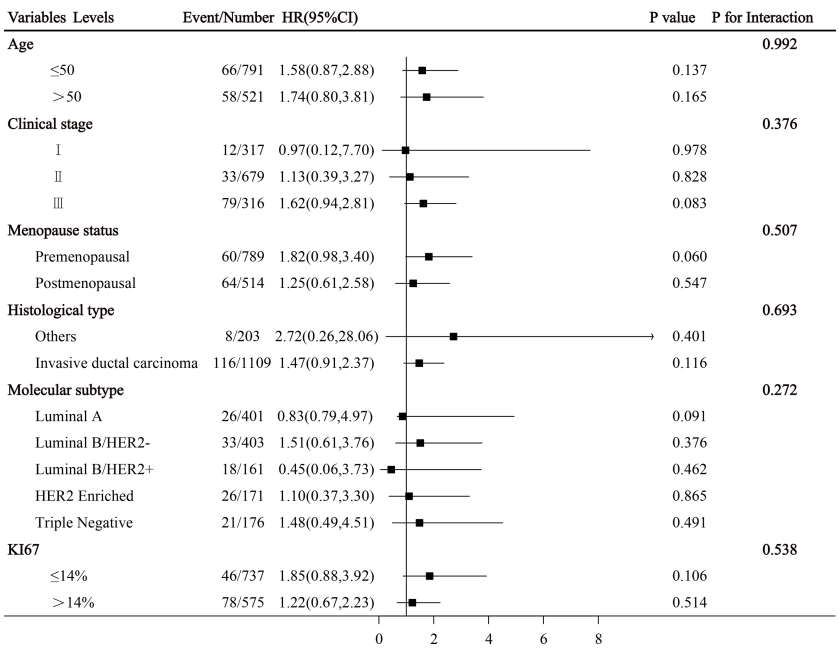


FIGURE 4 | Subgroup analysis of breast cancer-related clinical variables were illustrated in a forest plot.

CONCLUSION

The PIV appears to be an independent predictor of OS in patients with operable breast cancer. The proposed nomogram could be a useful tool for individualized assessment of prognosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

In the present study, all the procedures involving human participants were performed according to the ethical standards of the institutional research committee or the national research committee, or both, and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The Clinical Research Ethics Committee of SYSUCC approved this study (number: GZR2021-117). All participants in the study provided informed consent.

AUTHOR CONTRIBUTIONS

FL, H-XL, and LG contributed to literature search, study design, data analysis, writing, and critical revision. S-YX, H-YH, X-YC, and T-CJ: data acquisition, methodology, and data analysis. H-YH and L-PZ: visualization, investigation, and software. All authors: writing—reviewing, supervision, and editing.

FUNDING

H-XL is supported by the National Natural Science Foundation of China (Grant No. 81773103) and the Natural Science Foundation of Guangdong Province (2017A030313617). LG is supported by the National Natural Science Foundation of China (Grant Nos. 81772877 and 81572848).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.830138/full#supplementary-material

Supplementary Figure 1 | The optimal cut-off of PIV for overall survival in the whole cohort by using maximally selected rank statistics.

Supplementary Figure 2 | The forest plot of the results of multivariable regression analysis.

Supplementary Figure 3 | Kaplan-Meier survival curves with breast cancer after surgery between the high-PIV group and low-PIV group **(A)** in the training set and **(B)** in the validation set.

Supplementary Figure 4 | Time-dependent ROC curve compared with PIV and the TNM staging system.

Supplementary Figure 5 | Kaplan-Meier survival curves of breast cancer patients after surgery between the high-NLR group and low-NLR group in the whole cohort.

Supplementary Figure 6 | Time-dependent ROC curve compared with PIV and **(A)** NLR, **(B)** PLR, **(C)** SII.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2020. *CA Cancer J Clin* (2020) 70(1):7–30. doi: 10.3322/caac.21590
2. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast Cancer. *Lancet* (2021) 397(10286):1750–69. doi: 10.1016/S0140-6736(20)32381-3
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660
4. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, et al. Risk Factors and Preventions of Breast Cancer. *Int J Biol Sci* (2017) 13(11):1387–97. doi: 10.7150/ijbs.21635
5. Trapani D, Ginsburg O, Fadelu T, Lin NU, Hassett M, Ilbawi AM, et al. Global Challenges and Policy Solutions in Breast Cancer Control. *Cancer Treat Rev* (2022) 104:102339. doi: 10.1016/j.ctrv.2022.102339
6. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2022. *CA Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708
7. Yeo SK, Guan JL. Breast Cancer: Multiple Subtypes Within a Tumor? *Trends Cancer* (2017) 3(11):753–60. doi: 10.1016/j.trecan.2017.09.001
8. Landskron G, de la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic Inflammation and Cytokines in the Tumor Microenvironment. *J Immunol Res* (2014) 2014:149185. doi: 10.1155/2014/149185
9. Greten FR, Grivennikov SI. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity* (2019) 51(1):27–41. doi: 10.1016/j.immuni.2019.06.025
10. Scheurlen KM, Billeter AT, O'Brien SJ, Galandiuk S. Metabolic Dysfunction and Early-Onset Colorectal Cancer - How Macrophages Build the Bridge. *Cancer Med* (2020) 9(18):6679–93. doi: 10.1002/cam4.3315
11. Ma X, Jia C, Fu D, Chu M, Ding X, Wu X, et al. Analysis of Hematological Traits in Polled Yak by Genome-Wide Association Studies Using Individual SNPs and Haplotypes. *Genes (Basel)* (2019) 10(6):463. doi: 10.3390/genes10060463
12. Guo W, Lu X, Liu Q, Zhang T, Li P, Qiao W, et al. Prognostic Value of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio for Breast Cancer Patients: An Updated Meta-Analysis of 17079 Individuals. *Cancer Med* (2019) 8(9):4135–48. doi: 10.1002/cam4.2281
13. Graziano V, Grassadonia A, Iezzi L, Vici P, Pizzuti L, Barba M, et al. Combination of Peripheral Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio Is Predictive of Pathological Complete Response After Neoadjuvant Chemotherapy in Breast Cancer Patients. *Breast* (2019) 44:33–8. doi: 10.1016/j.breast.2018.12.014
14. Ethier JL, Desautels D, Templeton A, Shah PS, Amir E. Prognostic Role of Neutrophil-to-Lymphocyte Ratio in Breast Cancer: A Systematic Review and Meta-Analysis. *Breast Cancer Res* (2017) 19(1):2. doi: 10.1186/s13058-016-0794-1
15. Zhang Y, Sun Y, Zhang Q. Prognostic Value of the Systemic Immune-Inflammation Index in Patients With Breast Cancer: A Meta-Analysis. *Cancer Cell Int* (2020) 20:224. doi: 10.1186/s12935-020-01308-6
16. Hua X, Long ZQ, Zhang YL, Wen W, Guo L, Xia W, et al. Prognostic Value of Preoperative Systemic Immune-Inflammation Index in Breast Cancer: A Propensity Score-Matching Study. *Front Oncol* (2020) 10:580. doi: 10.3389/fonc.2020.00580
17. Nikolaou K, Sarris M, Talianidis I. Molecular Pathways: The Complex Roles of Inflammation Pathways in the Development and Treatment of Liver Cancer. *Clin Cancer Res* (2013) 19(11):2810–6. doi: 10.1158/1078-0432.CCR-12-1961
18. Fuca G, Guarini V, Antoniotti C, Morano F, Moretto R, Corallo S, et al. The Pan-Immune-Inflammation Value Is a New Prognostic Biomarker in Metastatic Colorectal Cancer: Results From a Pooled-Analysis of the Valentino and TRIBE First-Line Trials. *Br J Cancer* (2020) 123(3):403–9. doi: 10.1038/s41416-020-0894-7
19. Peng RR, Liang ZG, Chen KH, Li L, Qu S, Zhu XD. Nomogram Based on Lactate Dehydrogenase-To-Albumin Ratio (LAR) and Platelet-To-Lymphocyte Ratio (PLR) for Predicting Survival in Nasopharyngeal Carcinoma. *J Inflamm Res* (2021) 14:4019–33. doi: 10.2147/JIR.S322475
20. Ligorio F, Fuca G, Zattarin E, Lobefaro R, Zambelli L, Leporati R, et al. The Pan-Immune-Inflammation-Value Predicts the Survival of Patients With Human Epidermal Growth Factor Receptor 2 (HER2)-Positive Advanced Breast Cancer Treated With First-Line Taxane-Trastuzumab-Pertuzumab. *Cancers (Basel)* (2021) 13(8):1964. doi: 10.3390/cancers13081964
21. Zhang J, Zhao B, Jin F. The Assessment of 8th Edition AJCC Prognostic Staging System and a Simplified Staging System for Breast Cancer: The Analytic Results From the SEER Database. *Breast J* (2019) 25(5):838–47. doi: 10.1111/tbj.13347
22. Carlson RW, Hudis CA, Pritchard KINational Comprehensive Cancer Network Breast Cancer Clinical Practice Guidelines in O, American Society of Clinical Oncology Technology Assessment on the Use of Aromatase I and St Gallen International Expert Consensus on the Primary Therapy of Early Breast C. Adjuvant Endocrine Therapy in Hormone Receptor-Positive Postmenopausal Breast Cancer: Evolution of NCCN, ASCO, and St Gallen Recommendations. *J Natl Compr Canc Netw* (2006) 4(10):971–9. doi: 10.6004/jnccn.2006.0082
23. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *J Clin Oncol* (2013) 31(31):3997–4013. doi: 10.1200/JCO.2013.50.9984
24. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol* (2018) 36(20):2105–22. doi: 10.1200/JCO.2018.77.8738
25. Dunn GP, Old LJ, Schreiber RD. The Three Es of Cancer Immunoediting. *Annu Rev Immunol* (2004) 22:329–60. doi: 10.1146/annurev.immunol.22.012703.104803
26. Anichini A, Perotti VE, Sgambelluri F, Mortarini R. Immune Escape Mechanisms in Non Small Cell Lung Cancer. *Cancers (Basel)* (2020) 12(12):3605. doi: 10.3390/cancers12123605
27. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell* (2011) 144(5):646–74. doi: 10.1016/j.cell.2011.02.013
28. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. *Cell* (2010) 140(6):883–99. doi: 10.1016/j.cell.2010.01.025
29. Haemmerle M, Taylor ML, Gutschner T, Pradeep S, Cho MS, Sheng J, et al. Platelets Reduce Anoikis and Promote Metastasis by Activating YAP1 Signaling. *Nat Commun* (2017) 8(1):310. doi: 10.1038/s41467-017-00411-z
30. Gregory AD, Houghton AM. Tumor-Associated Neutrophils: New Targets for Cancer Therapy. *Cancer Res* (2011) 71(7):2411–6. doi: 10.1158/0008-5472.CAN-10-2583
31. Zhou SL, Zhou ZJ, Hu ZQ, Huang XW, Wang Z, Chen EB, et al. Tumor-Associated Neutrophils Recruit Macrophages and T-Regulatory Cells to Promote Progression of Hepatocellular Carcinoma and Resistance to Sorafenib. *Gastroenterology* (2016) 150(7):1646–58 e17. doi: 10.1053/j.gastro.2016.02.040
32. Yu PF, Huang Y, Han YY, Lin LY, Sun WH, Rabson AB, et al. TNFalpha-Activated Mesenchymal Stromal Cells Promote Breast Cancer Metastasis by Recruiting CXCR2(+) Neutrophils. *Oncogene* (2017) 36(4):482–90. doi: 10.1038/onc.2016.217
33. Szkandera J, Gerger A, Liegl-Atzwanger B, Absenger G, Stotz M, Samonigg H, et al. Validation of the Prognostic Relevance of Plasma C-Reactive Protein Levels in Soft-Tissue Sarcoma Patients. *Br J Cancer* (2013) 109(9):2316–22. doi: 10.1038/bjc.2013.595
34. Nguyen AV, Wu YY, Lin EY. STAT3 and Sphingosine-1-Phosphate in Inflammation-Associated Colorectal Cancer. *World J Gastroenterol* (2014) 20(30):10279–87. doi: 10.3748/wjg.v20.i30.10279
35. Hu B, Yang XR, Xu Y, Sun YF, Sun C, Guo W, et al. Systemic Immune-Inflammation Index Predicts Prognosis of Patients After Curative Resection

for Hepatocellular Carcinoma. *Clin Cancer Res* (2014) 20(23):6212–22. doi: 10.1158/1078-0432.CCR-14-0442
36. Mouchli M, Reddy S, Gerrard M, Boardman L, Rubio M. Usefulness of Neutrophil-to-Lymphocyte Ratio (NLR) as a Prognostic Predictor After Treatment of Hepatocellular Carcinoma. Review Article. *Ann Hepatol* (2021) 22:100249. doi: 10.1016/j.aohep.2020.08.067
37. Al Jarroudi O, El Bairi K, Abda N, Zaimi A, Jaouani L, Chibani H, et al. Neutrophil-To-Lymphocyte and Platelet-to-Lymphocyte Ratios as Predictors of Outcomes in Inflammatory Breast Cancer. *Biomark Med* (2021) 15 (14):1289–98. doi: 10.2217/bmm-2020-0717
38. Cupp MA, Cariolou M, Tzoulaki I, Aune D, Evangelou E, Berlanga-Taylor AJ. Neutrophil to Lymphocyte Ratio and Cancer Prognosis: An Umbrella Review of Systematic Reviews and Meta-Analyses of Observational Studies. *BMC Med* (2020) 18(1):360. doi: 10.1186/s12916-020-01817-1
39. Moldoveanu D, Pravongviengkham V, Best G, Martinez C, Hijal T, Meguerditchian AN, et al. Dynamic Neutrophil-To-Lymphocyte Ratio: A Novel Prognosis Measure for Triple-Negative Breast Cancer. *Ann Surg Oncol* (2020) 27(10):4028–34. doi: 10.1245/s10434-020-08302-2
40. Moon G, Noh H, Cho IJ, Lee JI, Han A. Prediction of Late Recurrence in Patients With Breast Cancer: Elevated Neutrophil to Lymphocyte Ratio (NLR) at 5 Years After Diagnosis and Late Recurrence. *Breast Cancer* (2020) 27 (1):54–61. doi: 10.1007/s12282-019-00994-z
41. Orlandini LF, Pimentel FF, Andrade JM, Reis F, Mattos-Arruda L, Tiezzi DG. Obesity and High Neutrophil-to-Lymphocyte Ratio Are Prognostic Factors in Non-Metastatic Breast Cancer Patients. *Braz J Med Biol Res* (2021) 54(10): e11409. doi: 10.1590/1414-431X2021e11409
42. Cho U, Park HS, Im SY, Yoo CY, Jung JH, Suh YJ, et al. Prognostic Value of Systemic Inflammatory Markers and Development of a Nomogram in Breast Cancer. *PloS One* (2018) 13(7):e0200936. doi: 10.1371/journal.pone.0200936
43. Krenn-Pilko S, Langsenlehner U, Thurner EM, Stojakovic T, Pichler M, Gerger A, et al. The Elevated Preoperative Platelet-to-Lymphocyte Ratio Predicts Poor Prognosis in Breast Cancer Patients. *Br J Cancer* (2014) 110 (10):2524–30. doi: 10.1038/bjc.2014.163
44. Zhu M, Chen L, Kong X, Wang X, Li X, Fang Y, et al. The Systemic Immune-Inflammation Index Is an Independent Predictor of Survival in Breast Cancer Patients. *Cancer Manag Res* (2022) 14:775–820. doi: 10.2147/CMAR.S346406
45. Jiang L, Fang J, Ding J. High Systemic Immune-Inflammation Index Predicts Poor Survival in Patients With Human Epidermal Growth Factor Receptor-2 Positive Breast Cancer Receiving Adjuvant Trastuzumab. *Cancer Manag Res* (2020) 12:475–84. doi: 10.2147/CMAR.S231444
46. Liu C, Huang Z, Wang Q, Sun B, Ding L, Meng X, et al. Usefulness of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio in Hormone-Receptor-Negative Breast Cancer. *Onco Targets Ther* (2016) 9:4653–60. doi: 10.2147/OTT.S106017
47. Jiang C, Lu Y, Zhang S, Huang Y. Systemic Immune-Inflammation Index Is Superior to Neutrophil to Lymphocyte Ratio in Prognostic Assessment of Breast Cancer Patients Undergoing Neoadjuvant Chemotherapy. *BioMed Res Int* (2020) 2020:7961568. doi: 10.1155/2020/7961568
48. Hu Y, Wang S, Ding N, Li N, Huang J, Xiao Z. Platelet/Lymphocyte Ratio Is Superior to Neutrophil/Lymphocyte Ratio as a Predictor of Chemotherapy Response and Disease-Free Survival in Luminal B-Like (HER2(-)) Breast Cancer. *Clin Breast Cancer* (2020) 20(4):e403–9. doi: 10.1016/j.clbc.2020.01.008
49. Takeuchi H, Kawanaka H, Fukuyama S, Kubo N, Hiroshige S, Yano T. Comparison of the Prognostic Values of Preoperative Inflammation-Based Parameters in Patients With Breast Cancer. *PloS One* (2017) 12(5):e0177137. doi: 10.1371/journal.pone.0177137
50. Sahin AB, Cubukcu E, Ocak B, Deligonul A, Oyucu Orhan S, Tolunay S, et al. Low Pan-Immune-Inflammation-Value Predicts Better Chemotherapy Response and Survival in Breast Cancer Patients Treated With Neoadjuvant Chemotherapy. *Sci Rep* (2021) 11(1):14662. doi: 10.1038/s41598-021-94184-7
51. Kim HJ, Park HS, Go YJ, Koh WU, Kim H, Song JG, et al. Effect of Anesthetic Technique on the Occurrence of Acute Kidney Injury After Total Knee Arthroplasty. *J Clin Med* (2019) 8(6):778. doi: 10.3390/jcm8060778
52. Chen X, Hong X, Chen G, Xue J, Huang J, Wang F, et al. The Pan-Immune-Inflammation Value Predicts the Survival of Patients With Anaplastic Lymphoma Kinase-Positive Non-Small Cell Lung Cancer Treated With First-Line ALK Inhibitor. *Transl Oncol* (2022) 17:101338. doi: 10.1016/j.tranon.2021.101338
53. Demir H, Demirci A, Eren SK, Beypinar I, Davarci SE, Baykara M. A New Prognostic Index in Young Breast Cancer Patients. *J Coll Phys Surg Pak* (2022) 32(1):86–91. doi: 10.29271/jcpsp.2022.01.86
54. Gambichler T, Said S, Abu Rached N, Scheel CH, Susok L, Stranzenbach R, et al. Pan-Immune-Inflammation Value Independently Predicts Disease Recurrence in Patients With Merkel Cell Carcinoma. *J Cancer Res Clin Oncol* (2022). doi: 10.1007/s00432-022-03929-y
55. Sato S, Shimizu T, Ishizuka M, Suda K, Shibuya N, Hachiya H, et al. The Preoperative Pan-Immune-Inflammation Value Is a Novel Prognostic Predictor for With Stage I-III Colorectal Cancer Patients Undergoing Surgery. *Surg Today* (2022). doi: 10.1007/s00595-021-02448-6
56. Hothorn T, Zeileis A. Generalized Maximally Selected Statistics. *Biometrics* (2008) 64(4):1263–9. doi: 10.1111/j.1541-0420.2008.00995.x
57. Zhang B, Xu H, Zhang H, Liu Q, Ye Y, Hao J, et al. Prognostic Value of N-Terminal Pro-B-Type Natriuretic Peptide in Elderly Patients With Valvular Heart Disease. *J Am Coll Cardiol* (2020) 75(14):1659–72. doi: 10.1016/j.jacc.2020.02.031
58. Jawitz NG, Raman V, Jawitz OK, Shimpi RA, Wood RK, Hartwig MG, et al. Utilization Trends and Volume-Outcomes Relationship of Endoscopic Resection for Early Stage Esophageal Cancer. *Ann Surg* (2021). doi: 10.1097/SLA.0000000000004834
59. Corti F, Lonardi S, Intini R, Salati M, Fenocchio E, Belli C, et al. The Pan-Immune-Inflammation Value in Microsatellite Instability-High Metastatic Colorectal Cancer Patients Treated With Immune Checkpoint Inhibitors. *Eur J Cancer* (2021) 150:155–67. doi: 10.1016/j.ejca.2021.03.043

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 14 April 2022
doi: 10.3389/fonc.2022.869397

Contralateral Axillary Lymph Node Metastasis of Breast Cancer: Retrospective Analysis and Literature Review

Liang Zhang[1], *Xin zhao Wang*[1], *Chao Li*[1], *Qian Yu*[2], *Zhaoyun Liu*[1] *and Zhi yong Yu*[1]*

[1] Department of Breast Surgery, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China, [2] Interventional Radiology, University of Chicago, Chicago, IL, United States

OPEN ACCESS

Edited by:
Yiding Chen,
Zhejiang University, China

Reviewed by:
Pier Carlo Rassu,
Azienda Sanitaria Locale Alessandria, Italy
Christos Markopoulos,
National and Kapodistrian University of Athens, Greece

***Correspondence:**
Zhi yong Yu
drzhiyongyu65@gmail.com

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *04 February 2022*
Accepted: *22 March 2022*
Published: *14 April 2022*

Citation:
Zhang L, Wang Xz, Li C, Yu Q, Liu Z and Yu Zy (2022) Contralateral Axillary Lymph Node Metastasis of Breast Cancer: Retrospective Analysis and Literature Review. Front. Oncol. 12:869397. doi: 10.3389/fonc.2022.869397

Background: Contralateral axillary lymph node metastasis (CAM) is classified as distant metastasis in guidelines, but the prognosis is better than that of stage IV patients. It is controversial to classify CAM as a distant metastasis or a regional metastasis, and the optimal treatment strategy for CAM is unknown.

Patients and Methods: Breast cancer patients who were confirmed by pathology and treated at Shandong Cancer Hospital between January 2012 and July 2021 were included in our study. We retrospectively reviewed the medical records of the patients for their clinical features, pathological diagnosis, treatment strategy, and follow-up data. Survival analysis was calculated by Kaplan–Meier analysis, and patient matching was performed by case–control matching.

Results: A total of 60 patients were included, and there were 49 metachronous CAM cases and 11 synchronous CAM cases. The prognosis of isolated CAM patients was better than that of patients with other distant metastases in terms of CAM-OS and PFS with significant differences (median CAM-OS 71.0 vs. 30.0 months, P=0.022; median PFS 42.0 vs. 11.0 months, P=0.009) and OS without significant differences (median OS 126.0 vs. 79.0 months, P=0.111). The five-year survival rate of isolated CAM patients was 67.4%, and the five-year disease-free survival (DFS) rate was 52.9%. The prognosis of CAM patients was similar to that of N3M0 patients in terms of OS (mean OS 82.4 vs. 65.6 months, P=0.537) and DFS (mean PFS 54.5 vs. 52.6 months, P=0.888). Axillary lymph node dissection (ALND) or low-middle level ALND significantly improved the OS (mean OS 237.4 vs. 111.0 months, P=0.011), CAM-OS (mean CAM-OS 105.2 vs. 46.6 months, P = 0.002), and PFS (mean PFS 92.3 vs. 26.9 months, P = 0.001) of isolated CAM patients. Axillary radiotherapy improved PFS, CAM-OS, and OS but without significant differences

(mean PFS 80.0 vs. 46.6 months, P = 0.345; mean CAM-OS 86.8 vs. 72.1 months, P = 0.338; mean OS 147.6 vs. 133.0 months, P = 0.426).

Conclusion: CAM should be diagnosed as local recurrence and treated with aggressive and curative rather than palliative strategies. Contralateral axillary surgery and radiotherapy are recommended for isolated CAM patients.

Keywords: contralateral axillary lymph node metastasis (CAM), breast cancer staging, local recurrence, treatment strategy, breast carcinoma (BC)

INTRODUCTION

The presence of contralateral axillary lymph node metastasis (CAM) without other organ involvement in breast cancer is rare with a reported incidence ranging between 0.81 and 6% of the total population (1–5).

The regional lymph nodes of the breast include the ipsilateral axillary, subclavian, supraclavicular, and internal mammary lymph nodes, but contralateral axillary lymph nodes are not included. CAM larger than 0.2 mm is classified as M1 (stage IV) rather than stage III according to the TNM classification in the seventh edition of the American Joint Commission on Cancer (AJCC) (6). However, the prognosis of CAM patients is better than that of stage IV patients (7).

The optimal treatment strategies are controversial, especially when CAM is the primary event of recurrence after primary tumor treatment. There is no standard treatment guideline for CAM, and patients need individualized treatment. At present, the impact of different treatment strategies on the prognosis of CAM is not clear. To date, the relevant literature consists of small-scale studies or case reports, and the details and integrity of the literature data vary greatly (8).

The mechanism of isolated CAM is different from that of CAM with ipsilateral mammary recurrence, and the occurrence of isolated CAM occurs much earlier. Isolated CAM may be an occult contralateral nodal metastasis of the primary breast cancer remaining *in situ* during the treatment, while CAM with ipsilateral mammary recurrence should be regarded as a regional metastasis recurrent breast tumor (8).

Whether CAM is regarded as a distant metastasis or a regional metastasis to the contralateral breast is currently controversial. There is a lack of large-scale clinical studies on the treatment and prognosis of CAM due to its low morbidity. It is difficult to develop a treatment strategy when CAM is the first event after treatment failure of the primary tumor, especially without other distant organ metastasis. In the present study, we aimed to evaluate the clinicopathologic characteristics of the tumor and the prognosis of patients who suffered from CAM, and we also aimed to clarify the stage and therapeutic approaches of CAM.

MATERIALS AND METHODS

The present study was a single-center, retrospective study. Breast cancer patients who were confirmed by pathology and treated at Shandong Cancer Hospital between January 2012 and July 2021 were included in our study. Patients who were initially diagnosed as N3M0 and CAM patients were included in the study. CAM was defined as synchronous CAM if the cases were diagnosed at the same time as the primary tumor or within 1 year after the initial diagnosis of the primary tumor. If CAM was detected over 1 year after the initial diagnosis of the primary tumor, we defined the cases as metachronous CAM. The diagnostic methods of CAM included pathological diagnosis of operation/biopsy, fine needle aspiration cytology, and imaging diagnosis. In addition to the contralateral axillary lymph nodes, patients with metastasis of other sites were also included in this study. The clinical, pathological, and prognostic data of all patients were collected in this study.

Estrogen receptor (ER) and progesterone receptor (PR) testing was performed by immunohistochemistry (IHC). Cancers with 1%–100% of cells positive for ER/PR expression were considered ER-/PR-positive, and cancers with <1% staining were considered negative. HER2 testing was performed using methodology outlined in the ASCO/CAP HER2 testing guideline.

Continuous data are expressed as medians and intervals, and categorical data are expressed as counts and percentages. The therapeutic effect was evaluated by overall survival (OS), overall survival after CAM diagnosis (CAM-OS), disease-free survival (DFS), and progression-free survival (PFS). Cam-OS was defined as the time from the diagnosis of CAM to death. Survival analysis was calculated by Kaplan–Meier analysis. Case–control matching was performed by molecular type, year of diagnosis, and age of diagnosis.

RESULTS

Initial Clinic-Pathological Characteristics and Metastasis

A total of 60 CAM patients were selected from 1247 advanced breast cancer patients in this study. The clinical and pathological characteristics at the time of the initial diagnosis are summarized in **Table 1**.

All of the patients were female. The onset age ranged from 23 to 69 years, and the median/mean onset age was 44.0/44.9 years. The primary tumor pathological type was definite in 49 patients, including 42 invasive ductal carcinomas, 1 myeloid carcinoma, 2 lipid secreting carcinomas, and 4 invasive lobular carcinomas. There was a significant difference in OS, CAM-OS, and PFS

TABLE 1 | Clinical and pathological characteristics of patients at initial diagnosis.

Variable	Number	Percent
Primary Side of Tumor	60	
Left	47	78.3%
Right	13	21.7%
Menstrual status	60	
Menopausal	18	30.0%
Premenopausal	42	70.0%
Primary Tumor Location	19	
Inner Upper Quadrant	2	10.5%
Outer Lower Quadrant	4	21.1%
Outer Upper Quadrant	9	47.4%
Central Region	3	15.8%
Inflammatory Breast Cancer	1	5.3%
Histopathological Grade	26	
I	0	0
II	14	53.8%
II-III	4	15.4%
III	8	30.8%
Stage	49	
I	0	0
IIa	3	6.1%
IIb	7	14.3%
IIIa	12	24.5%
IIIb	3	6.1%
IIIc	22	44.9%
IV	2	4.1%
T Stage	49	
0	1	2.0%
1	3	6.1%
2	23	46.9%
3	6	12.2%
4	16	32.7%
N Stage	54	
0	5	9.3%
1	12	22.2%
2	11	20.4%
3	26	48.1%
Molecular Subtype	49	
Luminal A	10	20.4%
Luminal B	9	18.4%
HER2 Enriched	15	30.6%
Triple Negative	15	30.6%

among patients with different molecular types at the initial diagnosis (P<0.001, P=0.003, and P=0.001, respectively; **Figures 1A–C**).

There were 49 metachronous CAMs and 11 synchronous CAMs. The median time from the initial diagnosis to the occurrence of CAM was 30.5 months, and the mean time was 45.5 months (range 0-185 months), including 2 cases diagnosed with CAM at the initial diagnosis, 4 cases diagnosed with CAM within 6 months, and 11 cases diagnosed with CAM within 12 months.

The molecular types of the contralateral axillary lymph nodes were definite in 17 cases (**Table 2**).

At the time of initial diagnosis, 34 patients had isolated CAM without other distant metastasis, and 26 patients had complicated other distant metastasis, including 17 patients with bone metastasis, 4 patients with lung metastasis, 3 patients with brain metastasis, and 11 patients with liver metastasis.

Treatment for CAM and Prognosis

There were 2 patients who refused any treatment after CAM. Of the remaining cases, 52 patients received chemotherapy, 8 patients received anti-HER2 therapy, 6 patients received contralateral axillary radiotherapy, and 16 patients received endocrine therapy. A total of 20 patients underwent contralateral axillary lymph node dissection (ALND) or low-middle level ALND, and 3 patients underwent surgical castration. Detailed information on the pathological results of lymph nodes at different levels was queried in 12 patients (**Table 3**). Contralateral mastectomy was performed in 5 patients, and no tumor was found in the gland.

The prognosis of isolated CAM patients was better than that of patients with other distant metastases in terms of CAM-OS and PFS with significant differences (median CAM-OS 71.0 vs. 30.0 months, P=0.022; median PFS 42.0 vs. 11.0 months, P=0.009) and OS without significant differences (median OS 126.0 vs. 79.0 months, P=0.111, **Figures 2A–C**).

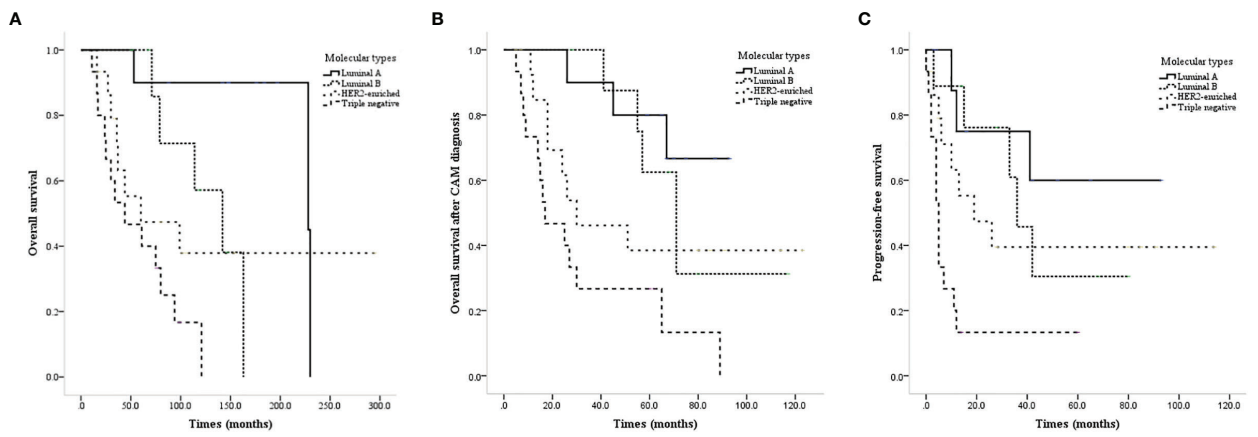


FIGURE 1 | Survival curves of CAM patients based on different molecular types at the initial diagnosis. **(A)** OS of CAM patients based on different molecular types at the initial diagnosis. **(B)** CAM-OS of CAM patients based on different molecular types at the initial diagnosis. **(C)** PFS of CAM patients based on different molecular types at the initial diagnosis.

TABLE 2 | Molecular types of primary tumors and contralateral axillary lymph nodes.

	ER%	PR%	HER2	Ki67%	Molecular Type	CAM-ER%	CAM-PR%	CAM-HER2	CAM-Ki67%	Molecular Type
1	—	—	—	10	Triple Negative	—	—	—	5	Triple Negative
2	—	—	—	90	Triple Negative	—	—	—	90	Triple Negative
3	—	—	—	50	Triple Negative	—	—	—	85	Triple Negative
4	95	20	1+	30	Luminal B	—	—	—	30	Triple Negative
5						—	—	—		Triple Negative
6	30	50	—	15	Luminal A	80	—	1+	30	Luminal B
7						90	30	—	50	Luminal B
8	10	10	—	50	Luminal B	70	70	—	40	Luminal B
9	60	80	1+	10	Luminal A	50	60	1+	65	Luminal B
10	90	90	—	45	Luminal B	90	—	—	40	Luminal B
11	80	20	—	10	Luminal A	90	—	—	35	Luminal B
12	50	60	—	40	Luminal B	70	60	1+	50	Luminal B
13						—	60	1+	75	Luminal B
14	—	—	3+		HER2-Enriched	—	—	3+	60	HER2-Enriched
15	—	—	3+	15	HER2-Enriched	—	—	3+	60	HER2-Enriched
16	—	—	3+	35	HER2-Enriched	—	—	3+	15	HER2-Enriched
17	10	—	1+	30	Luminal B	—	—	3+	30	HER2-Enriched

TABLE 3 | Metastatic status of contralateral axillary lymph nodes.

	Level 1 lymph nodes	Level 2 lymph nodes	Level 3 lymph nodes
1	9/12	2/2	—
2	6/13	0/4	—
3	5/14	0/4	—
4	5/10	1/2	0/6
5	4/8	1/2	—
6	3/5	7/7	3/5
7	1/16	0/2	0/2
8	1/14	—	0/1
9	1/12	0/3	—
10	1/12	—	—
11	1/10	—	—
12	1/11	0/2	0/1

For the isolated CAM patients, 22 patients developed tumor progression after CAM treatment with a mean PFS of 34.4 months, and 18 patients survived during the follow-up. The five-year survival rate of isolated CAM patients was 67.4%, and the five-year disease-free survival (DFS) rate was 52.9%.

ALND or low-middle level ALND significantly improved the OS (mean OS 237.4 vs. 111.0 months, P=0.011; **Figure 3A**), CAM-OS (mean CAM-OS 105.2 vs. 46.6 months, P = 0.002; **Figure 3B**), and PFS (mean PFS 92.3 vs. 26.9 months, P = 0.001; **Figure 3C**) of isolated CAM patients. Axillary radiotherapy improved PFS, CAM-OS, and OS but without a significant difference (mean PFS 80.0 vs. 46.6 months, P = 0.345; mean CAM-OS 86.8 vs. 72.1 months, P = 0.338; mean OS 147.6 vs. 133.0 months, P = 0.426; **Figures 4A**–**C**).

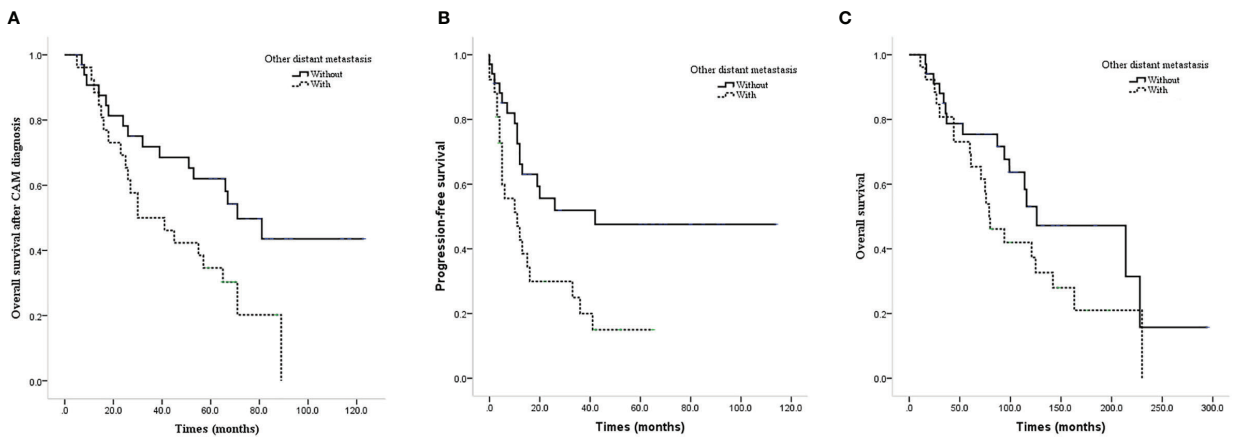


FIGURE 2 | Survival curves of CAM patients with or without other distant metastases. **(A)** CAM-OS of CAM patients with or without other distant metastases. **(B)** PFS of CAM patients with or without other distant metastases. **(C)** OS of CAM patients with or without other distant metastases.

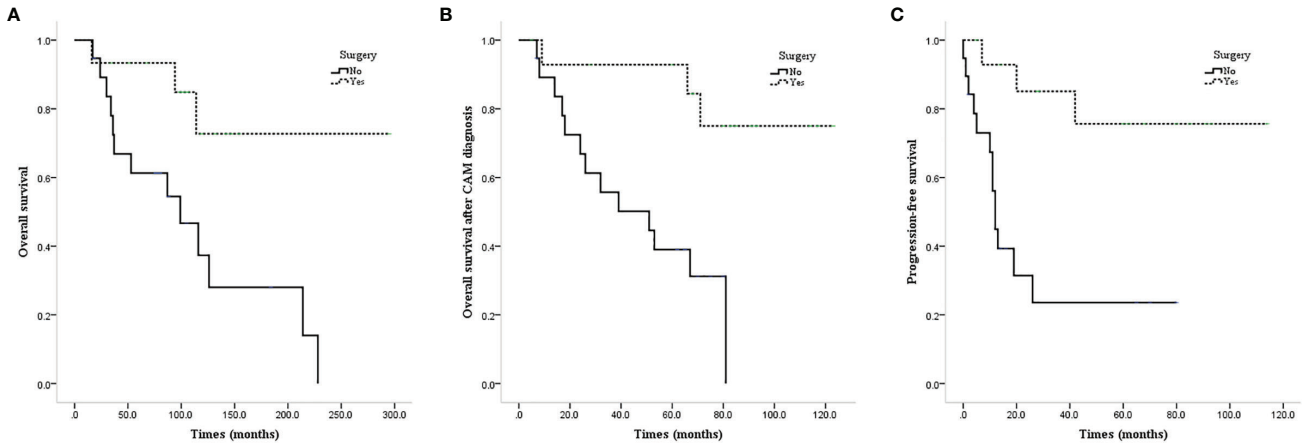


FIGURE 3 | Survival curves of CAM patients based on undergoing axillary surgery or not. **(A)** OS of CAM patients based on undergoing axillary surgery or not. **(B)** CAM-OS of CAM patients based on undergoing axillary surgery or not. **(C)** PFS of CAM patients based on undergoing axillary surgery or not.

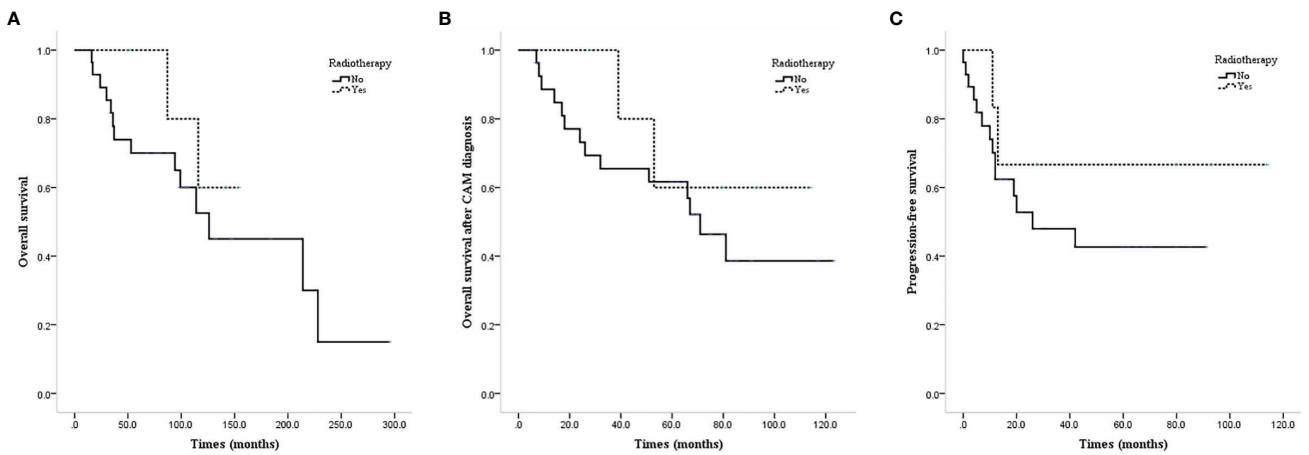


FIGURE 4 | Survival curves of CAM patients based on undergoing axillary radiotherapy or not. **(A)** OS of CAM patients based on undergoing axillary radiotherapy or not. **(B)** CAM-OS of CAM patients based on undergoing axillary radiotherapy or not. **(C)** PFS of CAM patients based on undergoing axillary radiotherapy or not.

Comparison of Prognosis Between N3M0 and CAM Patients

A total of 538 patients who were initially diagnosed with N3M0 were screened, and the molecular type was clear in 478 patients. Case–control matching was performed between the 17 CAM patients with definite molecular types of the contralateral axillary lymph nodes and N3M0 patients with definite molecular types by molecular type, year of diagnosis ±2, and age of diagnosis ±2. Finally, a total of 16 pairs of patients were successfully matched 1:1.

The prognosis of CAM patients after diagnosis of CAM was similar to that of N3M0 patients after initial diagnosis in terms of OS (mean OS 82.4 vs. 65.6 months, P=0.537, **Figure 5A**) and DFS (mean DFS 54.5 vs. 52.6 months, P=0.888, **Figure 5B**).

DISCUSSION

Although ipsilateral axillary lymph node metastasis is relatively common in breast cancer, CAM is rare. CAM can be classified into synchronous and metachronous CAM. The former, which is much rarer, exists when the primary tumor is diagnosed, and the latter appears after the treatment of the primary tumor (7, 9).

There are 3 possible sources for CAM as follows: 1) contralateral metastasis from the primary breast cancer; 2) metastasis from an occult primary in the ipsilateral breast; and 3) cancerization and metastasis from an extramammary site, such as adenocarcinoma of the uterus, gastrointestinal tract, ovary, thyroid, kidney, lymphoma, melanoma, squamous cell carcinoma of lung, squamous cell carcinoma of skin, or neurogenic tumor (10).

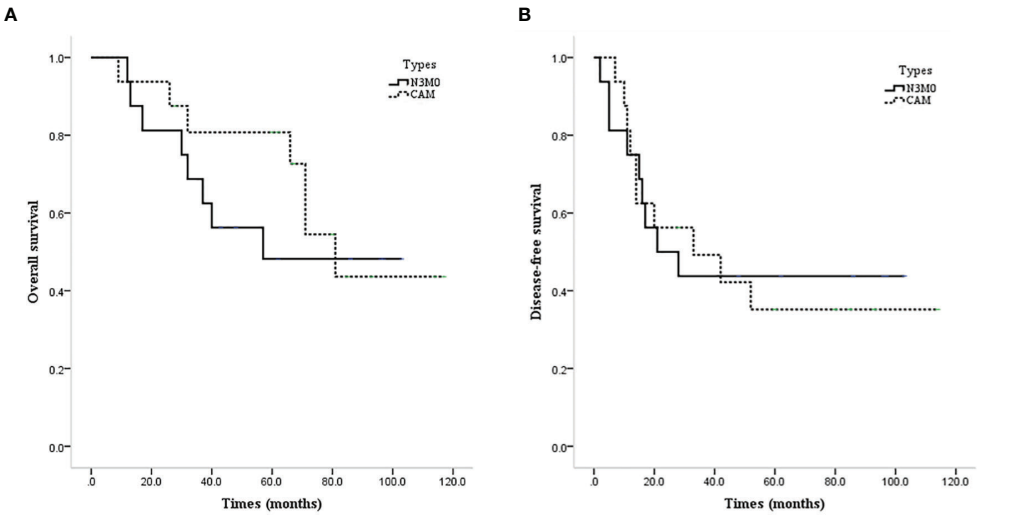


FIGURE 5 | Survival curves of CAM and N3M0 patients. **(A)** OS of CAM and N3M0 patients. **(B)** DFS of CAM and N3M0 patients.

Ultrasound and MRI are performed on the ipsilateral breast of the CAM to determine whether there is a second primary tumor. The accuracy of MRI is higher than that of ultrasound. FDG PET/CT and lymphoscintigraphy are also used to detect the contralateral axillary lymph node metastasis of a second primary tumor (1). New breast primary tumors are in 33–75% of cases after resection of the ipsilateral breast of the CAM and careful pathological sectioning (10).

The actual incidence rate of CAM is difficult to assess. On the one hand, it is difficult to assess whether there is occult breast cancer due to a lack of magnetic resonance imaging, leading to the overestimated morbidity of CAM. On the other hand, some patients are unwilling to be reviewed or lost to follow-up, resulting in underestimated morbidity.

Ipsilateral supraclavicular lymph node metastasis was regarded as distant metastasis before the sixth edition of the AJCC cancer staging manual. However, Brito et al. (11) have shown that the DFS and OS of patients with ipsilateral supraclavicular lymph node metastasis are similar to those of patients with stage IIIB disease and significantly better than those of patients with stage IV disease. Therefore, ipsilateral supraclavicular lymph node metastasis is divided into locoregional metastasis. In the present study, we found that the DFS and OS of CAM patients were similar to those of N3M0 patients and significantly better than those of patients with other distant metastases.

The occurrence of CAM is closely related to the degree of malignancy on primary tumor histopathology and changes in the lymphatic drainage pathway (4). The changes in the physiological lymphatic drainage pathway can be caused by tumor invasion of the skin, blockage of lymphatic vessels by tumor thrombi, injury caused by radiotherapy, or surgical treatment by autopsy. Haagensen et al. (12) postulated that there may be deep lymphatic drainage through the deep fascia of the chest wall to the contralateral axillary. Using lymphography, the change in the lymphatic drainage pathway to the contralateral lymph nodes (such as axillary, internal mammary, or supraclavicular lymph nodes) can be found after breast or axillary surgery. Among 330 patients, Tokmak et al. (13) showed that 2 cases (0.6%) had lymphatic imaging of the contralateral axilla. Lizarraga et al. (14) demonstrated that 7.5% (8/107) of the patients who did not undergo surgery had contralateral lymphatic imaging. Lymphatic drainage outside the ipsilateral axillary fossa existed in 20-57% of primary breast cancer patients and in 0-2% of all patients at the initial diagnosis (8). However, the proportion was 18-70% after a previous operation or radiotherapy of the breast or axilla, and 14.7% of patients had contralateral axillary lymph drainage (8).

The occurrence of distant metastasis arises from circular tumor cells in the body. The change in lymphatic drainage may suggest that CAM is a local rather than a systemic manifestation (1, 15). The change in lymphatic drainage is more important than the invasiveness of tumors for CAM (9). According to this theory, CAM could be treated actively rather than conservatively.

Wang et al. (2) observed that CAM is associated with aggressive tumors and has poor prognosis, and they suggested that CAM is more likely to be distant metastases through lymphatic routes than local metastases of second primary tumors. CAM is well controlled by comprehensive treatment, including chemotherapy and radiotherapy, while the effect of axillary lymph node resection is insufficient. Mastectomy is not recommended for CAM. However, Gingerich et al. (9) suggested that CAM is secondary to lymphatic rather than hematogenous spread, according to the altered lymphatic drainage and aberrant pathways caused by surgery or radiotherapy. Based on this theory, the treatment of CAM can be aggressive and curative rather than palliative. We found that level 1 CAM was prior to

that of level 2 and level 3 CAM, indicating that CAM may occur through chest wall lymphatic drainage rather than deep lymphatic drainage.

Chemotherapy and indicated endocrine therapy are indispensable if distant metastasis is considered or potential micrometastasis through skin lymphatic drainage outside the area of surgery and radiotherapy is needed for treatment (16). Morcos et al. (3) showed that the median DFS of 7 patients who only received endocrine therapy reached 24 months, including the longest time of 45 months of 1 case. Wang et al. (2) found that patients may obtain more survival benefits from chemotherapy or endocrine therapy than ALND or mastectomy. Neoadjuvant chemotherapy may test the sensitivity of treatment and improve the resectability of surgery (16). The PFS of CAM patients was significantly improved by radiotherapy (10 vs. 22 months) because radiotherapy can treat occult breast cancer on the same side and eradicate potential minute lesions in dermal lymphatics that may spread from the contralateral primary tumor (2). Because anti-HER2 treatment greatly improved the prognosis of breast cancer patients with CAM (3), anti-HER2 treatment should be considered in the treatment strategy of HER2-enriched cases (17, 18).

Surgery followed by radiotherapy is a reasonable and feasible scheme for patients without distant metastasis (16). Contralateral mastectomy is not recommended for the low incidence of contralateral occult breast cancer, except for some special cases, such as genetic breast cancer and CAM with different pathological and immunohistochemical features from the primary tumor (2, 3). None of the 9 patients who underwent ALND had axillary lymph node recurrence during the follow-up of 48 months (3, 10), and Huston et al. (10) showed that the DFS times of 2 patients after ALND were 29 months and 32 months. However, Wang et al. (2) considered that ALND was not effective because there was no statistical significance. The condition of the primary tumor and the timing of CAM should be considered in the formulation of therapeutic strategies (19).

Due to the insufficient number of cases and unclear immunohistochemical results, the effects of chemotherapy and endocrine and targeted therapy were not statistically analyzed. However, we still suggest that patients should receive more aggressive treatment to improve prognosis. We recommend axillary surgery for isolated CAM patients due to the improved local control and prolonged survival. In general, we do not recommend contralateral mastectomy because there was no lesion found in the resected breasts of all five patients. Although there was no significant difference, we suggest that radiotherapy should be performed to improve prognosis.

The survival time of patients with CAM varied. Most of the patients with synchronous CAM were at an advanced stage and had a worse prognosis than patients with early breast cancer, but patients with metachronous CAM had a longer survival time if there was no distant metastasis diagnosed. Chkheidze et al. (7) found that the mean CAM-OS was 27 months (range 7-40 months), the median OS after primary diagnosis was 32 months (range 13-124 months), and the median interval from CAM to distant metastasis was 18.5 months (range 5-33 months). The mean 5-year OS rate was 23% in patients with bone metastases and only 13% in patients with visceral metastasis (20). At present, Magnoni et al. (19) and Nash et al. (21) confirmed that the prognosis of CAM is not similar to that of metastatic breast cancer, but Guru et al. (22) suggested that CAM is comparable to stage IV disease in its natural history.

Moossdorff et al. (8) showed that 22 of 48 patients were followed up for a mean time of 50.3 months with an OS of 82.6% and a DFS of 65.2%. The mean follow-up time was 69.2 months for patients with isolated CAM, and the OS and DFS were 76.9% and 46.1%, respectively. Magnoni et al. (19) reported that the estimated OS was 72% at 5 years (95% CI 54–83), and the estimated DFS was 61% at 5 years (95% CI 44–74). The prognosis of patients with CAM was better than that of patients with distant metastasis, and it was comparable to that of patients with regional recurrence (5-year DFS, 56-84%). Postlewait et al. (23) showed that inflammatory breast cancer patients with isolated CAM had statistically similar survival to those with stage III disease. Therefore, it seems unjustified to classify CAM as distant metastasis.

In our analysis, the prognosis of CAM was much better than that of distant metastasis and similar to that of N3M0. This is the first study to directly compare the prognosis of N3M0 and CAM patients. At the same time, the five-year survival and five-year DFS rates of isolated CAM were similar to those of local recurrence, indicating that CAM should be considered local recurrence rather than distant metastasis.

Our study was a retrospective analysis with few patients included, and there were some restrictions for the analysis results. The subgroup analysis was limited due to insufficient cases, and the analysis results may be biased due to individual cases. Owing to the long span of cases and the change in treatment concept, the patients included later were treated more actively, resulting in the deviation of subgroup analysis.

CONCLUSION

A systemic examination should be completed to assess the status of lymph nodes for patients with recurrent breast cancer, especially for the contralateral axillary lymph nodes. Breast MRI should be completed to exclude occult breast cancer for CAM patients. Because the prognosis of CAM patients is similar to that of N3M0 patients and significantly better than that of patients with distant organ involvement, CAM should be treated as local recurrence with aggressive and curative rather than palliative strategies. The prognosis of CAM patients could be improved by ALND or low-middle level ALND and radiotherapy. Most isolated CAM will develop into distant metastasis combined with other sites, and active comprehensive treatment can control disease progression more effectively.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Affiliated Cancer Hospital of Shandong First Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LZ: manuscript writing, data collection, and statistical analysis. XW: manuscript writing, data collection, and statistical analysis. CL: revision and manuscript writing. QY: revision and manuscript writing. ZL: data collection and manuscript reviewing. ZY: guarantor, revision, manuscript writing, and supervision. The authors read and approved the final manuscript.

REFERENCES

1. Strazzanti A, Gangi S, Trovato C, Pacini N, Basile F. Contralateral Lymph Node Metastasis in a Woman With New Primary Breast Cancer: Systemic Desease or Locoregional Diffusion? *Int J Surg Case Rep* (2018) 53:400–2. doi: 10.1016/j.ijscr.2018.11.001
2. Wang W, Yuan P, Wang J, Ma F, Zhang P, Li Q, et al. Management of Contralateral Axillary Lymph Node Metastasis From Breast Cancer: A Clinical Dilemma. *Tumori* (2014) 100(6):600–4. doi: 10.1177/1778.19258
3. Morcos B, Jaradat I, El-Ghanem M. Characteristics of and Therapeutic Options for Contralateral Axillary Lymph Node Metastasis in Breast Cancer. *Eur J Surg Oncol* (2011) 37(5):418–21. doi: 10.1016/j.ejso.2011.01.024
4. Zhou C, Richir MC, Leenders MW, Langenhorst BL, Knol HP, Schreurs WH, et al. Contralateral Axillary Lymph Node Metastases at the Time of Primary Breast Cancer Diagnosis: Curative or Palliative Intent? *Case Rep Surg* (2013) 2013:389013. doi: 10.1155/2013/389013
5. Devitt JE, Michalchuk AW. Significance of Contralateral Axillary Metastases in Carcinoma of the Breast. *Can J Surg* (1969) 12(2):178–80.
6. Edge SB, Compton CC. The American Joint Committee on Cancer: The 7th Edition of the AJCC Cancer Staging Manual and the Future of TNM. *Ann Surg Oncol* (2010) 17(6):1471–4. doi: 10.1245/s10434-010-0985-4
7. Chkheidze R, Sanders MAG, Haley B, Leitch AM, Sahoo S. Isolated Contralateral Axillary Lymph Node Involvement in Breast Cancer Represents a Locally Advanced Disease Not Distant Metastases. *Clin Breast Cancer* (2018) 18(4):298–304. doi: 10.1016/j.clbc.2017.10.019
8. Moossdorff M, Vugts G, Maaskant-Braat AJ, Strobbe LJ, Voogd AC, Smidt ML, et al. Contralateral Lymph Node Recurrence in Breast Cancer: Regional Event Rather Than Distant Metastatic Disease. A Systematic Review of the Literature. *Eur J Surg Oncol* (2015) 41(9):1128–36. doi: 10.1016/j.ejso.2015.05.015
9. Gingerich J, Kapenhas E, Morgani J, Heimann A. Contralateral Axillary Lymph Node Metastasis in Second Primary Breast Cancer: Case Report and Review of the Literature. *Int J Surg Case Rep* (2017) 40:47–9. doi: 10.1016/j.ijscr.2017.08.025
10. Huston TL, Pressman PI, Moore A, Vahdat L, Hoda SA, Kato M, et al. The Presentation of Contralateral Axillary Lymph Node Metastases From Breast Carcinoma: A Clinical Management Dilemma. *Breast J* (2007) 13(2):158–64. doi: 10.1111/j.1524-4741.2007.00390.x
11. Brito RA, Valero V, Buzdar AU, Booser DJ, Ames F, Strom E, et al. Long-Term Results of Combined-Modality Therapy for Locally Advanced Breast Cancer With Ipsilateral Supraclavicular Metastases: The University of Texas M.D. Anderson Cancer Center Experience. *J Clin Oncol* (2001) 19(3):628–33. doi: 10.1200/JCO.2001.19.3.628
12. Haagensen CD. *The Lymphatics in Cancer* Vol. xvi. . Philadelphia: Saunders (1972). p. 583.
13. Tokmak H, Kaban K, Muslumanoglu M, Demirel M, Aktan S. Management of Sentinel Node Re-Mapping in Patients Who Have Second or Recurrent Breast Cancer and had Previous Axillary Procedures. *World J Surg Oncol* (2014) 12:205. doi: 10.1186/1477-7819-12-205
14. Lizarraga IM, Scott-Conner CE, Muzahir S, Weigel RJ, Graham MM, Sugg SL, et al. Management of Contralateral Axillary Sentinel Lymph Nodes Detected on Lymphoscintigraphy for Breast Cancer. *Ann Surg Oncol* (2013) 20 (10):3317–22. doi: 10.1245/s10434-013-3151-y
15. Kim JY, Lee H, Kim TS, Kang HS, Kim SK. Unusual Contralateral Axillary Lymph Node Metastasis in a Second Primary Breast Cancer Detected by FDG PET/CT and Lymphoscintigraphy. *Nucl Med Mol Imaging* (2017) 51(4):350–3. doi: 10.1007/s13139-017-0485-6
16. Kiluk JV, Prowler V, Lee MC, Khakpour N, Laronga C, Cox CE, et al. Contralateral Axillary Nodal Involvement From Invasive Breast Cancer. *Breast* (2014) 23(3):291–4. doi: 10.1016/j.breast.2014.03.004
17. Emens LA. Breast Cancer Immunotherapy: Facts and Hopes. *Clin Cancer Res* (2018) 24(3):511–20. doi: 10.1158/1078-0432.CCR-16-3001
18. Yuan Y, Hu S, Qian Y, Zhang L. The Successful Application of Pyrotinib in the Treatment of Primary Trastuzumab-Resistant HER-2-Positive Breast Cancer With Bilateral Axillary Lymph Node Metastasis: A Case Report. *Ann palliative Med* (2021) 10(6):7138–45. doi: 10.21037/apm-21-1364
19. Magnoni F, Colleoni M, Mattar D, Corso G, Bagnardi V, Frassoni S, et al. Contralateral Axillary Lymph Node Metastases From Breast Carcinoma: Is it Time to Review TNM Cancer Staging? *Ann Surg Oncol* (2020) 27(11):4488–99. doi: 10.1245/s10434-020-08605-4
20. Giordano SH, Buzdar AU, Smith TL, Kau SW, Yang Y, Hortobagyi GN, et al. Is Breast Cancer Survival Improving? *Cancer* (2004) 100(1):44–52. doi: 10.1002/cncr.11859
21. Nash A, Thomas SM, Plichta JK, Fayanju OM, Hwang ES, Greenup RA, et al. Contralateral Axillary Nodal Metastases: Stage IV Disease or a Manifestation of Progressive Locally Advanced Breast Cancer? *Ann Surg Oncol* (2021) 28 (10):5544–52. doi: 10.1245/s10434-021-10461-9
22. Guru SD, Loprinzi CL, Yan E, Hoskin TL, Jakub JW. Contralateral Axillary Metastases in Breast Cancer: Stage IV Disease or a Locoregional Event? *Am Surg* (2019) 85(12):1391–6. doi: 10.1177/000313481908501235
23. Postlewait L, Teshome M, Adesoye T, DeSnyder SM, Lim B, Kuerer HM, et al. Contralateral Axillary Metastasis in Patients With Inflammatory Breast Cancer. *Ann Surg Oncol* (2021) 28(13):8610–21. doi: 10.1245/s10434-021-10148-1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 14 April 2022
doi: 10.3389/fonc.2022.869864

Axillary Lymph Node Dissection Can Be Omitted in Breast Cancer Patients With Mastectomy and False-Negative Frozen Section in Sentinel Lymph Node Biopsy

Jing Si[1,2], Rong Guo[3], Huan Pan[4], Xiang Lu[1], Zhiqin Guo[5], Chao Han[1], Li Xue[1], Dan Xing[1], Wanxin Wu[5] and Caiping Chen[1,2]*

[1] Department of Breast Disease, The First Hospital of Jiaxing, The Affiliated Hospital of Jiaxing University, Jiaxing, China, [2] Cancer Research Center, The First Hospital of Jiaxing, The Affiliated Hospital of Jiaxing University, Jiaxing, China, [3] Department of Breast Surgery, Breast Cancer Center of the Third Affiliated Hospital of Kunming Medical University, Cancer Hospital of Yunnan Province, Kunming, China, [4] Department of Central Laboratory, The First Hospital of Jiaxing, The Affiliated Hospital of Jiaxing University, Jiaxing, China, [5] Department of Pathology, The First Hospital of Jiaxing, The Affiliated Hospital of Jiaxing University, Jiaxing, China

OPEN ACCESS

Edited by:
Yiding Chen,
School of Medicine,
Zhejiang University, China

Reviewed by:
Pier Carlo Rassu,
Azienda Sanitaria Locale Alessandria,
Italy
Mauro Giuseppe Mastropasqua,
University of Bari Medical School, Italy

***Correspondence:**
Jing Si
sijing1008@163.com

Specialty section:
This article was submitted to
Breast Cancer,
a section of the journal
Frontiers in Oncology

Received: 05 February 2022
Accepted: 14 March 2022
Published: 14 April 2022

Citation:
Si J, Guo R, Pan H, Lu X, Guo Z, Han C, Xue L, Xing D, Wu W and Chen C (2022) Axillary Lymph Node Dissection Can Be Omitted in Breast Cancer Patients With Mastectomy and False-Negative Frozen Section in Sentinel Lymph Node Biopsy. Front. Oncol. 12:869864. doi: 10.3389/fonc.2022.869864

Background: The IBCSG 23-01 and AMAROS trials both reported that axillary lymph node dissection (ALND) did not change survival rates in breast cancer patients with positive nodes detected by sentinel lymph node biopsy (SLNB). The aim of this study was to determine whether breast cancer patients with mastectomy and false-negative frozen section (FS) in SLNB could forgo ALND.

Materials and Methods: This was a retrospective study of cN0 patients diagnosed with primary invasive breast cancer treated by mastectomy and SLNB at our institute between January 2010 and December 2014. Patients with false-negative FS in SLNB were separated by the following management of axillary lymph node dissection in the non-ALND group (nonprocess or axillary radiation only) and ALND group (with or without radiation).

Results: A total of 212 patients were included, 86 and 126 patients in the non-ALND and ALND groups, respectively. The positive rate of non-sentinel lymph nodes (SLNs) was 15.87% (20/126) in the ALND group. In multivariate analysis, we found that patients with larger tumor size (>2 cm) (OR, 1.989; p = 0.030) and multifocal lesions (OR, 3.542; p = 0.029) tended to receive ALND. The positivity of non-SLNs in the ALND group was associated with SLN macrometastasis (OR, 3.551; p = 0.043) and lymphovascular invasion (OR, 6.158; p = 0.003). Also, removing more SLNs (≥3) was related to negativity in non-SLNs (OR, 0.255; p = 0.016). After a median follow-up of 59.43 months, RFS and OS of the two groups were similar (p = 0.994 and 0.441). In subgroup analysis, we found that 97 patients who met the inclusive criteria of the IBCSG 23-01 trial had similar RFS and OS between the non-ALND and ALND groups (p = 0.856 and 0.298). The positive rate of non-SLNs was 9.62% (5/52). Also, in 174

patients who met the criteria of the AMAROS trial, RFS and OS in the non-ALND and ALND groups were similar (p = 0.930 and 0.616). The positive rate of non-SLNs was 18.27% (19/104).

Conclusion: ALND can be carefully omitted in selected breast cancer patients with mastectomy and false-negative FS in SLNB. SLNB is relatively sufficient in the IBCSG 23-01-eligible patients, and axillary radiation was an effective option in the AMAROS-eligible patients.

Keywords: axillary lymph node dissection, sentinel lymph node biopsy, false-negative, frozen section, metastasis

INTRODUCTION

Axillary lymph node (ALN) metastasis is one of the significant prognostic factors in early breast cancer. The status of ALN can affect postoperative treatment options, including adjuvant chemotherapy and adjuvant radiotherapy (RT) (1). Over the last two decades, sentinel lymph node biopsy (SLNB) is considered the standard of care for axillary staging in early breast cancer patients with cN0, with axillary lymph node dissection (ALND) only reserved for patients with positive SLNs (2, 3). Frozen section (FS) is the most widely used method in the intraoperative assessment of SLNs, which has the advantage to proceed immediately to ALND in patients with positive SLNs, avoiding a second axillary surgery (4, 5). However, the sensitivity of FS is limited, with a false-negative rate of up to approximately 20%, which may lead to patients being recalled for ALND (6–14).

In recent years, the role of ALND in the treatment of patients with a limited number of positive SLNs has been relegated. Several clinical trials have been conducted on omitting ALND in patients with SLN metastasis (15). The IBCSG 23-01 trial showed that ALND could be omitted in cT1-2 patients with SLN micrometastasis (16, 17). Subsequently, the American College of Surgeons Oncology Group (ACOSOG) Z0011 trial showed that ALND could be avoided without affecting recurrence rate or survival in patients with cT1-2 and 1-2-positive SLNs treated with breast conversing surgery (BCS) (18). Furthermore, the EORTC 10981-22023 AMAROS trial showed that axillary RT could be an alternative to ALND in patients with cT1-2 with no significant differences in recurrence rate or overall survival (19). Thus, with the results of these trials and the fact that ALND may lead to higher complications and poorer quality of life, the role of ALND in early breast cancer patients with positive SLNs is controversial, especially its contribution to survival.

Interestingly, we found that most patients in these trials received BCS. Although BCS is mostly performed in patients with early breast cancer, mastectomy is still inevitable due to various factors, such as tumor burden, tumor location, patient preference, etc. According to previous studies, approximately 70% of patients received mastectomy in China (20, 21). Thus, the aim of this study was to evaluate axillary management and clinical outcomes of cN0 early breast cancer patients treated with mastectomy and found to have false-negative FS in SLNB. Furthermore, the study aimed to validate the results of both the IBCSG 23-01 and AMAROS trials in our population and determine whether breast cancer patients with mastectomy and false-negative frozen section in SLNB could forgo ALND.

MATERIALS AND METHODS

We retrospectively reviewed the medical records of cN0 patients diagnosed with primary invasive breast cancer who were treated by mastectomy and SLNB at our institute between January 2010 and December 2014. Patients were excluded if: (1) patients were male; (2) neoadjuvant chemotherapy was received prior to surgery; (3) patients had bilateral breast cancer; or (4) patients had a history of breast cancer. The study was approved by the Ethics Committee of Jiaxing University.

Patients in this study received mastectomy and SLNB at our institution. SLNB was performed at the same time as mastectomy. Methyl blue method was used to identify SLNs. All patients were injected with approximately 2 ml of methyl blue in the subcutaneous layer around the tumor after anesthesia and were gently massaged for 5 min from the tumor to the ipsilateral axillary region to facilitate the transmission of methyl blue. Blue-stained SLNs were tested. Clinically suspicious nodes in the surgical field were also resected as SLNs. The number of SLNs for FS was decided by surgeons. Intraoperative FS assessment of SLNs was performed accordingly. SLNs more than 4 mm were bisected through long axis. Half of the nodes were embedded and frozen at −20°C. SLNs less than or equal to 4 mm were completely frozen. Sections were cut and stained with hematoxylin and eosin (H&E) for frozen examination. Histological assessment with hematoxylin–eosin staining performed postoperatively served as the gold standard. A positive SLN was defined as the presence of either micrometastasis (>200 cells or >0.2 mm, but <2.0 mm) or macrometastasis (>2.0 mm) identified on hematoxylin–eosin staining. The definition of false-negative FS in SLNB was that, in intraoperative FS assessment, SLNs were all negative, however, in postoperatively histological assessment, one or more SLNs were shown positive with micrometastasis or macrometastasis. Patients with false-negative FS in SLNB could either perform ALND or not according to surgeons' choices. Levels I and II ALND were performed according to a standard ALND procedure in patients who underwent ALND. In the current

article, patients with false-negative FS in SLNB were separated by the following management of axillary lymph node dissection in the non-ALND group (nonprocess or radiation only including axillary area) and ALND group (with or without radiation including axillary area). Immunohistochemistry (IHC) for ER and PR was performed, and cases with more than 1% were considered positive staining. HER2 positivity was defined as cases where IHC was 3+ or 2+ with fluorescence *in situ* hybridization (FISH) positivity. In addition, IHC for Ki67 was also performed and cases with more than 14% were considered positive staining.

Date and status at last follow-up were collected. Recurrence events were recorded as local–regional and distant. Time to recurrence and time to death were measured from date of surgery and censored at the date of last follow-up for event-free patients. Time to recurrence was censored at time of death. Loss to follow-up was defined as patients with less than 2-year follow-up after surgery.

The clinicopathological characteristics were compared between patients in the non-ALND group and ALND group using chi-square test for categorical variables. Time-to-event outcomes were estimated using Kaplan–Meier methods and were compared across groups using the log-rank test. Two-tailed p-values were adopted, and $p < 0.05$ was considered significant. All statistical analysis was performed using SPSS statistical software version 18.0 (IBM, Chicago, IL, USA).

RESULTS

A total of 1,470 cN0 patients who received mastectomy and SLNB were included, of which 14.42% (212/1470) had false-negative FS in SLNB. The median number of SLNs removed was 3, ranging from 1 to 12. In patients with false-negative FS, 86 (40.57%) and 126 (59.43%) were in the non-ALND and ALND groups, respectively. The positive rate of non-SLNs was 15.87% (20/126) in the ALND group. The baseline characteristics of the cohort with false-negative FS are shown in **Table 1**. We found that patients with larger tumor size (>2 cm) ($p = 0.025$), multiple number of foci ($p = 0.017$), and macrometastasis in SLNs ($p = 0.045$) had a tendency of receiving ALND. In multivariate analysis, patients with larger tumor size (>2 cm) (OR, 1.989; $p = 0.030$) and multifocal lesions (OR, 3.542; $p = 0.029$) had a tendency of receiving ALND (**Table 1**).

Moreover, we analyzed the factors associated with non-SLN positivity in the ALND group (**Table 2**). We found that the positivity of non-SLNs in the ALND group was associated with SLN macrometastasis (OR, 3.551; $p = 0.043$) and lymphovascular invasion (OR, 6.158; $p = 0.003$). Also, removing more SLNs (≥3) was related to negativity in non-SLNs in these patients (OR, 0.255, $p = 0.016$) (**Table 2**).

We further analyzed the adjuvant therapy in the non-ALND and ALND groups. We found that the receipt of hormonal therapy (60.47% [52/86] vs. 53.97% [68/126], $p = 0.349$), adjuvant radiotherapy including axillary area (11.63% [10/86] vs. 12.70% [16/126], $p = 0.816$), and adjuvant chemotherapy (74.42% [64/86] vs. 78.57% [99/126], $p = 0.481$) was similar in the non-ALND and ALND groups.

In this cohort, 190 (89.62%) patients were included in the survival analysis. The median follow-up was 59.43 months, which was 4.95 years. Overall survival rate was 96.32% (183/190) for the total population in survival analysis. There were 20 (10.53%) recurrences: 5 local and regional; 13 distant; and 2 concurrent local and distant. The 5-year RFS rate and OS rate in the non-ALND and ALND groups are shown in **Table 3**. Moreover, Kaplan–Meier analysis showed that the RFS and OS of patients in the non-ALND and ALND groups were similar ($p = 0.994$ and 0.441, respectively) (**Figures 1A, B**).

In subgroup analysis, we found that 97 patients met the inclusive criteria of the IBCSG 23-01 trial, of which, 46.39% (45/97) of patients were in the non-ALND group with 5 patients receiving radiation only, and 53.61% (52/97) of patients were in the ALND group, respectively. Similar RFS and OS were shown between the non-ALND and ALND groups ($p = 0.856$ and 0.298, respectively) (**Figures 2A, B**). The positive rate of non-SLNs was 9.62% (5/52) in the ALND group. In addition, we found 174 patients who met the

TABLE 1 | Baseline characteristics of clinicopathological factors in cN0 patients who received mastectomy and had false-negative FS in SLNB.

Variables	Total = 212	Non-ALND = 86	ALND *N* = 126	Univariate *p*-value	Multivariate OR (95% CI), *p*-value
Age				0.563	
≤50	116	45	71		
>50	96	41	55		
Grade				0.243	
I	9	5	4		
II	155	66	89		
III	48	15	33		
Number of foci				0.017	
One	189	82	107		Ref
Multiple	23	4	19		3.542 (1.138–11.026), $p = 0.029$
Size on pathology				0.025	
≤2 cm	139	64	75		Ref
>2 cm	73	22	51		1.989 (1.070–3.697), $p = 0.030$
LVI				0.469	
No	156	61	95		
Yes	56	25	31		
Number of SLNs				0.312	
<3	70	25	45		
≥3	142	61	81		
SLN metastasis				0.045	
Micrometastasis	118	55	63		Ref
Macrometastasis	94	31	63		1.759 (0.981–3.154), $p = 0.058$
ER				0.227	
Negative	24	7	17		
Positive	188	79	109		
HER2				0.670	
Negative	173	69	104		
Positive	39	17	22		
Ki67				0.763	
≤14%	69	29	40		
>14%	143	57	86		

TABLE 2 | Factors associated with positivity of non-SLNs in the ALND group.

Variables	Negative non-SLNs *N* = 106	Positive non-SLNs *N* = 20	Univariate *p*-value	Multivariate OR (95% CI), *p*-value
Age			0.894	
≤50	60	11		
>50	46	9		
Grade			0.243	
I	3	1		
II	75	14		
III	28	5		
Number of foci				
One	89	18	0.489	
Multiple	17	2		
Size on pathology				
≤2 cm	62	13	0.586	
>2 cm	44	7		
LVI			0.004	
No	85	10		Ref
Yes	21	10		6.158 (1.865–20.338), $p = 0.003$
Number of SLNs			0.002	
<3	35	14		Ref
≥3	71	6		0.255 (0.084–0.773), $p = 0.016$
SLN metastasis			0.015	
Micrometastasis	58	5		Ref
Macrometastasis	48	15		3.551 (1.038–12.149), $p = 0.043$
ER			0.618	
Negative	15	2		
Positive	91	18		
HER2			0.338	
Negative	86	18		
Positive	20	2		
Ki67			0.855	
≤14%	34	6		
>14%	72	14		

criteria of the AMAROS trial, of which 40.23% (70/174) were in the non-ALND group with 9 receiving radiation only and 59.77% (104/174) were in the ALND group. We found that RFS and OS were similar between the non-ALND and ALND groups ($p = 0.930$ and 0.616, respectively) (**Figures 3A, B**). The positive rate of non-SLNs was 18.27% (19/104) in the ALND group.

DISCUSSION

SLNB is a minimally invasive procedure to determine the axillary status in patients with clinically negative lymph nodes. Currently, guidelines still recommend ALND if positive SLNs are found, except for patients who fit the criteria of several trials, including the ACOSOG Z0011 trial, IBCSG 23-01 trial, and EORTC 10981-22023 AMAROS trial, who can cautiously omit ALND (1, 15, 16, 18, 19). Therefore, accurate intraoperative SLN assessment is very important in order to avoid reoperation for patients with SLN metastasis. Among the various methods of intraoperative diagnosis of SLN status, FS is most commonly used. Previous studies showed that the false-negative rates of FS ranged from 13% to 43%, and the false-negative results occurred more frequently in cases with micrometastasis (5, 22–24). In our study, FS had a false-negative rate of 14.42% in patients who received mastectomy and SLNB, and 55.66% (118/212) were micrometastasis, which was consistent with previous studies. Our next focus is whether these patients with false-negative FS need to be recalled for ALND.

In recent years, several clinical trials have been focused on omitting ALND in patients with SLN metastasis (15). With the application of the criteria in these trials, ALND can be avoided in more patients with positive SLNs. However, most patients in these trials received BCS, including all the patients in the ACOSOG Z0011 trial, 91% of patients in the IBCSG 23-01 trial, and 82% of patients in the EORTC 10981-22023 AMAROS trial (16, 18, 19). Thus, with relatively solid evidence for patients who received BCS, the following questions remain to be resolved: (1) can patients who received mastectomy be managed the same way as BCS patients with regard to ALND and (2) is the use of intraoperative FS still needed if ALND can be omitted.

Previous studies have shown that omitting ALND is safe and has high survival rates in patients who received mastectomy with ≤2 positive SLNs (25, 26). Also, adding axillary radiotherapy can be a treatment option for patients with high-risk factors (25–27). In this current study, we retrospectively enrolled patients who received mastectomy with false-negative FS in SLNB. Several factors associated with the choice of ALND procedure and positive non-SLNs in patients who received ALND were found, including larger tumor size (>2 cm), multifocal lesions, lymphovascular invasion, SLN macrometastasis, and less-removed SLNs (<3). These were clinicopathological risk factors that could impact the decision of ALND or additional axillary radiotherapy, which were also consistent with previous studies of nomograms and models including these factors to evaluate the risk of further nodal involvement and might be used to select patients with positive SLNs in whom ALND may be safely omitted (28–30). In addition, we found that, with a median follow-up of 4.95 years, there were no significant differences in RFS and OS compared between the non-ALND and ALND group ($p = 0.994$ and 0.441, respectively). Also, in the IBCSG 23-01-eligible and AMAROS-eligible subgroups, survival outcomes were similar between non-ALND and ALND patients. Thus, with low axillary recurrence rate and relatively high survival outcome in patients with false-negative FS in SLNB without axillary clearance, we believe that omission of ALND may be considered in selected patients, especially with few clinicopathological high-risk factors.

TABLE 3 | Five-year RFS rate and OS rate in the ALND and non-ALND groups.

	Total (*N* = 190)	Non-ALND group (*N* = 79)	ALND group (*N* = 111)	*p*-value
5-Year RFS rate	87.99%	88.41%	87.71%	0.799
5-Year OS rate	96.94%	98.53%	95.74%	0.208

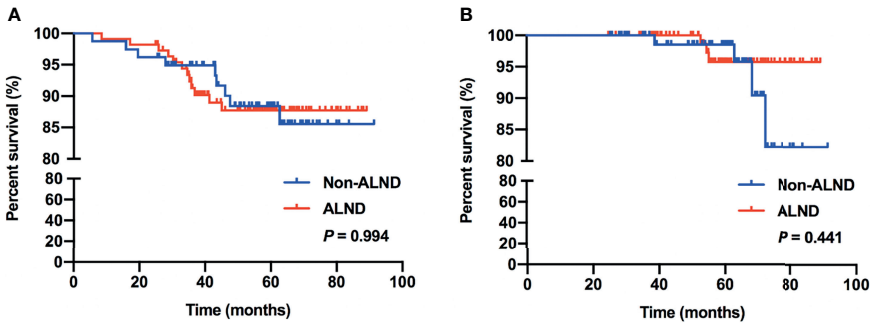


FIGURE 1 | RFS **(A)** and OS **(B)** between the non-ALND and ALND groups.

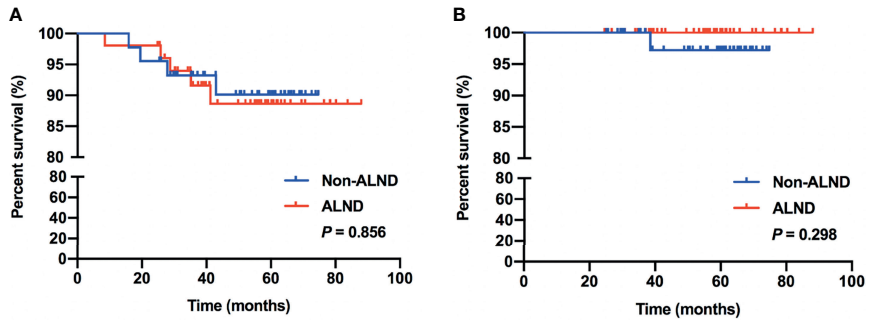


FIGURE 2 | RFS **(A)** and OS **(B)** in patients who met the inclusive criteria of the IBCSG 23-01 trial.

On the other hand, under the circumstance that ALND can be omitted in selected patients, the need for intraoperative FS is controversial. FS remains the most common method of intraoperative assessment of SLNs with the advantage to proceed immediately to ALND in patients with positive SLNs avoiding further axillary surgery. However, with the possibility of omitting ALND in patients with positive SLNs, the role of FS is less important. Previous reports showed a significant decline in intraoperative assessment of SLNs from 69%–92% to 26%–45% before and after the disclosure of the ACOSOG Z0011 trial (31, 32). In addition, Bishop et al. reported that FS for SLNs dropped from 69% to 2% in their institute (33). Jorns et al. also reported a marked decline of FS from 74% to 25% pre-Z0011 and post-Z0011 (34). Nevertheless, if avoiding ALND in patients with positive SLNs is recommended, the indications regarding patients who meet the criteria of the above trials are still discordant, especially in patients who received mastectomy. What we believe we should admit is that, although ALND can be omitted in most patients who meet the criteria of certain trials, those with high-risk factors could have resulted in undertreatment. Thus, we suggest that intraoperative FS should not be routinely performed in all breast cancer patients; whereas, in selected high-risk patients who might be offered ALND

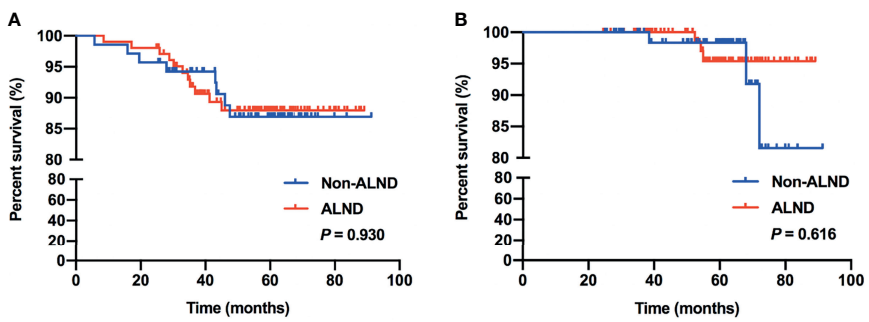


FIGURE 3 | RFS **(A)** and OS **(B)** in patients who met the inclusive criteria of the AMAROS trial.

after balancing the benefit from this procedure, intraoperative FS continues to be very useful in the intraoperative assessment of SLNs.

In addition, previous studies have been focused on increasing SLN detection rate in patients who need intraoperative assessment of SLNs. Several reports showed association between the method of SLN detection and sentinel lymph node identification, recommended combined method (radioactive tracer and blue dye), which could provide higher detection rate (35–37). Moreover, we found that removing more SLNs (≥3) was related to negativity in non-SLNs. Whereas, Dutta et al. indicated that no more than 4 SLNs should be removed because all patients with axillary metastasis were identified within the first 4 SLNs (38). Thus, we need to balance SLN detection rate and unnecessary SLN removal in clinical practice (39).

There were several limitations to this current study. Firstly, it was a retrospective study, which led to lower level of evidence. However, this study had a relatively large dataset with uniform inclusion and exclusion criteria. Secondly, few patients received axillary radiotherapy only in our institute, which provide limitations in our analysis. We analyzed survival outcomes in patients who received axillary radiotherapy only and patients who received ALND in the AMAROS-eligible subgroup, showing no significant difference. However, to avoid bias, we did not want to provide a conclusion based only on such a few cases of axillary radiotherapy. Further assessment is needed to accurately select patients in whom ALND can be safely avoided.

CONCLUSION

In this study, we confirmed that there was no difference in patients' survival regardless of additional ALND among early breast cancer patients who underwent mastectomy and false-negative intraoperative FS in SLNB. SLNB is relatively sufficient in patients who met the criteria of the IBCSG 23-01 trial, and axillary radiation was an effective option in patients who met the criteria of the AMAROS trial. Although further studies are needed, omission of ALND may be considered in selected patients. Thus, ALND can be carefully omitted in breast cancer patients with mastectomy and false-negative frozen section in SLNB.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JS wrote the article. RG analyzed data. HP, XL, ZG, CH, LX, and DX collected data. WW and CC gave administration support. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (Grant No. 81902674).

REFERENCES

1. Gradishar WJ, Anderson BO, Abraham J, Aft R, Agnese D, Allison K, et al. Breast Cancer, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* (2020) 18:452–78. doi: 10.6004/jnccn.2020.0016
2. Glechner A, Wockel A, Gartlehner G, Thaler K, Strobelberger M, Griebler U, et al. Sentinel Lymph Node Dissection Only Versus Complete Axillary Lymph Node Dissection in Early Invasive Breast Cancer: A Systematic Review and Meta-Analysis. *Eur J Cancer* (2013) 49:812–25. doi: 10.1016/j.ejca.2012.09.010
3. Veronesi U, Paganelli G, Viale G, Luini A, Zurrida S, Galimberti V, et al. A Randomized Comparison of Sentinel-Node Biopsy With Routine Axillary Dissection in Breast Cancer. *N Engl J Med* (2003) 349:546–53. doi: 10.1056/NEJMoa012782
4. van der Noordaa MEM, Vrancken Peeters M, Rutgers EJT. The Intraoperative Assessment of Sentinel Nodes - Standards and Controversies. *Breast* (2017) 34 Suppl 1:S64–9. doi: 10.1016/j.breast.2017.06.031
5. Layfield DM, Agrawal A, Roche H, Cutress RI. Intraoperative Assessment of Sentinel Lymph Nodes in Breast Cancer. *Br J Surg* (2011) 98:4–17. doi: 10.1002/bjs.7229
6. Qiao G, Cong Y, Zou H, Lin J, Wang X, Li X, et al. False-Negative Frozen Section of Sentinel Lymph Node Biopsy in a Chinese Population With Breast Cancer. *Anticancer Res* (2016) 36:1331–7.
7. Poling JS, Tsangaris TN, Argani P, Cimino-Mathews A. Frozen Section Evaluation of Breast Carcinoma Sentinel Lymph Nodes: A Retrospective Review of 1,940 Cases. *Breast Cancer Res Treat* (2014) 148:355–61. doi: 10.1007/s10549-014-3161-x
8. Jaka RC, Zaveri SS, Somashekhar SP, Sureshchandra, Parameswarani RV, et al. Value of Frozen Section and Primary Tumor Factors in Determining Sentinel Lymph Node Spread in Early Breast Carcinoma. *Indian J Surg Oncol* (2010) 1:27–36. doi: 10.1007/s13193-010-0008-8
9. Vanderveen KA, Ramsamooj R, Bold RJ. A Prospective, Blinded Trial of Touch Prep Analysis Versus Frozen Section for Intraoperative Evaluation of Sentinel Lymph Nodes in Breast Cancer. *Ann Surg Oncol* (2008) 15:2006–11. doi: 10.1245/s10434-008-9944-8
10. Godazande G, Moradi S, Naghshvar F, Shojaee L. Is Necessary Intraoprative Frozen Section In Sentinel Lymph Node Biopsy For Breast Cancer Patients? *Asian Pac J Cancer Prev* (2020) 21:647–51. doi: 10.31557/APJCP.2020.21.3.647
11. Andersson Y, Frisell J, Sylvan M, Boniface J, Bergkvist L, et al. Causes of False-Negative Sentinel Node Biopsy in Patients With Breast Cancer. *Br J Surg* (2013) 100:775–83. doi: 10.1002/bjs.9085
12. Kim T, Giuliano AE, Lyman GH. Lymphatic Mapping and Sentinel Lymph Node Biopsy in Early-Stage Breast Carcinoma: A Metaanalysis. *Cancer* (2006) 106:4–16. doi: 10.1002/cncr.21568
13. Lee SA, Lee HM, Lee HW, Yang BS, Park JT, Ahn SG, et al. Risk Factors for a False-Negative Result of Sentinel Node Biopsy in Patients With Clinically Node-Negative Breast Cancer. *Cancer Res Treat* (2018) 50:625–33. doi: 10.4143/crt.2017.089
14. Cipolla C, Graceffa G, Cabibi D, Gangi G, Latteri M, Valerio MR, et al. Current Role of Intraoperative Frozen Section Examination of Sentinel Lymph Node in Early Breast Cancer. *Anticancer Res* (2020) 40:1711–7. doi: 10.21873/anticanres.14124
15. Yan M, Abdi MA, Falkson C. Axillary Management in Breast Cancer Patients: A Comprehensive Review of the Key Trials. *Clin Breast Cancer* (2018) 18: e1251–9. doi: 10.1016/j.clbc.2018.08.002

16. Galimberti V, Cole BF, Viale G, Veronesi P, Vicini E, Intra M, et al. Axillary Dissection Versus No Axillary Dissection in Patients With Breast Cancer and Sentinel-Node Micrometastases (IBCSG 23-01): 10-Year Follow-Up of a Randomised, Controlled Phase 3 Trial. *Lancet Oncol* (2018) 19:1385–93. doi: 10.1016/S1470-2045(18)30380-2
17. Galimberti V, Cole BF, Zurrida S, Viale G, Luini A, Veronesi P, et al. Axillary Dissection Versus No Axillary Dissection in Patients With Sentinel-Node Micrometastases (IBCSG 23-01): A Phase 3 Randomised Controlled Trial. *Lancet Oncol* (2013) 14:297–305. doi: 10.1016/S1470-2045(13)70035-4
18. Giuliano AE, Ballman KV, McCall L, Beitsch PD, Brennan MB, Kelemen PR, et al. Effect of Axillary Dissection vs No Axillary Dissection on 10-Year Overall Survival Among Women With Invasive Breast Cancer and Sentinel Node Metastasis: The ACOSOG Z0011 (Alliance) Randomized Clinical Trial. *JAMA* (2017) 318:918–26. doi: 10.1001/jama.2017.11470
19. Donker M, van Tienhoven G, Straver ME, Meijnen P, van de Velde CJH, Mansel RE, et al. Radiotherapy or Surgery of the Axilla After a Positive Sentinel Node in Breast Cancer (EORTC 10981-22023 AMAROS): A Randomised, Multicentre, Open-Label, Phase 3 Non-Inferiority Trial. *Lancet Oncol* (2014) 15:1303–10. doi: 10.1016/S1470-2045(14)70460-7
20. Huang NS, Liu MY, Chen JJ, Yang BL, Xue JY, Quan CL, et al. Surgical Management of Breast Cancer in China: A 15-Year Single-Center Retrospective Study of 18,502 Patients. *Med (Baltimore)* (2016) 95:e4201. doi: 10.1097/MD.0000000000004201
21. Yang B, Ren G, Song E, Pan D, Zhang J, Wang Y, et al. Current Status and Factors Influencing Surgical Options for Breast Cancer in China: A Nationwide Cross-Sectional Survey of 110 Hospitals. *Oncologist* (2020) 25: e1473–80. doi: 10.1634/theoncologist.2020-0001
22. Lu Q, Tan EY, Ho B, Teo C, Seah MDW, Chen JJC, et al. Achieving Breast Cancer Surgery in a Single Setting With Intraoperative Frozen Section Analysis of the Sentinel Lymph Node. *Clin Breast Cancer* (2013) 13:140–5. doi: 10.1016/j.clbc.2012.11.005
23. Liu LC, Lang JE, Lu Y, Roe D, Hwang SE, Ewing CA, et al. Intraoperative Frozen Section Analysis of Sentinel Lymph Nodes in Breast Cancer Patients: A Meta-Analysis and Single-Institution Experience. *Cancer* (2011) 117:250–8. doi: 10.1002/cncr.25606
24. Liang C, Li L, Zhu M, Hu J, Yu Y. The Guiding Significance of the Number of Positive Sentinel Lymph Nodes in Frozen Section for Intraoperative Axillary Dissection in Early Breast Cancer. *Cancer Manag Res* (2021) 13:4803–10. doi: 10.2147/CMAR.S308796
25. Ortega Exposito C, Falo C, Pernas S, Carton SP, Gil MG, Ortega R, et al. The Effect of Omitting Axillary Dissection and the Impact of Radiotherapy on Patients With Breast Cancer Sentinel Node Macrometastases: A Cohort Study Following the ACOSOG Z0011 and AMAROS Trials. *Breast Cancer Res Treat* (2021) 189:111–20. doi: 10.1007/s10549-021-06274-9
26. Galimberti V, Chifu C, Rodriguez Perez S, Veronesi P, Intra M, Botteri E, et al. Positive Axillary Sentinel Lymph Node: Is Axillary Dissection Always Necessary? *Breast* (2011) 20(Suppl 3):S96–98. doi: 10.1016/S0960-9776(11) 70303-4
27. Kim BK, Park BW, Hur MH, Lee HB, Park MH, Jeong J, et al. Omission of Axillary Lymph Node Dissection in Patients Who Underwent Total Mastectomy With 1 or 2 Metastatic Lymph Nodes. *Ann Surg Treat Res* (2020) 98:283–90. doi: 10.4174/astr.2020.98.6.283
28. Coutant C, Olivier C, Lambaudie E, Fondrinier E, Marchal F, Guillemin F, et al. Comparison of Models to Predict Nonsentinel Lymph Node Status in Breast Cancer Patients With Metastatic Sentinel Lymph Nodes: A Prospective Multicenter Study. *J Clin Oncol* (2009) 27:2800–8. doi: 10.1200/ JCO.2008.19.7418
29. Rouzier R, Uzan C, Rousseau A, Guillot E, Zilberman S, Meyer C, et al. Multicenter Prospective Evaluation of the Reliability of the Combined Use of Two Models to Predict Non-Sentinel Lymph Node Status in Breast Cancer Patients With Metastatic Sentinel Lymph Nodes: The MSKCC Nomogram and the Tenon Score. Results of the NOTEGS Study. *Br J Cancer* (2017) 116:1135–40. doi: 10.1038/bjc.2017.47
30. Viale G, Giobbie-Hurder A, Gusterson BA, Maiorano E, Mastropasqua MG, Sonzogni A, et al. Adverse Prognostic Value of Peritumoral Vascular Invasion: Is It Abrogated by Adequate Endocrine Adjuvant Therapy? Results From Two International Breast Cancer Study Group Randomized Trials of Chemoendocrine Adjuvant Therapy for Early Breast Cancer. *Ann Oncol* (2010) 21:245–54. doi: 10.1093/annonc/mdp317
31. Wright GP, Mater ME, Sobel HL, Knoll GM, Oostendorp LD, Melnik MK, et al. Measuring the Impact of the American College of Surgeons Oncology Group Z0011 Trial on Breast Cancer Surgery in a Community Health System. *Am J Surg* (2015) 209:240–5. doi: 10.1016/j.amjsurg.2014.07.001
32. Caudle AS, Hunt KK, Tucker SL, Hoffman K, Gainer SM, Lucci A, et al. American College of Surgeons Oncology Group (ACOSOG) Z0011: Impact on Surgeon Practice Patterns. *Ann Surg Oncol* (2012) 19:3144–51. doi: 10.1245/s10434-012-2531-z
33. Bishop JA, Sun J, Ajkay N, Sanders MA. Decline in Frozen Section Diagnosis for Axillary Sentinel Lymph Nodes as a Result of the American College of Surgeons Oncology Group Z0011 Trial. *Arch Pathol Lab Med* (2016) 140:830–5. doi: 10.5858/arpa.2015-0296-OA
34. Jorns JM, Kidwell KM. Sentinel Lymph Node Frozen-Section Utilization Declines After Publication of American College of Surgeons Oncology Group Z0011 Trial Results With No Change in Subsequent Surgery for Axillary Lymph Node Dissection. *Am J Clin Pathol* (2016) 146:57–66. doi: 10.1093/ ajcp/aqw078
35. Xu L, Yang J, Du Z, Liang F, Xie Y, Long Q, et al. Redefining Criteria to Ensure Adequate Sentinel Lymph Node Biopsy With Dual Tracer for Breast Cancer. *Front Oncol* (2020) 10:588067. doi: 10.3389/fonc.2020.588067
36. Radovanovic Z, Golubovic A, Plzak A, Stojiljkovic B, Radovanovic D, et al. Blue Dye Versus Combined Blue Dye-Radioactive Tracer Technique in Detection of Sentinel Lymph Node in Breast Cancer. *Eur J Surg Oncol* (2004) 30:913–7. doi: 10.1016/j.ejso.2004.08.003
37. McMasters KM, Tuttle TM, Carlson DJ, Brown CM, Noyes RD, Glaser RL, et al. Sentinel Lymph Node Biopsy for Breast Cancer: A Suitable Alternative to Routine Axillary Dissection in Multi-Institutional Practice When Optimal Technique Is Used. *J Clin Oncol* (2000) 18:2560–6. doi: 10.1200/ JCO.2000.18.13.2560
38. Dutta R, Kluftinger A, MacLeod M, Kindrachuk G, Baliski C. Revisiting the "10% Rule" in Breast Cancer Sentinel Lymph Node Biopsy: An Approach to Minimize the Number of Sentinel Lymph Nodes Removed. *Am J Surg* (2012) 203:623–7. doi: 10.1016/j.amjsurg.2012.01.010.
39. Viale G, Mastropasqua MG, Maiorano E, Mazzarol G. Pathologic Examination of the Axillary Sentinel Lymph Nodes in Patients With Early-Stage Breast Carcinoma: Current and Resolving Controversies on the Basis of the European Institute of Oncology Experience. *Virchows Arch* (2006) 448:241–7. doi: 10.1007/s00428-005-0103-3

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 04 May 2022
doi: 10.3389/fonc.2022.819531

Nomogram Predicts the Role of Primary Tumor Surgery on *De Novo* Stage-IV Breast Cancer Patients: A SEER-Based Competing Risk Analysis Model

Hanxiao Cui[1†], Luyao Dai[1†], Yuanhang Bao[1], Liqun Hu[1], Zhangjian Zhou[1], Meng Wang[1], Shuai Lin[1], Hao Wu[2], Xiaobin Ma[1*] and Huafeng Kang[1*]*

[1] Department of Oncology, The Second Affiliated Hospital, Xi'an Jiaotong University, Xi'an, China, [2] School of Basic Medical Sciences, Xi'an Key Laboratory of Immune Related Diseases, Xi'an Jiaotong University, Xi'an, China

OPEN ACCESS

Edited by:
Yiding Chen,
Zhejiang University, China

Reviewed by:
Takahiro Kogawa,
Cancer Institute Hospital of Japanese Foundation for Cancer Research, Japan
Yi Ren,
Duke University, United States

***Correspondence:**
Huafeng Kang
kanghuafeng1973@126.com
Xiaobin Ma
binbinmxb@sohu.com
Hao Wu
lfshwu@xjtu.edu.cn

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *21 November 2021*
Accepted: *13 April 2022*
Published: *04 May 2022*

Citation:
Cui H, Dai L, Bao Y, Hu L, Zhou Z, Wang M, Lin S, Wu H, Ma X and Kang H (2022) Nomogram Predicts the Role of Primary Tumor Surgery on De Novo Stage-IV Breast Cancer Patients: A SEER-Based Competing Risk Analysis Model. Front. Oncol. 12:819531. doi: 10.3389/fonc.2022.819531

Objective: The efficacy of primary tumor surgery on survival in female patients with *de novo* stage IV breast cancer (BC) remains unclear. Our study endeavored to develop comprehensive competing risk nomograms to predict clinical outcomes and guide precision treatment in these patients.

Participants and Methods: A total of 12281 patients who had distant metastasis at initial BC diagnosis between 2010 and 2017 in the Surveillance Epidemiology and End Results (SEER) database, were enrolled in this study. First, we assessed the impacts of primary tumor surgery on overall survival (OS) and breast cancer-specific survival (BCSS) using the Kaplan-Meier curves. Then subgroup analyses stratified by different metastatic patterns were performed using Cox and competing risk models (CRM). Based on the filtered independent prognostic parameters by CRM, we established two nomograms to predict the probability of breast cancer-specific death (BCSD) at 1-,2- and 3-year intervals. Furthermore, calibration curves and area under the curves (AUC) were conducted for validation.

Results: Kaplan-Meier analysis revealed that surgery was associated with better OS and BCSS (P<0.001). Subgroup analyses demonstrated that in bone-only metastases pattern, relative to breast-conserving surgery (BCS), patients receiving mastectomy had worse prognosis and the poorest survival belonged to non-surgery individuals (BCSS: mastectomy: HR=1.35; 95%CI=1.15-1.60; non-surgery: 2.42; 2.08-2.82; OS: mastectomy: 1.44; 1.23-1.68; non-surgery: 2.40; 2.08-2.78). Additionally, no survival difference was observed between BCS and reconstruction recipients (BCSS: HR=1.10; 95%CI=0.85-1.43; OS: 1.11; 0.86-1.44). Furthermore, patients undergoing BCS possessed similar BCSS with mastectomy recipients as well as reconstruction recipients in viscera metastases pattern, whereas non-surgery individuals had a worse survival (mastectomy: HR=1.04; 95%CI=0.92-1.18; reconstruction: 0.86; 0.69-1.06; non-surgery: 1.83; 1.63-2.05). Two competing risk nomograms of distinct metastatic patterns were established to comprehensively predict the survival of patients. Calibration

curves indicated the terrific consistency of the models. Moreover, the AUC values in the training and validation sets were in the range of 0.70–0.80, exhibiting good specificity and sensitivity.

Conclusion: The surgery implementation was associated with a lower probability of BCSD in *de novo* stage-IV BC patients. Our nomograms could offer a relatively accurate and individualized prediction of the cumulative incidence rate of BCSD after primary tumor resection.

Keywords: SEER, *de novo* stage-IV breast cancer, surgery, competing risk model, nomogram, metastatic pattern

INTRODUCTION

According to the most recent report from the International Agency for Research on Cancer (IARC), new cases of breast cancer (BC) rapidly grew to 2.26 million in 2020. Besides, it has officially overtaken lung cancer as the major component of malignant tumors worldwide and maintained the leading cause of cancer-related death in females (1, 2). Approximately 5-8% of BC patients exhibit distant metastases at initial diagnosis (3). In addition, stage-IV BC is considered to be incurable with a relatively short median OS despite tremendous advances in systemic therapeutics. In view of these unfavorable prognoses, the chief objective of treatment is to mitigate symptoms, improve the quality of life and ameliorate survival (4, 5). It is generally accepted that systemic therapeutics, including chemo, endocrine, and targeted therapy, are the fundamental and effective treatments for MBC (6). However, due to the lack of consensus, the essential role of primary tumor resection in MBC patients is still controversial.

A multitude of retrospective studies has demonstrated that surgical resection of primary tumors extended the life expectancy of MBC patients (7–12). Nevertheless, four prospective randomized trials showed contentious results (13–16). MF07-01 trial was the only trial that observed survival benefits from locoregional surgery, with a remarkable improvement of 5-year OS (13). However, no statistical differences were found between primary tumor surgery and prognosis in the other trials (NCT00193778, ABCSG-28 POSYTIVE, and ECOG ACRIN 2018) (14–16). The discrepancy in outcomes may be ascribed to different metastatic patterns (13, 17). Furthermore, we are awaiting the results of several well-designed prospective trials, which are still following-up.

In our study, we meticulously probed the effectiveness of locoregional surgery in different metastatic patterns among *de novo* stage-IV BC patients using data from the Surveillance, Epidemiology, and End Results (SEER) database. Moreover, two nomograms considering competitive events were established, making up for a few limitations of retrospective study and offering precise prediction of survival outcomes.

METHODS

Patient Selection

The following inclusion criteria were used: 1) primary BC and 2) stage IV BC diagnosed from 2010 to 2017.The exclusion criteria comprised of the following: 1) not diagnosed by histology, 2) with unknown metastatic status and surgery data, 3) diagnosed by autopsy and death certificate, 4) with incomplete survival data, 5) without complete clinicopathological data, and 6) male patients.

Of the 12281 individuals in our study, 4689 cases undergoing primary tumor surgery were subdivided into the BCS, mastectomy, and reconstruction groups. Additionally, age at diagnosis, year of diagnosis, race, marriage, grade, histology, tumor size, lymph nodes status, subtype, metastatic pattern, radiation, chemotherapy, surgery information, and survival data were obtained from the database. Detailed information is exhibited in **Figure 1**.

Ethics Statement

The SEER database was set up by the National Cancer Institute of America, which covers approximately 30% of the U.S. population. We signed an agreement to access the SEER research data for this study. In addition, as the database is publicly available, our study was exempt from the ethical board of The Second Affiliated Hospital of Xi'an Jiaotong University.

Endpoints

The follow-up period ended in November 2020 and the median follow-up time was 25 months (1-107 months). In addition, the primary endpoints of this study were BCSS and BCSD, which were defined as the interval from diagnosis to death due to BC. The subordinate outcome was OS, which referred to the interval from BC diagnosis to death of any cause.

Statistical Analysis

We employed descriptive statistics to analyze the clinicopathological characteristics. Age at diagnosis, year of diagnosis, race, marriage, grade, histology, tumor size, lymph nodes status, subtype, metastatic pattern, radiation, chemotherapy, surgery information, and survival data were selected as variables. We conducted a chi-squared test of these variables to compare the variations between distinct surgical procedures. Kaplan-Meier analysis and log-rank test were completed to plot curves and compare the divergency in OS and BCSS. Subgroup analyses of different metastatic patterns were performed using Cox proportional hazard model and competing risk model to explore the independent prognostic factors. Based on the filtered variables, we constructed two competing risk nomograms to predict the probability of 1-, 2-, and 3-year BCSD. In addition, calibration curves and AUC values were used to assess

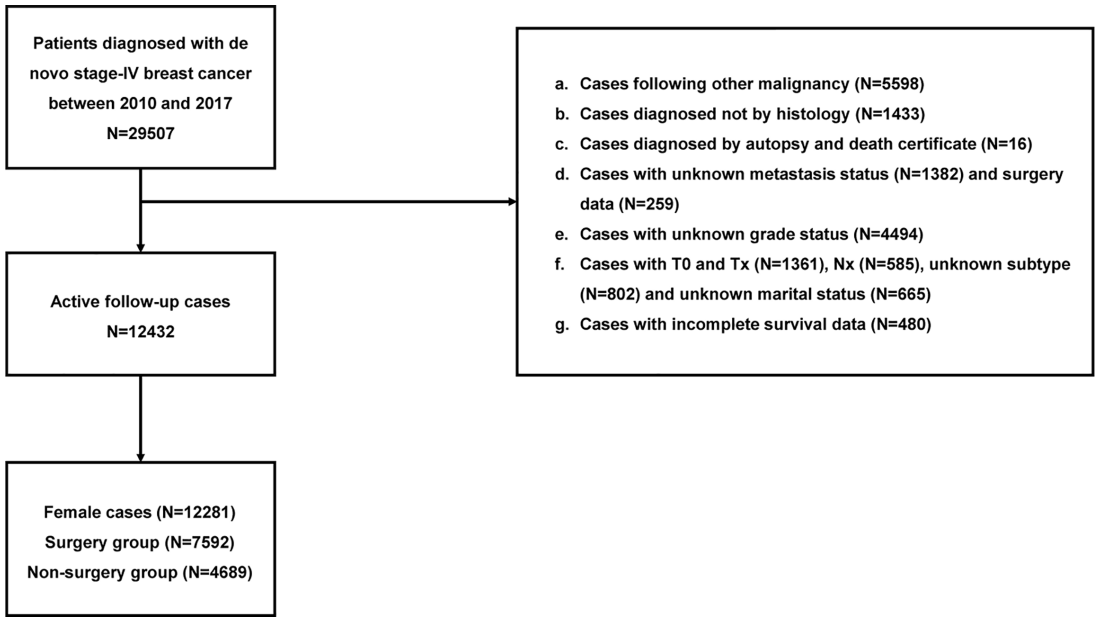


FIGURE 1 | Flow chart of patient selection.

the reliability of the model. All statistical assays were completed using R software 4.0.3. Statistical significance was defined as a two-sided P-value <0.05.

RESULTS

Clinicopathological Characteristics

A total of 12281 patients diagnosed with *de novo* stage-IV BC (2010–2017) were qualified for analyses. Of the 4689 (38.18%) individuals experiencing locoregional surgery, 1379, 2800, and 510 cases were subdivided into BCS, mastectomy, and reconstruction groups respectively, while the remaining 7592 patients (61.82%) avoided surgical interventions. Among these patients, 39.19% were 56-70 years old, 74.16% were white, 52.24% were in single status, 50.68% had poor differentiation (grade III-IV), 76.97% were invasive ductal carcinoma, 35.09% had T4 stage, 47.22% had N1 stage, 58.25% were luminal A subtype, 61.50% had viscera metastases, 63.55% did not receive radiotherapy, and 65.78% underwent chemotherapy. Significant differences were observed in age, race, marriage, grade, histology, T, N, subtype, metastatic status, radiotherapy, and chemotherapy between the four groups (**Table 1**).

Impact of Primary Tumor Surgery on Survival in *De Novo* MBC Patients

Kaplan-Meier curves showed that surgery improved both OS and BCSS (P<0.001; **Figure 2**). In addition, univariate Cox hazard proportional analysis revealed that age, race, marriage, grade, histology, T, N, subtype, metastatic status, radiotherapy, chemotherapy, and surgical procedures were related to survival (P<0.05; **Table 2**). Considering potential bias, we performed multivariate analysis and confirmed that primary tumor surgery was an independent protective factor for both BCSS (mastectomy: HR, 0.56; 95%CI, 0.52-0.59; BCS: HR, 0.47; 95% CI, 0.43-0.52; reconstruction: HR, 0.45; 95%CI, 0.39-0.52) and OS (mastectomy: HR, 0.56; 95%CI, 0.53-0.60; BCS: HR, 0.49; 95%CI, 0.44-0.53; reconstruction: HR, 0.43; 95%CI: 0.38-0.50).Furthermore, the metastatic pattern was also a crucial independent index that linked to prognosis (**Table 3**).

Subgroup Analyses of Metastatic Pattern

We then conducted subgroup analyses to explore selection strategies of surgical methods under different metastatic pattern circumstances. Results suggested that in bone-only metastasis pattern, in relation to BCS, patients receiving mastectomy had worse prognosis and the poorest survival belonged to non-surgery patients (BCSS: mastectomy: HR, 1.35; 95%CI, 1.15-1.60; non-surgery: HR, 2.42; 95%CI, 2.08-2.82; OS: mastectomy: HR, 1.44; 95%CI, 1.23-1.68; non-surgery: HR, 2.40; 95%CI, 2.08-2.78). Additionally, no survival difference was observed between BCS and reconstruction recipients (BCSS: HR, 1.10; 95%CI: 0.85-1.43; OS: HR, 1.11; 95%CI: 0.86-1.44). Furthermore, patients undergoing BCS had similar BCSS with mastectomy recipients together with reconstruction recipients in viscera metastasis pattern, whereas non-surgery individuals had a worse survival (mastectomy: HR, 1.04; 95%CI, 0.92-1.18; reconstruction: HR, 0.86; 95%CI, 0.69-1.06; non-surgery: HR, 1.83; 95%CI, 1.63-2.05). However, OS benefits were identified in reconstruction group compared with patients in BCS group (HR: 0.80; 95%CI: 0.65-0.99) (**Table 4**).

TABLE 1 | The baseline characteristics of *de novo* stage-IV BC patients with different surgical methods.

	Non-surgery N (%)	Mastectomy N (%)	Reconstruction N (%)	BCS N (%)	P
Age					<0.001
<=40	558 (7.35)	281 (10.04)	113 (22.16)	127 (9.21)	
41-55	1961 (25.83)	807 (28.82)	220 (43.14)	420 (30.46)	
56-70	3023 (39.82)	1086 (38.79)	149 (29.22)	555 (40.25)	
>70	2050 (27.00)	626 (22.36)	28 (5.49)	277 (20.09)	
Race					0.003
Black	1322 (17.41)	496 (17.71)	69 (13.53)	196 (14.21)	
White	5618 (74.00)	2029 (72.46)	391 (76.67)	1070 (77.59)	
Other	652 (8.59)	275 (9.82)	50 (9.80)	113 (8.19)	
Marriage					<0.001
Married	3405 (44.85)	1397 (49.89)	319 (62.55)	744 (53.95)	
Single	4187 (55.15)	1403 (50.11)	191 (37.45)	635 (46.05)	
Grade					<0.001
I-II	4099 (53.99)	1124 (40.14)	229 (44.90)	605 (43.87)	
III-IV	3493 (46.01)	1676 (59.86)	281 (55.10)	774 (56.13)	
Histology					<0.001
IDC	5837 (76.88)	2105 (75.18)	407 (79.80)	1104 (80.06)	
ILC	731 (9.63)	249 (8.89)	43 (8.43)	116 (8.41)	
Others	1024 (13.49)	446 (15.93)	60 (11.76)	159 (11.53)	
T					<0.001
T1	891 (11.74)	179 (6.39)	52 (10.20)	304 (22.04)	
T2	2466 (32.48)	862 (30.79)	208 (40.78)	710 (51.49)	
T3	1379 (18.16)	623 (22.25)	123 (24.12)	175 (12.69)	
T4	2856 (37.62)	1136 (40.57)	127 (24.90)	190 (13.78)	
N					<0.001
N0	1745 (22.98)	316 (11.29)	60 (11.76)	380 (27.56)	
N1	4019 (52.94)	1037 (37.04)	195 (38.24)	548 (39.74)	
N2	760 (10.01)	643 (22.96)	119 (23.33)	227 (16.46)	
N3	1068 (14.07)	804 (28.71)	136 (26.67)	224 (16.24)	
Subtype					<0.001
HER2-positive	678 (8.93)	312 (11.14)	49 (9.61)	128 (9.28)	
Luminal A	4597 (60.55)	1491 (53.25)	287 (56.27)	779 (56.49)	
Luminal B	1362 (17.94)	482 (17.21)	111 (21.76)	244 (17.69)	
Triple Negative	955 (12.58)	515 (18.39)	63 (12.35)	228 (16.53)	
Metastatic status					<0.001
Bone only	2800 (36.88)	1081 (38.61)	239 (46.86)	608 (44.09)	
Viscera	4792 (63.12)	1719 (61.39)	271 (53.14)	771 (55.91)	
Radiation					
No/Unknown	5393 (71.04)	1510 (53.93)	232 (45.49)	670 (48.59)	
Yes	2199 (28.96)	1290 (46.07)	278 (54.51)	709 (51.41)	
Chemotherapy					<0.001
No/Unknown	2993 (39.42)	705 (25.18)	82 (16.08)	422 (30.60)	
Yes	4599 (60.58)	2095 (74.82)	428 (83.92)	957 (69.40)	

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

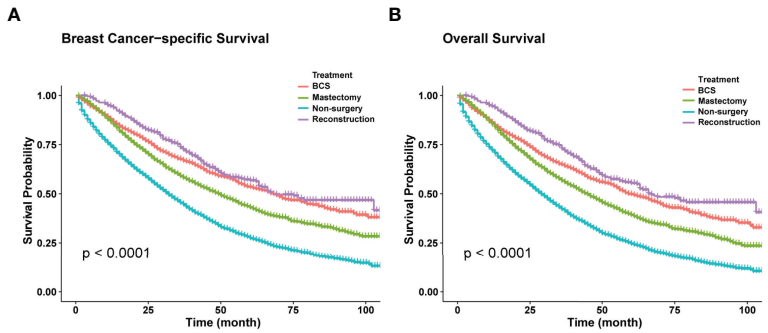


FIGURE 2 | **(A)** Kaplan-Meier curves of BCSS in different surgical methods. **(B)** Kaplan-Meier curves of OS in different surgical methods.

TABLE 2 | Univariate and multivariate Cox hazard proportional model analysis of *de novo* stage-IV BC patients.

	Univariate analysis						Multivariate analysis					
	BCSS			OS			BCSS			OS		
	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Age												
<=40		Reference			Reference			Reference			Reference	
41-55	1.20	1.09-1.33	<0.001	1.20	1.09-1.32	<0.001	1.14	1.03-1.27	0.010	1.13	1.03-1.25	0.013
56-70	1.41	1.28-1.55	<0.001	1.46	1.33-1.60	<0.001	1.25	1.13-1.38	<0.001	1.29	1.17-1.42	<0.001
>70	1.94	1.76-2.15	<0.001	2.17	1.97-2.39	<0.001	1.55	1.40-1.72	<0.001	1.70	1.53-1.88	<0.001
Race												
Black		Reference			Reference			Reference			Reference	
White	0.70	0.66-0.75	<0.001	0.71	0.67-0.75	<0.001	0.80	0.75-0.86	<0.001	0.80	0.75-0.84	<0.001
Other	0.62	0.56-0.69	<0.001	0.61	0.56-0.68	<0.001	0.72	0.65-0.80	<0.001	0.70	0.64-0.78	<0.001
Marital status												
Married		Reference			Reference			Reference			Reference	
Single	1.39	1.33-1.46	<0.001	1.43	1.37-1.50	<0.001	1.21	1.15-1.27	<0.001	1.23	1.17-1.29	<0.001
Grade												
I-II		Reference			Reference			Reference			Reference	
III-IV	1.44	1.37-1.51	<0.001	1.38	1.31-1.44	<0.001	1.46	1.38-1.54	<0.001	1.40	1.33-1.48	<0.001
Histology												
IDC		Reference			Reference			Reference			Reference	
ILC	0.97	0.89-1.06	0.523	0.97	0.89-1.05	0.462	1.21	1.10-1.32	<0.001	1.16	1.06-1.26	<0.001
Others	1.12	1.04-1.20	0.002	1.12	1.05-1.20	<0.001	1.12	1.05-1.20	0.001	1.12	1.05-1.20	<0.001
T												
T1		Reference			Reference			Reference			Reference	
T2	1.06	0.97-1.16	0.184	1.07	0.98-1.16	0.119	1.09	1.00-1.19	0.044	1.10	1.01-1.20	0.022
T3	1.24	1.13-1.37	<0.001	1.22	1.12-1.34	<0.001	1.19	1.08-1.31	<0.001	1.18	1.07-1.29	<0.001
T4	1.58	1.45-1.72	<0.001	1.57	1.44-1.70	<0.001	1.37	1.26-1.49	<0.001	1.35	1.24-1.47	<0.001
N												
N0		Reference			Reference						Reference	
N1	0.99	0.93-1.05	0.718	0.96	0.90-1.02	0.141				0.98	0.92-1.04	0.468
N2	0.95	0.87-1.03	0.218	0.91	0.84-0.99	0.024				1.05	0.97-1.15	0.217
N3	1.08	1.00-1.16	0.058	1.03	0.96-1.11	0.427				1.06	0.98-1.15	0.137
Subtype												
HER2 Positive		Reference			Reference			Reference			Reference	
Luminal A	1.10	1.01-1.21	0.032	1.10	1.01-1.19	0.030	1.09	0.99-1.20	0.074	1.04	0.95-1.14	0.365
Luminal B	0.80	0.72-0.89	<0.001	0.78	0.70-0.86	<0.001	0.82	0.74-0.91	<0.001	0.78	0.71-0.87	<0.001
Triple Negative	2.87	2.60-3.17	<0.001	2.70	2.46-2.97	<0.001	2.88	2.61-3.19	<0.001	2.68	2.44-2.95	<0.001
Metastatic status												
Bone only		Reference			Reference			Reference			Reference	
Viscera	1.47	1.40-1.55	<0.001	1.45	1.38-1.52	<0.001	1.34	1.27-1.42	<0.001	1.33	1.27-1.40	<0.001
Radiation												
No/Unknown		Reference			Reference			Reference			Reference	
Yes	0.89	0.84-0.93	<0.001	0.87	0.83-0.91	<0.001	1.11	1.06-1.17	<0.001	1.09	1.03-1.14	0.001
Chemotherapy												
No/Unknown		Reference			Reference			Reference			Reference	
Yes	0.72	0.69-0.76	<0.001	0.68	0.65-0.71	<0.001	0.68	0.64-0.72	<0.001	0.66	0.63-0.70	<0.001
Surgery												
Non-surgery		Reference			Reference			Reference			Reference	
BCS	0.48	0.44-0.53	<0.001	0.49	0.45-0.53	<0.001	0.47	0.43-0.52	<0.001	0.49	0.44-0.53	<0.001
Mastectomy	0.63	0.59-0.67	<0.001	0.63	0.60-0.67	<0.001	0.56	0.52-0.59	<0.001	0.56	0.53-0.60	<0.001
Reconstruction	0.41	0.35-0.47	<0.001	0.38	0.33-0.44	<0.001	0.45	0.39-0.52	<0.001	0.43	0.38-0.50	<0.001

HR, hazard ratio; CI, confidence interval.

Among the 12281 patients, 7241 (58.96%) died in this retrospective study. The cumulative incidence of breast cancer-specific death (BCSD) was 53.72% (6597/12281), while that of other cause-specific death was 5.24% (644/12281). Considering the potential bias caused by competing events, competing risk model (CRM) analyses were also performed. In the univariate analysis, BCS and reconstruction recipients had a relatively lower cumulative incidence rate of BCSD than those with mastectomy and non-surgery interventions, no matter in bone-only or viscera metastatic patterns (**Figure 3**; **Table 5**). Multivariate analyses demonstrated that ten variables (age, race, marriage, grade, histology, T, N, subtype, chemotherapy, and surgery) were still independent predictive indices in the bone-only metastatic pattern while nine (age, race, marriage, grade, T, subtype, brain metastases, chemotherapy, and surgery) in the viscera metastases pattern (**Table 6**).

TABLE 3 | Univariate analysis of different metastatic patterns.

	BCSS						OS					
	Bone-only			Viscera			Bone-only			Viscera		
	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Age												
<=40		Reference			Reference			Reference			Reference	
41-55	1.08	0.91-1.29	0.381	1.28	1.13-1.45	<0.001	1.11	0.93-1.31	0.243	1.26	1.11-1.42	<0.001
56-70	1.38	1.17-1.63	<0.001	1.44	1.28-1.62	<0.001	1.47	1.25-1.72	<0.001	1.47	1.31-1.65	<0.001
>70	1.97	1.66-2.34	<0.001	1.95	1.72-2.21	<0.001	2.25	1.91-2.66	<0.001	2.15	1.91-2.43	<0.001
Race												
Black		Reference			Reference			Reference			Reference	
White	0.66	0.59-0.74	<0.001	0.75	0.70-0.81	<0.001	0.66	0.60-0.73	<0.001	0.75	0.70-0.81	<0.001
Other	0.62	0.52-0.75	<0.001	0.62	0.55-0.70	<0.001	0.60	0.50-0.71	<0.001	0.62	0.55-0.70	<0.001
Marital status												
Married		Reference			Reference			Reference			Reference	
Single	1.35	1.24-1.46	<0.001	1.41	1.33-1.50	<0.001	1.42	1.31-1.54	<0.001	1.44	1.36-1.52	<0.001
Grade												
I-II		Reference			Reference			Reference			Reference	
III-IV	1.42	1.31-1.55	<0.001	1.32	1.24-1.40	<0.001	1.36	1.25-1.47	<0.001	1.27	1.20-1.35	<0.001
Histology												
IDC		Reference			Reference			Reference			Reference	
ILC	1.22	1.09-1.37	<0.001	0.94	0.82-1.08	0.376	1.19	1.07-1.33	0.002	0.95	0.84-1.08	0.440
Others	1.18	1.05-1.33	0.006	1.11	1.02-1.21	0.015	1.17	1.04-1.31	0.008	1.12	1.04-1.22	0.005
T												
T1		Reference			Reference			Reference			Reference	
T2	1.05	0.91-1.20	0.538	1.08	0.97-1.21	0.178	1.09	0.95-1.24	0.223	1.07	0.96-1.18	0.245
T3	1.29	1.11-1.50	<0.001	1.20	1.07-1.36	0.002	1.28	1.10-1.48	0.001	1.18	1.05-1.32	0.005
T4	1.55	1.34-1.78	<0.001	1.50	1.35-1.67	<0.001	1.57	1.37-1.80	<0.001	1.46	1.32-1.62	<0.001
N												
N0		Reference			Reference			Reference			Reference	
N1	0.98	0.88-1.10	0.764	0.95	0.87-1.03	0.176	0.95	0.86-1.06	0.361	0.91	0.85-0.99	0.022
N2	0.97	0.84-1.12	0.697	0.90	0.81-1.00	0.046	0.93	0.81-1.06	0.272	0.87	0.79-0.96	0.006
N3	1.19	1.04-1.35	0.009	0.96	0.88-1.06	0.453	1.12	0.99-1.27	0.069	0.93	0.85-1.02	0.114
Subtype												
HER2-positive		Reference			Reference			Reference			Reference	
Luminal A	1.58	1.23-2.03	<0.001	1.19	1.08-1.31	<0.001	1.55	1.23-1.96	<0.001	1.18	1.08-1.30	<0.001
Luminal B	1.14	0.87-1.50	0.348	0.80	0.71-0.89	<0.001	1.08	0.83-1.39	0.568	0.77	0.69-0.87	<0.001
Triple Negative	4.44	3.38-5.82	<0.001	2.63	2.37-2.93	<0.001	4.18	3.23-5.40	<0.001	2.48	2.24-2.75	<0.001
Brain												
No					Reference						Reference	
Yes				2.04	1.87-2.23	<0.001				1.99	1.92-2.17	<0.001
Radiation												
No/Unknown		Reference			Reference			Reference			Reference	
Yes	0.92	0.85-1.00	0.056	0.92	0.86-0.98	0.011	0.89	0.82-0.96	0.005	0.90	0.85-0.96	0.001
Chemotherapy												
No/Unknown		Reference			Reference			Reference			Reference	
Yes	0.71	0.65-0.77	<0.001	0.65	0.61-0.69	<0.001	0.67	0.62-0.73	<0.001	0.61	0.58-0.65	<0.001
Surgery												
BCS		Reference			Reference			Reference			Reference	
Mastectomy	1.54	1.31-1.81	<0.001	1.16	1.02-1.31	0.019	1.53	1.31-1.79	<0.001	1.14	1.02-1.28	0.027
Reconstruction	0.98	0.75-1.27	0.861	0.77	0.62-0.96	0.019	0.91	0.71-1.18	0.478	0.71	0.57-0.87	0.001
Non-surgery	2.33	2.01-2.70	<0.001	1.88	1.68-2.09	<0.001	2.34	2.03-2.69	<0.001	1.83	1.65-2.03	<0.001

Construction of the Nomogram Using CRM

According to the 7:3 ratio, we assigned the patients into the training and validation sets, respectively. The cutoff value was set in light of a CRM-related literature (18). Based on the screened variables, two nomograms considering metastatic patterns were developed to make precise predictions of 1-, 2- and 3-year BCSD. The probability of BCSD at these intervals can be estimated by the scale corresponding to the total score (**Figure 4**). Using the nomograms, we could prognosticate the BCSD of a given patient (bone-only metastases: 1-year=12.60%, 2-year=26.20%, and 3-year=41.60%; viscera metastases: 1-year=18.40%, 2-year=34.90%, 3-year=47.80%). Moreover, 30% of patients in the entire cohort were pitched on for internal validation. The calibration curves revealed high coherence between the nomogram-predicted and actual BCSD after one, two, and three years (**Figure 5**). The AUC values were in a range of 0.70-0.80 in both the training (bone-only metastases: 1-year=0.76, 2-year=0.75, 3-year=0.74; viscera metastases: 1-year=0.75, 2-year=0.76, 3-year=0.75) and validation sets (bone-only metastases: 1-year=0.74,

TABLE 4 | Multivariate analysis for independent predictive factors of different metastatic patterns.

	BCSS						OS					
	Bone-only			Viscera			Bone-only			Viscera		
	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Age												
<=40		Reference			Reference			Reference			Reference	
41-55	0.97	0.81-1.15	0.700	1.22	1.08-1.39	0.002	0.98	0.83-1.17	0.840	1.19	1.06-1.35	0.004
56-70	1.12	0.95-1.33	0.183	1.30	1.15-1.47	<0.001	1.19	1.00-1.40	0.046	1.32	1.17-1.49	<0.001
>70	1.47	1.23-1.76	<0.001	1.61	1.41-1.83	<0.001	1.67	1.40-1.98	<0.001	1.74	1.53-1.97	<0.001
Race												
Black		Reference			Reference			Reference			Reference	
White	0.77	0.69-0.86	<0.001	0.82	0.76-0.88	<0.001	0.75	0.68-0.84	<0.001	0.81	0.75-0.87	<0.001
Other	0.75	0.62-0.91	0.003	0.71	0.63-0.81	<0.001	0.71	0.59-0.85	<0.001	0.70	0.62-0.79	<0.001
Marital status												
Married		Reference			Reference			Reference			Reference	
Single	1.15	1.05-1.25	0.002	1.24	1.16-1.32	<0.001	1.18	1.09-1.29	<0.001	1.25	1.18-1.32	<0.001
Grade												
I-II		Reference			Reference			Reference			Reference	
III-IV	1.52	1.39-1.67	<0.001	1.39	1.30-1.49	<0.001	1.49	1.36-1.63	<0.001	1.36	1.27-1.45	<0.001
Histology												
IDC		Reference			Reference			Reference			Reference	
ILC	1.25	1.11-1.41	<0.001	1.09	0.95-1.25	0.209	1.24	1.10-1.39	<0.001	1.07	0.94-1.22	0.328
Others	1.16	1.03-1.31	0.014	1.09	1.00-1.19	0.041	1.15	1.02-1.29	0.019	1.11	1.02-1.20	0.017
T												
T1		Reference			Reference			Reference			Reference	
T2	1.08	0.93-1.24	0.307	1.11	0.99-1.24	0.073	1.12	0.98-1.28	0.088	1.10	0.99-1.22	0.078
T3	1.20	1.03-1.41	0.021	1.15	1.02-1.30	0.024	1.24	1.07-1.43	0.005	1.15	1.02-1.29	0.021
T4	1.31	1.13-1.52	<0.001	1.38	1.23-1.54	<0.001	1.38	1.20-1.59	<0.001	1.36	1.23-1.52	<0.001
N												
N0		Reference			Reference						Reference	
N1	1.02	0.92-1.14	0.711	0.99	0.91-1.07	0.788				0.97	0.89-1.04	0.382
N2	1.19	1.02-1.37	0.023	1.05	0.94-1.17	0.408				1.02	0.92-1.14	0.648
N3	1.37	1.20-1.58	<0.001	0.99	0.90-1.10	0.920				0.98	0.89-1.07	0.630
Subtype												
HER2-positive		Reference			Reference			Reference			Reference	
Luminal A	1.56	1.19-1.99	0.001	1.04	0.94-1.16	0.418	1.41	1.11-1.80	0.005	1.00	0.90-1.11	0.996
Luminal B	1.19	0.89-1.54	0.210	0.76	0.68-0.86	<0.001	1.07	0.83-1.38	0.610	0.73	0.66-0.82	<0.001
Triple Negative	4.60	3.49-6.06	<0.001	2.59	2.32-2.88	<0.001	4.03	3.11-5.22	<0.001	2.40	2.16-2.66	<0.001
Brain												
No					Reference						Reference	
Yes				1.91	1.73-2.10	<0.001				1.89	1.72-2.08	<0.001
Radiation												
No/Unknown					Reference			Reference			Reference	
Yes				0.96	0.90-1.03	0.280	1.11	1.02-1.20	0.018	0.95	0.89-1.02	0.154
Chemotherapy												
No/Unknown		Reference			Reference			Reference			Reference	
Yes	0.74	0.67-0.81	<0.001	0.67	0.62-0.72	<0.001	0.73	0.67-0.80	<0.001	0.64	0.60-0.69	<0.001
Surgery												
BCS		Reference			Reference			Reference			Reference	
Mastectomy	1.35	1.15-1.60	<0.001	1.04	0.92-1.18	0.507	1.44	1.23-1.68	<0.001	1.03	0.92-1.16	0.600
Reconstruction	1.10	0.85-1.43	0.519	0.86	0.69-1.06	0.154	1.11	0.86-1.44	0.406	0.80	0.65-0.99	0.040
Non-surgery	2.42	2.08-2.82	<0.001	1.83	1.63-2.05	<0.001	2.40	2.08-2.78	<0.001	1.76	1.58-1.96	<0.001

2-year=0.73, 3-year=0.70; viscera metastases: 1-year=0.74, 2-year=0.75, 3-year=0.75) (**Figure 6**).

DISCUSSION

Using the data from the SEER database, we constructed a Cox proportional hazard model and a competing risk model in 12281 patients diagnosed with *de novo* stage-IV BC from 2010 to 2017. Based on the variables filtered by multivariate analysis of CRM, which is widely employed in the study of oncology (19, 20), two nomograms considering metastatic patterns were constructed to predict the probability of BCSD at 1-, 2-, and 3-year intervals. As we know, this is the first large-scale SEER-based study to predict the impact of various surgical methods on survival under different metastatic patterns using competing risk analyses.

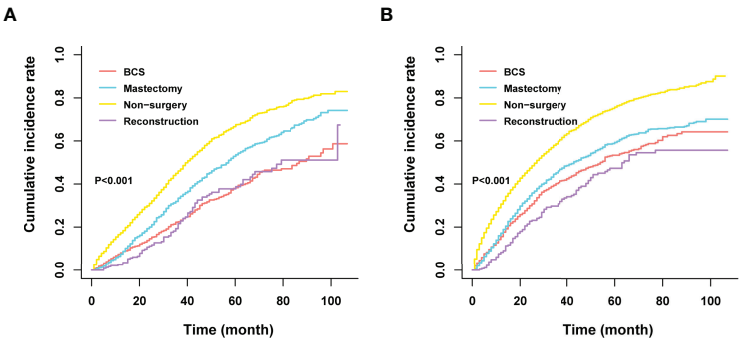


FIGURE 3 | **(A)** Cumulative incidence rate of BCSD in the bone-only metastatic pattern. **(B)** Cumulative incidence rate of BCSD in the viscera metastatic pattern.

Systemic therapy is generally considered the primary treatment for patients with MBC, while locoregional therapy such as surgery is implemented to control localized symptoms such as pain and bleeding (5, 6). To date, the influence of locoregional surgery on survival has not been determined yet. In this study, Kaplan-Meier curves revealed that primary tumor resection was associated with better BCSS and OS (P<0.001). As was illustrated in our study, the median survival time of the surgery group (BCSS, 56 months; 95% CI=53–59 months; OS, 50 months; 95% CI=48–53 months) was almost 1.8-fold that of the non-surgery group (BCSS=32 months, 95% CI=31-33 months; OS=29 months, 95% CI=28–30 months). Considering potential selection bias, univariate and multivariate Cox analyses were conducted, and the results (hazard ratio, HR) summarized the risk and protective indices of survival. As shown in **Table 2**, surgery played a pivotal role in improving both BCSS and OS. Similar results were observed in several retrospective studies (7–12). The most recent research based on SEER database exhibited striking improvements in OS (before: HR, 0.57; 95%CI: 0.54-0.61; P<0.001; after: HR, 0.56; 95%CI, 0.51-0.60; P<0.001) and BCSS (before: HR, 0.56; 95%CI, 0.52-0.59; P<0.001; after: HR, 0.52; 95%CI, 0.50-0.59; P<0.001) through surgical intervention among *de novo* stage-IV patients whether before or after propensity score matching (PSM) (12). Additionally, one large-scale NCDB-based study also witnessed a survival benefit in surgery recipients with stage-IV BC. Also, OS was remarkably prolonged in the surgery group after PSM (HR, 0.68; 95%CI, 0.63-0.72; P<0.001) (11). This could be explained by the primary tumor-induced immunosuppression. The removal of lesions promoted the recovery of immunological function, preventing distant dissemination of the tumor and dislodging potential chemo-resistant cells, which led to better survival (21, 22). However, a few studies have suggested that surgical benefits were due to confounding factors caused by the design of retrospective studies (23, 24).

Despite the support from many retrospective studies, definite evidence from prospective studies is still lacking. Four prospective randomized trials observed controversial results (13–16). MF07-01 was the only trial that demonstrated survival benefits from locoregional surgery, with a remarkable improvement of 5-year OS (HR, 0.66; 95% CI, 0.49-0.88; P=0.005), while no survival advantage was found in 3-year OS (13). The ABCSG-28 POSYTIVE trial (2010–2015) and NCT00193778 (2005–2013) trial were prospective randomized trials enrolling 90 and 350 untreated patients MBC patients respectively to evaluate the impact of primary tumor surgery on OS. Patients were randomly assigned to group A (surgery following systemic treatment) and group B (systemic treatment only). Neither trial showed statistical differences in survival between the two groups (P=0.267 and P=0.790, respectively). In the former trial, the primary tumor load and lymph node metastases in group A were more serious than those in group B. In the latter, only 2% HER2 positive patients received targeted therapy, and only a minority of patients used paclitaxel during early rescue chemotherapy (14, 15). These reasons may account for the discrepancy in the outcomes. ECOG ACRIN 2018 trial revealed that primary tumor treatment notably decreased locoregional progression rate, but OS and overall quality of life were similar in patients with or without surgery (16). The results of this study aroused wide concern because only 80% of surgery recipients attained clear margins. Moreover, subgroup analyses of the metastatic patterns were not carried out.

Previous studies have indicated that biological characteristics and prognoses may vary in distinct metastatic patterns (25–27). For further analysis, we divided metastatic patterns into bone-only and viscera metastases. In our study, the survival time of bone-only metastases patients was longer than that of viscera metastases individuals, with a median BCSS of 48 months (95%CI=46-50) in the former while 33 months (95%CI=31-34) in the latter. Additionally, patients with luminal A subtype are more likely to have bone metastases (luminal A: 48.10%, luminal B: 32.74%, HER2-positive: 16.60%, triple negative: 21.18%), whereas HER2-positive and triple negative BC had a higher proportion of viscera metastasis (luminal A: 51.90%, luminal B: 67.26%, HER2-positive: 83.23%, triple negative: 78.82%), which were consistent with previous studies (28–30). To further explore the role of different surgical methods on prognosis and remove the bias from other

TABLE 5 | Univariate competing risk analysis of different metastatic patterns.

	Univariate analysis (BCSD) (%)							
	Bone-only				Viscera			
	1-year	2-year	3-year	P	1-year	2-year	3-year	P
Age				<0.001				<0.001
<=40	6.55	15.66	26.89		13.04	30.89	41.18	
41-55	7.55	18.41	31.58		20.22	36.69	48.90	
56-70	11.71	25.11	38.37		24.86	40.80	51.95	
>70	23.39	35.02	47.25		34.81	49.37	59.60	
Race				<0.001				<0.001
Black	18.08	33.61	48.89		30.19	49.95	61.40	
White	12.45	23.49	35.78		24.34	39.60	50.60	
Other	8.42	22.12	34.98		19.20	32.72	44.40	
Marriage				<0.001				<0.001
Married	9.37	20.33	32.91		19.63	34.52	45.95	
Single	16.40	29.20	42.22		29.64	46.48	57.41	
Grade				<0.001				<0.001
I-II	10.69	20.19	31.76		20.55	33.88	44.97	
III-IV	16.56	32.20	46.78		28.08	45.85	57.04	
Histology				<0.001				0.085
IDC	12.67	24.34	36.64		24.53	40.54	51.77	
ILC	13.79	24.82	40.40		22.32	38.17	48.75	
Other	13.58	27.71	40.17		28.38	43.77	54.71	
T				<0.001				<0.001
T1	13.18	22.05	33.95		21.21	34.48	45.69	
T2	9.85	21.10	31.60		20.71	35.93	47.04	
T3	11.57	25.06	39.81		23.68	40.19	50.59	
T4	18.45	31.64	46.74		29.79	46.69	58.25	
N				0.005				0.498
N0	15.95	25.48	37.03		28.27	42.33	53.45	
N1	12.35	23.86	35.98		24.21	40.09	52.15	
N2	9.18	24.78	38.71		22.42	37.97	48.12	
N3	13.79	27.04	42.41		25.38	43.43	53.15	
Subtype				<0.001				<0.001
HER2-positive	13.65	22.16	29.09		21.05	34.63	44.44	
Luminal A	10.60	22.03	35.84		22.30	36.93	49.20	
Luminal B	11.00	19.20	30.46		16.15	26.35	37.50	
Triple Negative	38.17	63.25	72.77		44.01	71.07	80.21	
Brain								<0.001
No					22.65	38.43	49.83	
Yes					45.86	63.15	72.21	
Radiation				0.173				0.056
No/Unknown	13.87	25.74	38.80		25.50	41.91	52.61	
Yes	11.76	23.70	36.17		23.76	38.70	50.78	
Chemotherapy				<0.001				<0.001
No/Unknown	17.40	29.32	42.11		36.15	49.42	58.68	
Yes	9.76	21.63	34.36		20.28	37.30	49.24	
Surgery				<0.001				<0.001
BCS	7.92	14.42	22.17		15.39	29.30	39.50	
Mastectomy	7.35	19.74	32.78		17.05	34.12	45.14	
Reconstruction	2.52	11.14	21.55		8.21	22.73	30.74	
Non-surgery	17.13	30.34	44.59		30.26	46.21	57.89	

cause-specific death, subgroup analyses of the Cox model and competing risk model regarding metastatic patterns were performed. Results demonstrated that mastectomy showed an inferior prognosis to BCS in bone-only metastases patients, whereas the worst survival belonged to non-surgery individuals. Meanwhile, no survival difference was observed between BCS and reconstruction recipients. Furthermore, patients undergoing BCS possessed similar BCSS with mastectomy and reconstruction recipients in viscera metastases pattern, while non-surgery individuals had the poorer survival. One SEER-based research had similar results, but our study was more detailed in that we subdivided mastectomy into simple mastectomy and reconstruction after mastectomy (31). We conjectured that on the premise of ensuring the safety of tumor treatments, breast reconstruction and breast-conserving surgery increase the beauty and integrity, which bring confidence and self-acceptance to patients, so as to promote the patients' physical and

TABLE 6 | Multivariate analysis of BCSD using competing risk model.

	Multivariate analysis (BCSD)					
	Bone-only			Viscera		
	HR	95%CI	P	HR	95%CI	P
Age						
<=40		Reference			Reference	
41-55	1.00	0.86-1.18	0.962	1.26	1.12-1.41	<0.001
56-70	1.13	0.97-1.32	0.122	1.30	1.17-1.46	
>70	1.39	1.18-1.64	<0.001	1.47	1.30-1.65	
Race						
Black		Reference			Reference	
White	0.81	0.73-0.91	<0.001	0.85	0.79-0.91	<0.001
Other	0.81	0.67-0.97	0.021	0.75	0.66-0.84	<0.001
Marriage						
Married		Reference			Reference	
Single	1.10	1.01-1.19	0.031	1.22	1.15-1.29	<0.001
Grade						
I-II		Reference			Reference	
III-IV	1.50	1.37-1.64	<0.001	1.35	1.27-1.44	<0.001
Histology						
IDC		Reference			Reference	
ILC	1.27	1.13-1.43	<0.001			
Other	1.14	1.02-1.28	0.021			
T						
T1		Reference			Reference	
T2	1.04	0.90-1.20	0.567	1.08	0.97-1.21	0.143
T3	1.20	1.03-1.41	0.022	1.13	1.01-1.27	0.035
T4	1.28	1.10-1.49	0.002	1.34	1.20-1.49	<0.001
N						
N0		Reference			Reference	
N1	1.04	0.93-1.16	0.479			
N2	1.20	1.04-1.39	0.014			
N3	1.38	1.20-1.58	<0.001			
Subtype						
HER2-positive		Reference			Reference	
Luminal A	1.53	1.16-2.01	0.002	1.10	0.99-1.21	0.080
Luminal B	1.21	0.90-1.61	0.205	0.81	0.72-0.91	<0.001
Triple Negative	3.99	2.96-5.36	<0.001	2.51	2.26-2.79	<0.001
Brain						
No					Reference	
Yes				1.78	1.62-1.95	<0.001
Chemotherapy						
No/Unknown		Reference			Reference	
Yes	0.77	0.70-0.84	<0.001	0.72	0.67-0.77	<0.001
Surgery						
BCS		Reference			Reference	
Mastectomy	1.34	1.14-1.58	<0.001	1.07	0.94-1.20	0.303
Reconstruction	1.09	0.85-1.39	0.519	0.89	0.73-1.09	0.255
Non-surgery	2.20	1.89-2.56	<0.001	1.75	1.57-1.95	<0.001

psychological recovery, and consequently ameliorate survival. However, in sufferers with viscera metastases, BCS, reconstruction, and mastectomy had similar survival outcomes. We presume that due to the poor prognosis of viscera metastases patients, the survival advantages of BCS and reconstruction were attenuated.

Based on the independent predictive variables (bone-only metastases: age, race, marriage, grade, histology, T, N, subtype, chemotherapy, and surgery; viscera: age, race, marriage, grade, T, subtype, brain metastases, chemotherapy, and surgery) screened by multivariate competing risk analyses, two nomograms integrating demography, clinicopathology, and treatment information were constructed to accurately predict 1-, 2-, 3-year BCSD among stage-IV BC patients. Results of the model evaluation showed that fabulous consistency was witnessed between nomogram-predicted and actual BCSD in calibration curves. In addition, discrimination was assessed by AUC values, and the results reflected the fine sensitivity and specificity of the model. Recently, certain nomograms have been developed to predict the impact of locoregional surgery on survival among stage-IV BC patients. However, there existed a few evident limitations. For one hand, these models did not clearly

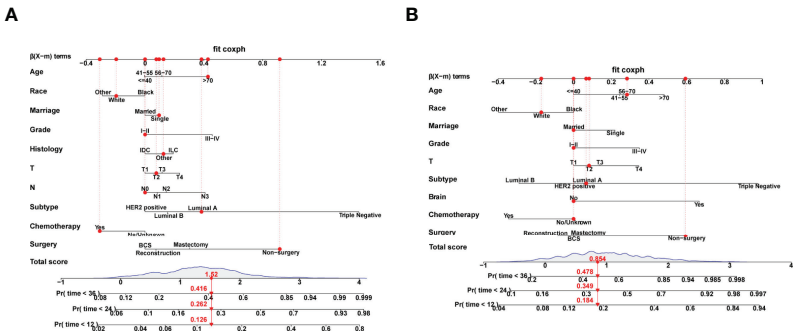


FIGURE 4 | **(A)** Competing risk nomogram in the bone-only metastatic pattern. **(B)** Competing risk nomogram in the viscera pattern.

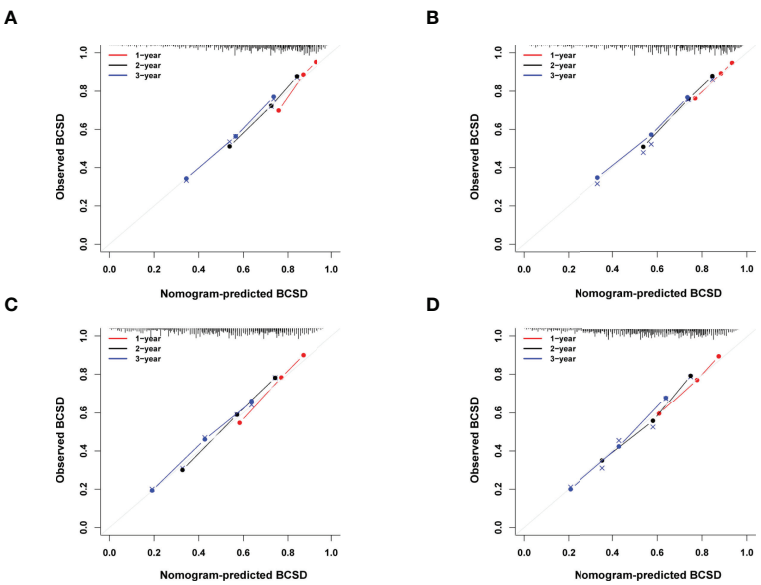


FIGURE 5 | **(A)** Calibration curves for predicting BCSD at 1-, 2- and 3-year intervals in the training set in bone-only metastatic pattern. **(B)** Calibration curves for predicting BCSD at 1-,2-and 3-year intervals in the validation set in bone-only metastatic pattern. **(C)** Calibration curves for predicting BCSD at 1-, 2- and 3-year intervals in the training set in viscera metastatic pattern. **(D)** Calibration curves for predicting BCSD at 1-, 2- and 3-year intervals in the validation set in viscera metastatic pattern.

discriminate different metastatic patterns, which were confirmed to be critical in survival outcomes. For another, they failed to take into account the influence of competitive events, as a result, they might overestimate the value of primary tumor surgery in stage IV BC individuals (32, 33).

This study also had several limitations that needed to be explained. First, there was no endocrine and targeted therapy in the SEER database. Second, the database lacked basic clinical data for patients, such as information on heart, lung, liver, and kidney function, as well as specific information on the metastatic focus, which might have significant impacts on treatment decisions and prognosis. Additionally, as the number of patients with *de novo* MBC was relatively small, the competitive risk model lacked effective external validation. Lastly, it was difficult to completely eliminate the bias using existing statistical methods due to the nature of retrospective cohort studies.

CONCLUSION

The primary tumor surgery was associated with a lower probability of BCSD in patients with *de novo* MBC. The nomograms could offer a relatively accurate prediction of the cumulative incidence of BCSD among patients with *de novo*

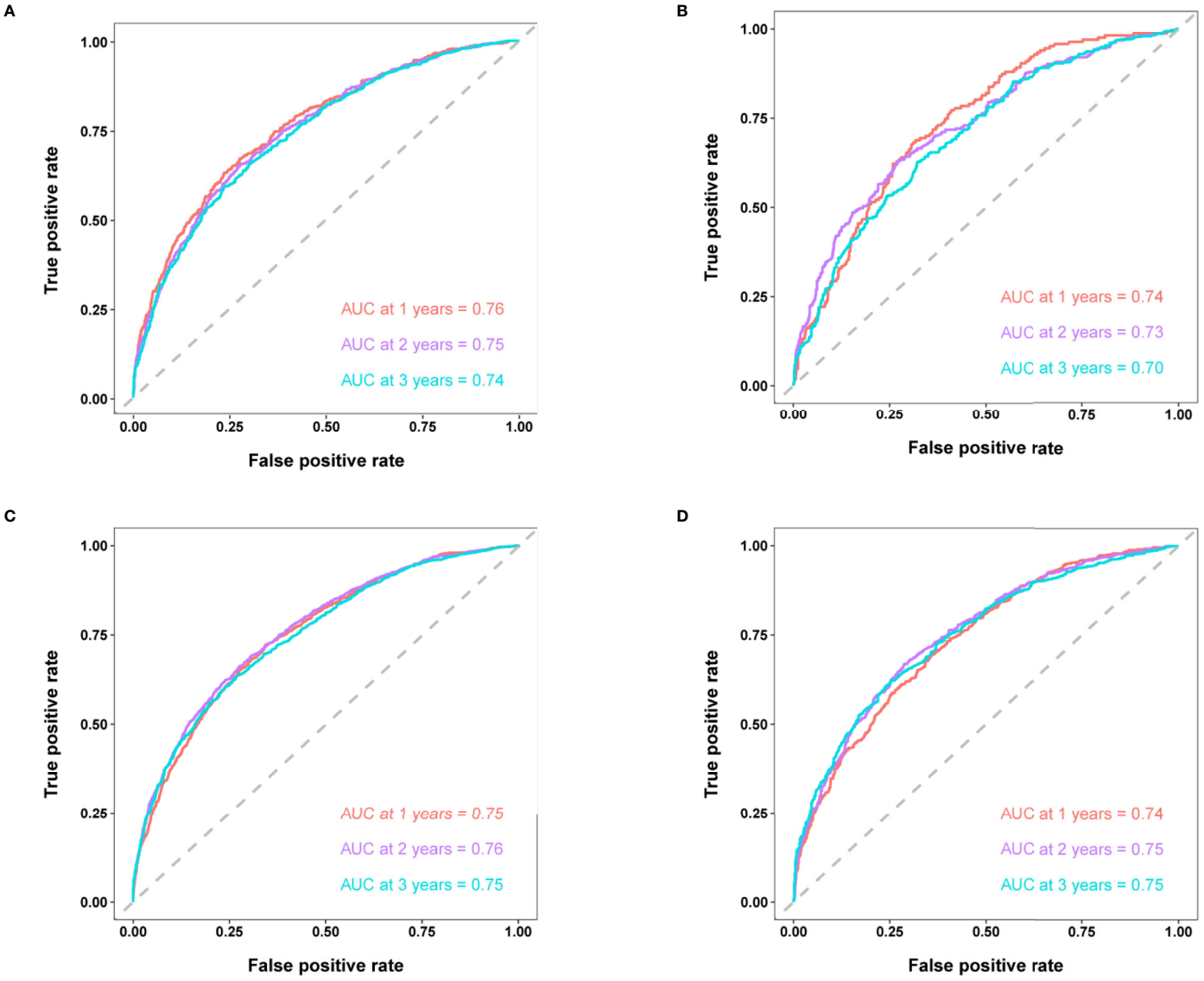


FIGURE 6 | **(A)** Time-dependent ROC curves of BCSD at 1-,2- and 3-year intervals in the training set in bone-only metastatic pattern. **(B)** Time-dependent ROC curves of BCSD at 1-,2- and 3-year intervals in the validation set in bone-only metastatic pattern. **(C)** Time-dependent ROC curves of BCSD at 1-,2- and 3-year intervals in the training set in viscera metastatic pattern. **(D)** Time-dependent ROC curves of BCSD at 1-,2- and 3-year intervals in the validation set in viscera metastatic pattern.

MBC. This will have a great significance in guiding the patients' decisions regarding personalized precision treatment. Finally, we hope that external verification based on Chinese patients can be realized in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

HK, ZZ, and XM designed the study and supervised the completion. HC contributed to data collection, data analysis and manuscript writing. LD, YB, and LH reviewed the background knowledge. MW, SL, and HW edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the National Natural Science Foundation of China (No. 82103129), Basic Research Program of Natural Science Foundation of Shaanxi Province (No. 2021JQ-422) (No. 2022JM-101), Prior Science and Technology Program for Overseas Chinese Talents of Shaanxi Province (No. 2020-015), and International Science and Technology Cooperation Program Project of Shaanxi Province (No. 2019KW-077) (No. 2022KW-01).

ACKNOWLEDGMENTS

We are grateful for the Surveillance, Epidemiology, and End Results database and all our colleagues in Department of Oncology, The Second Affiliated Hospital of Xi'an Jiaotong University.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.819531/full#supplementary-material

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660
2. Li N, Deng Y, Zhou L, Tian T, Yang S, Wu Y, et al. Global Burden of Breast Cancer and Attributable Risk Factors in 195 Countries and Territories, From 1990 to 2017: Results From the Global Burden of Disease Study 2017. *J Hematol Oncol* (2019) 12(1):e140. doi: 10.1186/s13045-019-0828-0
3. DeSantis CE, Fedewa SA, Goding Sauer A, Kramer JL, Smith RA, Jemal A. Breast Cancer Statistics, 2015: Convergence of Incidence Rates Between Black and White Women. *CA Cancer J Clin* (2016) 66(1):31–42. doi: 10.3322/caac.21320
4. Sánchez-Muñoz A, Pérez-Ruiz E, Ribelles N, Márquez A, Alba E. Maintenance Treatment in Metastatic Breast Cancer. *Expert Rev Anticancer Ther* (2008) 8(12):1907–12. doi: 10.1586/14737140.8.12.1907
5. Si Y, Yuan P, Hu N, Wang X, Ju J, Wang J, et al. Primary Tumor Surgery for Patients With *De Novo* Stage IV Breast Cancer can Decrease Local Symptoms and Improve Quality of Life. *Ann Surg Oncol* (2020) 27(4):1025–33. doi: 10.1245/s10434-019-08092-2
6. Lane WO, Thomas SM, Blitzblau RC, Plichta JK, Rosenberger LH, Fayanju OM, et al. Surgical Resection of the Primary Tumor in Women With *De Novo* Stage IV Breast Cancer: Contemporary Practice Patterns and Survival Analysis. *Ann Surg* (2019) 269(3):537–44. doi: 10.1097/sla.0000000000002621
7. Warschkow R, Güller U, Tarantino I, Cerny T, Schmied BM, Thuerlimann B, et al. Improved Survival After Primary Tumor Surgery in Metastatic Breast Cancer: A Propensity-Adjusted, Population-Based SEER Trend Analysis. *Ann Surg* (2016) 263(6):1188–98. doi: 10.1097/sla.0000000000001302
8. Ruiterkamp J, Ernst MF. The Role of Surgery in Metastatic Breast Cancer. *Eur J Cancer* (2011) 47(Suppl 3):S6–22. doi: 10.1016/s0959-8049(11)70142-3
9. Ma L, Mi Y, Cui S, Wang H, Fu P, Yin Y, et al. Role of Locoregional Surgery in Patients With *De Novo* Stage IV Breast Cancer: Analysis of Real-World Data From China. *Sci Rep* (2020) 10(1):e18132. doi: 10.1038/s41598-020-75119-0
10. Thomas A, Khan SA, Chrischilles EA, Schroeder MC. Initial Surgery and Survival in Stage IV Breast Cancer in the United States, 1988-2011. *JAMA Surg* (2016) 151(5):424–31. doi: 10.1001/jamasurg.2015.4539
11. Arciero C, Liu Y, Gillespie T, Subhedar P. Surgery and Survival in Patients With Stage IV Breast Cancer. *Breast J* (2019) 25(4):644–53. doi: 10.1111/tbj.13296
12. Lin Y, Huang K, Zeng Q, Zhang J, Song C. Impact of Breast Surgery on Survival of Patients With Stage IV Breast Cancer: A SEER Population-Based Propensity Score Matching Analysis. *PeerJ* (2020) 8:e8694. doi: 10.7717/peerj.8694
13. Soran A, Ozmen V, Ozbas S, Karanlik H, Muslumanoglu M, Igci A, et al. Randomized Trial Comparing Resection of Primary Tumor With No Surgery in Stage IV Breast Cancer at Presentation: Protocol MF07-01. *Ann Surg Oncol* (2018) 25(11):3141–9. doi: 10.1245/s10434-018-6494-6
14. Badwe R, Hawaldar R, Nair N, Kaushik R, Parmar V, Siddique S, et al. Locoregional Treatment Versus No Treatment of the Primary Tumour in Metastatic Breast Cancer: An Open-Label Randomised Controlled Trial. *Lancet Oncol* (2015) 16(13):1380–8. doi: 10.1016/s1470-2045(15)00135-7
15. Fitzal F, Bjelic-Radisic V, Knauer M, Steger G, Hubalek M, Balic M, et al. Impact of Breast Surgery in Primary Metastasized Breast Cancer: Outcomes of the Prospective Randomized Phase III ABCSG-28 POSYTIVE Trial. *Ann Surg* (2019) 269(6):1163–9. doi: 10.1097/sla.0000000000002771
16. Khan SA, Zhao F, Goldstein LJ, Cella D, Basik M, Golshan M, et al. Early Local Therapy for the Primary Site in *De Novo* Stage IV Breast Cancer: Results of a Randomized Clinical Trial (EA2108). *J Clin Oncol* (2022) 40(9):978–87. doi: 10.1200/jco.21.02006
17. Kono M, Fujii T, Matsuda N, Harano K, Chen H, Wathoo C, et al. Somatic Mutations, Clinicopathologic Characteristics, and Survival in Patients With Untreated Breast Cancer With Bone-Only and non-Bone Sites of First Metastasis. *J Cancer* (2018) 9(19):3640–6. doi: 10.7150/jca.26825
18. Li K, Wang B, Yang Z, Yu R, Chen H, Li Y, et al. Nomogram Predicts the Role of Contralateral Prophylactic Mastectomy in Male Patients With Unilateral Breast Cancer Based on SEER Database: A Competing Risk Analysis. *Front Oncol* (2021) 11:587797. doi: 10.3389/fonc.2021.587797
19. Haller B, Schmidt G, Ulm K. Applying Competing Risks Regression Models: An Overview. *Lifetime Data Anal* (2013) 19(1):33–58. doi: 10.1007/s10985-012-9230-8
20. Shen W, Sakamoto N, Yang L. Model to Predict Cause-Specific Mortality in Patients With Head and Neck Adenoid Cystic Carcinoma: A Competing Risk Analysis. *Ann Surg Oncol* (2017) 24(8):2129–36. doi: 10.1245/s10434-017-5861-z
21. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer. *N Engl J Med* (2004) 351(8):781–91. doi: 10.1056/NEJMoa040766

22. Danna EA, Sinha P, Gilbert M, Clements VK, Pulaski BA, Ostrand-Rosenberg S. Surgical Removal of Primary Tumor Reverses Tumor-Induced Immunosuppression Despite the Presence of Metastatic Disease. *Cancer Res* (2004) 64(6):2205–11. doi: 10.1158/0008-5472.can-03-2646
23. Cady B, Nathan NR, Michaelson JS, Golshan M, Smith BL. Matched Pair Analyses of Stage IV Breast Cancer With or Without Resection of Primary Breast Site. *Ann Surg Oncol* (2008) 15(12):3384–95. doi: 10.1245/s10434-008-0085-x
24. Dominici L, Najita J, Hughes M, Niland J, Marcom P, Wong YN, et al. Surgery of the Primary Tumor Does Not Improve Survival in Stage IV Breast Cancer. *Breast Cancer Res Treat* (2011) 129(2):459–65. doi: 10.1007/s10549-011-1648-2
25. Leung AM, Vu HN, Nguyen KA, Thacker LR, Bear HD. Effects of Surgical Excision on Survival of Patients With Stage IV Breast Cancer. *J Surg Res* (2010) 161(1):83–8. doi: 10.1016/j.jss.2008.12.030
26. Wang R, Zhu Y, Liu X, Liao X, He J, Niu L. The Clinicopathological Features and Survival Outcomes of Patients With Different Metastatic Sites in Stage IV Breast Cancer. *BMC Cancer* (2019) 19(1):e1091. doi: 10.1186/s12885-019-6311-z
27. Rogoz B, Houzé de l'Aulnoit A, Duhamel A, Houzé de l'Aulnoit D. Thirty-Year Trends of Survival and Time-Varying Effects of Prognostic Factors in Patients With Metastatic Breast Cancer-A Single Institution Experience. *Clin Breast Cancer* (2018) 18(3):246–53. doi: 10.1016/j.clbc.2017.08.012
28. Li Y, Wang S, Yang W, Liu H. Prognostic Significance of Molecular Subtype, Metastatic Site and Primary Tumor Surgery for Survival in Primary Metastatic Breast Cancer: A SEER-Based Study. *Med (Baltimore)* (2021) 100(27):e26619. doi: 10.1097/md.0000000000026619
29. Wang H, Zhang C, Zhang J, Kong L, Zhu H, Yu J. The Prognosis Analysis of Different Metastasis Pattern in Patients With Different Breast Cancer Subtypes: A SEER Based Study. *Oncotarget* (2017) 8(16):26368–79. doi: 10.18632/oncotarget.14300
30. Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, et al. Metastatic Behavior of Breast Cancer Subtypes. *J Clin Oncol* (2010) 28 (20):3271–7. doi: 10.1200/jco.2009.25.9820
31. Li K, Zhou C, Yu Y, Niu L, Zhang W, Wang B, et al. Metastatic Pattern Discriminates Survival Benefit of Type of Surgery in Patients With *De Novo* Stage IV Breast Cancer Based on SEER Database. *Front Surg* (2021) 8:e696628. doi: 10.3389/fsurg.2021.696628
32. Zhao W, Wu L, Zhao A, Zhang M, Tian Q, Shen Y, et al. A Nomogram for Predicting Survival in Patients With *De Novo* Metastatic Breast Cancer: A Population-Based Study. *BMC Cancer* (2020) 20(1):e982. doi: 10.1186/s12885-020-07449-1
33. Zheng Y, Zhong G, Yu K, Lei K, Yang Q. Individualized Prediction of Survival Benefit From Locoregional Surgical Treatment for Patients With Metastatic Breast Cancer. *Front Oncol* (2020) 10:e148. doi: 10.3389/fonc.2020.00148

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 18 May 2022
doi: 10.3389/fonc.2022.847858

One-Step Nucleic Acid Amplification System in Comparison to the Intraoperative Frozen Section and Definitive Histological Examination Among Breast Cancer Patients: A Retrospective Survival Study

Serena Bertozzi[1,2,3*], *Ambrogio P. Londero*[2,4*], *Michela Bulfoni*[5], *Luca Seriau*[1], *Diane Agakiza*[3], *Alberto Pasqualucci*[6,7], *Michela Andretta*[1], *Maria Orsaria*[5], *Laura Mariuzzi*[3,5] *and Carla Cedolini*[1,2]

[1] Breast Unit, University Hospital of Udine, Udine, Italy, [2] Ennergi Research, Lestizza, Italy, [3] Department of Medical Area (DAME), University of Udine, Udine, Italy, [4] Academic Unit of Obstetrics and Gynaecology, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Infant Health, University of Genoa, Genova, Italy, [5] Institute of pathology, University Hospital of Udine, Udine, Italy, [6] Department of Surgical and Biomedical Science, University of Perugia, Perugia, Italy, [7] Rashid Hospital, Trauma and Emergency Center, Dubai Health Authority, Dubai, United Arab Emirates

OPEN ACCESS

Edited by:
Youlia M. Kirova,
Institut Curie, France

Reviewed by:
Tanuja Shet,
Tata Memorial Hospital, India
Donatella Santini,
Sant'Orsola-Malpighi Polyclinic, Italy

***Correspondence:**
Serena Bertozzi
dr.bertozzi@gmail.com
Ambrogio P. Londero
ambrogio.londero@gmail.com

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: 03 January 2022
Accepted: 14 April 2022
Published: 18 May 2022

Citation:
Bertozzi S, Londero AP, Bulfoni M, Seriau L, Agakiza D, Pasqualucci A, Andretta M, Orsaria M, Mariuzzi L and Cedolini C (2022) One-Step Nucleic Acid Amplification System in Comparison to the Intraoperative Frozen Section and Definitive Histological Examination Among Breast Cancer Patients: A Retrospective Survival Study. Front. Oncol. 12:847858. doi: 10.3389/fonc.2022.847858

Simple Summary: Implementing intraoperative assessment of sentinel lymph nodes by one-step nucleic acid amplification in early breast cancer can reduce the surgical burden to the patient and the costs to the health system. However, only limited data are available in terms of long-term disease-free survival and overall survival. Therefore, this study aims to compare disease-free survival and overall survival between one-step nucleic acid amplification, frozen section, and definitive histology. These results could impact the healthcare community, adding further proof to the body of evidence supporting the broader adoption of this innovative technology that enables a safe reduction in patient surgical burden and healthcare costs.

Background: The one-step nucleic acid amplification (OSNA) system is a novel molecular technique, which consents to quick intraoperative detection of sentinel lymph node metastases by the amplification of cytokeratin 19 mRNA. Our study aims to evaluate the OSNA method in comparison with frozen section (FS) and definitive histological examination of the sentinel lymph node biopsy among early breast cancer patients considering disease-free survival (DFS) and overall survival (OS).

Methods: In this study, we included all women who underwent sentinel lymph node biopsy (SLNB) for breast cancers classified as TNM stage I and II in our center between January 2005 and January 2017, and the follow-up was collected up to January 2019. We divided patients among three groups based on SLNB evaluation: definitive histological examination, intra-operative FS, or OSNA.

Results: We included 2412 SLNBs: 727 by definitive histological examination, 697 by FS, and 988 by OSNA. Isolated tumor cells were found in 2.32% of cases, micrometastasis in 9.12%, and macrometastases in 13.64%. Surgical procedure duration was significantly shorter in OSNA than in FS (42.1 minutes ±5.1 vs. 70.1 minutes ±10.5, p <0.05). No significant differences have been observed among the three groups regarding OS, DSF, cumulative local, or distant metastases. In particular 5-year DFS was 96.38% in definitive histology (95% C.I. 95.02-97.75%), 96.37% in FS (95% C.I. 94.98-97.78%), and 96.51% in OSNA group (95% C.I. 95.32-97.72%).

Conclusions: No difference in OS and DFS was found comparing OSNA, FS, and definitive histology. Furthermore, reduced operative time was found in the OSNA group.

Keywords: sentinel lymph node biopsy, OSNA, breast cancer, survival, frozen section

INTRODUCTION

The sentinel lymph node biopsy (SLNB) procedure has dramatically revolutionized breast surgery during the last decades (1). In fact, SLNB with staging intent has progressively replaced complete axillary lymph node dissection (CALND), previously intended with a curative purpose. Probably in the future, even this procedure might be abandoned in favor of a non-surgical lymph node evaluation to predict patients' prognosis and better tailor subsequent therapies (1, 2).

For what concerns the technique, we assisted in a first evolution to reduce the number of interventions with the introduction of intraoperative frozen section evaluation of the SLNB. Performing in the same surgical session, the primary breast surgery, the SLNB, and eventually the CALND according to the intraoperative lymph node assessment reduces the patients' surgical burden and the healthcare system costs (3–5). Secondly, an intraoperative molecular-based lymph node staging has been adopted in place of the traditional morphological examination to minimize the operative time and enhance accuracy (6). In particular, the one-step nucleic acid amplification (OSNA) system consists of the amplification of cytokeratin (CK) 19 mRNA directly from the lysate to distinguish positive from negative samples (7–9). This second advance, besides ensuring a reduction in surgical sessions per patient and the costs for the healthcare system, allows reducing operating times and the pathologist workload (4, 6).

Although OSNA is considered the most accurate intraoperative lymph node staging technique (10), the literature lacks cohort studies, with everyday routine data, comparing survival analysis between OSNA and other lymph node staging methods (intraoperative frozen section or definitive histology).

Our study evaluates the OSNA method for the intraoperative analysis of sentinel lymph node biopsy compared with frozen section and definitive histological examination among patients affected by breast cancers classified as TNM stage I and II considering disease-free survival and overall survival.

Abbreviations: AJCC/UICC, American Joint Committee on Cancer/Union for International Cancer Control; BMI, body mass index; CALND, complete axillary lymph node dissection; CI, confidence interval; CK, cytokeratin; DFS, disease free survival; FS, frozen section; IQR, interquadrtile range; ITC, isolated tumor cell; mRNA, messenger ribonucleic acid; OS, overall survival; OSNA, One-step nucleic acid amplification; SLNB, sentinel lymph node biopsy; TNM, tumor, node, metastasis.

METHODS

Study Design and Subjects

All women were included in this retrospective cohort study who underwent SLNB for invasive breast cancers classified as TNM stage I and II in our center between January 2005 and January 2017. The follow-up was collected up to January 2019. According to Helsinki Declaration, the study was carried out and followed the dictates of the general authorization to process personal data for scientific research purposes by the Italian Data Protection Authority. We excluded all cases that underwent primary CALND, male breast cancer patients, women affected by intraductal neoplasia, benign breast diseases, as well as invasive breast cancers classified as TNM stage III or IV. The patient information was gathered from clinical files.

In all included cases, SLNB was performed. At the same time, breast cancer removal consisted of breast-conserving surgery or mastectomy when appropriate, followed or not by immediate breast reconstruction as previously described (5, 11, 12). Non-palpable breast lesions were removed by radio-guided occult lesion localization or wire hook localization as previously described (5, 13–15).

The cohort of included patients was divided into three groups according to SLNB histological assessment: group A consists of all cases in which SLNB was assessed by definitive histological examination, group B includes all cases in which SLNB was assessed by intraoperative frozen section (FS), and group C includes all cases in which SLNB was assessed by OSNA. Intraoperative FS was introduced in 2002 and is still performed in selected cases (more than three sentinel nodes, big-sized sentinel nodes, history of hematological disease, previous neoadjuvant chemotherapy, OSNA system unavailability). OSNA system was introduced in October 2011. Definitive histological examination was performed on any sentinel node removal under local anesthesia before planning final breast surgery.

Definitive Histological Examination

In the event of definitive histological examination, all biopsied lymph nodes were cut in parts of 2 mm thickness, formalin-fixed, and paraffin-embedded before undergoing an accurate in toto evaluation of 0.15-mm-spaced, hematoxylin-eosin-stained sections (16). Concurrently, an immunohistochemical assessment of a random portion of the considered nodes to search for an eventual positivity for cytokeratins was performed on pathologist request (17).

Intraoperative Frozen Section

In intraoperative FS, the sentinel nodes were cut in parts of 2 mm thickness, frozen, and optimal cutting temperature (OCT) embedded before undergoing intraoperative assessment. First, the pathologist performed a histological examination of 2 hematoxylin-eosin-stained sections (0.15-mm-spaced). Thereafter, the remnant sentinel lymph node tissue underwent traditional definitive in toto histological examination with evaluation of 0.15-mm-spaced, hematoxylin-eosin-stained sections, and immunohistochemical evaluation of a random nodal portion on pathologist request.

One-Step Nucleic Acid Amplification

The detailed OSNA assay has been previously described (18–20). First, all the collected sentinel lymph nodes were separately homogenized in an mRNA-stabilizing solution (Lynorhag, pH 3.5 Sysmex®). Then, an isothermal (65°C) CK19 amplification was performed using the Lynoamp amplification kit (Sysmex®) through a reverse transcriptase amplification assay (RT-LAMP) in a gene amplification detector RD-100i (Sysmex®). A standard positive control sample and a negative control sample were used for calibration in every assay. Our protocol complied with a previously described procedure (20). As previously defined, the results were given automatically in a semiquantitative way (18, 20–22). In brief, if the CK19 mRNA copy number/μl lysate was less than 250 copies/μl, the result was regarded as negative (-), indicating non-metastasis; copy numbers between 250 and 5000/μl were regarded as positive (+), indicating micrometastasis; and copy numbers of 5000/μl and greater as strongly positive (++), indicating macrometastasis.

Variables and Outcomes

The primary outcomes for this study were overall survival (OS), disease-free survival (DFS), cumulative local recurrences, and cumulative distant recurrences. In addition, the following information was collected: patient age, body mass index (BMI), tobacco smoke habit, family history of breast and ovarian cancer, previous use of estrogens, post-menopausal status, definitive type of breast surgery, definitive type of axilla surgery, definitive histological results, non-surgical treatments (e.g., neo-adjuvant or adjuvant chemotherapy), the presence of comedo-like necrosis, multifocality/multicentricity, extensive intraductal component, peritumoral vascular invasion, peritumoral inflammation, breast cancer molecular subtype, tumor grading, lymph node characteristics (e.g., presence of isolated tumor cells (ITCs), micrometastasis, extracapsular lymph node invasion, or lymph node bunching), tumor size, nodal status, and TNM stage.

The tumor stage was defined according to the VII edition of the TNM classification (AJCC/UICC) (23). Tumor histology was interpreted and classified according to the World Health Organization (24). Furthermore, Elston and Ellis's recommendations were used to evaluate the tumor grade (25). According to Rosen and Oberman's criteria, the peritumoral vascular invasion was considered, and the molecular subtype of breast cancer was evaluated as previously described (25, 26). In addition, the expression and quantification of ER, PR, Her-2/Neu, and the proliferative tumor fraction (Mib1/Ki67) were evaluated as previously described (26). In addition, the lymph node extracapsular invasion was defined as the extracapsular growth of tumor cells, invasion of perinodal fat, or extranodal location of tumor cells (26).

Statistical Methods

Statistical analysis was performed using R (version 3.6.2 – http://www.R-project.org/). The normal distribution of considered numeric variables was evaluated through the Kolmogorov-Smirnov test. Numeric variables were described with the mean (± standard deviation) or median and interquartile range (IQR), while categorical variables were described as percentages and absolute values. Moreover, the following statistical tests were applied when appropriate: Wilcoxon test, t-test, Kruskall-Wallis test, and one-way ANOVA for continuous variables, Fisher exact test, or chi-square test for categorical variables. The Kaplan-Meier analysis was used to analyze overall survival, disease-free survival, and cumulative local or distant recurrences. The differences between different groups were tested using the Log-rank test. Furthermore, the univariate and multivariate Cox proportional hazards regression analysis was performed considering as response variables OS and DFS.

RESULTS

We included in this study 2412 patients with invasive breast cancer classified as TNM stage I and II and operated on during the considered period. A definitive histological examination of SLNB was performed in 727 cases (group A), intra-operative FS in 697 patients (group B), and OSNA in 988 cases (group C).

Mean patient age resulted in 60.24 years (± 12.1), mean BMI was 25.23 kg/m2 (± 4.77), and 79.35% of women were in their post-menopausal period. The prevalence of familial cancer history and previous use of estrogens were respectively 30.29% and 35.28%. In most cases, definitive breast surgery was conservative in most cases (62.94%), while mastectomy was definitively performed in 37.06% of cases. Adjuvant hormonal therapy was administered in 84.82% of women, adjuvant radiotherapy in 62.16%, and adjuvant chemotherapy in 31.84% (759/2384).

ITCs were found in 2.32% of cases, micrometastasis in 9.12%, and macrometastases in 13.64%. The extracapsular lymph node

invasion was found in 0.5% of cases, and non-axillary loco-regional lymph node metastases were found in 1.37% of cases. Definitive CALND was performed in 22.18% of patients. Among 535 CALND, 312 were performed after detecting macrometastases, 173 micrometastases, 9 ITC, and 41 cases because of sentinel node detection failure. In addition, CALND was not performed in 47 patients with ITCs and 47 with micrometastases.

The most frequent histotype was invasive carcinoma non-special type (previously named invasive ductal carcinoma, 79.1%), followed by invasive lobular carcinoma (12.73%), other special types of invasive carcinoma (3.98%), and the combined ductal and lobular invasive carcinoma (4.19%). The most common molecular subtype was luminal A (50.08%), followed by luminal B (27.57%), basal-like (7.59%), luminal Her (5.85%), and Her-enriched (3.23%). In 5.68%, the molecular subtype was not specified. Tumor grading G2 accounted for 59.37% of cases. The majority of tumors were classified as T1 (85.66%), N0 (77.24%), and TNM stage I (75.17%).

All patients with definitive histological examination had two surgical interventions, while FS and OSNA were performed in the same surgical session as the primary breast tumor. In addition, the surgical operation was significantly longer in cases assessed intra-operatively by FS than by OSNA (70.1 minutes ±10.5 vs. 42.1 minutes ±5.1; $p < 0.05$).

In **Table 1**, we report the different characteristics of the three studied groups. The definitive histological examination group had a significantly higher prevalence of BCS than FS or OSNA ones. In addition, FS was associated with a lower prevalence of CALND than the definitive histological examination or OSNA (**Table 1**). The prevalence of Mib-1>20% was significantly higher in OSNA than FS and definitive histological examination. **Table 2** shows the differences in terms of tumor characteristics between the definitive histological examination and OSNA or FS groups. The prevalence of luminal A subtype was significantly higher in FS than in definitive histological examination and OSNA (**Table 2**). In addition, the prevalence of positive nodes was significantly higher in the OSNA group than in the other two groups (**Table 2**). Concurrently, the OSNA group had a significantly higher prevalence of N1 tumors (**Table 3**) and a higher prevalence of both macro- and micro-metastases (**Table 2**) than the other two groups.

Figure 1 shows the Kaplan-Meier analysis, and no significant differences have been observed in OS, DSF, cumulative local or distant metastases (**Figures 1A–D**). At 5 years follow-up the OS in definitive histological examination group, FS, and OSNA was respectively 99.16% (95% C.I. 98.49-99.83%), 99.12% (95% C.I. 98.43-99.82%), and 99.20% (95% C.I. 98.61-99.80%) while the DFS was respectively 96.38% (95% C.I. 95.02-97.75%), 96.37% (95% C.I. 94.98-97.78%), and 96.51% (95% C.I. 95.32-97.72%). The mortality rates in the definitive histological examination group, FS, and OSNA were respectively 1.681 deaths/1000 patients/year, 1.757 deaths/1000 patients/year, and 1.600 deaths/1000 patients/year. The local recurrence incidence rates in the definitive histology group, FS, and OSNA were respectively 3.383 cases/1000 patients/year, 4.740 cases/1000 patients/year, and 4.853 cases/1000 patients/year. The distant metastases recurrence incidence rates in the definitive histological examination group, FS, and OSNA were respectively 4.236 cases/1000 patients/year, 3.237 cases/1000 patients/year, and 2.753 cases/1000 patients/year. **Table 4** also shows univariate and multivariate cox analysis, and no significant differences have been found in OS and DFS among the three studied groups. The analysis in **Table 4** was stratified for N0, and no significant differences were found. DFS was assessed separately for macrometastases. In the univariate analysis and multivariate analysis, no significant differences were observed. The multivariate adjustment for DFS in the sub-group of macrometastases resulted for intraoperative FS of HR 2.44 (95% C.I. 0.44 - 13.38) (p=0.305) in reference to definitive histology and for Intraoperative OSNA of HR 1.05 (95% C.I. 0.18 - 6.1) (p=0.957). The multivariate adjustments were performed according to the most predictive factors and the possible confounders found in the univariate analysis. No other stratifications were performed due to the limited number of events.

TABLE 1 | Description of the population subdivided in the three considered groups (definitive histology, intraoperative frozen section, and OSNA).

	Definitive histology (727)	Intraoperative frozen section (697)	Intraoperative OSNA (988)	p
Age (years)	60.7 (± 12.0)	59.4 (± 11.4)	60.5 (± 12.6)	1
BMI (kg/m²)	25.6 (± 4.7)	25.1 (± 4.7)	24.9 (± 4.9)	1,2
Tobacco smoke	7.8% (53/676)	8.1% (52/644)	16.8% (90/537)	2,3
Familial cancer history	29.2% (42/144)	35.0% (82/234)	29.1% (237/814)	NS
Previous use of estrogens	33.6% (36/107)	31.1% (37/119)	39.3% (66/168)	NS
Post-menopausal status	81.4% (592/727)	80.1% (558/697)	77.3% (764/988)	2
Definitive breast surgical intervention				
BCS	74.7% (543/727)	63.1% (440/697)	54.1% (535/988)	1,2,3
Mastectomy	25.3% (184/727)	36.9% (257/697)	45.9% (453/988)	1,2,3
Definitive CALND	23.2% (169/727)	19.2% (134/697)	23.5% (232/988)	3
Non-surgical treatments				
Adjuvant radiotherapy	69.2% (496/717)	66.3% (460/694)	54.1% (526/973)	2,3
Adjuvant chemotherapy	34.3% (246/717)	29.1% (202/694)	32.0% (311/973)	1
Adjuvant hormonal therapy	83.3% (597/717)	85.0% (590/694)	85.8% (836/974)	NS

***Differences statistically significant (p < 0.05) between**, (1) definitive histology and intraoperative frozen section; (2) definitive histology and OSNA; (3) intraoperative frozen section and OSNA.*
BMI, body mass index; BCS, breast conservative surgery; CALND, complete axillary lymph node dissection.

TABLE 2 | Tumor characteristics considering the three groups (definitive histology, intraoperative frozen section, and OSNA).

	Definitive histology (727)	Intraoperative frozen section (697)	Intraoperative OSNA (988)	p
Histological type				
Invasive carcinoma non-special type	76.6% (557/727)	80.6% (562/697)	79.9% (789/988)	NS
Lobular invasive carcinoma	14.4% (105/727)	12.1% (84/697)	11.9% (118/988)	NS
Ductal and lobular invasive carcinoma	3.9% (28/727)	3.9% (27/697)	4.7% (46/988)	NS
Other invasive carcinoma	5.1% (37/727)	3.4% (24/697)	3.5% (35/988)	NS
Tumor characteristics				
Mib-1>20%	28.3% (196/693)	24.0% (151/628)	33.6% (320/953)	2,3
Comedo-like necrosis	4.1% (30/727)	9.9% (69/697)	8.3% (82/988)	1,2
Multifocality/multicentricity	16.6% (121/727)	14.2% (99/697)	17.1% (169/988)	NS
EIC	19.8% (144/727)	28.3% (197/697)	16.8% (166/988)	1,3
PVI	2.3% (17/727)	15.4% (107/697)	25.6% (253/988)	1,2,3
Peri-tumoral inflammation	3.3% (24/727)	0.6% (4/697)	0.5% (5/988)	1,2
Molecular subtype				
Luminal A	51.3% (356/694)	59.5% (377/634)	50.2% (475/947)	1,3
Luminal B	30.5% (212/694)	23.2% (147/634)	32.3% (306/947)	1,3
Luminal Her	6.1% (42/694)	6.0% (38/634)	6.4% (61/947)	NS
Her enriched	2.9% (20/694)	3.2% (20/634)	4.0% (38/947)	NS
Basal-like	9.2% (64/694)	8.2% (52/634)	7.1% (67/947)	NS
Lymph node characteristics				
Sentinel nodes removed >2	17.83% (64/359)	9.61% (67/697)	8.04% (78/970)	1,2
Ppositive sentinel nodes	22.0% (160/727)	17.1% (119/697)	27.3% (270/988)	1,2,3
ITC	5.6% (41/727)	2.2% (15/697)	—	1
Micrometastasis	6.5% (47/727)	5.2% (36/697)	13.9% (137/988)	2,3
Macrometastasis	15.5% (113/727)	11.9% (83/697)	13.5% (133/988)	1
Extracapsular lymph node invasion	0.6% (4/727)	0.3% (2/697)	0.6% (6/988)	NS
Non axilla locoregional lymph node metastasis	2.2% (16/727)	2.4% (17/697)	0.0% (0/988)	2,3

***Differences statistically significant (p < 0.05) between**, (1) definitive histology and intraoperative frozen section; (2) definitive histology and OSNA; (3) intraoperative frozen section and OSNA. EIC, extensive intraductal component; PVI, peritumoral vascular invasion; ITC, isolated tumor cells.*

TABLE 3 | TNM stage and tumor grading among the three considered groups (definitive histology, intraoperative frozen section, and OSNA).

	Definitive histology (727)	Intraoperative frozen section (697)	Intraoperative OSNA (988)	p
Tumor local extension				
T1	83.9% (610/727)	89.2% (622/697)	84.4% (834/988)	1,3
T2	16.1% (117/727)	10.8% (75/697)	15.3% (151/988)	1,3
T3	0.0% (0/727)	0.0% (0/697)	0.3% (3/988)	NS
Nodal status				
N0	78.0% (567/727)	82.9% (578/697)	72.7% (718/988)	1,2,3
N1	21.6% (157/727)	17.1% (119/697)	27.1% (268/988)	1,2,3
N2	0.4% (3/727)	0.0% (0/697)	0.2% (2/988)	NS
TNM stage				
I	72.1% (524/727)	79.9% (557/697)	74.1% (732/988)	1,3
II	27.9% (203/727)	20.1% (140/697)	25.9% (256/988)	1,3
Tumor grading				
G1	5.8% (42/727)	28.4% (198/697)	23.9% (236/988)	1,2,3
G2	69.6% (506/727)	53.7% (374/697)	55.9% (552/988)	1,2
G3	24.6% (179/727)	17.9% (125/697)	20.2% (200/988)	1,2

***Differences statistically significant (p < 0.05) between**: (1) definitive histology and intraoperative frozen section; (2) definitive histology and OSNA; (3) intraoperative frozen section and OSNA. TNM, Tumor-Node-Metastasis.*

DISCUSSION

Surgical procedure length was significantly shortened by the intraoperative OSNA technique. In addition, despite the long follow-up considered, no significant differences have been observed among the three groups (intraoperative OSNA or FS and definitive histology) regarding OS, DSF, cumulative local or distant metastases, apart from a non-significant increased risk of local recurrences related to FS and OSNA method.

Previous studies demonstrated a peak of local recurrences after SLNB between the third and the sixth year of follow-up, which are mostly included in our study (11). Only a limited number of studies performed a survival analysis considering OSNA, and none compared in the same population in all three groups we considered based on the SLNB evaluation technique (10, 27, 28). Recently, Shimazu and coworkers found a significantly improved DFS in N0 status detected by OSNA, if compared with traditional histology, suggesting the better

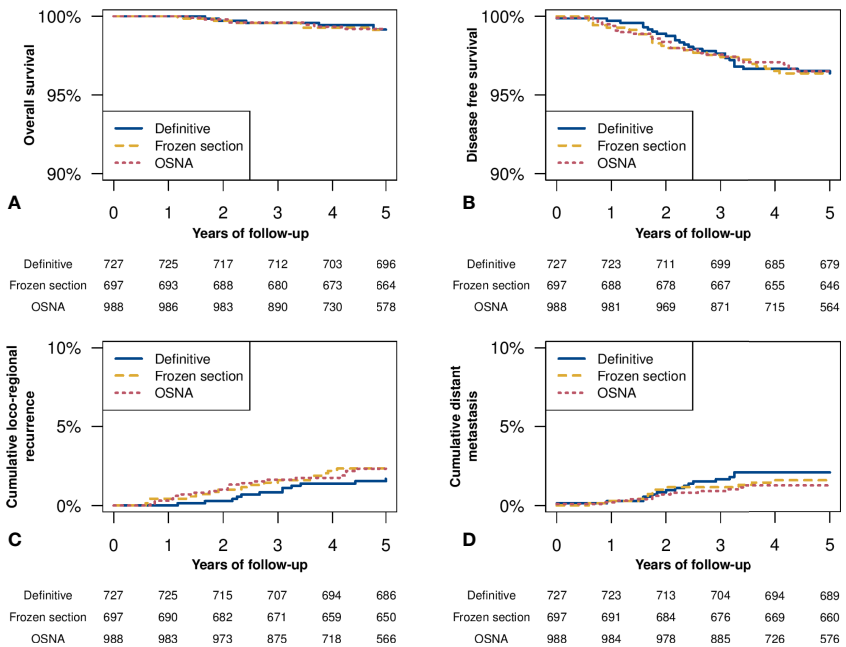


FIGURE 1 | Kaplan Meier analysis. **(A)** Overall survival among the studied groups (log-rank test p-value=0.806). **(B)** Disease free survival among the studied groups (log-rank test p-value=0.295). **(C)** Cumulative loco-regional recurrence among the studied groups (log-rank test p-value=0.152). **(D)** Cumulative distant metastases among the studied groups (log-rank test p-value=0.589).

TABLE 4 | Univariate and multivariate Cox analysis.

OS (All nodal status)	**HR (95% CI)**	**p**	**HR (95% CI) (*)**	**p**
Definitive histology	Reference	1.000	Reference	1.000
Intraoperative FS	1.05 (0.34 - 3.25)	0.937	2.53 (0.75 - 8.55)	0.135
Intraoperative OSNA	0.97 (0.33 - 2.90)	0.962	1.54 (0.44 - 5.35)	0.498
OS (Only N0)	**HR (95% CI)**	**p**	**HR (95% CI) (**)**	**p**
Definitive histology	Reference	1.000	Reference	1.000
Intraoperative FS	0.82 (0.22 - 3.05)	0.767	1.38 (0.34 - 5.65)	0.651
Intraoperative OSNA	1.25 (0.4 - 3.96)	0.701	1.91 (0.51 - 7.06)	0.335
DFS (All nodal status)	**HR (95% CI)**	**p**	**HR (95% CI) (*)**	**p**
Definitive histology	Reference	1.000	Reference	1.000
Intraoperative FS	1.01 (0.58 - 1.74)	0.982	0.99 (0.53 - 1.86)	0.973
Intraoperative OSNA	0.96 (0.57 - 1.62)	0.888	0.84 (0.47 - 1.52)	0.573
DFS (Only N0)	**HR (95% CI)**	**p**	**HR (95% CI) (**)**	**p**
Definitive histology	Reference	1.000	Reference	1.000
Intraoperative FS	1.08 (0.57 - 2.06)	0.807	1.18 (0.56 - 2.49)	0.658
Intraoperative OSNA	1.02 (0.55 - 1.91)	0.943	1.14 (0.56 - 2.29)	0.717
DFS (Nodal macrometastases)	**HR (95% CI)**	**p**	**HR (95% CI) (**)**	**p**
Definitive histology	Reference	1.000	Reference	1.000
Intraoperative FS	0.98 (0.31 - 3.08)	0.971	2.44 (0.44 - 13.38)	0.305
Intraoperative OSNA	0.51 (0.15 - 1.74)	0.281	1.05 (0.18 - 6.1)	0.957

(*) Multivariate Cox analysis adjusted for woman age, histological type, molecular subtype, nodal status, TNM stage, tumor grading, Mib-1>20%, comedo-like necrosis, multifocality/multicentricity, EIC, PVI, type of breast surgery, type of axilla surgery.

(**) Multivariate Cox analysis adjusted for woman age, histological type, molecular subtype, TNM stage, tumor grading, Mib-1>20%, comedo-like necrosis, multifocality/multicentricity, EIC, PVI, type of breast surgery, type of axilla surgery.

staging efficiency of the OSNA method (28). Our data did not confirm this advantage. Instead, we showed a non-significant increased recurrence risk in the case of intraoperative OSNA, in comparison with definitive histology, which may be explained by the higher incidence of unfavorable prognostic factors found in the group of patients who underwent OSNA evaluation of their SLNB. Indeed, the OSNA method resulted significantly associated with tumor features, which are usually expressions of a more aggressive biological behavior of the disease, such as tumor multifocality/multicentricity, extensive intraductal

component, peritumoral vascular invasion, comedo-like necrosis, and Mib-1>20% (29). And in our opinion, this fact simply reflects the progressive extension of SLNB indications.

In the literature, the OSNA system allowed a more efficient detection of micrometastasis, consequently decreasing the number of false-negative histological examinations resulting from the small size of micrometastases, which may not be included in any microscopical section (28, 30, 31). Also, in our experience, a significant increase in the prevalence of micrometastasis was found compared to FS and definitive histology. In addition, the prevalence of macrometastases was similar to the definitive histology, while FS had a significantly lower prevalence of macrometastases than definitive histology. This last finding could be due to a significantly higher detection rate of definitive histology than FS or simply to a better selection of subjects undergoing FS than definitive histology. Along with the increased number of detected micrometastases compared to FS and definitive histology, the OSNA technique also increased the number of diagnosed macrometastases than FS, correlating with a higher prevalence of node-positive disease and consequently a higher prevalence of secondary CALNDs. However, Hintzen and coworkers recently demonstrated that the increased rate of CALND after OSNA could be limited by broader adoption of the criteria that emerged from the Z0011 and AMAROS trials for axilla treatment (32–34). In particular, they found that the use of the OSNA method, in association with these emerging criteria for axilla treatment, does not lead to more CALNDs, axilla radiotherapy, or adjuvant systemic therapies (34).

As expected, the OSNA technique resulted in an evident improvement in breast surgery in our center. In particular, in accordance with the literature (4), it succeeded in significantly reducing the surgical time from a mean operation length of 70.1 (± 10.5) minutes in the case of FS to a mean operation length of 42.1 (± 5.1) minutes using OSNA. However, this result required accurate compliance with some technical premises, such as the strong limitation of the number of excised nodes. Consequently, both OSNA and FS correlated with a smaller number of excised sentinel nodes than definitive histological examination, resulting in nearly one single node. Furthermore, recently Saruta and coworkers found that in Japan, the adoption of the OSNA technique, in addition to reducing the burden on the patient (limiting the number of surgeries and the duration of surgical procedures), also reduced the breast cancer healthcare costs per patient (35).

The main limitations of this study are the retrospective and non-randomized nature of the chart review and the unavailability of detailed data about cost-effectiveness and side effects. Among the strengths of this study, we can emphasize the broad cohort and the remarkable follow-up data. In addition, another essential strength is the uniform management due to regular multidisciplinary meetings in a single-center experience.

Our findings add further proof to the body of evidence supporting the wider adoption of this innovative technology that enables a safe reduction in patient surgical burden and healthcare costs. The reduction in costs also comprises a lower workload for the pathologist than intraoperative FS and definitive histology.

In conclusion, no difference was found in OS and DFS when comparing OSNA, FS, and definitive histology. At the same time, the OSNA system was advantageous in reducing single-session surgical operating time and the pathologist workload.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the data that support the findings of this study are available, but restrictions apply to the availability of these data, which was used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Internal Review Board. Requests to access the datasets should be directed to serena.bertozzi@asufc.sanita.fvg.it.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Internal Review Board of the Department of Medical Area (University of Udine). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Substantial contributions to conception and design or acquisition of data or to analysis and interpretation of data: SB, AL, MB, LS, DA, AP, MA, MO, LM, and CC. Drafting the article or revising it critically for important intellectual content: SB, AL, MB, LS, DA, AP, MA, MO, LM, and CC. All authors have read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors would like to thank the whole staff for collaborating in patient management, data collection, article selection, reading, and paper writing and reviewing.

REFERENCES

1. Bertozzi S, Cedolini C, Londero AP, Baita B, Giacomuzzi F, Capobianco D, et al. Sentinel Lymph Node Biopsy in Patients Affected by Breast Ductal Carcinoma *in Situ* With and Without Microinvasion: Retrospective Observational Study. *Med (Baltimore)* (2019) 98:e13831. doi: 10.1097/MD.0000000000013831
2. Bertozzi S, Londero AP. The Sentinel Lymph Node Biopsy for Breast Cancer Over the Years. *Eur J Gynaecol Oncol* (2016) 37:13–6. doi: 10.12892/ejgo2773.2016
3. Madsen EVE, van Dalen J, van Gorp J, van Oort PMP, van Dalen T. Frozen Section Analysis of Sentinel Lymph Nodes in Patients With Breast Cancer Does Not Impair the Probability to Detect Lymph Node Metastases. *Virchows Arch* (2012) 460:69–76. doi: 10.1007/s00428-011-1171-1

4. Cattin F, Castriotta L, Zumerle G, Scarpa E, Leo CA, Semprini G, et al. The Breast Cancer: A Comparison Among Different Diagnostic and Therapeutic Protocols. *Breast J* (2013) 19:560–2. doi: 10.1111/tbj.12171
5. Cedolini C, Bertozzi S, Seriau L, Londero AP, Concina S, Cattin F, et al. Eight-Year Experience With the Intraoperative Frozen Section Examination of Sentinel Lymph Node Biopsy for Breast Cancer in a North-Italian University Center. *Int J Clin Exp Pathol* (2014) 7:364–71.
6. Szychta P, Westfal B, Maciejczyk R, Smolarz B, Romanowicz H, Krawczyk T, et al. Intraoperative Diagnosis of Sentinel Lymph Node Metastases in Breast Cancer Treatment With One-Step Nucleic Acid Amplification Assay (OSNA). *Arch Med Sci* (2016) 12:1239–46. doi: 10.5114/aoms.2016.62902
7. Schem C, Maass N, Bauerschlag DO, Carstensen MH, Löning T, Roder C, et al. One-Step Nucleic Acid Amplification-A Molecular Method for the Detection of Lymph Node Metastases in Breast Cancer Patients; Results of the German Study Group. *Virchows Arch* (2009) 454:203–10. doi: 10.1007/s00428-008-0703-9
8. AIOM. *Linee guida - Neoplasia della mammella*. Milan, Italy: AIOM (2018).
9. Londero AP, Bertozzi S, Cedolini C eds. The Sentinel Lymph Node: Detection, Procedures and Clinical Implications. In: *Cancer Etiology, Diagnosis and Treatments*. New York: Nova Medicine & Health.
10. Osako T, Iwase T, Ushijima M, Yonekura R, Ohno S, Akiyama F. A New Molecular-Based Lymph Node Staging Classification Determines the Prognosis of Breast Cancer Patients. *Br J Cancer* (2017) 117:1470–7. doi: 10.1038/bjc.2017.311
11. Bernardi S, Bertozzi S, Londero AP, Gentile G, Giacomuzzi F, Carbone A. Incidence and Risk Factors of the Intraoperative Localization Failure of Nonpalpable Breast Lesions by Radio-Guided Occult Lesion Localization: A Retrospective Analysis of 579 Cases. *World J Surg* (2012) 36:1915–21. doi: 10.1007/s00268-012-1577-1
12. Bertozzi S, Londero AP, Giacomuzzi F, Angione V, Carbone A, Petri R, et al. Applicability of Two Different Validated Models to Predict Axillary non-Sentinel Lymph Node Status by Sentinel Node Biopsy in a Single Italian Center. *Breast Cancer* (2015) 22:350–5. doi: 10.1007/s12282-013-0485-z
13. Cedolini C, Bertozzi S, Seriau L, Londero AP, Concina S, Moretti E, et al. Feasibility of Concervative Breast Surgery and Intraoperative Radiation Therapy for Early Breast Cancer: A Single-Center, Open, non-Randomized, Prospective Pilot Study. *Oncol Rep* (2014) 31:1539–46. doi: 10.3892/or.2014.3018
14. Bernardi S, Bertozzi S, Londero AP, Giacomuzzi F, Angione V, Dri C, et al. Nine Years of Experience With the Sentinel Lymph Node Biopsy in a Single Italian Center: A Retrospective Analysis of 1,050 Cases. *World J Surg* (2012) 36:714–22. doi: 10.1007/s00268-011-1420-0
15. Bernardi S, Bertozzi S, Londero AP, Gentile G, Angione V, Petri R. Influence of Surgical Margins on the Outcome of Breast Cancer Patients: A Retrospective Analysis. *World J Surg* (2014) 38:2279–87. doi: 10.1007/s00268-014-2596-x
16. Weaver DL. Pathology Evaluation of Sentinel Lymph Nodes in Breast Cancer: Protocol Recommendations and Rationale. *Mod Pathol* (2010) 23 Suppl 2: S26–32. doi: 10.1038/modpathol.2010.36
17. Giobuin SM, Kavanagh DO, Myers E, Doherty AO, Quinn CM, Crotty T, et al. The Significance of Immunohistochemistry Positivity in Sentinel Nodes Which are Negative on Haematoxylin and Eosin in Breast Cancer. *Eur J Surg Oncol* (2009) 35:1257–60. doi: 10.1016/j.ejso.2009.04.004
18. Pegolo E, Puppin C, Gerometta A, Damante G, Puglisi F, Di Loreto C. One-Step Nucleic Acid Amplification (OSNA) for Intraoperative Evaluation of Sentinel Lymph Node Status in Breast Cancer: A Comparative Study Between CK19 Protein Expression and CK19 mRNA Level in Primary Tumors and Lymph Node Metastasis. *Virchows Arch* (2013) 463:7–15. doi: 10.1007/s00428-013-1440-2
19. Tamaki Y, Akiyama F, Iwase T, Kaneko T, Tsuda H, Sato K, et al. Molecular Detection of Lymph Node Metastases in Breast Cancer Patients: Results of a Multicenter Trial Using the One-Step Nucleic Acid Amplification Assay. *Clin Cancer Res* (2009) 15:2879–84. doi: 10.1158/1078-0432.CCR-08-1881
20. Tsujimoto M, Nakabayashi K, Yoshidome K, Kaneko T, Iwase T, Akiyama F, et al. One-Step Nucleic Acid Amplification for Intraoperative Detection of Lymph Node Metastasis in Breast Cancer Patients. *Clin Cancer Res* (2007) 13:4807–16. doi: 10.1158/1078-0432.CCR-06-2512
21. Feldman S, Krishnamurthy S, Gillanders W, Gittleman M, Beitsch PD, Young PR, et al. A Novel Automated Assay for the Rapid Identification of Metastatic Breast Carcinoma in Sentinel Lymph Nodes. *Cancer* (2011) 117:2599–607. doi: 10.1002/cncr.25822
22. Snook KL, Layer GT, Jackson PA, de Vries CS, Shousha S, Sinnett HD, et al. Multicentre Evaluation of Intraoperative Molecular Analysis of Sentinel Lymph Nodes in Breast Carcinoma. *Br J Surg* (2011) 98:527–35. doi: 10.1002/bjs.7347
23. J Brierley, MK Gospodarowicz, C Wittekind eds. *TNM Classification of Malignant Tumours. Eighth edition*. Chichester, West Sussex, UK; Hoboken, NJ: John Wiley & Sons, Inc (2017).
24. Lokuhetty D, White VA, Watanabe R, Cree IA, WHO Classification of Tumours Editorial Board, et al. *Breast Tumours, Fifth edition*. Lyon, France: International Agency for Research on Cancer (2019).
25. Londero AP, Bernardi S, Bertozzi S, Angione V, Gentile G, Dri C, et al. Synchronous and Metachronous Breast Malignancies: A Cross-Sectional Retrospective Study and Review of the Literature. *BioMed Res Int* (2014) 2014:250727. doi: 10.1155/2014/250727
26. Cedolini C, Bertozzi S, Londero AP, Bernardi S, Seriau L, Concina S, et al. Type of Breast Cancer Diagnosis, Screening, and Survival. *Clin Breast Cancer* (2014) 14:235–40. doi: 10.1016/j.clbc.2014.02.004
27. Peg V, Sansano I, Vieites B, Bernet L, Cano R, Córdoba A, et al. Role of Total Tumour Load of Sentinel Lymph Node on Survival in Early Breast Cancer Patients. *Breast* (2017) 33:8–13. doi: 10.1016/j.breast.2017.02.011
28. Shimazu K, Miyake T, Okuno J, Naoi Y, Tanei T, Shimoda M, et al. One-Step Nucleic Acid Amplification Can Identify Sentinel Node-Negative Breast Cancer Patients With Excellent Prognosis. *Anticancer Res* (2019) 39:1447–54. doi: 10.21873/anticanres.13261
29. Bertozzi S, Londero AP, Bernardi S, Cedolini C. Applicability of the Notthingham Prognostic Index for Predicting the Survival of Triple-Negative Invasive Breast Cancer in a Single Italian Center. *Eur J Gynaecol Oncol* (2019) 40:787–90. doi: 10.12892/ejgo4942.2019
30. Tanis PJ, van Sandick JW, Nieweg OE, Valdés Olmos RA, Rutgers EJT, Hoefnagel CA, et al. The Hidden Sentinel Node in Breast Cancer. *Eur J Nucl Med Mol Imaging* (2002) 29:305–11. doi: 10.1007/s00259-001-0732-y
31. Weaver DL, Ashikaga T, Krag DN, Skelly JM, Anderson SJ, Harlow SP, et al. Effect of Occult Metastases on Survival in Node-Negative Breast Cancer. *N Engl J Med* (2011) 364:412–21. doi: 10.1056/NEJMoa1008108
32. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. Early Breast Cancer: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up†. *Ann Oncol* (2019) 30:1194–220. doi: 10.1093/annonc/mdz173
33. Lyman GH, Somerfield MR, Bosserman LD, Perkins CL, Weaver DL, Giuliano AE. Sentinel Lymph Node Biopsy for Patients With Early-Stage Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. *J Clin Oncol* (2017) 35:561–4. doi: 10.1200/JCO.2016.71.0947
34. Hintzen KFH, de Rooij L, Schouten N, van Bastelaar J, Cörvers S, Janssen A, et al. Molecular Analysis of Sentinel Lymph Nodes in Patients With Breast Cancer Using One-Step Nucleic Acid Amplification (OSNA): Does Not Lead to Overtreatment in the Current Era of De-Escalating Axillary Management. *Surg Oncol* (2020) 35:224–8. doi: 10.1016/j.suronc.2020.09.010
35. Saruta Y, Puig-Junoy J. Cost and Budget Impact Analysis of an Accurate Intraoperative Sentinel Lymph Node Diagnosis for Breast Cancer Metastasis. *Appl Health Econ Health Policy* (2016) 14:323–35. doi: 10.1007/s40258-016-0235-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

CLINICAL TRIAL
published: 26 May 2022
doi: 10.3389/fonc.2022.850155

Effectiveness of the Sanyin Formula Plus Chemotherapy on Survival in Women With Triple-Negative Breast Cancer: A Randomized Controlled Trial

Chunyu Wu[1†], Chenping Sun[1†], Guangyu Liu[2,3], Yuenong Qin[1], Xiaohong Xue[4], Xueqing Wu[5], Qun Wang[6], Jin Liu[6], Zhen Ye[1,7], Qiong Li[4], Wenchao Qu[5], Yi Wang[1,7], Shuai Zhang[1], Zhiming Shao[2,3,8] and Sheng Liu[9*]*

[1] Department of Breast Surgery (Integrated Traditional and Western Medicine), Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [2] Department of Breast Surgery, Key Laboratory of Breast Cancer in Shanghai, Fudan University Shanghai Cancer Center, Shanghai, China, [3] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China, [4] Department of Breast Surgery, Yueyang Hospital of Integrated Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [5] Department of Breast, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [6] Department of Surgery (Thyroid and Breast Surgery), Shanghai Traditional Chinese Medicine Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [7] School of Longhua Clinical Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [8] Shanghai Cancer Center and Institutes of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai, China, [9] Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

OPEN ACCESS

Edited by:
Xiaosong Chen,
Shanghai Jiao Tong University, China

Reviewed by:
Raksha Bhat,
Baylor College of Medicine, United States
Yiwei Tong,
Shanghai Jiao Tong University, China

***Correspondence:**
Zhiming Shao
zhimingshao@yahoo.com
Sheng Liu
sliu_tcm@163.com

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: 07 January 2022
Accepted: 29 April 2022
Published: 26 May 2022

Citation:
Wu C, Sun C, Liu G, Qin Y, Xue X, Wu X, Wang Q, Liu J, Ye Z, Li Q, Qu W, Wang Y, Zhang S, Shao Z and Liu S (2022) Effectiveness of the Sanyin Formula Plus Chemotherapy on Survival in Women With Triple-Negative Breast Cancer: A Randomized Controlled Trial. Front. Oncol. 12:850155. doi: 10.3389/fonc.2022.850155

Purpose: To evaluate the efficacy of the Sanyin formula (SYF) plus conventional standard chemotherapy in operable triple-negative breast cancer (TNBC) patients, a randomized controlled trial was implemented at 5 hospitals and cancer centers in China between May 23, 2016, and October 31, 2019.

Materials and Methods: Female patients aged 18 to 80 years with operable TNBC after definitive surgery were screened and enrolled. The exclusion criteria included metastatic disease, other tumors, or locally advanced disease. Patients were randomly divided into groups SYF plus conventional standard chemotherapy and placebo plus conventional standard chemotherapy at a ratio of 1:1. The primary endpoint of the investigation was disease-free survival (DFS), and secondary endpoints included overall survival (OS) and toxicity.

Results: A total of 252 operable female TNBC patients were randomized to receive SYF plus conventional standard chemotherapy (N = 127) or a placebo plus conventional standard chemotherapy (N = 125). At a median follow-up of 51 months, 5-year DFS time was longer in those assigned to SYF plus conventional standard chemotherapy compared with placebo plus conventional standard chemotherapy (94.2%vs 85.5%, hazard ratio [HR] = 0.40; 95%CI, 0.17-0.97; $P = 0.034$). The absolute benefit for 5-year DFS was 8.7% in the SYF plus conventional standard chemotherapy group. No statistically significant difference was observed in OS between the two groups ($P = 0.23$). Patients with negative

node status benefited more from SYF plus conventional standard chemotherapy treatment (HR = 0.21, *P*-interaction = 0.013) in accordance with the exploratory subgroup analyses of DFS.

Conclusions: The results of the present study suggest that the traditional Chinese medicine SYF plus conventional chemotherapy regimens is an effective alternative adjuvant chemotherapy strategy for female operable TNBC patients.

Clinical Trial Registration: https://www.chictr.org.cn/searchproj.aspx, identifier ChiCTR-IPR-16008590.

Keywords: Sanyin formula, traditional Chinese medicine, triple-negative breast cancer, a randomized controlled trial, survival

INTRODUCTION

Triple-negative breast cancer (TNBC) is infiltrating breast cancer with negative estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expressions, which renders it unresponsive to conventional anti-hormonal therapy and anti-HER2-targeted treatments (1). Although TNBC accounts for 15-20% of all female breast cancers, the metastatic occurrence rate of TNBC is the highest, and the overall survival rate is the lowest (2). Due to the lack of approved targeted therapies, surgery, cytotoxic chemotherapy and DNA damaging agents are the current established standard treatments for TNBC (3). Although chemotherapy can significantly improve the clinical outcome of TNBC patients, the recurrence rate is still relatively high, and TNBC tumors are usually resistant to chemotherapy agents (4–6). Therefore, considering the limited treatment options and relatively high invasiveness and recrudescence of TNBC, the development of new TNBC treatment options is crucial.

In China, traditional Chinese medicine (TCM) is widely used in the treatment of cancers (7). The progress of traditional Chinese medicine in the prevention and treatment of cancer has attracted the attention of many countries worldwide in recent years. The US National Cancer Institute Office of Cancer Complementary and Alternative Medicine has increased the incentives for international cooperation and has engaged in extension cooperation in traditional Chinese medicine and cancer research with the Cancer Institute of the China Academy of Chinese Medical Sciences and institutes at the China Academy of Sciences and Chinese Academy of Medical Sciences (8). In contrast to Western medicine, TCM has multiple targets, which gives it various advantages in the treatment of cancers. For example, all-*trans* retinoic acid (ATRA) increases the cellular uptake of arsenic trioxide (ATO) by upregulating aquaporin 9, while ATO inhibits the carcinogenic function of Pin1 by noncovalent binding with the Pin1 active site. ATRA/ATO therapy cooperatively ablates Pin1, which can block many cancer-driving pathways and, finally, inhibit the growth of cancer cells (9). Thus, ATRA plus ATO synergistic targeted therapy was recommended by NCCN as the first choice for acute promyelocytic leukemia (APL) treatment in 2014 (10). A meta-analysis suggested that TCM in combination with Western medicine has advantages over Western medicine in treating TNBC (11). These advantages include reducing the side effects of radiotherapy and chemotherapy, improving the quality of life of patients, preventing tumor metastasis and recurrence, and improving the survival rate of patients. Therefore, the therapeutic strategy of combining traditional Chinese medicine with Western medicine is a new and beneficial strategy for the clinical treatment of TNBC patients.

The Sanyin formula (SYF), which is a traditional Chinese medicine formula that is composed of 9 traditional Chinese medicines, is effective in reducing the recurrence and metastasis of TNBC patients during long-term clinical treatment in our hospital. In our previous prospective cohort study, we investigated the clinical efficacy of SYF for TNBC and found that SYF increased the 2-year disease free survival (DFS) (12). The 2-year DFS was 88.7% for the SYF plus conventional standard chemotherapy group, which was greater than that of the nonexposure control group (82.5%) ($P < 0.05$). The absolute benefit for the 2-year DFS was 6.2% for the SYF group. SYF reduced the disease-related recurrence and metastasis rate by 11.0% (OR = 0.89, 95% CI 0.37-0.96), with a statistically significant difference ($P < 0.05$). However, we do not know the longer-term benefits of SYF in TNBC patients because clinical data over periods of more than 2 years were not obtained. Additionally, this prospective cohort study was not a double-blind randomized controlled trial, and researcher bias may have been present. Therefore, we designed a multicenter, randomized, double-blind, placebo-controlled (RDBPC) trial to observe and verify the clinical efficacy and safety of SYF for operable TNBC patients. The randomized controlled trial (RCT) has been registered and approved in the Chinese Clinical Trial Registry (ChiCTR) (No. ChiCTR-IPR-16008590).

Abbreviations: AC-T, adriamycin and cyclophosphamide plus paclitaxel (Taxol); CEF-T, cyclophosphamide, epirubicin, fluorouracil and docetaxel; ChiCTR: Chinese Clinical Trial Registry; DFS, disease-free survival; EC, epirubicin plus cyclophosphamide; EC-P, epirubicin and cyclophosphamide plus paclitaxel (Taxol); ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; ORR, objective response rate; OS: overall survival; PR: progesterone receptor; RCT, randomized controlled trial; RDBPC, randomized, double-blind, placebo-controlled trial; SYF, Sanyin formula; TCM, traditional Chinese medicine; TNBC, triple-negative breast cancer.

MATERIALS AND METHODS

Study Design and Participants

The randomized double-blind placebo control trial was a randomized and multicenter clinical trial that was carried out in 5 cancer centers and hospitals in China (**Supplement Table 1**). The study protocol was approved by the independent institutional review committees of the participating centers and hospitals (**Supplementary Table 1**). This investigation followed the guidelines of the Consolidated Standards of Reporting Trials (CONSORT). The study was performed according to the ethical principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines. All patients provided written informed consent. Trial registration: Chinese Clinical Trial Registry (ChiCTR), ChiCTR-IPR-16008590. Registered 3 June 2016, https://www.chictr.org.cn/searchproj.aspx.

All patients were screened between May 23, 2016, and October 31, 2019. Female patients aged 18 to 80 years with operable, primary invasive TNBC were included in this study. The estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) statuses were identified according to our previous study (13). The sample size of the study was estimated using Power and Sample Size at http://powerandsamplesize.com/Calculators/ in a compare two proportions manner with the following formula according to a previous study (14).

$$n_A = kn_B, \quad n_B = \left(\frac{p_A(1-p_A)}{k} + p_B(1-p_B)\right)\left(\frac{z_{1-\alpha}+z_{1-\beta}}{p_A-p_B}\right)^2$$

$$1-\beta = \varnothing\left(\frac{|p_A-p_B|}{\sqrt{\frac{p_A(1-p_A)}{n_A}+\frac{p_B(1-p_B)}{n_B}}} - z_{1-\alpha}\right)$$

Here, $\kappa=n_A/n_B$ is the matching ratio, $\varnothing$ is the standard normal distribution function, $\varnothing^{-1}$ is the standard normal quantile function, α is type I error, and β is type II error, meaning $1-\beta$ is power. In this trial, $\kappa = 1$, $\alpha = 0.05$, and $\beta = 0.2$. Substituting the formula, the calculated amount of each group is N = 123. Approximately 252 cases were eventually included.

Inclusion Criteria

The inclusion criteria were as follows: (1) primary breast cancer cases after surgical treatment were clearly diagnosed as malignant epithelial tumors of the breast (breast cancer) by pathological examination, and the results of ER, PR, and Her-2 immunohistochemistry were all negative; (2) newly diagnosed patients had breast cancer before chemotherapy or within 3 months after chemotherapy, and there was no recurrence or metastasis; (3) Karnofsky score ≥ 60 points; (4) patients were female and 18-80 years old with an estimated survival time > 6 months; (5) patients had no severe organic or functional diseases and no drug or food allergies; and (6) patients were willing to accept treatment, observation, and various examinations.

Exclusion Criteria

The exclusion criteria were as follows: (1) patients who did not meet the inclusion criteria; (2) patients for whom tumor markers continued to increase, there were undiagnosed masses in the pelvic or abdominal cavity or organs, or PET indicated recurrence and metastasis; (3) patients who had an obstruction and could not take traditional Chinese medicine; (4) women who were breastfeeding, pregnant, or about to become pregnant; (5) patients with allergies to multiple drugs; (6) patients with severe primary diseases of the cardiovascular, cerebrovascular, liver, kidney, or hematopoietic system or mental illness; and (7) subjects who participated in other drug tests.

Randomization and Blinding

The eligible female cases after breast cancer surgery were randomly assigned to receive the Sanyin formula or placebo (one-tenth dose of SYF) at a ratio of 1:1. The randomization method was implemented by biostatisticians who had no knowledge of the data management or data analysis of this experiment from Shanghai BioGuider Medicinal Technology Co., Ltd. using the SAS 9.3 package in a central random system. After blinding, the blinder mailed one sealed copy of the blind codes to the hospital research office. The other copy was kept by the sponsor. Patients who met the inclusion criteria were randomly assigned after obtaining signed written informed consent. The random number and confirmation code information was entered into the DAS for the IWRS system after the blind coding was completed, and this information was used for the random number application and drug distribution. Data collection and management were managed and preserved by Shanghai BioGuider Medicinal Technology Co., Ltd. (Shanghai, China). All patients were randomly divided into two groups according to the above stratification.

Procedures

The baseline characteristics of the participants were recorded before randomization. All patients received SYF or placebo based on the results of the randomization. All patients received SYF or placebo for at least 2 years. The medicine (dry powder, 2 bags) was administered after being fully dissolved in a suitable amount of hot water (approximately 50-60 ml). The oral dosage was 14 g bid. The detailed protocol was carried out according to our previous study (13). The prescription composition and preparation of SYF and placebo were as follows.

Prescription Composition

The Sanyin formula is a traditional Chinese medicine formula that is composed of the following 9 traditional Chinese herbs: *Codonopsis pilosula* Nannf. (Chinese name: Dangshen), *Atractylodes macrocephala* Koidz. (Chinese name: Baizhu), *Poria cocos (Schw.)* Wolf. (Chinese name: Fuling), *Salviae chinensis* Herba (Chinese name: Shijianchuan), *Curcuma phaeocaulis* Valeton (Chinese name: Ezhu), *Epimedium brevicornu* Maxim. (Chinese name: Yinyanghuo), *Solanum nigrum* Linn. (Chinese name: Longkui), *Scutellariae barbatae* D. Don (Chinese name: Banzhilian), and *Prunella vulgaris* Linn.

(Chinese name: Xiakucao). The total daily dose was 180 g. All Chinese herbal medicines were purchased from Shanghai Kangqiao Traditional Chinese Medicine Pieces Co., Ltd. and were identified by expert traditional Chinese pharmacists at Longhua Hospital.

Preparation of SYF and the Placebo

SYF and the placebo were prepared by Tiangjiang Pharmaceutical Co., Ltd. The prescription composition (180 g), as specified above, was added at a ratio of 1:13 to pure water (2340 ml). Then, the mixture was heated to boiling and kept slightly boiling for 1 hour. The filtrate was obtained after filtration. The obtained residue was decocted again. The filtrates from the two extractions were combined. The filtrate was concentrated under reduced pressure to a liquid with a specific gravity of 1.10-1.12 (65 ± 5°C). Spray drying (process parameters: inlet air temperature 160°C-180°C, outlet air temperature 95°C-105°C) was used to obtain a dry extract powder. The dry extract was ground into 12-40 mesh granules. These particles were packed into aluminum foil bags that had been preprinted with corresponding labels. There was a total of 7.0 g Chinese medicine granules in each bag.

The placebo was obtained by mixing excipients with SYF at a ratio of 9:1. The excipients were composed of the following: lactose, caramel color, sunset yellow basic color, lemon yellow basic color, and a bittering agent, among other compounds. After the excipients were adjusted, they were mixed with the SYF mixture and packaged.

Drug Packaging and Delivery

Drug packaging was carried out by staff who were unrelated to this study and worked at Shanghai BioGuider Medicinal Technology Co. Ltd. The drugs were packaged according to the treatment group that corresponded to the random number that was generated by the software. In this trial, a central random system was used to deliver drugs according to the visit period. Each subject received a completely consistent treatment regimen according to their unique random number. The package number of the drug was entered into the DAS for the IWRS system after blind coding was completed and was used for the drug distribution.

Outcomes

The primary endpoint was DFS, and DFS events were identified in accordance with previous work (13). Briefly, the DFS events included noninvasive and invasive breast cancer recurrences, second primary noninvasive and invasive breast cancers, and second primary non-breast cancers, as well as death from any cause. Secondary endpoints included overall survival (OS) and toxicity. OS was defined as the time from random assignment to death from any cause.

Unblinding

The unblinding date was June 28, 2021. The unblinding table was mailed to the researchers by a third-party data management company (Shanghai BioGuider Medicinal Technology Co. Ltd).

Safety Evaluation

In order to assess drug safety or any adverse effects, patients were observed for safety metrics throughout the study. Blood, urine, stool routine, liver and kidney function, abdominal B-ultrasound, and electrocardiogram were performed every 3 months. If the investigator finds any adverse events (AEs), they should be graded according to the World Health Organization (WHO) criteria for presentation and indicators of acute and subacute toxicity (grades 0, 1, 2, 3, and 4). The correlation between toxic or side effects and drugs was carefully analyzed. In the event of serious adverse reactions, the trial will be terminated by the comprehensive decision of the subjects or investigators.

Statistical Analysis

Data were analyzed from June 29, 2021, to April 22, 2022. For continuous and categorical factors, the Wilcoxon rank-sum test and the $\chi 2$ test were used to evaluate differences between the SYF group and placebo group. The distributions of survival outcomes were calculated by the Kaplan–Meier method. The stratified log-rank test was used to compare the survival curves of the two groups. The stratified Cox proportional hazards model was used to calculate the hazard ratios and 95% confidence intervals (95% CIs). The Cox regression analysis was carried out to analyze the possible interaction between the indicators of the subgroups and the prognosis. Kaplan–Meier curves were adopted to estimate the DFS and OS probabilities at 1, 3, and 5 years. A Z-test was employed to compare the 1-, 3-, and 5-year survival rates between the two groups. All analyses were performed with SPSS statistical software, version 19.0 (IBM Corp., Armonk, NY). The results were plotted using OriginPro statistical software, version 2021b (OriginLab Corp., Northampton, MA). A two-sided *P*-value or *P*-interaction < 0.05 was considered statistically significant according to usual practice.

RESULTS

Patients Characteristics

A total of 322 TNBC patients were screened at 5 hospitals and cancer centers in China between May 23, 2016, and October 31, 2019. Among them, 252 patients were enrolled and randomly divided into two groups: 127 in the SYF treatment group and 125 in the placebo group (**Figure 1**). The baseline characteristics of the 252 patients who completed the investigation were well balanced between the two groups (**Table 1**). The median age of the employed patients was 51 years (interquartile range, 44-60 years) at the randomization time. The main pathological type of the enrolled patients was invasive ductal carcinoma (> 90%) (**Table 1**). Most cases were early-stage TNBC cases (node-negative rate: 62%) (**Table 1**). The chemotherapy regimen for these patients was mainly EC-P (**Supplementary Table 2**). The proportions of EC-P, EC, and CEF-T cells in the placebo group were 46%, 12%, and 11%, respectively. The rates of EC-P, EC, and CEF-T in the SYF groups were 48%, 14%, and 9%, respectively. Approximately two-thirds of the patients underwent a mastectomy to remove the tumor

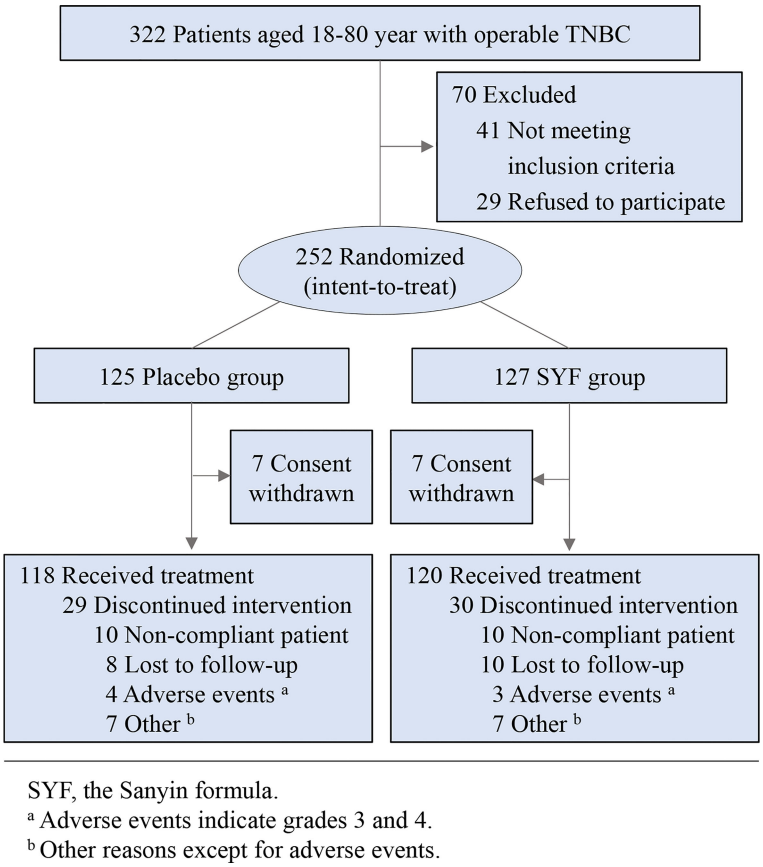


FIGURE 1 | Flow diagram of the study.

mass, and the others underwent BCS (**Table 1**). Tumor size, histological grade, and Ki67 proliferation index were similar between the two groups (**Table 1**). Approximately 77% of the patients completed all trials (**Table 2**).

Efficacy

All patients underwent a minimum of 42 months of follow-up. At a median follow-up of 51 months, 24 (9.5%) DFS events were observed in these female patients (**Table 3**). The results of the Kaplan-Meier curves of DFS suggested that the 5-year DFS time was longer in those assigned to SYF plus conventional standard chemotherapy compared with placebo plus conventional standard chemotherapy (94.2%vs 85.5%, $P = 0.034$) (**Figure 2**). The absolute benefit for 5-year DFS was 8.7% in the SYF plus conventional standard chemotherapy group (**Figure 2**). Only 7 DFS events among 127 TNBC cases were found in the SYF plus chemotherapy group, while 17 events were found among 125 patients in the placebo plus chemotherapy group. The HR of the SYF plus chemotherapy group was 0.40 (95% CI, 0.17-0.97), with a statistically significant difference (stratified log-rank $P = 0.035$) (**Figure 2**).

Although there was no significant difference in the Kaplan-Meier curves of OS between the two treatment groups, the number of death events in the SYF plus chemotherapy group was less than that in the placebo plus chemotherapy group (2 vs. 5, HR, 0.38; 95% CI, 0.074-1.98; stratified log-rank $P = 0.23$) (**Figure 3**). The absolute benefit for the 5-year OS rate in the SYF plus chemotherapy group was 2.2% ($P = 0.26$). However, adequate evaluation of the efficacy of SYF plus chemotherapy on OS in female patients with TNBC requires more events and long-term follow-up.

Node-negative patients benefited more from SYF plus chemotherapy treatment (HR = 0.21; 95% CI, 0.045-0.94, P-interaction = 0.013) in accordance with the exploratory subgroup analyses of DFS (**Figure 4**). Patients of older age, with large tumor size, and a high-grade histological appeared to have benefited more from SYF plus chemotherapy treatment (**Figure 4**). There were no marked differences among the treatment intervention times (before or after chemotherapy) or the types of surgeries (BCS and mastectomy).

Safety

There was no significant difference in treatment-related adverse events (grades 2 to 4) between the two treatment groups throughout the trial. The two treatments were generally well tolerated. There were no treatment-related deaths or life-

TABLE 1 | Patient baseline characteristics.

Characteristic	No. (%) of patients		
	Total (N = 252)	Placebo (N = 125)	SYF (N = 127)
Age, median (IQR), year	51 (44-60)	51 (44-60)	52 (45-61)
Pathological type			
Invasive ductal carcinoma	227 (90.1)	112 (89.6)	115 (90.6)
Others	25 (9.9)	13 (10.4)	12 (9.4)
Age, months			
<50	105 (41.7)	55 (44.0)	50 (39.4)
≥50	147 (58.3)	70 (56.0)	77 (60.6)
Tumor size			
T1	133 (52.8)	58 (46.4)	75 (59.1)
T2-3	104 (41.3)	60 (48.0)	44 (34.6)
Unknown	15 (5.9)	7 (5.6)	8 (6.3)
Histological grade			
I-II	58 (23.0)	28 (22.4)	30 (23.6)
III	171 (67.9)	87 (69.6)	84 (66.2)
Unknown	23 (9.1)	10 (8.0)	13 (10.2)
Ki67 proliferation index (%)			
≤30	56 (22.2)	31 (24.8)	25 (19.7)
>30	188 (74.6)	88 (70.4)	100 (78.7)
Unknown	8 (3.2)	6 (4.8)	2 (1.6)
Node status			
Negative	157 (62.3)	82 (65.6)	75 (59.1)
Positive	68 (27.0)	30 (24.0)	38 (29.9)
Unknown	27 (10.7)	13 (10.4)	14 (11.0)
Administration			
Before chemotherapy	102 (40.6)	50 (40.0)	52 (40.9)
After chemotherapy	150 (59.4)	75 (60.0)	75 (59.1)
Surgery			
BCS	82 (32.5)	45 (36.0)	37 (29.1)
Mastectomy	166 (65.9)	76 (60.8)	90 (70.9)
Unknown	4 (1.6)	4 (3.2)	0 (0.0)

threatening events during the consecutive experimental observation period. Upper limb edema, pain, and diarrhea were observed in the SYF plus chemotherapy group, while liver function injury, weakness, pain, and diarrhea were observed in the placebo group. The overall treatment-related adverse event rates of the experimental group and the placebo group were 2.4% and 3.2%, respectively (**Table 2**).

DISCUSSION

The purpose of this RCT was to determine whether SYF has additional benefits in TNBC adjuvant therapy. The results of the present study indicated that SYF adjuvant therapy plus conventional standard chemotherapy has clear benefits compared with conventional standard chemotherapy.

According to our previous prospective investigation, SYF can increase 2-year DFS in patients with TNBC. The absolute return of 2-year DFS in the SYF plus chemotherapy group was 6.2%, and SYF plus chemotherapy reduced the rate of breast cancer recurrence and metastasis by 11% ($P < 0.05$). In the present study, the absolute return of 5-year DFS in SYF plus chemotherapy group was 8.7%. The 5-year DFS time was longer in those assigned to SYF plus conventional standard chemotherapy compared with placebo plus conventional standard chemotherapy (94.2%vs 85.5%, hazard ratio [HR] = 0.40; 95%CI, 0.17-0.97; $P = 0.034$). The results of the present RCT study not only confirmed this benefit but also further

TABLE 2 | Summary of clinical trial termination.

Reasons	No. (%) of patients	
	Placebo (N = 125)	SYF (N = 127)
Total	29 (23.2)	30 (23.6)
Non-compliant patient	10 (8.0)	10 (7.9)
Lost to follow-up	8 (6.4)	10 (7.9)
Withdrawal of informed consent	7 (5.6)	7 (5.5)
Adverse reactions	4 (3.2)	3 (2.4)

TABLE 3 | First DFS event by treatment.

DFS event	No. (%) of patients	
	Placebo (N = 125)	SYF (N = 127)
Local and regional recurrence	6 (4.8)	1 (0.8)
Contralateral breast tumor	2 (1.6)	2 (1.6)
Distant metastasis	9 (7.2)	4 (3.1)
Death	5 (4.0)	2 (1.6)
Total	17 (13.6)	7 (5.5)

showed that SYF adjuvant therapy can maintain this benefit for a long time. The risk of metastasis and recurrence increases with the prolonged course of the patient's disease. The survival benefit from SYF seems to increase over time. This may be realized by the inhibition of SYF in cancer cell metastasis. In subsequent subgroup analyses, TNBC patients with lymph node-negative benefit more than cases with lymph node-positive (*P*-interaction = 0.013). We will discuss this benefit in the subsequent subgroup analysis. The 5-year DFS in this trial was significantly higher than that in our previous prospective study. We believe that this may be due to two factors. One may be that the number of samples in the previous study was smaller than that in this trial, and the other may be that the tumor burden of participants in this trial was low. The present investigation involved not only a sufficient sample size but also a longer follow-up time. More importantly, this trial was a multicenter, randomized, double-blind, placebo-controlled trial that was more reliable than our previous prospective investigation. Additionally, the 5-year DFS was 94.2% in this study, which was superior to the estimated 86.5% 5-year DFS in our previous PATTERN (adjuvant platinum and taxane in triple-negative breast cancer) trial (15). The results of the present study indicated that SYF adjuvant therapy plus chemotherapy has additional significant benefits compared with recommended conventional standard chemotherapy, even compared with the current best recommended chemotherapy regimens (15). It should be noted that the patients who tend to seek traditional Chinese medicine adjuvant therapies may have a stronger desire for survival than ordinary nontraditional Chinese medicine adjuvant therapy patients (16). Thus, this potential psychological suggestion effect should not be ignored.

According to exploratory subgroup analysis, the patients who are more sensitive to the SYF combination regimen may exhibit similar characteristics, such as old age, larger tumor size, and higher grade of pathology. The subgroup analysis also showed that the benefits of the SYF regimen were significant in patients with lymph node-negative TNBC when compared to in cases with lymph node-positive (*P*-interaction = 0.013). We hypothesized that SYF can effectively inhibit the invasion and metastasis of breast cancer cells in these node-negative patients.

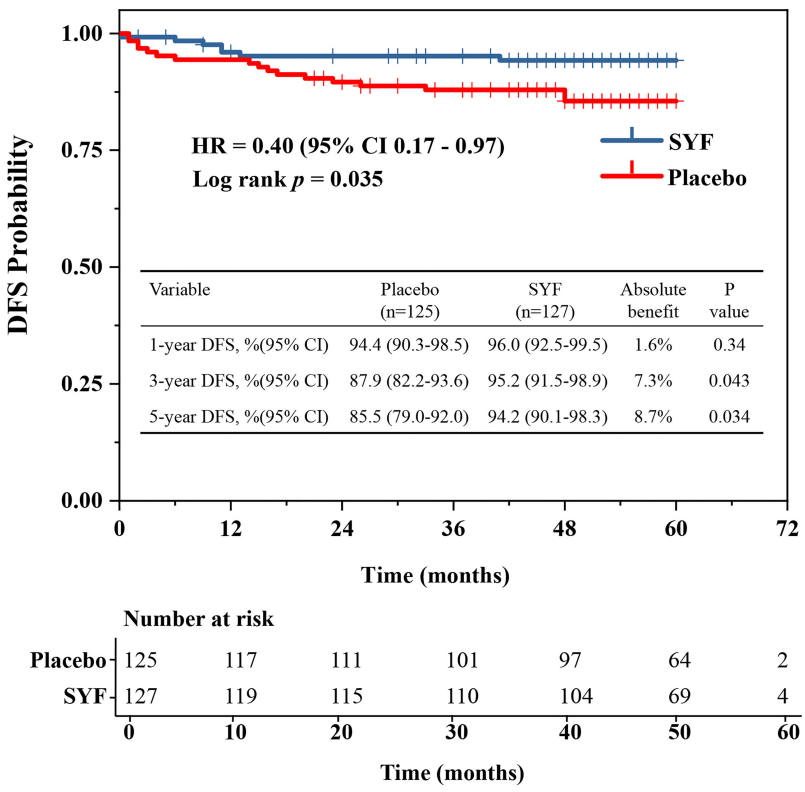


FIGURE 2 | Disease-Free Survival.

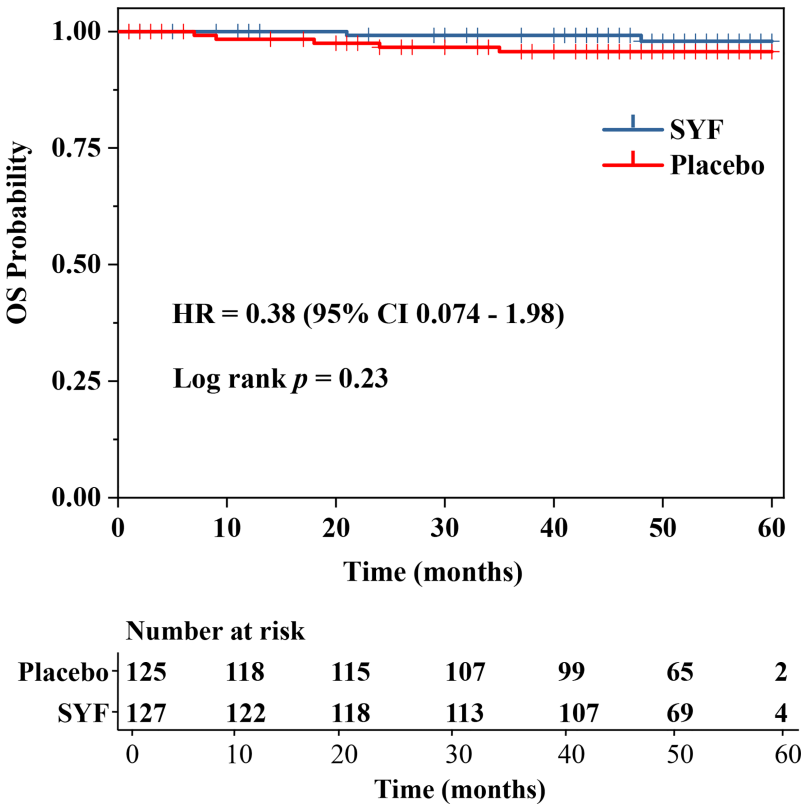


FIGURE 3 | Overall Survival.

A negative lymph node status generally suggests no clinically detectable metastasis or less metastasis, while a positive node status indicates high metastatic capability. A previous study found that lymph node metastasis is not only a marker of late diagnosis of breast cancer but also a marker of the invasive phenotype (17). Early intervention with SYF could effectively inhibit the migration, invasion, and metastasis of breast cancer cells in these patients with negative lymph node status. Although the HR of SYF was also lower (< 0.5) in lymph node-positive patients, the benefit of SYF adjuvant therapy was not significant ($P > 0.19$). There are two possible reasons for this phenomenon. The first is that SYF adjuvant therapy has no additional benefit for TNBC patients with metastases. As discussed above, a positive lymph node status usually indicates a high possibility of metastasis of cancer cells. Indeed, there is a correlation between lymph node metastasis and the tumor immune microenvironment in breast cancer (18). These lymph node-positive patients seem to develop clinically undetectable or detectable metastases. Unfortunately, SFY was ineffective in the treatment of micrometastasis of tumor cells. The second reason is that there may be too few lymph node-positive cases or too few DFS events. Due to the exaggerated 95% CI data range among the subgroups with too few samples, the phenomenon may actually be caused by the small sample size (19). Increasing the number of TNBC cases or prolonging the follow-up time may resolve this issue. In addition, we found that TNBC patients with age ≤ 35 years and regional lymph node stage N1 may be the benefit group of SYF in our previous cohort trial (13). But there were 73 and 75 cases in the exposed and non-exposed groups in our previous study, respectively (13). Therefore, although with a significant *P*-value, we believed that the results analyzed with quite a few cases were not very highly reliable. Although a larger sample size than that in our previous trial was included, a similar situation would occur if the subgroup analysis was performed according to more subgroup types in the present RCT investigation. Therefore, limited subgroup analyses were performed in this study. In the future, more direct experimental evidence should be supplied to evaluate this hypothesis. Another RCT trial with more clinical centers and more patients has been approved by the Shanghai Hospital Development Center (No. SHDC2020CR1050B). The investigation is expected to uncover these confusions, which provide more evidence and reference for the precise treatment of TNBC.

There are several limitations of this study. First, the chemotherapy regimen in this trial was not the only fixed regimen. Although epirubicin and cyclophosphamide (EC) followed by paclitaxel (EC-P) was the principal chemotherapy regimen (> 45% of the total cases) in the present study, which is recommended in the National Comprehensive Cancer Network

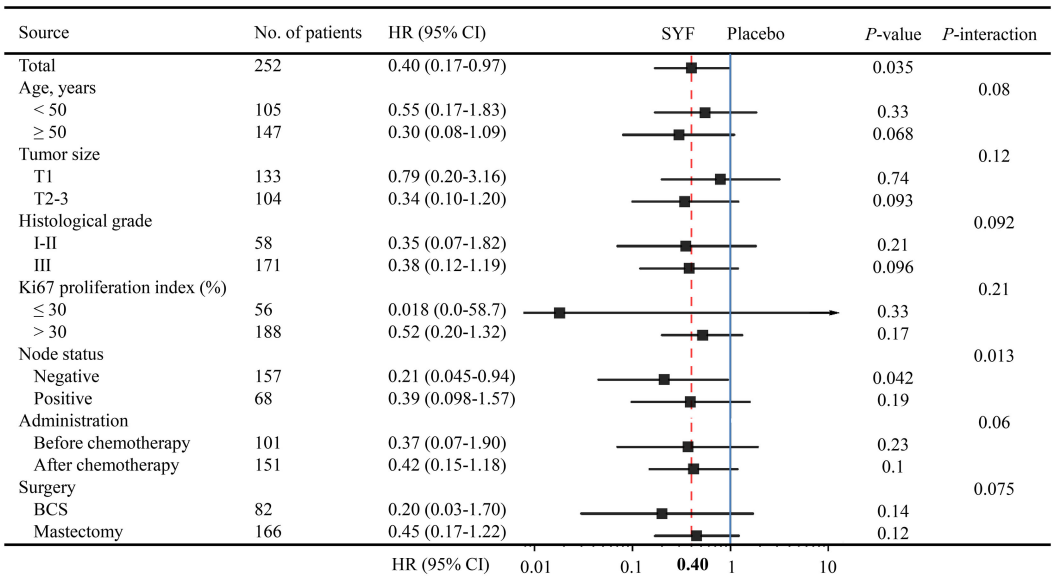


FIGURE 4 | Forest plots of the exploratory subgroup analysis of DFS.

guidelines (ECOG 1199) (20), there were also other chemotherapy regimens, such as CEF-T and AC-T (**Supplementary Table 2**). Second, the sample size of the current study was still relatively small, and the follow-up time was not long enough. Stratifying patients is difficult, especially in OS analysis. Third, due to the high heterogeneity of TNBC, it can be further subdivided into multiple subtypes (21–23). The present study did not perform a TNBC subtype analysis, and additional trials should be carried out to follow the TNBC subtypes. Identifying TNBC patients who may benefit from immunotherapy in advance, and then realizing precise immunotherapy, will be the key to improving the prognosis of TNBC patients. Based on the new TNBC classification, our recent FUTURE trial found that advanced immunomodulatory TNBC patients responded well to immunotherapy (combination regimen consisting of famitinib, camrelizumab, and nab-paclitaxel) and achieved the best objective response rate (ORR) (24). Finally, the patients who participated in this study were early-stage and operable TNBC patients. Whether the advantage of SYF is applicable to patients with advanced-stage TNBC still needs to be determined by further clinical trials.

CONCLUSIONS

In summary, the present RDBPC trial found that compared with conventional chemotherapy regimens, the traditional Chinese medicine SYF plus conventional chemotherapy regimens may be an alternative adjuvant chemotherapy strategy for women with operable TNBC. However, high-level evidence still needs to be collected and examined before traditional Chinese medicine SYF plus chemotherapy regimens can be made the new standard of care.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Chinese Clinical Trial Registry. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception and design: SL and ZS. Collection and assembly of data: CW, GL, QL, WQ, JL, ZY, YW, and SZ. Analysis and interpretation of the data: CW and CS. Drafting of the article: CW and CS. Critical revision for important intellectual content: GL, YQ, XX, XW, and QW. Provision of study materials or patients: GL, YQ, XX, XW, and QW. Obtaining of funding: SL. Final approval of the article: All authors.

FUNDING

This study was supported by grants from the Shanghai Municipal Health Commission (Grant Number ZY3-LCPT-2-1002), and Science and Technology Commission of Shanghai Municipality (Grant Number 20Z21900300), as

well as Shanghai Hospital Development Center (Grant Number SHDC2020CR1050B).

ACKNOWLEDGMENTS

We thank the staff who worked at Tiangjiang Pharmaceutical Company for the preparation of SYF and the placebo. We thank the staff who worked in Shanghai BioGuider Medicinal Technology Company for randomization, blinding, and unblinding and for the drug packaging and delivery. We also thank Prof. Jihan Huang for his assistance with the statistical analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.850155/full#supplementary-material

Supplementary Table 1 | Recruitment of patients by hospital and cancer center.

Supplementary Table 2 | Summary of main chemotherapy regimens. EC-P, epirubicin and cyclophosphamide plus paclitaxel (Taxol); EC, epirubicin plus cyclophosphamide; CEF-T, cyclophosphamide, epirubicin, fluorouracil and docetaxel; AC-T, adriamycin and cyclophosphamide plus paclitaxel (Taxol).

REFERENCES

1. Foulkes WD, Smith IE, Reisfilho JS. Triple–negative Breast Cancer. *N Engl J Med* (2010) 363:1938–48. doi: 10.1056/NEJMra1001389
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2018) 68:394–424. doi: 10.3322/caac.21492
3. Rapoport BL, Nayler S, Demetriou GS, Moodley SD, Benn CA. Triple Negative Breast Cancer Pathologic Diagnosis and Current Chemotherapy Treatment Options. *Oncol Hematol Rev* (2014) 10:35–42. doi: 10.17925/OHR.2014.10.1.25
4. Kelland L. The Resurgence of Platinum–Based Cancer Chemotherapy. *Nat Rev Cancer* (2007) 7:573–84. doi: 10.1038/nrc2167
5. Martin GVM. Neoadjuvant Treatments for Triple–Negative Breast Cancer (TNBC). *Ann Oncol* (2012) 23:35–9. doi: 10.1093/annonc/mds193
6. Joensuu H, Gligorov J. Adjuvant Treatments for Triple–Negative Breast Cancers. *Ann Oncol* (2012) 23:40–5. doi: 10.1093/annonc/mds194
7. Liu J, Wang S, Zhang Y, Fan HT, Lin HS. Traditional Chinese Medicine and Cancer: History, Present Situation, and Development. *Thorac Cancer* (2015) 6:561–9. doi: 10.1111/1759-7714.12270
8. Jia L, Lin H, Oppenheim J, Howard OMZ, Li J, Fan H, et al. US National Cancer Institute–China Collaborative Studies on Chinese Medicine and Cancer. *J Natl Cancer Inst Monogr* (2017) 2017:58–61. doi: 10.1093/jncimonographs/lgx007
9. Kozono S, Lin YM, Seo HS, Pinch B, Lian X, Qiu C, et al. Arsenic Targets Pin1 and Cooperates With Retinoic Acid to Inhibit Cancer–Driving Pathways and Tumor–Initiating Cells. *Nat Commun* (2018) 9:3069. doi: 10.1038/s41467-018-05402-2
10. Chen Z, Chen SJ. Poisoning the Devil. *Cell* (2017) 168:556–60. doi: 10.1016/j.cell.2017.01.029
11. Wang Y, Yao C, He F, Zeng J. Meta–analysis of Effectiveness of Traditional Chinese Medicine or its Combination With Western Medicine in the Treatment of Triple Negative Breast Cancer. *Trop J Pharm Res* (2019) 18:639–45. doi: 10.4314/tjpr.v18i3.28
12. Wang Y, Li JW, Qin YN, Sun CP, Chen JJ, Ruan YY, et al. Clinical Observation on the Effect of Chinese Medicine–"TCM Formula" Intervention on Recurrence and Metastasis of Triple Negative Breast Cancer. *Compl Ther Med* (2020) 52:102456. doi: 10.1016/j.ctim.2020.102456
13. Chen J, Qin Y, Sun C, Hao W, Zhang S, Wang Y, et al. Clinical Study on Postoperative Triple–Negative Breast Cancer With Chinese Medicine. *Medicine* (2018) 97:e11061. doi: 10.1097/MD.0000000000011061
14. Chow S, Shao J, Wang H. Sample Size Calculations in Clinical Research. In: *Chapman & Hall/CRC Biostatistics Series, 2nd Ed*, vol. 89. New York: Taylor & Francie (2008). doi: 10.1201/9781584889830
15. Yu KD, Ye FG, He M, Fan L, Ma D, Mo M, et al. Effect of Adjuvant Paclitaxel and Carboplatin on Survival in Women With Triple–Negative Breast Cancer: A Phase 3 Randomized Clinical Trial. *JAMA Oncol* (2020) 6:1390–6. doi: 10.1001/jamaoncol.2020.2965
16. Chan H, Chui YY, Chan C, Cheng KK, Shiu AT, So WK, et al. Exploring the Influence of Traditional Chinese Medicine on Self–Care Among Chinese Cancer Patients. *Eur J Oncol Nurs* (2014) 18:445–51. doi: 10.1016/j.ejon.2014.05.005
17. Jatoi I, Hilsenbeck SG, Clark GM, Osborne CK. Significance of Axillary Lymph Node Metastasis in Primary Breast Cancer. *J Clin Oncol* (1999) 17:2334–40. doi: 10.1200/jco.1999.17.8.2334
18. Takada K, Kashiwagi S, Asano Y, Goto W, Kouhashi R, Yabumoto A, et al. Prediction of Lymph Node Metastasis by Tumor–Infiltrating Lymphocytes in T1 Breast Cancer. *BMC Cancer* (2020) 20:598. doi: 10.21203/rs.3.rs-23989/v3
19. Cocks K, Torgerson DJ. Sample Size Calculations for Pilot Randomized Trials: A Confidence Interval Approach. *J Clin Epidemiol* (2013) 66:197–201. doi: 10.1016/j.jclinepi.2012.09.002
20. Sparano JA, Wang M, Martino S, Jones V, Perez EA, Saphner T, et al. Weekly Paclitaxel in the Adjuvant Treatment of Breast Cancer. *N Engl J Med* (2008) 358:1663–71. doi: 10.1056/nejmc081136
21. Laurentiis MD, Cianniello D, Caputo R, Stanzione B, Arpino G, Cinieri S, et al. Treatment of Triple Negative Breast Cancer (TNBC): Current Options and Future Perspectives. *Cancer Treat Rev* (2010) 36:80–6. doi: 10.1016/S0305-7372(10)70025-6
22. Jiang YZ, Liu Y, Xiao Y, Hu X, Jiang L, Zuo WJ, et al. Molecular Subtyping and Genomic Profiling Expand Precision Medicine in Refractory Metastatic Triple-Negative Breast Cancer: The FUTURE Trial. *Cell Res* (2021) 31(2):178–86. doi: 10.1038/s41422-020-0375-9
23. Jiang YZ, Ma D, Suo C, Shi J, Xue M, Hu X, et al. Genomic and Transcriptomic Landscape of Triple-Negative Breast Cancers: Subtypes and Treatment Strategies. *Cancer Cell* (2019) 35(3):428–40. doi: 10.1016/j.ccell.2019.02.001
24. Chen L, Jiang YZ, Wu SY, Wu J, Di GH, Liu GY, et al. Famitinib With Camrelizumab and Nab-Paclitaxel for Advanced Immunomodulatory Triple-Negative Breast Cancer (FUTURE-C-PLUS): An Open-Label, Single-Arm, Phase 2 Trial. *Clin Cancer Res* (2022) CCR-21-4313-E.2021. doi: 10.1158/1078-0432.CCR-21-4313

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Oncology

CASE REPORT
published: 30 May 2022
doi: 10.3389/fonc.2022.877014

Cutaneous Breast Cancer Metastasis Is Effectively Treated With Intralesional Interleukin-2 and Imiquimod: A Case Report and Brief Literature Review

Ashley Drohan[1†], *Dejan Vidovic*[2†], *Penelope Jane Barnes*[3], *Carman Anthony Giacomantonio*[2] *and Lucy Kathryn Helyer*[2*]

[1] Department of Surgery, University of Toronto, Toronto, ON, Canada, [2] Department of Surgery, Dalhousie University, Halifax, NS, Canada, [3] Department of Pathology, Dalhousie University, Halifax, NS, Canada

OPEN ACCESS

Edited by:
Kunwei Shen,
Shanghai Jiao Tong University, China

Reviewed by:
Greta Volpedo,
The Ohio State University, United States
Wenjing Peng,
Texas Tech University, United States

***Correspondence:**
Lucy Kathryn Helyer
lhelyer@dal.ca

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *16 February 2022*
Accepted: *28 April 2022*
Published: *30 May 2022*

Citation:
Drohan A, Vidovic D, Barnes PJ, Giacomantonio CA and Helyer LK (2022) Cutaneous Breast Cancer Metastasis Is Effectively Treated With Intralesional Interleukin-2 and Imiquimod: A Case Report and Brief Literature Review. Front. Oncol. 12:877014. doi: 10.3389/fonc.2022.877014

Breast cancer is the most common non-cutaneous cancer affecting women worldwide and is a major cause of cancer-related morbidity and mortality in females. While many women are diagnosed with early-stage disease, a subset of women may present with isolated cutaneous metastases or recurrent locoregional cutaneous metastatic disease. There is a paucity of evidence for effective treatments for cutaneous breast cancer metastases. Herein, we present a case of hormone receptor negative, HER2 positive cutaneous breast cancer metastasis treated with intralesional IL-2 and topical imiquimod, which was well tolerated with only minor low grade side effects. We also present a brief literature review of immunotherapy for cutaneous breast cancer metastasis to frame the discussion around using minimally invasive local therapies for this disease. Together, this limited data suggests that intralesional IL-2 and imiquimod may be considered as a safe option when treating a patient with cutaneous breast cancer metastases.

Keywords: cutaneous breast cancer, intralesional, interleukin-2 (IL-2), IL2, imiquimod, intralesional immunotherapy, intratumoral, cutaneous breast cancer metastasis

INTRODUCTION

Breast cancer is the most common non-cutaneous cancer affecting women worldwide. Approximately 5% of patients present with *de novo* stage IV disease (1), with the most common sites of distant metastasis being bone, lung, and liver (2). Although cutaneous breast cancer metastases are rare (3), 69% of skin metastases among females with cancer are from a primary breast malignancy (4). While a minority of patients present with isolated cutaneous metastasis, most commonly they occur in the setting of distant metastatic disease. Currently, there is no well-defined treatment algorithm for cutaneous metastatic breast cancer.

Intralesional interleukin-2 (IL-2) injections have been used successfully to treat cutaneous metastases in other cancers, including melanoma (5) and porocarcinoma (6). However, the use of

IL-2 in the treatment of breast cancer is not well-described, with a single case study published in 2021 reporting a pathologic complete response after intralesional IL-2 injections in a patient with triple-negative breast cutaneous cancer metastasis (7). Few others have had some success in treating cutaneous metastatic breast cancer with imiquimod, a topical Toll-like receptor 7 (TLR7) agonist (8–10). Herein, we describe the treatment of a patient with ER^-/PR^- $HER2^+$ cutaneous metastatic breast cancer using intralesional IL-2 and topical imiquimod to generate a durable complete clinical response.

CASE DESCRIPTION

A Case of ER^-/PR^- $HER2^+$ Cutaneous Metastatic Breast Cancer

Our patient, a now 81-year-old female, was diagnosed with ER^-/PR^- $HER2^+$ left-sided breast cancer at the age of 54 (1994). She was initially treated with 6 cycles of neoadjuvant 5-fluorouracil, doxorubicin, cyclophosphamide (FAC), followed by lumpectomy, and adjuvant radiotherapy. Five years later, in 1999, she presented with a locoregional recurrence to the left chest wall and axilla, treated with a mastectomy, axillary dissection and adjuvant docetaxel, which was switched to 5-fluorouracil/folinic acid (FUFA) after two cycles due to disease progression. After a period of relative stability, at the age of 67 (2007), she was treated for a left chest wall recurrence with capecitabine and trastuzumab for a total of five years (2007-2012). Trastuzumab was discontinued within the first year of treatment (2007), due to cardiac toxicity. After experiencing stable disease for a period of five years, in 2012 surveillance computed tomography (CT) detected radiographic evidence of soft tissue metastases in the left chest wall, as well as axillary lymphadenopathy. Given the paucity of treatment options at that time, she was switched to vinorelbine. After one year of therapy, surveillance imaging demonstrated disease progression, and her regimen was then changed to paclitaxel with palliative intent. Additionally, she was treated with left upper chest wall radiotherapy. At the time, a repeat multigated acquisition (MUGA) scan revealed an ejection fraction (EF) of 65%; thus, the decision was made to start T-DM1. She remained on T-DM1 for only approximately 6 months, as it was ultimately stopped due to a significant decrease in EF. Additionally, she re-demonstrated disease progression with further development of contralateral (right) axillary lymphadenopathy that was treated with palliative radiotherapy to good effect. At this point, all systemic chemotherapeutic treatments were discontinued, and from 2015 to 2020 she was solely on zoledronic acid. She experienced a five-year period of relatively stable disease, with no further development of metastases on imaging.

After five years of stable disease, at the age of 79, she developed cutaneous lesions over the left chest wall. Excisional biopsy demonstrated viable adenocarcinoma consistent with her previous ER^-/PR^- $HER2^+$ metastatic breast cancer (**Supplementary Figure 1**), with a prominent chronic inflammatory infiltrate composed of lymphocytes and histiocytes (**Figure 1A**). Staging CT did not reveal any distant metastatic disease, and a positron emission tomography-computed tomography (PET-CT) scan demonstrated a solitary 12mm PET avid lesion on the left pectoral muscle, consistent with her physical exam findings (**Figures 1B, C**). Her case was reviewed by a multidisciplinary team, and it was decided to proceed with intralesional IL-2 treatment.

Over the course of the next 14 weeks, she was treated with intralesional IL-2 every two weeks for a total of 7 treatments (**Figure 2**). Each treatment consisting of IL-2 contains 8 million international units (IU) per dose. At her third IL-2 treatment, physical exam revealed the chest wall nodule was now slightly more prominent, suggesting partial response to IL-2: thus, imiquimod cream was added to the biweekly IL-2 injections with the aim to promote and maintain a stronger anti-tumor immune response. Imiquimod is a TLR7 agonist and immunoadjuvant that has been used successfully for the treatment of in-transit melanoma and squamous cell carcinoma (11), and with which we have also had some success in treating primary cutaneous malignancies. It is dosed as daily topical application to the affected area for a total of five days, starting on the day of IL-2 injection. Our patient remained on biweekly IL-2 and imiquimod until the end of 14 weeks (**Figure 2**). Both treatments were very well tolerated, with side effects limited to grade 1/2 self-limiting fever, local injection site erythema, and fatigue all lasting less than 48 hours.

At the 14-week timepoint, a cutaneous chest wall punch biopsy was performed that revealed only a lymphocytic lichenoid infiltrate with vacuolar ulceration, and no evidence of metastatic or recurrent disease (**Figure 3A**). At the three-month follow-up, her physical exam revealed no evidence of cutaneous recurrence, however a palpable nodule was noted in the left flank. A CT scan revealed it was 10mm in size, and a fine-needle aspirate (FNA) demonstrated presence of scant adenocarcinoma cells in the specimen. She was treated with three intralesional IL-2 treatments, again dosed every two weeks. By then, the nodule had clinically completely resolved (**Figure 3B**), and her final PET-CT scan two weeks after the last injection revealed complete resolution of the left chest wall lesion and left flank (**Figure 3C**). At last follow-up, 24 weeks after completing therapy, she remained disease-free with no clinically evident lesions.

DISCUSSION

Herein, we present a case of ER^-/PR^- $HER2^+$ cutaneous metastatic breast cancer treated successfully using a local immunotherapy. Our patient underwent ten intralesional IL-2 treatments biweekly, and five topical imiquimod treatments for a five-day course starting on the third cycle of IL-2 treatments and continuing for five cycles, for a total of 32 weeks of treatment. Both therapies were tolerated very well with minor grade 1/2 adverse events. By the last follow-up 24 weeks after the last cycle of treatment, she had no clinical or radiological evidence of disease. This represents for the first time, to our knowledge,

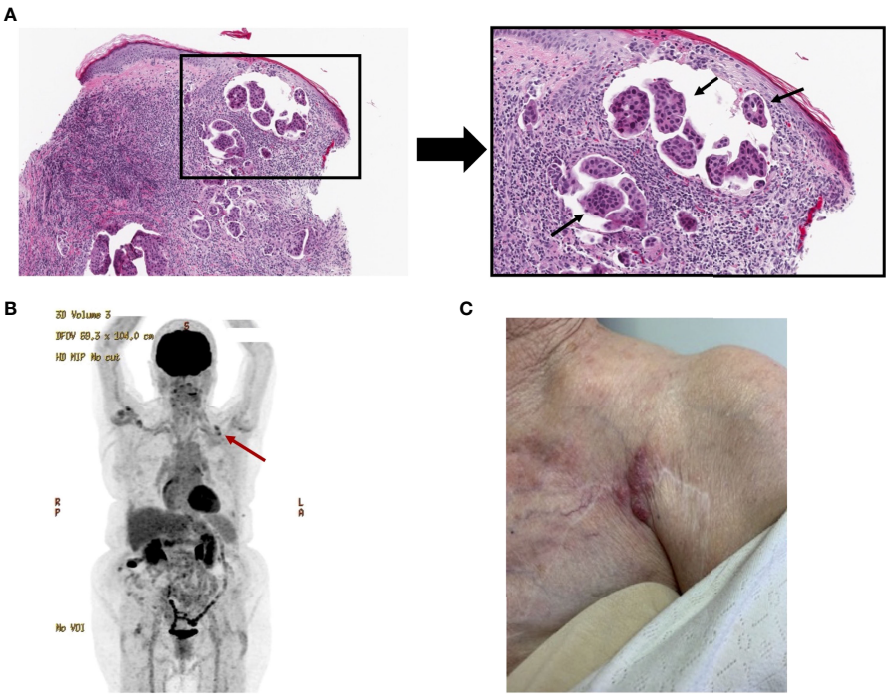


FIGURE 1 | Initial presentation of an 81-year-old female with cutaneous breast cancer metastasis. **(A)** Histological H&E-stained image of cutaneous breast cancer metastasis, under 100X magnification (left) and higher power view, 200X magnification (right) of the metastatic adenocarcinoma, highlighted with arrows. This biopsy was obtained approximately at the first treatment timepoint. **(B)** PET-CT, obtained at the first treatment timepoint, revealing PET-avid uptake overlying the left pectoral muscle, highlighted with red arrow. **(C)** Image of the lesion pre-treatment.

the treatment of cutaneous breast cancer metastasis with intralesional IL-2 and topical imiquimod immunotherapy.

In the last decade, there has been interest in the use of immunotherapies to treat cancer. Numerous immunotherapeutic agents have been trialed in many different cancers, such as chimeric antigen receptor T cell (CAR-T cell) therapies, immune checkpoint inhibitors, oncolytic viruses, and tumor vaccines. Immunotherapy in breast cancer is also an actively evolving field, illustrated by the recent FDA approval of pembrolizumab (a PD-1 inhibitor) (12) to be used as both a neoadjuvant therapy in combination with chemotherapy, or as adjuvant treatment alone for high-risk early stage triple-negative breast cancer (TNBC).

A significant challenge in the development and prospect of immunotherapy being used in breast cancer is the notion that compared to other cancers like melanoma or non-small cell

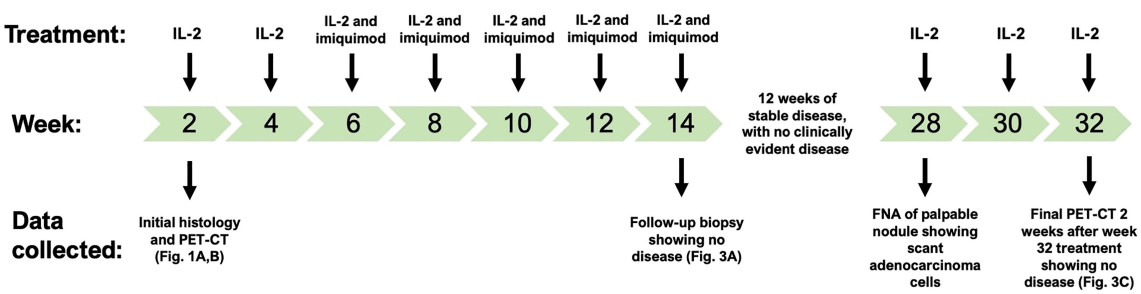


FIGURE 2 | Treatment timeline for intralesional IL-2 injections and topical imiquimod. The patient was initially treated with IL-2 monotherapy, but showed only a partial response, after which imiquimod was added. The initial histology and PET-CT (**Figure 1**) were collected at week 2, the first treatment. Following 12 weeks of clinical stability with no evidence of disease, an FNA positive for adenocarcinoma led to three more IL-2 treatments, which lead to a complete clinical and radiological response (**Figure 3**).

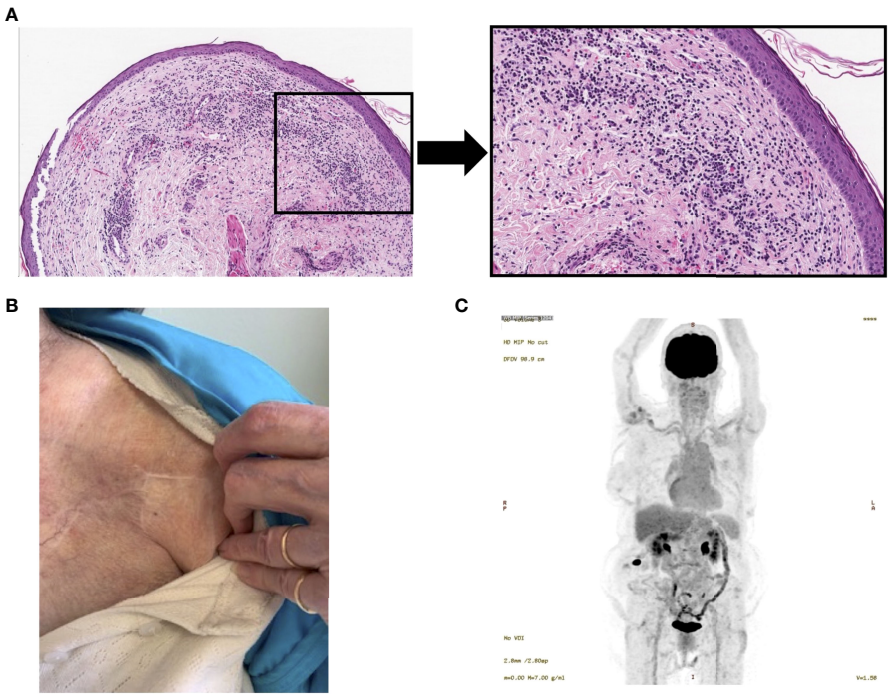


FIGURE 3 | Resolution of cutaneous metastasis following intralesional IL-2 and topical imiquimod. **(A)** Punch biopsy of injected site at the 14-week timepoint, 100X magnification (left) and representative section, 200X magnification (right) showing no evidence of adenocarcinoma, with chronic immune infiltrates. **(B)** Image of lesion post-treatment, showing complete clinical response. **(C)** Post-treatment PET-CT (2 weeks after the final treatment; week 34) revealing no evidence of PET-avid lesions overlying the left pectoral muscle.

lung cancer, breast cancer is predominantly immunologically quiescent (13). There are several steps required for the generation of anti-tumor immune responses, including adequate T cell trafficking into tumors, immunogenic cell death with neoantigen generation, and adequate neoantigen presentation. All or some are required to generate robust and potentially systemic anti-tumor immunity (14). Certainly, breast carcinomas that have a high mutational burden lend to having higher response rates and progression-free survival following immunotherapy. This is thought to be due to improved neoantigen stimulation of the immune system (15).

Within the landscape of breast cancer, there is evidence that ER^-/PR^- HER2+ tumors (i.e. the biomarker profile of our patient's disease) have higher mutational burden than hormone receptor positive tumors (16, 17), which is consistent with the observed clinical trial data leading to approval of pembrolizumab. In addition, approximately only 11% of breast cancers have a lymphocyte-predominant phenotype (greater than 50% tumor infiltrating lymphocytes, TILs) (18). $HER2^+$ and TNBC tumors have the highest proportion of a lymphocyte-predominant phenotype (18). Clinically, patients with $HER2^+$ cancers with higher percentages of TILs have a higher percentage of pathological complete responses following neoadjuvant trastuzumab and lapatinib, compared to HER2- patients (19). Our patient had histopathology suggestive of a significant inflammatory infiltrate, which may have aided in the generation of a complete response.

There is a paucity of data examining the immune microenvironment of cutaneous breast cancer metastases. However, other primary cutaneous malignancies, such as melanoma and cutaneous squamous cell carcinoma (cSCC) are regularly treated with local and/or intralesional immunotherapies (5). For cutaneous metastatic breast cancer, some intralesional therapies have been attempted in the past to varying success. Intralesional interferon alpha (IFNa) and interferon gamma (IFNg) achieved between a 43-71% lesional complete response rate, with evidence of anti-tumor immune responses in noninjected lesions (20). On the other end of the spectrum, intralesional injection of adenoviral vectors encoding IL-2 was not able to generate any conventional clinical responses (21). Similarly, a recent preclinical trial using c-Met-targeted CAR-T cells injected intralesionally in patients with cutaneous breast cancer metastases did not generate any clinical responses (22). Of note, a recent phase II trial using topical imiquimod monotherapy for cutaneous breast cancer metastases achieved a 20% partial response rate, also revealing that the cytokine response in the tumor microenvironment was enriched with activated $CD8^+$ and $CD4^+$ T cells, with increased local anti-tumor cytokine expression (23). Similarly, another recent phase II trial using systemic paclitaxel combined with topical imiquimod for cutaneous

metastatic breast cancer generated a 36% complete response rate and 72% overall response rate (10). Other, smaller case studies have reported similar favorable findings using imiquimod with some patients experiencing complete responses (24). Together, this data illustrates imiquimod may have a favorable use in this setting. Intralesional IL-2, on the other hand, has not widely been used in cutaneous metastatic breast cancer, outside of a recent case report in 2021 (7). Certainly, in our patient, intralesional IL-2 monotherapy was able to generate a partial response initially. It was not until the addition of imiquimod that our patient experienced a complete response, which was then bolstered with additional IL-2 injections to a durable complete response.

Certainly, the use of both drugs led to a synergistic response in our patient. While the exact mechanism of action of these two drugs in this context has not been extensively studied, we hypothesize that the IL-2 likely provided a short-lived pulse of T cell-mediated anti-tumor immune activity, and the addition of imiquimod synergized the response further by acting as a strong local immune activator *via* TLR7 stimulation. Together, the two drugs were able to overcome immune quiescence through active stimulation of both arms of the immune system. In doing so, a robust anti-tumor immunity was mounted which led to tumor clearance.

In conclusion, our case has demonstrated the successful treatment of HER2+ cutaneous breast metastases with intralesional IL-2 and imiquimod. At present, our patient has tolerated 32 total weeks of treatment with minimal side effects and has no clinical or radiographic evidence of disease recurrence 24 weeks after her last treatment. These results suggest that intralesional IL-2 and topical imiquimod may be a durable treatment option among patients with cutaneous metastasis from breast cancers. While our patient experienced very minimal side effects, further study into safety profile of these drugs, especially in combination, is required. In addition, a potential drawback of this approach for both practitioners and patients are the frequency and duration of treatments. As this is a study of only a single case, further research to fully investigate this approach is required.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

AD, DV, and LH contributed to data collection, project conception, and design of this manuscript and wrote sections of the manuscript. LH also contributed to project funding. PB contributed to data collection and manuscript editing. CG contributed to project design and manuscript editing. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.877014/full#supplementary-material

Supplementary Figure 1 | Excisional biopsy of local recurrence showing HER2 positive (3+) immunohistochemical staining of a subcutaneous tumor deposit, 100X magnification (left) and 200X magnification (right).

REFERENCES

1. Bryan S, Masoud H, Weir HK, Woods R, Lockwood G, Smith L, et al. Cancer in Canada: Stage at Diagnosis. *Health Rep* (2018) 29:21–5.
2. Wang R, Zhu Y, Liu X, Liao X, He J, Niu L. The Clinicopathological Features and Survival Outcomes of Patients With Different Metastatic Sites in Stage IV Breast Cancer. *BMC Cancer* (2019) 19:1091. doi: 10.1186/s12885-019-6311-z
3. Johnson C, Friedmann DP, Gade A, Dhawan N, Hinchee-Rodriguez K, Mishra V, et al. Cutaneous Manifestation as Initial Presentation of Metastatic Breast Cancer: A Systematic Review. *Cutis* (2021) 107:E29–36. doi: 10.12788/cutis.0223
4. Brownstein M, Helwig E. Patterns of Cutaneous Metastasis. *Arch Dermatol* (1972) 105:862–8. doi: 10.1001/archderm.1972.01620090034008
5. Vidovic D, Giacomantonio C. Insights Into the Molecular Mechanisms Behind Intralesional Immunotherapies for Advanced Melanoma. *Cancers* (2020) 12:1321. doi: 10.3390/cancers12051321
6. Drohan A, Melvin J, Murphy J, Giacomantonio C, Helyer L. Resolution of Metastatic Subungal Eccrine Porocarcinoma Treated With Intralesional Interleukin-2. *Curr Oncol Tor Ont* (2020) 28:220–5. doi: 10.3390/curroncol28010024
7. Gorman BG, Jakub JW, Hobday TJ, Brewer JD. Intratumoral Interleukin-2 Injections Without Systemic Therapy for Isolated Cutaneous Metastatic Breast Cancer. *Dermatol Surg Off Publ Am Soc Dermatol Surg Al* (2021) 47:1119–21. doi: 10.1097/DSS.0000000000003050
8. Henriques L, Palumbo M, Guay M, Bahoric B, Basik M, Kavan P, et al. Imiquimod in the Treatment of Breast Cancer Skin Metastasis. *J Clin Oncol Off J Am Soc Clin Oncol* (2014) 32:e22–25. doi: 10.1200/JCO.2012.46.4883
9. Nguyen AL, Chong EG, Lee J, Mirshahidi S, Mirshahidi H. A Case Report of Imiquimod Topical Therapy as Treatment for Cutaneous Metastasis of Breast Cancer. *Rare Tumors* (2021) 13:2036361320975748. doi: 10.1177/2036361320975748
10. Salazar LG, Lu H, Reichow JL, Childs JS, Coveler AL, Higgins DM, et al. Topical Imiquimod Plus Nab-Paclitaxel for Breast Cancer Cutaneous Metastases: A Phase 2 Clinical Trial. *JAMA Oncol* (2017) 3:969–73. doi: 10.1001/jamaoncol.2016.6007
11. Vidovic D, Simms GA, Pasternak S, Walsh M, Peltekian K, Stein J, et al. Case Report: Combined Intra-Lesional IL-2 and Topical Imiquimod Safely and Effectively Clears Multi-Focal, High Grade Cutaneous Squamous Cell Cancer in a Combined Liver and Kidney Transplant Patient. *Front Immunol* (2021) 12:1321. doi: 10.3389/fimmu.2021.678028

12. Schmid P, Cortes J, Pusztai L, McArthur H, Kummel S, Bergh J, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. *N Engl J Med* (2020) 382:810–21. doi: 10.1056/NEJMoa1910549
13. Adams S, Gatti-Mays ME, Kalinsky K, Korde LA, Sharon E, Amiri-Kordestani L, et al. Current Landscape of Immunotherapy in Breast Cancer: A Review. *JAMA Oncol* (2019) 5:1205–14. doi: 10.1001/jamaoncol.2018.7147
14. Chen DS, Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* (2013) 39:1–10. doi: 10.1016/j.immuni.2013.07.012
15. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. *N Engl J Med* (2014) 371:2189–99. doi: 10.1056/NEJMoa1406498
16. Budczies J, Bockmayr M, Denkert C, Klauschen F, Lennerz JK, Gyorffy B, et al. Classical Pathology and Mutational Load of Breast Cancer – Integration of Two Worlds. *J Pathol Clin Res* (2015) 1:225–38. doi: 10.1002/cjp2.25
17. Luen S, Virassamy B, Savas P, Salgado R, Loi S. The Genomic Landscape of Breast Cancer and Its Interaction With Host Immunity. *Breast Edinb Scotl* (2016) 29:241–50. doi: 10.1016/j.breast.2016.07.015
18. Stanton SE, Adams S, Disis ML. Variation in the Incidence and Magnitude of Tumor-Infiltrating Lymphocytes in Breast Cancer Subtypes: A Systematic Review. *JAMA Oncol* (2016) 2:1354–60. doi: 10.1001/jamaoncol.2016.1061
19. Salgado R, Denkert C, Campbell C, Savas P, Nuciforo P, Aura C, et al. Tumor-Infiltrating Lymphocytes and Associations With Pathological Complete Response and Event-Free Survival in HER2-Positive Early-Stage Breast Cancer Treated With Lapatinib and Trastuzumab: A Secondary Analysis of the NeoALTTO Trial. *JAMA Oncol* (2015) 1:448–54. doi: 10.1001/jamaoncol.2015.0830
20. Habif DV, Ozzello L, De Rosa CM, Cantell K, Lattes R. Regression of Skin Recurrences of Breast Carcinomas Treated With Intralesional Injections of Natural Interferons Alpha and Gamma. *Cancer Invest* (1995) 13:165–72. doi: 10.3109/07357909509011686
21. Stewart AK, Lassam NK, Quirt IC, Bailey DJ, Rotten LE, Krajden M, et al. Adenovector-Mediated Gene Delivery of Interleukin-2 in Metastatic Breast Cancer and Melanoma: Results of a Phase 1 Clinical Trial. *Gene Ther* (1999) 6:350–63. doi: 10.1038/sj.gt.3300833
22. Tchou J, Zhao Y, Levine BL, Zhang PJ, Davis MM, Melenhorst JJ, et al. Safety and Efficacy of Intratumoral Injections of Chimeric Antigen Receptor (CAR) T Cells in Metastatic Breast Cancer. *Cancer Immunol Res* (2017) 5:1152–61. doi: 10.1158/2326-6066.CIR-17-0189
23. Adams S, Kozhaya L, Martiniuk F, Meng T, Chiriboga L, Liebes L, et al. Topical TLR7 Agonist Imiquimod can Induce Immune-Mediated Rejection of Skin Metastases in Patients With Breast Cancer. *Clin Cancer Res Off J Am Assoc Cancer Res* (2012) 18:6748–57. doi: 10.1158/1078-0432.CCR-12-1149
24. Krishnasamy SR, Almazan TH, Suero-Abreu GA, Jung JY. Successful Treatment of Cutaneous Metastatic Breast Cancer With Topical Treatments That Potentially Synergize With Systemic Therapy: A Case Series. *JAAD Case Rep* (2018) 4:711–5. doi: 10.1016/j.jdcr.2018.04.013

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 23 June 2022
doi: 10.3389/fonc.2022.833093

Association of Molecular Biomarker Heterogeneity With Treatment Pattern and Disease Outcomes in Multifocal or Multicentric Breast Cancer

Shuai Li, Jiayi Wu, Ou Huang, Jianrong He, Weiguo Chen, Yafen Li, Xiaosong Chen and Kunwei Shen**

Department of General Surgery, Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

OPEN ACCESS

Edited by:
Cristian Scatena, University of Pisa, Italy

Reviewed by:
Hiroko Yamashita, Hokkaido University, Japan
Marco Invernizzi, University of Eastern Piedmont, Italy
Feng Wang, Affiliated Hospital of Nantong University, China

***Correspondence:**
Xiaosong Chen
chenxiaosong0156@hotmail.com
Kunwei Shen
kwshen@medmail.com.cn

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *10 December 2021*
Accepted: *20 May 2022*
Published: *23 June 2022*

Citation:
Li S, Wu J, Huang O, He J, Chen W, Li Y, Chen X and Shen K (2022) Association of Molecular Biomarker Heterogeneity With Treatment Pattern and Disease Outcomes in Multifocal or Multicentric Breast Cancer. Front. Oncol. 12:833093. doi: 10.3389/fonc.2022.833093

Purpose: This study aimed to evaluate the rates of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki67 heterogeneity in multifocal or multicentric breast cancer (MMBC) and its association with treatment pattern and disease outcomes.

Methods: MMBC patients with ER, PR, HER2, and Ki67 results for each tumor focus were retrospectively analyzed using Kappa test and categorized into the homogeneous group (Homo group) and the heterogeneous group (Hetero group). Chi-square tests were performed to compare the clinical features and treatment options between the groups. Disease-free survival (DFS) and overall survival (OS) rates were estimated from Kaplan–Meier curves and compared between two groups.

Results: A total of 387 patients were included, and 93 (24.0%) were classified into the Hetero group. Adjuvant endocrine therapy was more frequently assigned for patients in the Hetero group than in the Homo group (84.9% vs. 71.7%, $p = 0.046$). There was no difference in terms of adjuvant anti-HER2 therapy (28.3% vs. 19.6%, $p = 0.196$) and chemotherapy (69.9% vs. 69.8%, $p = 0.987$) usage between the two groups. At a median follow-up of 36 months, DFS rates were 81.2% for the Hetero group and 96.5% for the Homo group ($p = 0.041$; adjusted *HR*, 2.95; 95% CI, 1.04–8.37). The estimated 3-year OS rates for the groups were 95.8% and 99.5%, respectively ($p = 0.059$; adjusted *HR*, 5.36; 95% CI, 0.97–29.69).

Conclusion: Heterogeneity of ER, PR, HER2, or Ki67 was present in 24.0% patients with MMBC. Biomarkers heterogeneity influenced adjuvant endocrine therapy usage and was associated with worse disease outcomes, indicating further clinical evaluation.

Keywords: biomarkers, breast neoplasms, intertumoral heterogeneity, multifocal, multicentric, prognosis

INTRODUCTION

Breast cancer is a heterogeneous group of diseases in which individual patient differs in morphological features, molecular profiles, therapeutic responses, and prognosis (1). Morphological variability such as pathological type and histological grade has been well documented for decades and forms the basis for histological classification of breast cancer. More recently, different molecular phenotypes of breast cancer have been defined by genetic or immunohistochemistry testing. For example, the well-defined 2013 St Gallen subtypes of breast cancer were based on the expressions of estrogen (ER) and progesterone (PR) receptors, human epidermal growth factor receptor 2 (HER2), and Ki67 proliferative index, which provide prognostic information and can be used to tailor systemic adjuvant therapy (2).

The molecular heterogeneity can occur either between different tumors within the same patient (intertumoral heterogeneity) or within the same tumor (intratumoral heterogeneity) (1). Heterogeneous expressions of ER, PR, HER2, and Ki67 have been widely reported between core needle biopsy and surgical samples, between different regions of a primary tumor, between a primary tumor and a matched metastatic lesion, or between metastatic lesions (3–9). Beyond spatial heterogeneity, heterogeneity can be observed as the natural evolution of a tumor or as consequences of anticancer treatments (10–12).

Multifocal/multicentric breast cancer (MMBC) has become more frequently diagnosed with the popular breast cancer screening program and the advancement of imaging methods (13, 14). In a previous study that evaluated the heterogeneity of ER, PR, HER2, and Ki67 between different foci in MMBC, the heterogeneity of these molecular markers was present in 4.4%, 15.9%, 9.7%, and 15.0% cases (13). MMBC with biomarkers heterogeneity represents a situation in breast cancer treatment where there are few guidelines to direct care. However, there are few studies investigating the therapeutic and prognostic impact of such heterogeneity. Herein, we performed this retrospective study to evaluate the rates of ER, PR, HER2, and Ki67 heterogeneity in patients with MMBC and its impacts on systemic adjuvant therapy decision-making and disease outcomes.

METHODS

Study Population

Patients who received surgery and were diagnosed with multifocal or multicentric breast cancer at Department of General Surgery, Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine from January 2009 to December 2018 were retrospectively analyzed. Clinicopathological characteristics, adjuvant treatment, and follow-up data were retrieved from Shanghai Jiao Tong University Breast Cancer Database (SJTU-BCDB). The eligibility criteria were as follows: (1) at least one invasive tumor focus; (2) no distant metastasis at diagnosis; and (3) ER, PR, HER2, and Ki67 both tested between different tumor foci. Those who received neo-adjuvant therapy and those with only *in situ* tumor foci were excluded from the present study. Patients who did not have all samples tested for biomarkers were also exploratorily evaluated for disease outcomes.

Histopathology Assessments

Histopathology analysis for different tumor foci on surgical specimens were independently performed and reviewed by two pathologists at the Department of Pathology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (15, 16). In this study, multifocality was defined as the presence of more than one focus of carcinoma in one breast quadrant (MFBC), and multicentricity was defined as the presence of a focus in a different breast quadrant from the main lesion (MCBC) (13). Immunohistochemistry (IHC) of ER, PR, Ki67, and HER2 were performed on 4-μm slices of formalin-fixed paraffin-embedded (FFPE) specimens with primary antibodies against ER (SP1, 1:100, Dako, Denmark), PR (PgR 636, 1:100, Dako, Denmark), HER2 (4B5, Roche, Switzerland), Ki67 (MIB-1, 1:100, Dako, Denmark) by Ventana autostain system, BenchMark XT as previously described (15). In brief, the tissue sections were incubated with primary antibody of ER, PR, and Ki67 for 32 min at 42°C and of HER2 for 16 min at 42°C, which were then counterstained with hematoxylin. ER/PR was considered positive if there were ≥1% of the tumor cells with nuclear staining (16). HER2 was scored as 0 to 3+ by IHC, and those with IHC 2+ were further examined with fluorescence *in situ* hybridization (FISH) according to the ASCO/CAP guidelines, where HER2 positivity was defined as either IHC 3+ or IHC 2+ with FISH amplification (17–19). The Ki67 index was scored as the percentage of positively nuclear staining cells among at least 500–2,000 uniformly distributed cells or 2,000 cells from the hotspot and negative areas (20). Molecular subtypes were determined based on 2013 St Gallen system: luminal A-like (ER+/PR ≥ 20%/HER2-/Ki67 < 20%), luminal B-like (HER2−) (ER+/HER2−/Ki67 ≥ 20% or ER+/PR < 20%/HER2 or ER−/PR+/HER2−), luminal B-like (HER2+) (ER+ or PR+/HER2+), HER2+ (ER−/PR−/HER2+), and triple negative (ER−/PR−/HER2−) (2). Patients with concordant status of ER, PR, HER2, and Ki67 among all tumor foci were categorized into the homogeneous group (Homo group), while the heterogeneous group (Hetero group) was defined as the existence of at least one discordance for ER, PR, HER2, or Ki67 between different foci. The main focus referred to the largest tumor focus, and the other foci were named minor foci. Distance between the main and minor foci was assessed on pathological specimens, which was defined as the shortest distance between the edges of two tumor foci.

Treatment and Follow-Up

Adjuvant treatment decisions were made through multidisciplinary team (MDT) meetings attended by surgical oncologists, medical oncologists, radiation oncologists, and pathologists (21). Decision was tailored according to the tumor biological features, stage at diagnosis, patient medical complications, and preferences. The patients were followed up every 3 months during the first 2 years after surgery, every 6 months from the third to the fifth year and once per year hereafter till death. DFS was defined as the period from the date of surgery to first local-regional relapse, contralateral breast cancer, secondary new malignant tumor, distant relapse, or death. OS was calculated from the date of surgery to the date of

death. For patients who were free from DFS/OS events at the time of last follow-up, DFS/OS were calculated as the period from the date of surgery to the date of last follow-up.

Statistics

Kappa tests were performed to evaluate the concordance rates of pathological type, histological grade, ER, PR, HER2, Ki67, and molecular subtype between the larger tumor focus and the smaller focus. For tumors with three or four foci, the results were considered concordant only when the biomarkers status of all tumor foci were concordant. The clinical features and adjuvant therapy options were compared between the Homo group and the Hetero group using chi-square test or Fisher's exact test. DFS and OS rates were estimated from Kaplan–Meier curves and compared between the two groups *via* log-rank test. Cox proportional hazard model was used to calculate the hazard ratios for relapse and death. Clinical features and disease outcomes were also compared between MFBC and MCBC. Two-side $p < 0.05$ was considered statistically significant. All the statistical procedures were performed on SPSS (version 26.0).

RESULTS

Baseline Clinicopathological Characteristics

There were 8,210 stage II–III breast cancer patients who received surgery from January 2009 to December 2018 at Ruijin Hospital, among which 584 (7.11%) women were diagnosed with multifocal or multicentric breast cancers and 387 were included in the study (**Figure 1**). There were 52, 76, and 69 cases who were excluded from the study, as they had *in situ* foci only, received neo-adjuvant therapy, or lacked molecular markers data, respectively. Physical examination, sonography, mammography, and MRI identified 16.9%, 66.8%, 33.4%, and 77.2% of these patients, respectively (**Supplementary Figure S1**). As shown in the **Supplementary Figure S2**, the median distance between the main and minor foci was 12.6 [interquartile range (IQR), 7.2–20.0] mm, which showed no significant difference between the two groups (12.4 mm vs. 15.0 mm, $p = 0.082$).

The demographic and clinicopathological characteristics for the cohort are summarized in **Table 1**. The median age for the patients was 55 (IQR, 46–64) years, and 41.5% patients were pre/peri-menopausal at diagnosis. Patients with two foci accounted for 91.5% and 65.4% had multifocal diseases. Comparisons of MCBC and MFBC are summarized in **Supplementary Table S1**. Thirty-four (8.8%) patients received breast-conserving surgery, and sentinel lymph node biopsy was performed in 115 (31.3%) patients. There were 151 (39.0%) patients whose main tumor foci were larger than 2.0 cm, and 144 (37.2%) patients had positive axillary lymph nodes (ALN). A total of 77 (19.9%) and 140 (36.2%) were diagnosed with non-IDC in the main and minor tumor foci, respectively (**Supplementary Figure S3**). Luminal A-like, luminal B-like (HER2-), luminal B-like (HER2+), HER2 +, and triple negative breast cancers were present in 117 (30.2%), 135 (34.9%), 50 (12.9%), 47 (12.1%), and 38 (9.9%) patients, respectively. There were significant differences in terms of

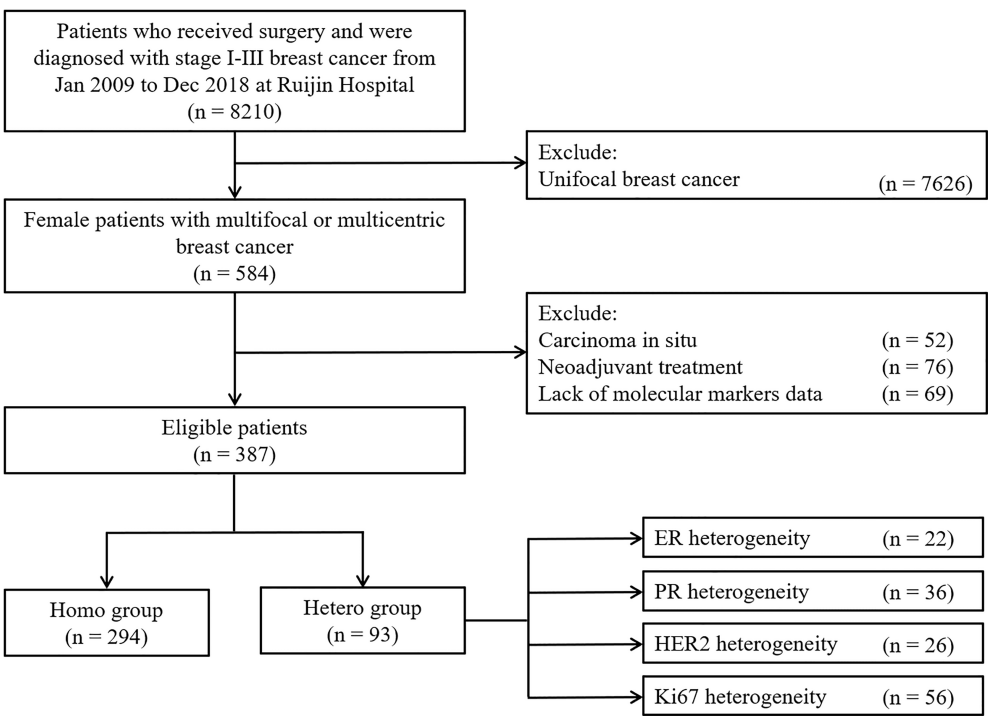


FIGURE 1 | Flow chart of 387 patients in the study.

TABLE 1 | Baseline clinical and pathological characteristics.

Characteristics	Total N = 387 (%)	Homo N = 294 (%)	Hetero N = 93 (%)	*p*-value
Age (y/o)	**55 (46–64)**	**55 (46–64)**	**55 (47–65)**	**0.619**
Menstrual status				**0.538**
Pre/Peri-	160 (41.5)	124 (42.3)	36 (38.7)	
Post-	226 (58.5)	169 (57.7)	57 (51.3)	
Number of foci				**0.378**
2	354 (91.5)	271 (92.2)	83 (89.2)	
3/4	33 (8.5)	23 (7.8)	10 (10.8)	
Location of foci				**0.842**
Multifocal	253 (65.4)	193 (65.6)	60 (64.5)	
Multicentric	134 (34.6)	101 (34.4)	33 (35.5)	
Breast surgery				**0.727**
BCS	34 (8.8)	25 (8.5)	9 (9.7)	
Mastectomy	353 (91.2)	269 (91.5)	84 (90.3)	
Axillary surgery				**0.733**
SLNB	115 (31.3)	89 (30.9)	26 (32.9)	
ALND	252 (68.7)	199 (69.1)	53 (67.1)	
Pathological type[a]				**0.656**
IDC	310 (80.1)	237 (80.6)	73 (78.5)	
Non-IDC	77 (19.9)	57 (19.4)	20 (21.5)	
Pathological type[b]				**<0.001**
IDC	247 (63.8)	211 (71.8)	36 (38.7)	
Non-IDC	140 (36.2)	83 (28.2)	57 (61.3)	
Tumor size[a]				**0.754**
≤2.0 cm	236 (61.0)	178 (60.5)	58 (62.4)	
>2.0 cm	151 (39.0)	116 (39.5)	35 (37.6)	
ALN status				**0.521**
Negative	243 (62.8)	182 (61.9)	61 (65.5)	
Positive	144 (37.2)	112 (38.1)	32 (34.4)	
Histological grade[a]				**0.739**
I	24 (6.2)	20 (6.8)	4 (4.3)	
II	183 (47.3)	141 (48.0)	42 (45.2)	
III	96 (24.8)	71 (24.1)	25 (26.9)	
NA	84 (21.7)	62 (21.1)	22 (23.6)	
Molecular subtype[a]				**<0.001**
LA	117 (30.2)	103 (35.0)	14 (15.1)	
LB (HER2–)	135 (34.9)	87 (29.6)	48 (51.6)	
LB (HER2+)	50 (12.9)	34 (11.6)	16 (17.2)	
HER2+	47 (12.1)	39 (13.3)	8 (8.6)	
TNBC	38 (9.9)	31 (10.5)	7 (7.5)	

[a]*Main focus.*
[b]*Minor focus.*
ALN, axillary lymph node; ALND, axillary lymph node dissection; BCS, breast-conserving surgery; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; LA, Luminal A-like; LB, Luminal B-like; NA, not available; SLNB, sentinel lymph node biopsy; TNBC, triple negative breast cancer; y/o, years old.

pathological type of the minor tumor focus ($p < 0.001$) and molecular subtype of the main tumor focus ($p < 0.001$) between the Hetero group and the Homo group.

Rates of Molecular Markers Heterogeneity

As shown in **Table 2**, concordance rates of ER, PR, HER2, and Ki67 among different tumor foci were 94.3%, 90.7%, 93.3%, and 87.1%, respectively (all p values <0.001). Among the whole cohort, a total of 93 (24.0%) patients showed intertumoral heterogeneity of molecular markers, and the remaining 294 (76.0%) were homogeneous. There were 60 (23.7%) patients with MFBC and 33 (24.0%) with MCBC who were classified to the Hetero group ($p = 0.842$, **Supplementary Tables S1–3**).

The molecular subtypes were identical within the same patient in 310 (80.1%) of the 387 cases using the 2013 St Gallen standard, with 104 luminal A-like tumors, 93 luminal B-like (HER2–) tumors, 42 luminal B-like (HER2+) tumors, 39 HER2-enriched tumors, and 32 triple negative breast cancers (**Table 3**). Molecular subtypes differed among different tumor foci in 77 (19.9%) patients, including 46 (18.2%) and 31 (23.1%) with MFBC and MCBC, respectively ($p = 0.336$, **Supplementary Tables S1, 4, 5**).

Heterogeneity of Molecular Markers and Adjuvant Therapy

There were 150 patients with recorded MDT-recommended adjuvant therapies, and the compliance was 95.3%, 96.0%, and 97.3% to chemotherapy, endocrine therapy, and anti-HER2 therapy, respectively. A total of 45 (84.9%) out of 330 patients with at least two invasive tumor foci in the Hetero group received adjuvant endocrine therapy, which was significantly higher than that of patients in the Homo group (71.7%, $p = 0.046$,

TABLE 2 | Concordance rates of pathological type, histological grade, ER, PR, HER2, and Ki67 status.

Main focus	Minor focus			Concordancerate (%)	Kappa	*p*-value
Pathological type	IDC		Non-IDC	78.0	0.473	<0.001
IDC	236		74			
Non-IDC	11		66			
Histological grade	I	II	III	88.4	0.772	<0.001
I	15	3	0			
II	3	139	5			
III	1	16	59			
ER	Negative		Positive	94.3	0.841	<0.001
Negative	79		8			
Positive	14		286			
PR	Negative		Positive	90.7	0.798	<0.001
Negative	121		18			
Positive	18		230			
HER2	Negative		Positive	93.3	0.819	<0.001
Negative	279		11			
Positive	15		82			
Ki67	< 20%		≥ 20%	87.1	0.743	<0.001
< 20%	163		8			
≥ 20%	42		174			

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Figure 2A). There were no significant differences in the usage rates of adjuvant anti-HER2 therapy (28.3% vs. 19.6%, $p = 0.196$, **Figure 2B**) and chemotherapy (69.9% vs. 69.8%, $p = 0.987$, **Figure 2C**) between the two groups.

As shown in **Figure 2D**, endocrine therapy was more frequently utilized among patients with HR heterogeneity than HR-negative patients (75.0% vs. 0.0%, $p < 0.001$), while the rates were comparable among patients with at least one HR+ tumor foci (75.0% vs. 92.1%, $p = 0.140$). Similarly, HER2 heterogeneity was associated with higher rate of anti-HER2 therapy compared with HER2-negative patients (72.7% vs. 0.0%, $p < 0.001$), and once again, no significant difference was observed among patients with at least one HER2+ tumor focus (72.7% vs. 77.5%, $p = 0.711$, **Figure 2E**).

Heterogeneity of Molecular Markers and Disease Outcomes

At a median follow-up of 35 (IQR, 19–57) months, 21 DFS events and 5 deaths were recorded (**Table 4**). Patients in the Hetero group had significantly worse DFS (81.2% vs. 96.5%, $p = 0.041$) and comparable OS (95.8% vs. 99.5%, $p = 0.059$) than those in the Homo group (**Table 5** and **Figure 3**). After adjusting age, tumor size, ALN status, molecular subtype, and systemic treatments in multivariate models, patients in the Hetero group had significantly worse DFS (adjusted *HR*, 2.95; 95% CI, 1.04–8.37) and comparable OS (adjusted *HR*, 5.36; 95% CI, 0.97–29.69) than those in the Homo group (**Supplementary Table S1**).

DISCUSSION

The study was designed to evaluate the rates of molecular markers heterogeneity and its associations with systemic adjuvant therapy and disease outcomes in MMBC. Molecular markers showed good concordance among different tumor foci. Heterogeneity of ER, PR, HER2, and Ki67 were present in 24.0% MMBC, which was associated with more adjuvant endocrine therapy usage ($p = 0.046$) and shorter DFS ($p = 0.041$), indicating the necessity of molecular assessments for different tumor foci in patients with MMBC.

There were some published literatures that reported the rates of intertumoral biomarkers heterogeneity among different foci in MMBC (13, 22–25). For example, Buggi and colleagues enrolled 113 invasive multiple breast cancers, and they reported

TABLE 3 | Concordance rates of molecular subtypes[a].

Main focus	Minor focus					Concordance rate (%)	Kappa	*p*-value
	LA	LB (HER2–)	LB (HER2+)	HER2+	TNBC	80.1	0.830	<0.001
LA	104	6	1	3	3			
LB (HER2–)	32	93	4	1	5			
LB (HER2+)	4	4	42	0	0			
HER2+	0	0	2	39	6			
TNBC	3	1	1	1	32			

[a]*The cutoff value of Ki67 was 20% for differentiating luminal A-like and luminal B-like (HER2–).*

HER2, human epidermal growth factor receptor 2; HR, hormone receptor; LA, luminal A-like; LB, luminal B-like; TNBC, triple negative breast cancer.

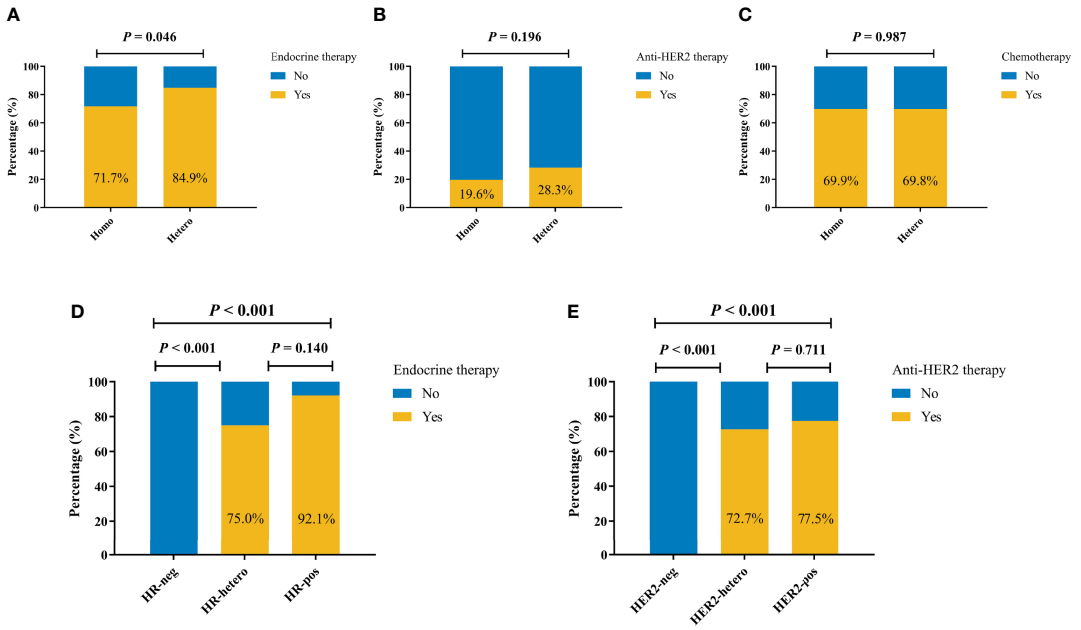


FIGURE 2 | Adjuvant systemic therapy by molecular markers status among 330 patients with at least two invasive tumor foci. Adjuvant endocrine therapy **(A)**, anti-HER2 therapy **(B)**, and chemotherapy **(C)** by molecular markers status. **(D)** Adjuvant endocrine therapy by HR status. **(E)** Adjuvant anti-HER2 therapy by HER2 status.

TABLE 4 | Details of DFS and OS events by status of molecular markers among 330 patients with at least two invasive tumor foci.

	Total N = 330 (%)	Homo N = 277 (%)	Hetero N = 53 (%)
DFS events			
No recurrence	309 (93.6)	261 (94.2)	48 (90.6)
Local-regional recurrence	5 (1.5)	4 (1.4)	1 (1.9)
Contralateral breast cancer	4 (1.2)	4 (1.4)	0 (0.0)
Second non-breast malignancy	2 (0.6)	1 (0.4)	1 (1.9)
Distant recurrence	8 (2.4)	6 (2.2)	2 (3.8)
Death without recurrence	2 (0.6)	1 (0.4)	1 (1.9)
OS events			
Alive	323 (97.9)	272 (98.2)	51 (96.2)
Death of any cause	7 (2.1)	5 (1.8)	2 (3.9)
Death with recurrence	5 (1.5)	4 (1.4)	1 (1.9)
Death without recurrence	2 (0.6)	1 (0.4)	1 (1.9)

DFS, disease-free survival; OS, overall survival.

mismatches on ER, PR, HER2, and Ki67 in 4.4%, 15.9%, 9.7%, and 15.0% cases (13). Similarly, the rate of ER, PR, HER2, and Ki67 heterogeneity in our cohort was 5.7%, 9.3%, 6.7%, and 22.9%, respectively. Moreover, molecular subtypes differed in 77 (19.9%) patients as classified by 2013 St Gallen system, comparable to the results of Pekar et al. (12.7%) (14). There might be some causes of molecular heterogeneity. First, heterogeneity of molecular markers was more frequent among patients whose minor tumor focus was invasive carcinoma of special type or carcinoma *in situ* in our study, making pathological differences a potential explanation. This finding will guide us to select patients for a second molecular evaluation in the present clinical practice. However, there would be other innate tumor properties beyond histopathology but crucial to tumor tumorigenesis and evolution, which control tumor heterogeneity. Recent studies have revealed that extensive genetic diversity caused by genome instability and mutation will affect key cancer pathways, eventually driving phenotypic variation (10–12, 26). In light of this, intratumor heterogeneity can lead to underestimation of the tumor genomics landscape from pathology only and may present major challenges to personalized medicine, which should be further evaluated by emerging technologies such as next-generation and single-cell sequencing (12). Moreover, non-mutational epigenetic reprogramming and cellular plasticity can also contribute to tumor heterogeneity (27, 28). Last but not the least, technical

TABLE 5 | Univariate analysis of prognostic factors affecting DFS and OS among 330 patients with at least two invasive tumor foci.

Characteristics	*p*-value	
	DFS	OS
Age (<50 y/o vs. ≥50 y/o)	0.741	0.277
Menstrual status (Pre/Peri- vs. Post-)	0.966	0.969
Number of foci (2 vs. 3/4)	0.264	0.557
Location of foci (Multifocal vs. Multicentric)	0.571	0.703
Histology type (IDC vs. non-IDC) [a]	0.802	0.789
Tumor size (≤2.0 vs. >2.0 cm) [a]	0.786	0.292
ALN status (Negative vs. Positive)	0.572	0.841
Histological grade (I vs. II vs. III vs. NA)[a]	0.637	0.892
Molecular subtype[a]	0.309	0.460
Chemotherapy (No vs. Yes)	0.698	1.000
Endocrine therapy (No vs. Yes)	0.102	0.458
Anti-HER2 therapy (No vs. Yes)	0.379	0.352
Group (Homo vs. Hetero)	0.041	0.059

[a]*Main focus.*

ALN, axillary lymph node; IDC, invasive ductal carcinoma; DFS, disease-free survival; NA, not available; OS, overall survival; y/o, years old.

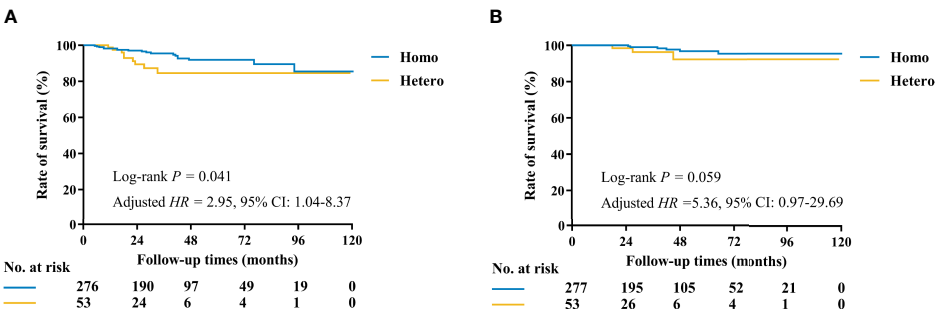


FIGURE 3 | Kaplan–Meier curves of DFS and OS by molecular markers status among 330 patients with at least two invasive tumor foci. **(A)** The estimated 3-year DFS rates for the Homo and Hetero groups were 96.5% and 81.2%, respectively (p = 0.041). **(B)** The estimated 3-year OS rates for the Homo and Hetero groups were 99.5% and 95.8%, respectively (p = 0.059).

issue and analytical artifact may also affect accuracy of molecular evaluation, which could be avoided by standardization.

Accurate biomolecular analysis is of great significance to make treatment options and to evaluate functional outcomes and quality of life of breast cancer patients in the era of precise medicine (2, 29–31). However, with limited knowledge on the consequences of molecular heterogeneity for therapeutic decision-making, it has been accepted that biomarkers can be assessed only in the largest individual tumor focus (32). This is based on the observations that molecular markers in MMBC are usually homogeneous. However, if the tumor foci demonstrate different pathological or histological features, biomarkers evaluation of the smaller focus will be necessary. According to Buggi et al., 14 out of the 113 (12.4%) patients received additional systemic treatments with the biomarkers analysis for the smaller tumor focus (13). In the present study, heterogeneity of biomarkers was also found to be significantly associated with the usage of adjuvant endocrine therapy among patients with at least two invasive tumor foci. Additional biomarkers evaluation of the other foci would potentially change the adjuvant treatment decisions, especially for those whose main tumor focus lacked HR or HER2. This would further impact breast cancer patient's survival.

To the best of the authors' knowledge, this was the largest study to evaluate the prognostic significance of intertumoral biomarkers heterogeneity. Our cohort revealed that patients in the Hetero group had clinically worse DFS and OS compared those in the Homo group, although the differences were not statistically significant. However, the results should be taken as exploratory only and interpreted with caution, particularly given the relatively small number of DFS and OS events and short follow-up. Consistent with our results, Pekar et al. concluded that patients with phenotypically heterogeneous MMBC had a significantly shorter breast-cancer-specific survival (HR = 2.87; 95% CI, 1.08–7.64, p = 0.034) and OS (HR = 2.80; 95% CI, 1.05–7.44, p = 0.039) (14). The inferior disease outcomes in the Hetero group were thought to be associated with the biology behavior itself and possible undertreatment or low treatment sensitivity. Taken together, these results suggested the necessity of evaluating molecular markers for different tumor foci in

patients with MMBC (33). However, we acknowledge that it is not a routine to test all tumor foci in the present clinical practice. Possibilities could be attributed to lack of evidence or economic reasons. In addition, it is important to point out that patients who have MMBC but do not have all foci tested may be more likely to have homogeneous tumors, since the finding of MMBC with discordant grades may prompt biomarker evaluation. Therefore, it could be useful to evaluate the clinical features and disease outcomes among patients who do not have all samples tested for biomarkers. However, this was a small group in our cohort, and only 3 out of 30 patients with multiple diseases who lacked biomarkers data experienced DFS events, indicating that a large cohort with more patients are needed to validate this recommendation.

The present study enrolled both multifocal and multicentric diseases, which were heterogeneous and could be further differentiated into MFBC and MFBC. Patients with MCBC were different to those with MFBC in terms of number of tumor foci, tumor size, and molecular subtype (**Supplementary Table S1**). However, the biomarker and subtype heterogeneity rates were comparable between the two subgroups (**Supplementary Table S1–S5**), while for DFS and OS, no significant differences were observed between the two groups (**Supplementary Figure S3**). To date, relatively few reports have directly compared the clinical–pathological features, treatment patterns, and survivals of patients with MFBC and MCBC, which warrant further research (34–36).

The incidence of MMBC increases with the advancement of preoperative imaging, and intertumoral molecular heterogeneity has attracted the attention of clinicians. For example, MRI can identify 74.6%, 54.2%, and 67.3% of MMBC that were not identified by physical examination, sonography, or mammography, respectively (**Supplementary Figure S1**). We performed the study for the first time to evaluate the rates of biomarkers heterogeneity in MMBC and its impacts on adjuvant therapy and survival. However, there were several limitations in the present study. First, this was a single-institutional retrospective study, so there might be selection bias and limited applicability. Further validation in other cohorts will provide us more insights to the therapeutic and prognostic role of biomarkers heterogeneity. Second, the number of DFS and OS events was very small, and survival did not differ significantly between the groups. Therefore, the evaluation of outcomes was exploratory and hypothesis generating and warranted larger study. Third, the present study enrolled patients over a 9-year period from January 2009 through December 2018, which might exert an influence to disease outcomes.

In conclusion, heterogeneity of ER, PR, HER2, or Ki67 was present in 24.0% patients with MMBC. Biomarkers heterogeneity was associated with more adjuvant endocrine therapy usage and worse disease outcomes, indicating the necessity of molecular assessments for different tumor foci in patients with MMBC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

XC conceived and designed the study. SL analyzed and interpreted the data for presentation and was a main contributor in writing the manuscript. XC revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by the National Natural Science Foundation of China (Grant Number: 81772797); Shanghai Municipal Education Commission—Gaofeng Clinical Medicine Grant Support (20172007); and Ruijin Hospital, Shanghai Jiao Tong University School of Medicine-"Guangci Excellent Youth Training Program" (GCQN-2017-A18). All these financial sponsors had no role in the study design, data collection, analysis, or interpretation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.833093/full#supplementary-material

REFERENCES

1. Zardavas D, Irrthum A, Swanton C, Piccart M. Clinical Management of Breast Cancer Heterogeneity. *Nat Rev Clin Oncol* (2015) 12:381–94. doi: 10.1007/s10555-017-9684-y
2. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ, et al. Strategies for Subtypes-Dealing With the Diversity of Breast Cancer: Highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol* (2011) 22:1736–47. doi: 10.1093/annonc/mdr304
3. Aurilio G, Disalvatore D, Pruneri G, Bagnardi V, Viale G, Curigliano G, et al. A Meta-Analysis of Oestrogen Receptor, Progesterone Receptor and Human Epidermal Growth Factor Receptor 2 Discordance Between Primary Breast Cancer and Metastases. *Eur J Cancer* (2014) 50:277–89. doi: 10.1016/j.ejca.2014.01.013
4. Yates LR. Intratumoral Heterogeneity and Subclonal Diversification of Early Breast Cancer. *Breast* (2017) 34 (Suppl 1):S36–42. doi: 10.1016/j.breast.2017.06.025
5. Yeo SK, Guan JL. Breast Cancer: Multiple Subtypes Within a Tumor? *Trends Cancer* (2017) 3:753–60. doi: 10.1016/j.trecan.2017.09.001
6. Falck AK, Bendahl PO, Chebil G, Olsson H, Ferno M, Ryden L. Biomarker Expression and St Gallen Molecular Subtype Classification in Primary Tumours, Synchronous Lymph Node Metastases and Asynchronous Relapses in Primary Breast Cancer Patients With 10 Years' Follow-Up. *Breast Cancer Res Treat* (2013) 140:93–104. doi: 10.1007/s10549-013-2617-8
7. Corso G, Magnoni F, Provenzano E, Girardi A, Iorfida M, De Scalzi AM, et al. Multicentric Breast Cancer With Heterogeneous Histopathology: A Multidisciplinary Review. *Future Oncol* (2020) 16:395–412. doi: 10.2217/fon-2019-0540
8. Ng CK, Martelotto LG, Gauthier A, Wen HC, Piscuoglio S, Lim RS, et al. Intra-Tumor Genetic Heterogeneity and Alternative Driver Genetic

Alterations in Breast Cancers With Heterogeneous HER2 Gene Amplification. *Genome Biol* (2015) 16:107. doi: 10.1186/s13059-015-0657-6
9. Muller KE, Marotti JD, Tafe LJ. Pathologic Features and Clinical Implications of Breast Cancer With HER2 Intratumoral Genetic Heterogeneity. *Am J Clin Pathol* (2019) 152:7–16. doi: 10.1093/ajcp/aqz010
10. Burrell RA, McGranahan N, Bartek J, Swanton C. The Causes and Consequences of Genetic Heterogeneity in Cancer Evolution. *Nature* (2013) 501:338–45. doi: 10.1038/nature12625
11. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumoral Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. *N Engl J Med* (2012) 366:883–92. doi: 10.1056/NEJMoa1113205
12. Dagogo-Jack I, Shaw AT. Tumour Heterogeneity and Resistance to Cancer Therapies. *Nat Rev Clin Oncol* (2018) 15:81–94. doi: 10.1038/nrclinonc.2017.166
13. Buggi F, Folli S, Curcio A, Casadei-Giunchi D, Rocca A, Pietri E, et al. Multicentric/multifocal Breast Cancer With a Single Histotype: Is the Biological Characterization of All Individual Foci Justified? *Ann Oncol* (2012) 23:2042–6. doi: 10.1093/annonc/mdr570
14. Pekar G, Gere M, Tarjan M, Hellberg D, Tot T. Molecular Phenotype of the Foci in Multifocal Invasive Breast Carcinomas: Intertumoral Heterogeneity is Related to Shorter Survival and may Influence the Choice of Therapy. *Cancer* (2014) 120:26–34. doi: 10.1002/cncr.28375
15. Chen X, Sun L, Mao Y, Zhu S, Wu J, Huang O, et al. Preoperative Core Needle Biopsy is Accurate in Determining Molecular Subtypes in Invasive Breast Cancer. *BMC Cancer* (2013) 13:390. doi: 10.1186/1471-2407-13-390
16. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. *J Clin Oncol* (2010) 28:2784–95. doi: 10.1200/JCO.2009.25.6529
17. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *J Clin Oncol* (2007) 25:118–45. doi: 10.1200/JCO.2006.09.2775
18. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *J Clin Oncol* (2013) 31:3997–4013. doi: 10.1200/JCO.2013.50.9984
19. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol* (2018) 36:2105–22. doi: 10.1200/JCO.2018.77.8738
20. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in Breast Cancer: Recommendations From the International Ki67 in Breast Cancer Working Group. *J Natl Cancer Inst* (2011) 103:1656–64. doi: 10.1093/jnci/djr393
21. Tong Y, Chen X, Fei X, Lin L, Wu J, Huang O, et al. Can Breast Cancer Patients With HER2 Dual-Equivocal Tumours be Managed as HER2-Negative Disease? *Eur J Cancer* (2018) 89:9–18. doi: 10.1016/j.ejca.2017.10.033
22. Garimella V, Long ED, O'Kane SL, Drew PJ, Cawkwell L. Oestrogen and Progesterone Receptor Status of Individual Foci in Multifocal Invasive Ductal Breast Cancer. *Acta Oncol* (2007) 46:204–7. doi: 10.1080/02841860600897884
23. Choi Y, Kim EJ, Seol H, Lee HE, Jang MJ, Kim SM, et al. The Hormone Receptor, Human Epidermal Growth Factor Receptor 2, and Molecular Subtype Status of Individual Tumor Foci in Multifocal/Multicentric Invasive Ductal Carcinoma of Breast. *Hum Pathol* (2012) 43:48–55. doi: 10.1016/j.humpath.2010.08.026
24. Boros M, Ilyes A, Nechifor Boila A, Moldovan C, Eniu A, Stolnicu S. Morphologic and Molecular Subtype Status of Individual Tumor Foci in Multiple Breast Carcinoma. A Study of 155 Cases With Analysis of 463 Tumor Foci. *Hum Pathol* (2014) 45:409–16. doi: 10.1016/j.humpath.2013.10.006
25. Singh K, Wang Y, Marketkar S, Kalife ET, Steinhoff MM. Comparison of Estrogen Receptor, Progesterone Receptor and HER2 Results in Concurrent Ipsilateral Samples With Invasive Breast Carcinoma; A Retrospective Study of 246 Biopsies From 119 Patients. *Hum Pathol* (2017) 65:123–32. doi: 10.1016/j.humpath.2017.04.016
26. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
27. Yuan S, Norgard RJ, Stanger BZ. Cellular Plasticity in Cancer. *Cancer Discovery* (2019) 9:837–51. doi: 10.1158/2159-8290.CD-19-0015
28. Hanahan D. Hallmarks of Cancer: New Dimensions. *Cancer Discovery* (2022) 12:31–46. doi: 10.1158/2159-8290.CD-21-1059
29. Venetis K, Invernizzi M, Sajjadi E, Curigliano G, Fusco N. Cellular Immunotherapy in Breast Cancer: The Quest for Consistent Biomarkers. *Cancer Treat Rev* (2020) 90:102089. doi: 10.1016/j.ctrv.2020.102089
30. Zemlin C, Stuhlert C, Schleicher JT, Wörmann C, Altmayer L, Lang M, et al. Longitudinal Assessment of Physical Activity, Fitness, Body Composition, Immunological Biomarkers, and Psychological Parameters During the First Year After Diagnosis in Women With Non-Metastatic Breast Cancer: The BEGYN Study Protocol. *Front Oncol* (2021) 11:762709. doi: 10.3389/fonc.2021.762709
31. Criscitiello C, Guerini-Rocco E, Viale G, Fumagalli C, Sajjadi E, Venetis K, et al. Immunotherapy in Breast Cancer Patients: A Focus on the Use of the Currently Available Biomarkers in Oncology. *Anticancer Agents Med Chem* (2022) 22:787–800. doi: 10.2174/1871520621666210706144112
32. Lester SC, Bose S, Chen YY, Connolly JL, de Baca ME, Fitzgibbons PL, et al. Protocol for the Examination of Specimens From Patients With Invasive Carcinoma of the Breast. *Arch Pathol Lab Med* (2009) 133:1515–38. doi: 10.5858/133.10.1515
33. Navale P, Bleiweiss IJ, Jaffer S, Nayak A. Evaluation of Biomarkers in Multiple Ipsilateral Synchronous Invasive Breast Carcinomas. *Arch Patho Lab Med* (2019) 143:190–6. doi: 10.5858/arpa.2017-0494-OA
34. Lynch SP, Lei X, Chavez-MacGregor M, Hsu L, Meric-Bernstam F, Buchholz TA, et al. Multifocality and Multicentricity in Breast Cancer and Survival Outcomes. *Ann Oncol* (2012) 23:3063–9. doi: 10.1093/annonc/mds136
35. Kadioğlu H, Özbaş S, Akcan A, Soyder A, Soylu L, Koçak S, et al. Comparison of the Histopathology and Prognosis of Bilateral Versus Unilateral Multifocal Multicentric Breast Cancers. *World J Surg Oncol* (2014) 12(1):266–74. doi: 10.1186/1477-7819-12-266
36. Ataseven B, Lederer B, Blohmer JU, Denkert C, Gerber B, Heil J, et al. Impact of Multifocal or Multicentric Disease on Surgery and Locoregional, Distant and Overall Survival of 6,134 Breast Cancer Patients Treated With Neoadjuvant Chemotherapy. *Ann Surg Oncol* (2015) 22:1118–27. doi: 10.1245/s10434-014-4122-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 13 July 2022
doi: 10.3389/fonc.2022.762906

Optimal Selection of Imaging Examination for Lymph Node Detection of Breast Cancer With Different Molecular Subtypes

Mingjie Zheng†, Yue Huang†, Jinghui Peng†, Yiqin Xia, Yangyang Cui, Xu Han, Shui Wang and Hui Xie**

Department of Breast Surgery, The First Affiliated Hospital With Nanjing Medical University, Nanjing, China

Objective: Axillary lymph node management is an important part of breast cancer surgery and the accuracy of preoperative imaging evaluation can provide adequate information to guide operation. Different molecular subtypes of breast cancer have distinct imaging characteristics. This article was aimed to evaluate the predictive ability of imaging methods in accessing the status of axillary lymph node in different molecular subtypes.

Methods: A total of 2,340 patients diagnosed with primary invasive breast cancer after breast surgery from 2013 to 2018 in Jiangsu Breast Disease Center, the First Affiliated Hospital with Nanjing Medical University were included in the study. We collected lymph node assessment results from mammography, ultrasounds, and MRIs, performed receiver operating characteristic (ROC) analysis, and calculated the sensitivity and specificity of each test. The C-statistic among different imaging models were compared in different molecular subtypes to access the predictive abilities of these imaging models in evaluating the lymph node metastasis.

Results: In Her-2 + patients, the C-statistic of ultrasound was better than that of MRI (0.6883 vs. 0.5935, p=0.0003). The combination of ultrasound and MRI did not raise the predictability compared to ultrasound alone (p=0.492). In ER/PR+HER2- patients, the C-statistic of ultrasound was similar with that of MRI (0.7489 vs. 0.7650, p=0.5619). Ultrasound+MRI raised the prediction accuracy compared to ultrasound alone (p=0.0001). In ER/PR-HER2- patients, the C-statistics of ultrasound was similar with MRI (0.7432 vs. 0.7194, p=0.5579). Combining ultrasound and MRI showed no improvement in the prediction accuracy compared to ultrasound alone (p=0.0532).

Conclusion: From a clinical perspective, for Her-2+ patients, ultrasound was the most recommended examination to assess the status of axillary lymph node metastasis. For ER/PR+HER2- patients, we suggested that the lymph node should be evaluated by ultrasound plus MRI. For ER/PR-Her2- patients, ultrasound or MRI were both optional examinations in lymph node assessment. Furthermore, more new technologies should be explored, especially for Her2+ patients, to further raise the prediction accuracy of lymph node assessment.

Keywords: breast cancer, molecular subtype, lymph node assessment, imaging examination, ROC (receiver operating characteristic) analysis

OPEN ACCESS

Edited by:
Yiding Chen,
Zhejiang University, China

Reviewed by:
Donatella Santini,
Sant'Orsola-Malpighi Polyclinic, Italy
Marcus Vetter,
University Hospital of Basel, Switzerland

****Correspondence:***
Shui Wang
ws0801@hotmail.com
Hui Xie
Hxie@njmu.edu.cn

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *23 August 2021*
Accepted: *30 May 2022*
Published: *13 July 2022*

Citation:
Zheng M, Huang Y, Peng J, Xia Y, Cui Y, Han X, Wang S and Xie H (2022) Optimal Selection of Imaging Examination for Lymph Node Detection of Breast Cancer With Different Molecular Subtypes. Front. Oncol. 12:762906. doi: 10.3389/fonc.2022.762906

INTRODUCTION

The progression of breast cancer is characterized by metastasis (1). The presence of regional lymph node metastasis in cancer patients correlates with dissemination to distant organs and a poorer prognosis (1). For breast cancer, modern strategies of axillary lymph node management involve stepwise approaches including fine needle aspiration or core needle biopsy, sentinel lymph node biopsy (SLNB), and axillary lymph node dissection (ALND). Based on preoperative imaging evaluation of axillary lymph nodes, clinicians take corresponding measures. Historically, ALND was regarded as the most accurate method for assessing regional metastatic spread (2). However, associated complications such as seroma, nerve injury, and lymphedema would bring unnecessary pain for pathologically node-negative patients (2, 3). Conversely, residual axillary disease would bring regional recurrence and a poorer prognosis. Therefore, accurate preoperative imaging evaluation of axillary lymph node status is of great importance for precision treatment of breast cancer patients.

Breast cancer is a highly heterogeneous disease. Based on gene expression profiles, it is currently categorized into three distinct molecular subtypes, including HER2 positive (Her2+), ER/PR positive/HER2 negative (ER/PR+Her2-), and triple-negative (ER/PR-Her2-) types (4). Molecular subtype classification of breast cancer is a regular process for individualized cancer management. Distinct molecular subtypes confer different treatment programs and different clinical prognosis (5). Moreover, some reports have indicated that characteristic imaging manifestation was also correlated with the three subtypes mentioned above. For instance, Wang et al. found that compared to HER2-positive breast cancer, HER2-negative breast cancer was more likely to have spiculated margins (6). However, the influence of breast cancer subtypes on the diagnostic performance of axillary imaging is unknown. This raised the speculation that the accuracy of imaging assessment of axillary lymph node metastasis might also be affected by the molecular subtype of primary tumors.

Therefore, in order to determine whether the imaging diagnostic performance of lymph nodes differ among various subtypes of breast cancer, we conducted a retrospective matched cohort study in 2,340 patients, with the goal to provide a more reliable imaging evaluation of lymph node status for each breast cancer subtype.

MATERIAL AND METHODS

Patient Population and Data Collection

Patients diagnosed with primary invasive breast cancer and positive axillary lymph nodes after breast surgery between 2013 and 2018 in Jiangsu Breast Disease Center, the First Affiliated Hospital with Nanjing Medical University were included in the study. Exclusion criteria were as follows: male breast cancer, patients without any imaging lymph node staging before surgery [i.e., mammography, ultrasound, breast magnetic resonance imaging (MRI)], and patients whose receptor status was missing. Then, the controls were age- and molecular subtype-matched to the cases, whose axillary lymph node were confirmed negative by surgery. The selection procedure is summarized in **Figure S1**. Data on patients, tumor characteristics, imaging, and histopathological outcome of the axillary lymph nodes were retrospectively collected. The study was approved by the Ethics Committee of Nanjing Medical University.

Clinical Nodal Status

Pre-operative nodal status was assessed by mammography, ultrasound, and MRI. The imaging results we adopted were performed in all of our patients before local or systemic treatment, including mass puncture biopsy. Mammography were obtained by clinical full-field digital mammography unit, which used molybdenum for target and filter (Selenia, Hologic, USA) (7). Lymph nodes considered abnormal had a size>2cm, increased density, rounded or irregular shape, spiculate margins or the absence of fatty hilum (8) (**Figure S2**). Ultrasound was performed using MyLab Twice (Esaote S.p.A., Genova, Italy) Color Doppler with a 4-13MHz linear transducer (iU22; Philips Medical Systems, Bothell, WA, USA) (8). A lymph node was considered abnormal if the cortex was either focally or diffusely thickened (> 3 mm thick) and the fatty hilum was deformed or absent (**Figure S3**). MRI was conducted using a bilateral eight-channel phased-array breast coil with a 3.0 T scanner (MAGNETOM Trio, Siemens, Germany) to obtain images (9). A positive lymph node was defined as: an irregular contour compared with the contralateral axilla, a node measuring greater than 1 cm, the thickened cortex was >3 mm or there was a loss of fatty hilum (10) (**Figure S4**).

The axillary images *via* mammography, ultrasound, or MRI was interpreted independently by one of five dedicated breast radiologists with more than 5 years of experience in breast imaging.

Axillary Lymph Node Management

Patients clinically diagnosed with negative nodes underwent SLNB. The SLNB procedure was performed using both the gamma probe to detect radioactivity and blue dye to detect lymphatic vessels. If one or more sentinel lymph nodes were confirmed with macro-metastasis, a completion ALND was then performed. In clinically node positive patients an ALND was performed directly.

Pathological Assessment of Axillary Lymph Node

SLNB samples were assessed by immediate frozen section and hematoxylin and eosin (H&E) staining. Then the lymph node was subsequently submitted for permanent section and stained with cytokeratin immunohistochemical (IHC), while all ALND samples were embedded in paraffin as permanent section for histological evaluation. Lymph nodes with isolated tumor cells were also considered node-negative and no additional lymph node surgery was performed. Meanwhile, for patients who underwent surgery after neoadjuvant therapy, lymph node

positivity was defined by the residual tumor cell, and lymph nodes with evidence of treatment response but no tumor cells were also defined as metastatic nodes in our research.

Pathological Type

Pathological type was determined based on American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines. Receptor status was considered positive if 10% of cells were stained positive by IHC staining (11); HER2 positive status was defined as 3+. A value of 2+ for HER2 amplification was then confirmed by fluorescence *in situ* hybridization (12). Three subtypes of breast cancer were finally distinguished for analysis based on receptor status (1): HER2+, (2) ER/PR+HER2-, (3) ER/PR-HER2- (13).

Statistical Analysis

To explore the potential predictive ability, we conducted receiver operating characteristic (ROC) analysis and calculated sensitivity and specificity. An analysis of variance (ANOVA) was used to compare the C-statistic among different imaging models, including mammography, ultrasound, MRI, and ultrasound+MRI models. (Analyzing receiver operating characteristic curves using SAS: Cary, NC: SAS Press 2007.)

RESULT

Demographics

A total of 2,340 patients were enrolled in this research And 1,170 lymph node positive patients were brought into experiment group, while the other 1,170 lymph node negative patients were age- and molecular subtype-matched into control groups. The baseline characteristics showed that age, menopausal age, height, weight, and the molecular subtype in the experiment group and control group were basically balanced (**Table 1**). 53.7% of cases in the experiment group and 53.6% of cases in the control group were Her2+; 33.4% of cases in the experiment group and 33.9% of cases in the control group were ER/PR+, Her2-; only 12.9% of cases in the experiment group and 12.5% of cases in the control group were ER/PR-, Her2-; 21.3% of patients in experiment group and only 4% of cases in control group received neoadjuvant chemotherapy. In total, the true positive rate of ultrasound in detecting lymph node properties reached 62.9% and the false positive rate was 26.6%. The true positive rate of mammography was only 22.2% and the false positive rate was 11.7%. The true positive rate of MRI reached 67.9% while the false positive rate was 33.1%.

TABLE 1 | Baseline characteristics of study participants.

	Case (n = 1170)	Control (n = 1170)
Age*	51.3 (11.1)	51.4 (11.1)
Menopausal Age	46.7 (6.7)	47.3 (6.7)
Height	160.3 (4.6)	159.8 (4.6)
Weight	61.3 (9.0)	59.9 (8.8)
Menopause, %	50.5	51.6
Pathologic type		
Her2+%	53.7	53.6
ER/PR+Her2- %	33.4	33.9
ER/PR-Her2-%	12.9	12.5
Neoadjuvant chemotherapy (positive)%	21.3	4.0
Ultrasound (positive) %	62.9	26.6
Mammogram (positive) %	22.2	11.7
MRI (positive) %	67.9	33.1

Values are means (SD) for continuous variables; percentages for categorical variables, and are standardized to the age distribution of the study population.
**Value is not age adjusted.*

Differences in Axillary Lymph Node Identification in Total Population by Different Imaging Examinations

To assess the predictive ability for axillary lymph node of mammography, ultrasound, and MRI, we calculated the sensitivity, specificity, and C-statistic using receiver operating characteristic (ROC) analysis. Mammography is a common imaging exam used for breast cancer screening and nearly every breast cancer patient would have one before surgery. The sensitivity of mammography was only 0.22368 while the specificity was 0.88351 and the C-statistic was 0.5536 (**Figure 1A**). Ultrasound is another common imaging examination in breast disease. The sensitivity and specificity of ultrasound were 0.63071 and 0.73 respectively, and the C-statistic was 0.6810(**Figure 1B**). The third imaging exam was MRI but it is not as commonly applied for breast cancer patients. The ROC Curve for MRI is explicated in **Figure 1C**, the sensitivity shows 0.68024, the specificity shows 0.67143 and the C-statistic show 0.6758. In **Figure 1D**, we compared the C-statistic of ultrasound, MRI, and ultrasound+MRI. It was found that ultrasound + MRI had the largest C -statistics, while MRI alone had the smallest. The C-statistic was statistically different for MRI and ultrasound ($p=0.0093$), as well as for ultrasound+MRI and ultrasound alone ($p<0.0001$).

Differences in Axillary Lymph Nodes Identification in Her-2+ Patients by Different Imaging Examinations

Breast cancer was divided into three types: Her2+, ER/PR +Her2-, and ER/PR-Her2-. We next conducted ROC curve in the Her2+ subtype to compare the predictive ability of mammography, ultrasound, and MRI. **Figure 2A** shows that the sensitivity of mammography was 0.2137, the specificity was 0.84444, and the C-statistic was 0.5291. **Figure 2B** shows that the sensitivity of ultrasound was 0.62477, the specificity was 0.68641, and the C-statistic was 0.6556. The ROC Curve for MRI is shown in **Figure 2C**, the sensitivity was 0.64844, the specificity was 0.55882, and the C-statistic was 0.6036. In **Figure 2D**, we compared the C-statistics of ultrasound, MRI, and ultrasound +MRI. There was a statistical difference between MRI and ultrasound ($P=0.0003$). However, no statistical difference was found between ultrasound+MRI and ultrasound alone($p=0.492$).

Differences in Axillary Lymph Nodes Identification in ER/PR+Her2-Patients by Different Imaging Examinations

The ROC curve was conducted in the ER/PR+Her2- subtype to compare the predictive ability of mammography, ultrasound,

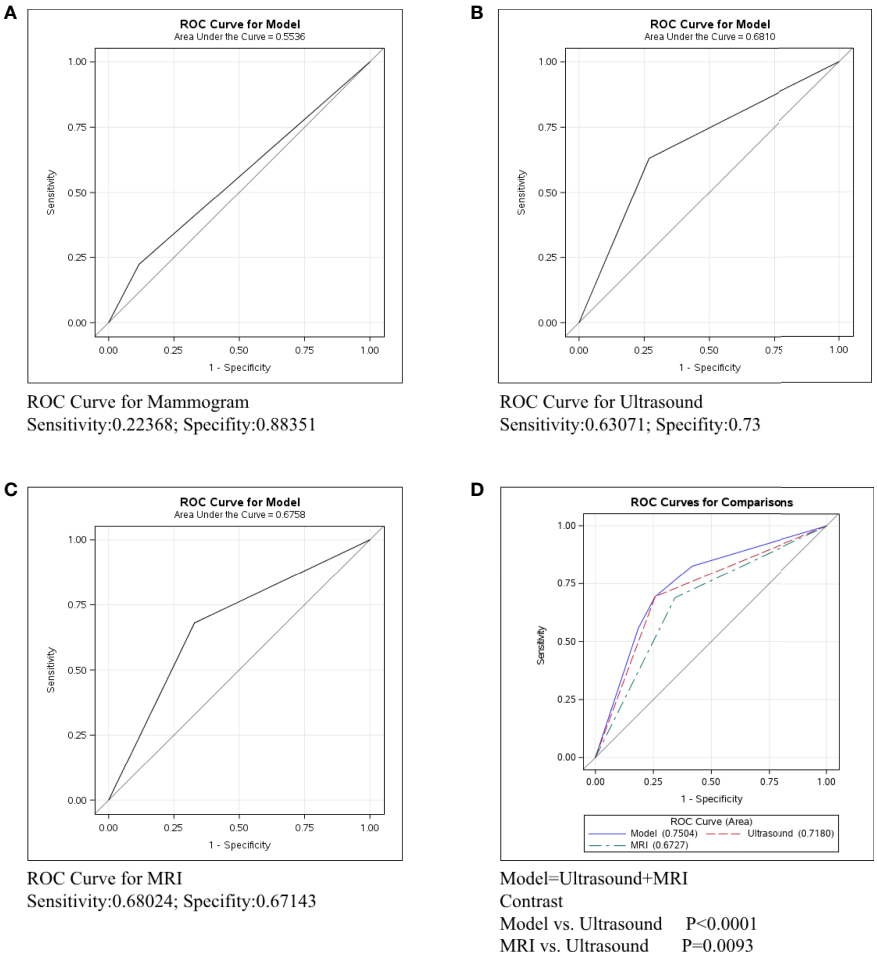


FIGURE 1 | ROC curve analysis for specific imaging examination in all molecular subtypes. The vertical axis is sensitivity, the horizontal axis is 1-specificity. AUC is a parameter used to measure the value of imaging examination in the prediction of axillary lymph nodes. **(A)** ROC curve for mammogram. **(B)** ROC curve for ultrasound. **(C)** ROC curve for MRI. **(D)** ROC curve for ultrasound+MRI.

and MRI in **Figure 3**. **Figure 3A** shows that the sensitivity of mammography was 0.19005, the specificity was 0.93023, and the C-statistic was 0.5601. **Figure 3B** shows that the sensitivity of ultrasound was 0.59040, the specificity was 0.82143, and the C-statistic was 0.7059. The ROC Curve for MRI is shown in **Figure 3C**, and the sensitivity was 0.67879, the specificity was 0.84, and the C-statistic was 0.7604. In **Figure 3D**, we compared the C-Statistics of ultrasound, MRI, and ultrasound+MRI. Although no statistical difference was found between MRI and ultrasound (p=0.5619), there was a statistical difference between ultrasound+MRI and ultrasound alone (p=0.0001).

Differences in Axillary Lymph Nodes Identification in ER/PR-Her2-Patients by Different Imaging Examinations

In **Figure 4**, the ROC curve was conducted in the ER/PR-Her2-subtype to compare the predictive ability of mammography, ultrasound, and MRI. **Figure 4A** shows that the sensitivity and specificity of mammography were 0.33673 and 0.93023, respectively, and the C-statistic was 0.6335. **Figure 4B** shows the ROC curve of the ultrasound and the sensitivity and specificity were 0.76984 and 0.68276, respectively, and the C-statistic was 0.7125. **Figure 4C** shows the sensitivity of MRI was 0.8, the specificity was 0.625, and the C-statistic was 0.7263. In **Figure 4D**, we also compared the C-Statistics of ultrasound, MRI, and ultrasound+MRI. However, there was no statistical difference between MRI and ultrasound alone (p=0.5579) and also no statistical difference between ultrasound+MRI and ultrasound alone(p=0.0532).

Differences in Axillary Lymph Node Identification in Different Molecular Types by Specific Imaging Examination

The accuracy of each imaging examination in different molecular subtypes was also compared. Mammography had the worst predictive power in assessing axillary lymph node status in

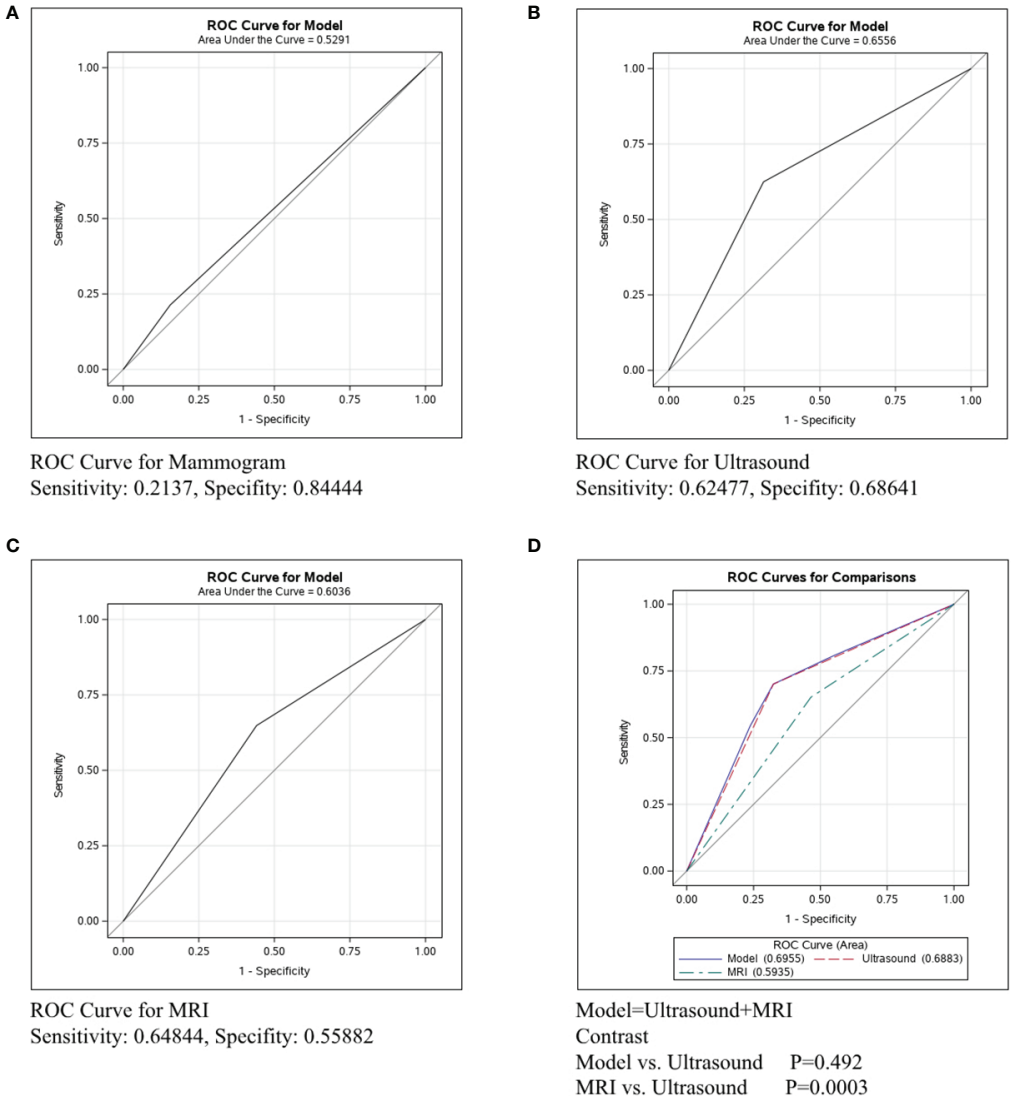


FIGURE 2 | ROC curve analysis for specific imaging examination in Her2+ subtype. The vertical axis is sensitivity, the horizontal axis is 1-specificity. AUC is a parameter used to measure the value of imaging examination in the prediction of axillary lymph nodes. **(A)** ROC curve for mammogram. **(B)** ROC curve for ultrasound. **(C)** ROC curve for MRI. **(D)** ROC curve for ultrasound+MRI.

breast cancer of any molecular type, as shown in **Figure 5**. The C-statistics were 0.5291, 0.5601, and 0.6335, respectively. **Figure 6** shows the accuracy of ultrasound, which was the best in ER/PR-Her2- patients with the C-statistic of 0.7125. In ER/PR+Her2- patients, the accuracy was next to that in ER/PR-Her2- patients, with the C-statistic of 0.7059. In Her2+ patients, accuracy was the worst, with the C-statistic of only 0.6556. **Figure 7** shows MRI had the best accuracy with a C-statistic of 0.7604 in ER/PR+Her2 -patients, while the worst accuracy was in HER2+ patients with the C-statistic of 0.6036. MRI accuracy in ER/PR-Her2- patients was moderate with the C-statistic of 0.7203.

DISCUSSION

The progression of cancer is characterized by metastasis. As the first organ to be involved during metastasis, the presence of regional lymph node metastasis correlates with dissemination to distant organs and a poorer prognosis (1, 14). Based on preoperative imaging evaluation of axillary, clinicians would choose fine needle aspiration, core needle biopsy, SLNB, or ALND to treat potential local metastases. Historically, ALND was regarded as the most accurate and radical method for assessing and controlling regional metastatic spread (2). However, excessive treatment would bring unnecessary pain

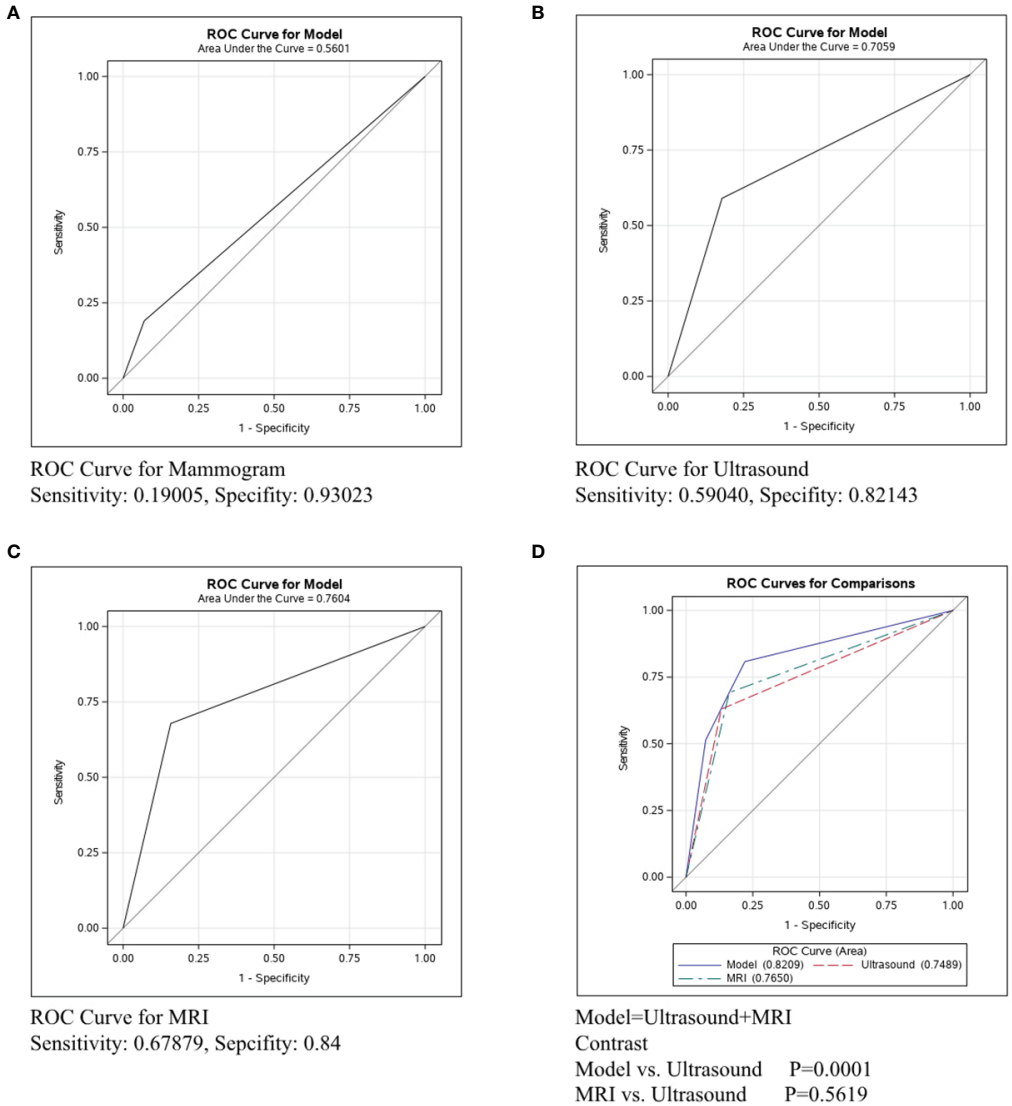


FIGURE 3 | ROC curve analysis for specific imaging examination in ER/PR+Her2-subtype. The vertical axis is sensitivity, the horizontal axis is 1-specificity. AUC is a parameter used to measure the value of imaging examination in the prediction of axillary lymph nodes. **(A)** ROC curve for mammogram. **(B)** ROC curve for ultrasound. **(C)** ROC curve for MRI. **(D)** ROC curve for ultrasound+MRI.

for pathologically node-negative patients, while residual lesions would bring potential recurrent risk. Therefore, accurate preoperative imaging evaluation of axillary lymph node involvement is very important for precision treatment of breast cancer patients.

The imaging methods we reviewed to assess the metastasis of axillary lymph nodes included mammography, ultrasound, and MRI. Mammography is the standard imaging modality for breast cancer screening, especially for postmenopausal women whose breast are almost entirely fatty (2, 15). However, our research showed that mammography was not reliable for the evaluation of lymph node metastasis (**Figure 1A**), This poor prediction may be from extremely low sensitivity but high specificity, which was consistent with the results from former studies (2). The low sensitivity may be attributed to the limited spatial resolution and the fact that the axillary area may not be fully visualized. Nevertheless, the high specificity of mammography can help raise the suspicion of malignancy detected by ultrasound or MRI. Ultrasound is usually the preferred method for the assessment of lymph node involvement in breast cancer patients (2, 16). It was reported that the sensitivity of ultrasound had a wide range, between 49% and 87%, and the specificity was between 55% and 97% (2). Our study reached a similar conclusion (**Figure 1B**). In the identification of lymph node metastasis, the evaluation standards include the size criteria as well as the morphologic criteria. Moreover, the Color Doppler allows for the visualization

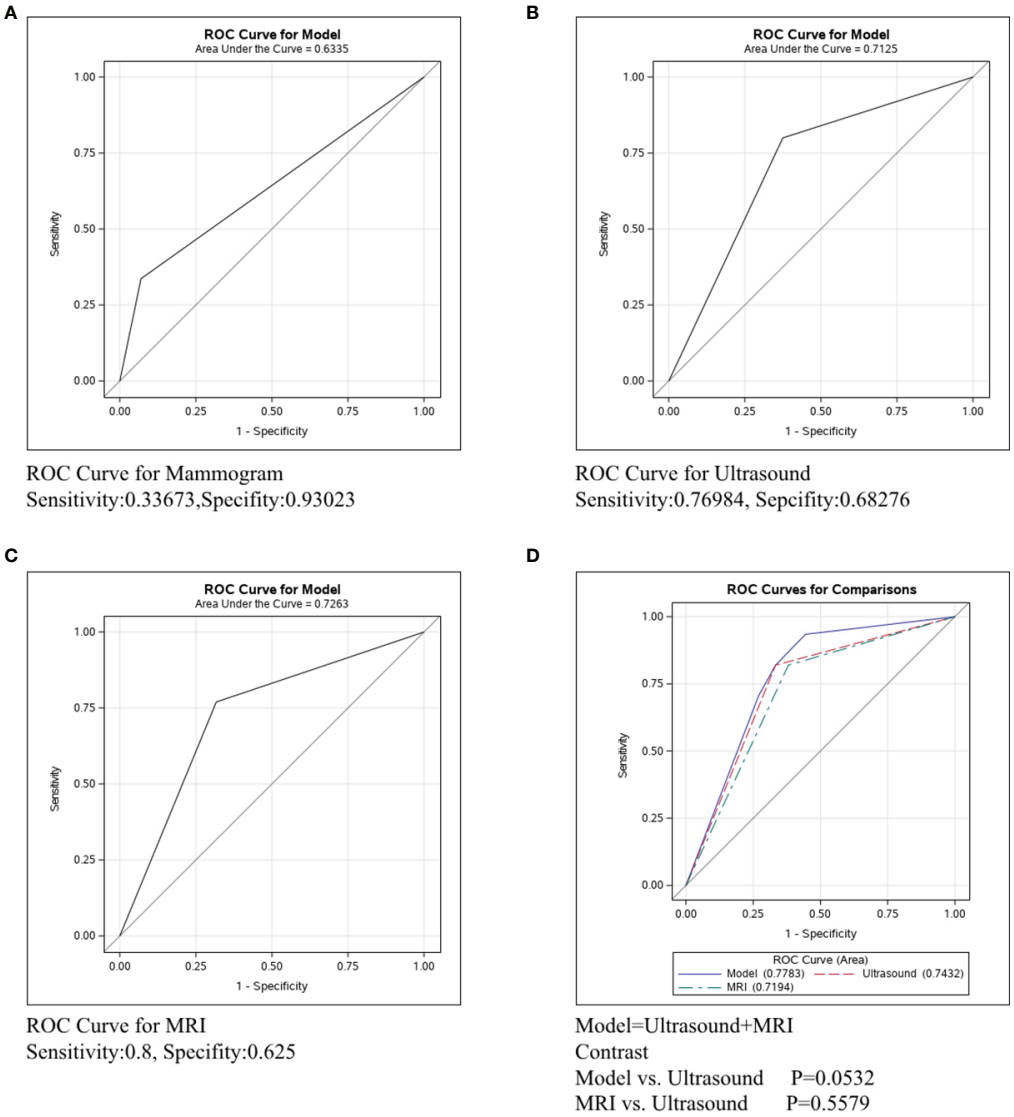


FIGURE 4 | ROC curve analysis for specific imaging examination in ER/PR-Her2- subtype. The vertical axis is sensitivity, the horizontal axis is 1-specificity. AUC is a parameter used to measure the value of imaging examination in the prediction of axillary lymph nodes. **(A)** ROC curve for mammogram. **(B)** ROC curve for ultrasound. **(C)** ROC curve for MRI. **(D)** ROC curve for ultrasound+MRI.

of intranodal vascular pattern and the abnormal cortical blood flow to help further increase the detection rates (17). MRI has a minor role in the diagnosis of breast cancer and metastatic lymph node in a clinical setting, mostly because of its high price and time-consuming features (18, 19). According to the literature, the pooled diagnostic sensitivity and specificity of MRI to detect axillary lymph node metastasis in patients with breast cancer were 75%-80% and 89%-91% respectively (19). As our research suggests, the sensitivity and specificity of MRI in detecting metastatic nodes were both weaker than ultrasound (**Figures 1B–D**), mainly because the dedicated breast coils may limit the complete visualization of the axilla. Moreover, the pulsation artifact from heart may occasionally obscure the images of lymph nodes (20).

Molecular subtype classification of breast cancer is a regular process for individualized cancer management. Previous studies have indicated that the molecular subtype was correlated with characteristic imaging manifestation of the lump (21). Therefore, we next explored whether the imaging diagnostic performance of lymph nodes differ among different molecular subtypes of breast cancer.

In Her2+ subtype, the C-statistics of mammography, ultrasound, and MRI were 0.5291, 0.6556 and 0.6036, respectively (**Figures 2A–C**). Clearly, ultrasound was the most

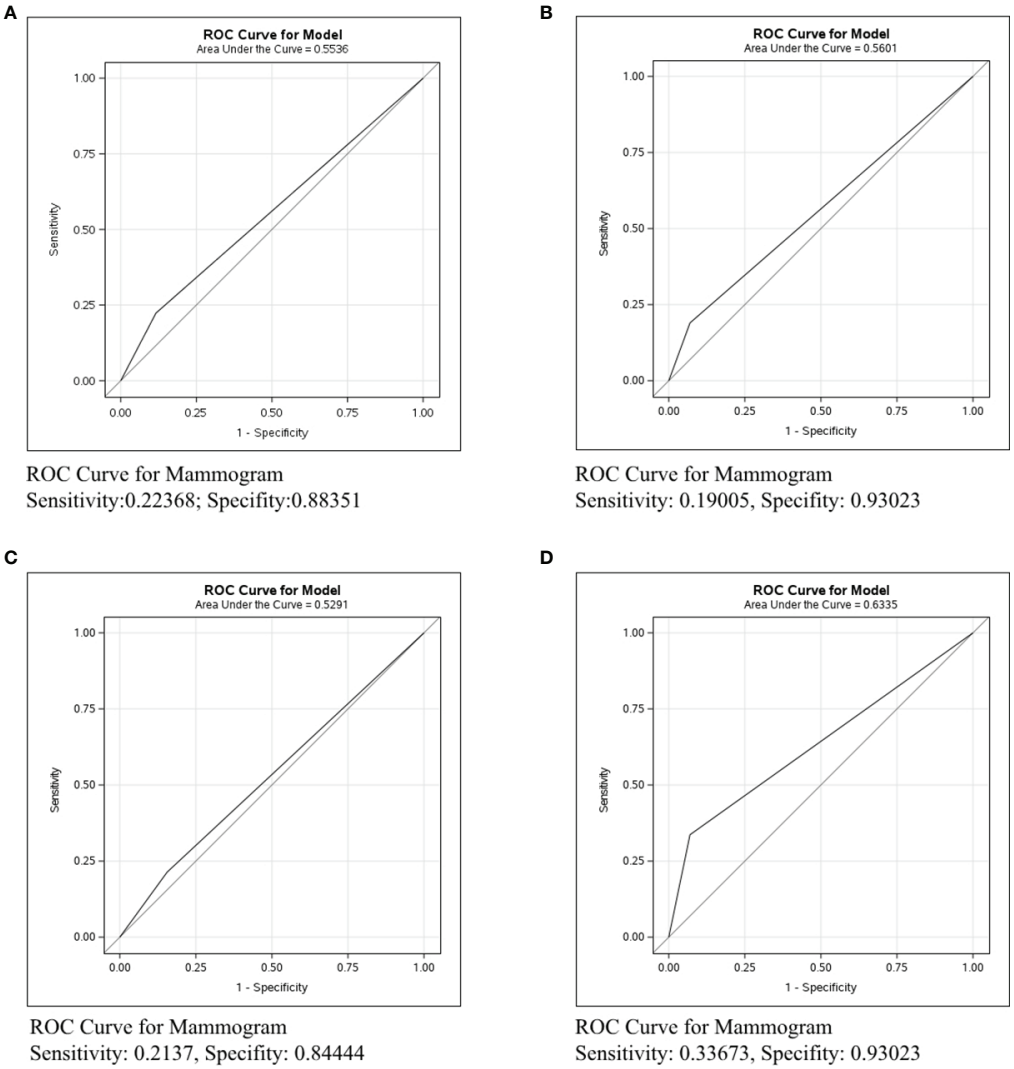


FIGURE 5 | ROC curve analysis for Mammogram in all molecular subtypes. The vertical axis is sensitivity, the horizontal axis is 1-specificity. AUC is a parameter used to measure the value of mammogram in the prediction of axillary lymph nodes. **(A)** ROC curve in all molecular subtypes. **(B)** ROC curve in ER/PR+Her2- negative subtype. **(C)** ROC curve in Her2+ subtype. **(D)** ROC curve in ER/PR-Her2- subtype.

precise examination for lymph node assessment. Moreover, no statistical difference was found between ultrasound+MRI and ultrasound alone for detecting metastatic lymph nodes (p=0.492, **Figure 2D**). To sum up, ultrasound was the most recommended examination in Her2+ patients and MRI was not strictly necessary for the diagnosis lymph node involvement in HER2+ breast cancer.

In ER/PR+Her2- patients, the C-statistics of mammography, ultrasound, and MRI were 0.5601, 0.7059, and 0.7604, respectively (**Figures 3A–C**). Our study indicates that the diagnostic effect of MRI and ultrasound were similar (p=0.5619, **Figure 3D**), while ultrasound+MRI increased the accuracy for lymph node assessment than ultrasound alone (p=0.0001, **Figure 3D**). We recommend ultrasound+MRI in ER/PR+Her2- patients for more accurate axillary assessment. Currently, since there are harmful side-effects of axillary surgery, minimizing, and even eliminating the axillary surgery is a clear trend. Related clinical trials include BOOG 2013-08 trial (22), SOUND trial (23),and INSEMA trial (24). According to the literature, less than 4 involved nodes (1-3 macro-metastases) and were considered to have little influence in breast cancer mortality, in which condition and the risk of disease progression depended mainly on the biological characteristic of the primary tumor (24). Based on this, to positively decrease the axillary side effect rates and improve the quality of life, the axillary surgery should be considered mainly on the basis of tumor traits rather than node involvement. As we know, the prognosis of ER/PR+Her2- subtype is best among three subtypes

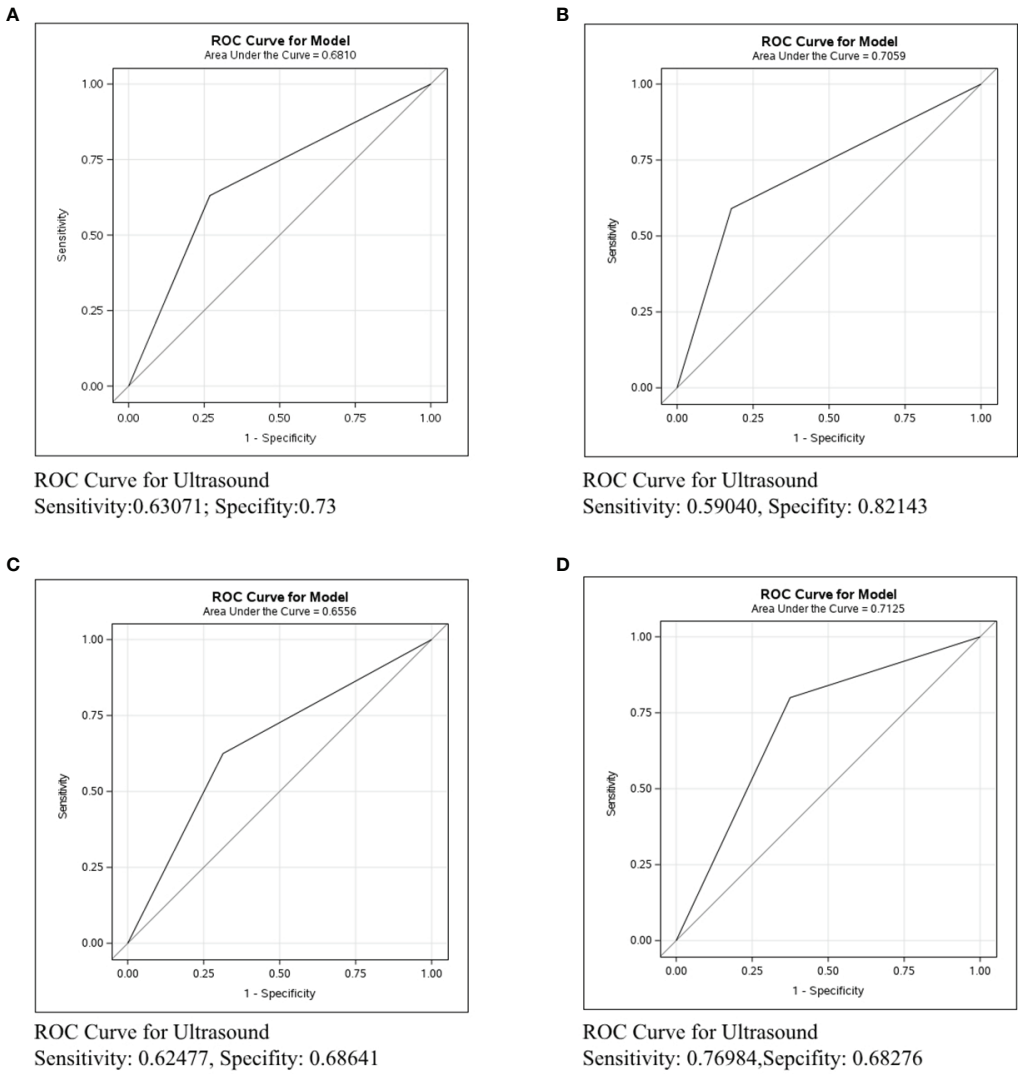


FIGURE 6 | ROC curve analysis for Ultrasound in all molecular subtypes. The vertical axis is sensitivity, the horizontal axis is 1-specificity. AUC is a parameter used to measure the value of ultrasound in the prediction of axillary lymph nodes. **(A)** ROC curve in all molecular subtypes. **(B)** ROC curve in ER/PR+Her2- subtype. **(C)** ROC curve in Her2+ subtype. **(D)** ROC curve in ER/PR-Her2- subtype.

(25). Meanwhile, the diagnostic accuracy of ultrasound+MRI in lymph node metastases was also highest in the ER/PR+Her2- subtype in our study. Therefore, we can reasonably assume that the lymph node negative ER/PR+Her2- patients diagnosed by imaging tests would rarely have massive positive lymph node pathologically(≥4 macro-metastases), and compared with axillary surgery, no axillary surgical intervention for clinically node negative breast cancer would bring non-inferior overall survival rates and better quality of life. In the future, we would like to design prospective studies with ER/PR+Her2- patients to explore the subtraction of axillary surgery in patients with negative lymph nodes by adequate imaging evaluation.

In ER/PR-Her2- patients, the C-statistics of mammography, ultrasound and MRI were 0.6335, 0.7125 and 0.7263, respectively (**Figures 4A–C**). The diagnosis effect of MRI and ultrasound was similar (p=0.5579, **Figure 4D**), while adding MRI did not increase the accuracy for lymph node assessment by ultrasound (p=0.0532, **Figure 4D**). Nonetheless, we can see a trend that adding MRI improved accuracy, and perhaps increasing the sample size could get a statistical difference (26). Therefore, in ER/PR-Her2- patients, ultrasound was the preferred imaging examination and if cost is not a regard, MRI examination may be also feasible. Next, we performed horizontal comparison. The lymph node assessment accuracy of mammography, ultrasound, and MRI were all worse in the Her2+ subtype than in ER/PR+Her2- or ER/PR-Her2- subtypes. In order to improve the detection rate of metastasis lesion, new technologies for axillary assessment such as contrast-

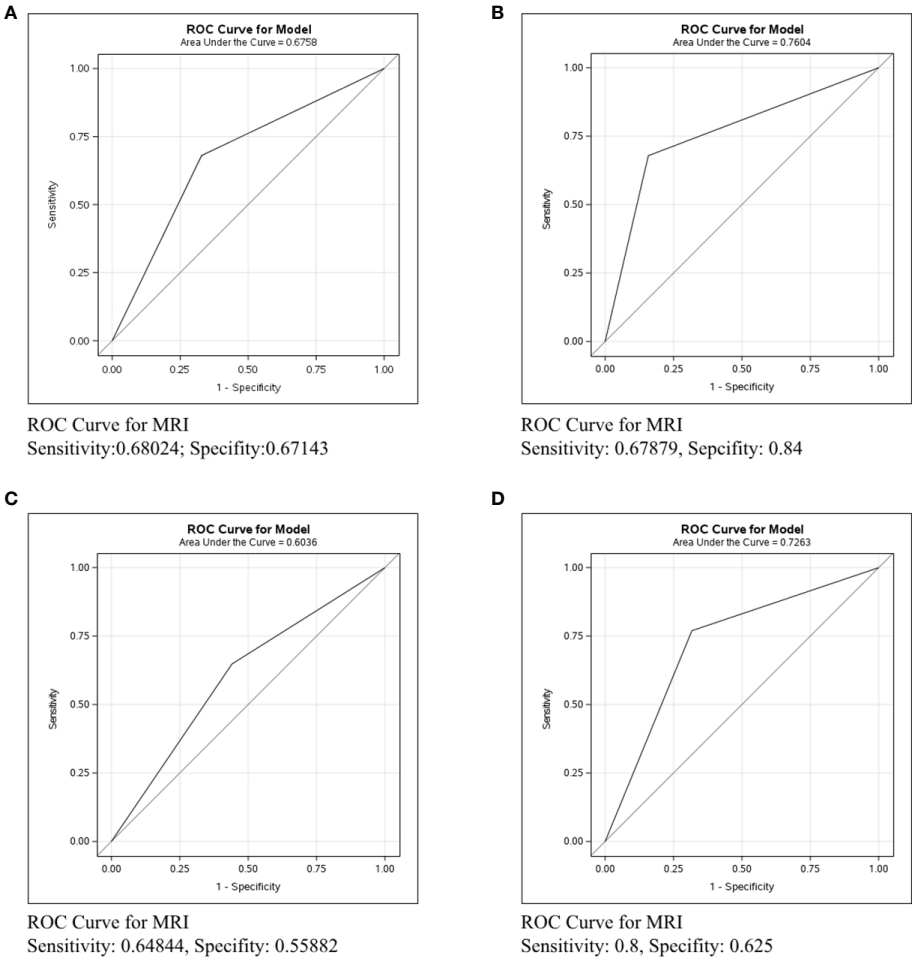


FIGURE 7 | ROC curve analysis for MRI in all molecular subtypes. The vertical axis is sensitivity, the horizontal axis is 1-specificity. AUC is a parameter used to measure the value of MRI in the prediction of axillary lymph nodes. **(A)** ROC curve in all molecular subtypes. **(B)** ROC curve in ER/PR+Her2- subtype. **(C)** ROC curve in Her2+ subtype. **(D)** ROC curve in ER/PR-Her2- subtype.

enhanced ultrasonography (27), digital breast tomosynthesis (DBT) (28), and the lymph PET (29) should be further explored, with an expected increase in the accuracy and predictability of axillary lymph nodes and increase in the benefit to more patients.

For the first time, our study explored the influence of breast cancer molecular subtypes on the diagnostic performance of three different axillary imaging. However, our research was a single center and retrospective study. The amount of data in hierarchical analysis is relatively small and a prospective study with a larger sample size is expected in the future.

CONCLUSION

From a clinical perspective, our job reviewed the diagnostic performance of three commonly used axillary imaging methods in different molecular subtypes of breast cancer. It may give some suggestion in the selection of lymph node assessment examinations and the subsequent axillary treatments. ER/PR+Her2- breast cancer may become a breakthrough in research on reducing axillary lymph node surgery due to its high imaging accuracy and good prognosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration, and has been approved by the Medical Ethics Review Committee of the first

affiliated hospital of Nanjing Medical University (reference number 2021-SR-182, Figure S5.

AUTHOR CONTRIBUTIONS

YX, YC and XH collected data. YH and JP analyzed the data. MZ, XH and SW designed the experiments. MZ and YH writed the paper and approved the submission and publication.

FUNDING

This study was financially supported by the National Natural Science Foundation of China (81702607, 81672612), Scientific Research Project of Jiangsu Province Association of Maternal and Child Health (FYX202018), Scientific Research Project of Jiangsu Women and Children Health Hospital for Youth Talents (FYRC202003).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.762906/full#supplementary-material

Supplementary Figure 1 | Flowchart of the patient selection process in this study.

Supplementary Figure 2 | Morphological features of mammograph that are predictors of lymph node malignancy. Size>2cm **(A, C)**, increased density **(B)**, rounded or irregular shape **(B, D)**, spiculated margins or the absent fatty hilum).

Supplementary Figure 3 | Ultrasonic morphological features that can predict lymph node malignancy. Focally or diffusely thickened cortex (> 3 mm thick) **(A, B)**, deformed or absent fatty hilum **(C, D)**, abnormal blood flow **(B, D)**.

Supplementary Figure 4 | Morphological features of MRI that can predict lymph node malignancy. Irregular contour compared with the contralateral axilla **(A–C)**, node measuring greater than 1 cm **(A, B**, thickened cortex >3 mm **(A, B)**, the loss of fatty hilum **(C)** Supplementary Figure 5 Ethics review.

REFERENCES

1. Pereira E, Kedrin D, Seano G, Gautier O, Meijer E, Jones D, et al. Lymph Node Metastases can Invade Local Blood Vessels, Exit the Node, and Colonize Distant Organs in Mice Science. (2018) 359(6382):1403–7. doi: 10.1126/science.aal3622
2. Choi HY, Park M, Seo M, Song E, Shin SY, Sohn YM. Preoperative Axillary Lymph Node Evaluation in Breast Cancer: Current Issues and Literature Review. *Ultrasound Q* (2017) 33(1):6–14. doi: 10.1097/RUQ.0000000000000277
3. Manca G, Rubello D, Tardelli E, Giammarile F, Mazzarri S, Boni G, et al. Sentinel Lymph Node Biopsy in Breast Cancer. *Clin Nucl Med* (2016) 41 (2):126–33. doi: 10.1097/rlu.0000000000000985
4. Vane MLG, van Nijnatten TJA, Nelemans PJ, Lobbes MBI, van Roozendaal LM, Kooreman LFS, et al. Does the Subtype of Breast Cancer Affect the Diagnostic Performance of Axillary Ultrasound for Nodal Staging in Breast Cancer Patients? *Eur J Surg Oncol* (2019) 45(4):573–7. doi: 10.1016/j.ejso.2019.01.012
5. Grimm L, Johnson K, Marcom P, Baker J, Soo MJR. Can Breast Cancer Molecular Subtype Help to Select Patients for Preoperative MR Imaging? Radiology (2015) 274(2):352–8. doi: 10.1148/radiol.14140594
6. Wang Y, Ikeda D, Narasimhan B, Longacre T, Bleicher R, Pal S, et al. Estrogen Receptor-Negative Invasive Breast Cancer: Imaging Features of Tumors With and Without Human Epidermal Growth Factor Receptor Type 2 Overexpression Radiology. (2008) 246(2):367–75. doi: 10.1148/radiol.2462070169
7. Jiang Y, Lou J, Wang S, Zhao Y, Wang C, Wang D. Evaluation of the Role of Dynamic Contrast-Enhanced MR Imaging for Patients With BI-RADS 3-4 Microcalcifications. *PLoS One* (2014) 9(6):e99669. doi: 10.1371/journal.pone.0099669
8. Valente SA, Levine GM, Silverstein MJ, Rayhanabad JA, Weng-Grumley JG, Ji L, et al. Accuracy of Predicting Axillary Lymph Node Positivity by Physical Examination, Mammography, Ultrasonography, and Magnetic Resonance Imaging. *Ann Surg Oncol* (2012) 19(6):1825–30. doi: 10.1245/s10434-011-2200-7
9. Liu HL, Zong M, Wei H, Wang C, Lou JJ, Wang SQ, et al. Added Value of Histogram Analysis of Apparent Diffusion Coefficient Maps for Differentiating Triple-Negative Breast Cancer From Other Subtypes of Breast Cancer on Standard MRI. *Cancer Manag Res* (2019) 11:8239–47. doi: 10.2147/CMAR.S210583
10. Boughey JC, Ballman KV, Hunt KK, McCall LM, Mittendorf EA, Ahrendt GM, et al. Axillary Ultrasound After Neoadjuvant Chemotherapy and Its Impact on Sentinel Lymph Node Surgery: Results From the American College of Surgeons Oncology Group Z1071 Trial (Alliance). *J Clin Oncol* (2015) 33 (30):3386–93. doi: 10.1200/jco.2014.57.8401
11. Hammond ME, Hayes DF, Wolff AC, Mangu PB, Temin S. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. *J Oncol Pract* (2010) 6(4):195–7. doi: 10.1200/JOP.777003
12. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *J Clin Oncol* (2013) 31(31):3997–4013. doi: 10.1200/jco.2013.50.9984
13. Burstein HJ, Curigliano G, Thurlimann B, Weber WP, Poortmans P, Regan MM, et al. Customizing Local and Systemic Therapies for Women With Early Breast Cancer: The St. Gallen International Consensus Guidelines for Treatment of Early Breast Cancer 2021. *Ann Oncol* (2021) 32(10):1216–35. doi: 10.1016/j.annonc.2021.06.023
14. Sleeman JP. The Lymph Node as a Bridgehead in the Metastatic Dissemination of Tumors. *Recent Results Cancer Res* (2000) 157:55–81. doi: 10.1007/978-3-642-57151-0_6
15. Muttarak M, Chaiwun B, Peh WCG. Role of Mammography in Diagnosis of Axillary Abnormalities in Women With Normal Breast Examination. *Australas Radiol* (2004) 48(3):306–10. doi: 10.1111/j.0004-8461.2004.01312.x
16. Sehgal CM, Weinstein SP, Arger PH, Conant EF. A Review of Breast Ultrasound. *J Mammary Gland Biol Neoplasia* (2006) 11(2):113–23. doi: 10.1007/s10911-006-9018-0
17. Yang WT, Chang J, Metreweli C. Patients With Breast Cancer: Differences in Color Doppler Flow and Gray-Scale US Features of Benign and Malignant Axillary Lymph Nodes. *Radiology* (2000) 215(2):568–73. doi: 10.1148/radiology.215.2.r00ap20568
18. Atallah D, Moubarak M, Arab W, El Kassis N, Chahine G, Salem C. MRI-Based Predictive Factors of Axillary Lymph Node Status in Breast Cancer. *Breast J* (2020) 26(11):2177–2182. doi: 10.1111/tbj.14089
19. Zhou P, Wei YQ, Chen GY, Guo L, Yan DY, Wang Y. Axillary Lymph Node Metastasis Detection by Magnetic Resonance Imaging in Patients With Breast Cancer: A Meta-Analysis. *Thorac Cancer* (2018) 9(8):989–96. doi: 10.1111/1759-7714.12774
20. Marino MA, Avendano D, Zapata P, Riedl CC, Pinker K. Lymph Node Imaging in Patients With Primary Breast Cancer: Concurrent Diagnostic Tools. *Oncologist* (2020) 25(2):E231–E42. doi: 10.1634/theoncologist.2019-0427
21. Wu M, Ma J. Association Between Imaging Characteristics and Different Molecular Subtypes of Breast Cancer. *Acad Radiol* (2017) 24(4):426–34. doi: 10.1016/j.acra.2016.11.012
22. van Roozendaal LM, Vane MLG, van Dalen T, van der Hage JA, Strobbe LJA, Boersma LJ, et al. Clinically Node Negative Breast Cancer Patients Undergoing Breast Conserving Therapy, Sentinel Lymph Node Procedure Versus Follow-

Up: A Dutch Randomized Controlled Multicentre Trial (BOOG 2013-08). *BMC Cancer* (2017) 17(1): 459. doi: 10.1186/s12885-017-3443-x
23. Gentilini O, Veronesi U. Abandoning Sentinel Lymph Node Biopsy in Early Breast Cancer? A New Trial in Progress at the European Institute of Oncology of Milan (SOUND: Sentinel Node vs Observation After Axillary UltraSouND). *Breast* (2012) 21(5):678–81. doi: 10.1016/j.breast.2012.06.013
24. Reimer T, Stachs A, Nekljudova V, Loibl S, Hartmann S, Wolter K, et al. Restricted Axillary Staging in Clinically and Sonographically Node-Negative Early Invasive Breast Cancer (C/It1-2) in the Context of Breast Conserving Therapy: First Results Following Commencement of the Intergroup-Sentinel-Mamma (INSEMA) Trial. *Geburtshilfe Und Frauenheilkunde* (2017) 77 (2):149–57. doi: 10.1055/s-0042-122853
25. Voduc KD, Cheang MCU, Tyldesley S, Gelmon K, Nielsen TO, Kennecke H. Breast Cancer Subtypes and the Risk of Local and Regional Relapse. *J Clin Oncol* (2010) 28(10):1684–91. doi: 10.1200/jco.2009.24.9284
26. Carey L, Winer E, Viale G, Cameron D, Gianni L. Triple-Negative Breast Cancer: Disease Entity or Title of Convenience? *Nat Rev Clin Oncol* (2010) 7 (12):683–92. doi: 10.1038/nrclinonc.2010.154
27. Hu Z, Cheng X, Li J, Jiang J, Jiang Z, Li H, et al. Preliminary Study of Real-Time Three-Dimensional Contrast-Enhanced Ultrasound of Sentinel Lymph Nodes in Breast Cancer. *Eur Radiol* (2020) 30(3):1426–35. doi: 10.1007/s00330-019-06494-0
28. Hofvind S, Holen ÅS, Aase HS, Houssami N, Sebuødegård S, Moger TA, et al. Two-View Digital Breast Tomosynthesis Versus Digital Mammography in a Population-Based Breast Cancer Screening Programme (To-Be): A Randomised, Controlled Trial. *Lancet Oncol* (2019) 20(6):795–805. doi: 10.1016/s1470-2045(19)30161-5
29. van Nijnatten TJA, Goorts B, Vöö S, de Boer M, Kooreman LFS, Heuts EM, et al. Added Value of Dedicated Axillary Hybrid 18F-FDG PET/MRI for Improved Axillary Nodal Staging in Clinically Node-Positive Breast Cancer Patients: A Feasibility Study. *Eur J Nucl Med Mol Imaging* (2017) 45(2):179–86. doi: 10.1007/s00259-017-3823-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Oncology

ORIGINAL RESEARCH
published: 19 July 2022
doi: 10.3389/fonc.2022.861210

A Nomogram Based on Hematological Parameters and Clinicopathological Characteristics for Predicting Local–Regional Recurrence After Breast-Conserving Therapy

Luhao Sun, Wei Zhao, Fukai Wang, Xiang Song, Xinzhao Wang, Chao Li† and Zhiyong Yu*†*

Breast Cancer Center, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China

OPEN ACCESS

Edited by:
Yiding Chen,
Zhejiang University, China

Reviewed by:
Eva Valentina Klocker,
University Hospital Graz, Austria
Yutian Zou,
Sun Yat-sen University Cancer Center (SYSUCC), China
Kai Chen,
Sun Yat-sen Memorial Hospital, China

***Correspondence:**
Chao Li
lichao19890305@126.com
Zhiyong Yu
drzhiyongyu@aliyun.com

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: *24 January 2022*
Accepted: *13 June 2022*
Published: *19 July 2022*

Citation:
Sun L, Zhao W, Wang F, Song X, Wang X, Li C and Yu Z (2022) A Nomogram Based on Hematological Parameters and Clinicopathological Characteristics for Predicting Local–Regional Recurrence After Breast-Conserving Therapy. Front. Oncol. 12:861210. doi: 10.3389/fonc.2022.861210

Objectives: The aim of this study was to identify the factors for local–regional recurrence (LRR) after breast-conserving therapy (BCT). We established a practical nomogram to predict the likelihood of LRR after BCT based on hematological parameters and clinicopathological features.

Methods: A retrospective analysis was performed on 2,085 consecutive breast cancer patients who received BCT in Shandong Cancer Hospital from 2006 to 2016, including 1,460 patients in the training cohort and 625 patients in the validation cohort. Univariate and multivariate analyses were performed based on hematological parameters (fibrinogen, platelets, mean platelet volume, neutrophils, monocytes, and lymphocytes) and clinicopathological characteristics to identify the independent factors for LRR. Subsequently, a nomogram for predicting LRR was established by logistic regression analysis. The nomogram was validated in 625 patients in the validation cohort.

Results: During the median follow-up period of 66 months, 44 (3.01%) patients in the training cohort and 19 (3.04%) patients in the validation cohort suffered from LRR. Multivariate analysis showed six independent factors related to LRR, including molecular subtype, pathological N stage, re-resection, radiotherapy or not, platelet count*MPV*fibrinogen (PMF), and neutrophil count/lymphocyte count ratio (NLR). Six variables were entered into logistic regression to establish the nomogram for predicting LRR. The nomogram of LRR showed excellent discrimination and prediction accuracy. The area under the receiver operating characteristic curve (AUC) was 0.89 ($p < 0.001$, 95% CI = 0.83, 0.95) in the training cohort and 0.88 ($p < 0.001$, 95% CI = 0.8, 0.96) in the validation cohort. Calibration curves for the prediction model in the training and validation cohorts both demonstrated satisfactory consistency between the nomogram-predicted and actual LRR.

Conclusion: The combination of hematological parameters and clinicopathological characteristics can predict LRR after BCT. The predictive nomogram based on preoperative and postoperative indicators of BCT might serve as a practical tool for individualized prognostication. More prospective studies should be performed to verify the model.

Keywords: breast-conserving therapy, local–regional recurrence, hematological parameters, clinicopathological characteristics, nomogram, predicting model

INTRODUCTION

Breast cancer is the most common malignancy in women and is also the main cause of female death (1). Multiple prospective randomized clinical trials have confirmed that breast-conserving therapy (BCT) plus radiotherapy is similar to mastectomy in terms of disease control and long-term overall survival (2–4). The BCT ratio in European and American countries has exceeded 60%. Although the BCT ratio in China is increasing, it is still at a low level, at only 20%–30%. The main reason is that a number of Chinese patients believe that BCT carries a risk of LRR compared to mastectomy. Therefore, it is necessary to study the LRR of the breast-conserving population in China. In this study, hematological parameters were innovatively added to predict LRR after BCT. Some previous studies have pointed out that hematological parameters (such as neutrophil count/lymphocyte count, lymphocyte count/monocyte count, and platelet count/lymphocyte count) have a satisfactory predictive effect on the recurrence of a few cancers, such as gastric cancer and bladder cancer (5–7). Therefore, we combined hematological parameters and clinicopathological features to predict the recurrence of breast cancer after BCT, and established a prediction model. Balancing survival and breast aesthetics, BCT has become the preferred local treatment for early invasive breast cancer (8). However, patients who received BCT and postoperative radiotherapy still suffered from LRR (3%–5%) in 10 years. Previous studies have found that clinicopathological characteristics (such as young age of onset, no radiotherapy, high nuclear grade, tumor stage, and molecular subtype) are factors for LRR after BCT (9, 10). For molecular subtype, according to the CSCO guidelines: Luminal A: HER-2 (−), ER (+), PR (+) and high expression, Ki67 low expression. Luminal B: HER-2 (−), ER (+), PR (−) or low expression, Ki67 high expression.

Hematological parameters (such as fibrinogen and platelets) have potential effects on the occurrence and development of tumors. Previous studies have reported that fibrinogen and platelets have synergistic effects in protecting tumor cells from NK cells (11, 12). Satoshi Takagi reported that platelets could promote the interaction between aggrus/podoplanin and CLEC-2 to promote tumor growth and metastasis (13). It also shown that platelets could promote immune escape adaptive immune responses by increasing the expression of PD-L1 in cancer cells (14). The mean platelet volume (MPV) level reflects the activity of platelets, which are elevated in patients with myocardial infarction and cancer (15). The tumor-induced systemic inflammatory response (SIR) can inhibit the function of T-cell immune monitoring and the immune response, causing tumor development and metastasis (16, 17). Inflammatory factors (neutrophils, monocytes, and lymphocytes) and platelets can be used to evaluate the host's antitumor immune response and effectively predict the prognosis of cancer (18). Since tumor-associated inflammation is a basic component of tumor microenvironment, it may affect the prognosis of tumor. In a clinical setting, the detection of elevated inflammatory factors in the systemic circulation is widely considered to be a prognostic factor for many malignancies (19).

Therefore, this retrospective study was performed for two purposes: the first was to identify the factors related to the LRR of breast cancer treated by BCT, and the second was to establish a nomogram for predicting LRR after BCT by clinicopathological characteristics and hematological parameters.

MATERIALS AND METHODS

Patient Population

The study retrospectively investigated the relationship between hematological parameters, clinicopathological features, and LRR at Shandong Cancer Hospital from 2006 to 2016. The eligibility criteria were as follows: (1) female patients with invasive carcinoma or ductal carcinoma *in situ* by pathology; (2) all patients were treated with BCT; (3) chemotherapy and radiotherapy were not received before the operation; (4) patients did not receive other anticancer treatment or blood transfusion before blood examination; and (5) all patients completed the analysis of hematological parameters after entering the hospital to the day before the operation.

Among all patients who received BCT from 2006 to 2016, 74 patients who had data loss and 104 patients who received neoadjuvant chemotherapy were excluded. Finally, 2,085 patients selected for the study were randomly divided into a training cohort (1,460) and a validation cohort (625) according to a 7:3 ratio (**Figure 1**).

The selected clinicopathological characteristics included the following: age of onset, menstrual status, pathological stage, presence of carcinoma *in situ*, molecular subtype, nuclear grade, re-resection, pathological T stage, pathological N stage, axillary surgery type, hormone receptor status, HER-2 status, chemotherapy, radiotherapy, and endocrine treatment, which are summarized in **Table 1**. The pathological stage was in accordance with the American Joint Commission on Cancer (AJCC) 7th edition staging standard. Histological grade was

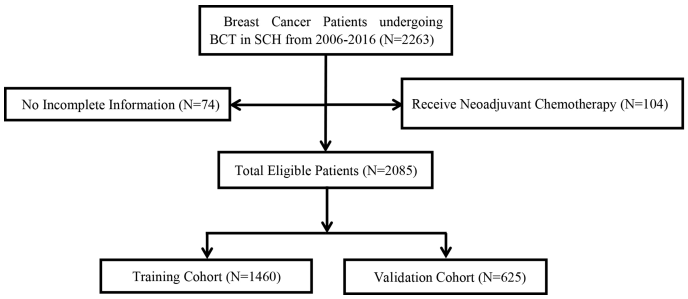


FIGURE 1 | Consort diagram for the study cohort. BCT, breast cancer therapy; SCH, Shandong cancer hospital; LRR, local–regional recurrence.

determined according to the World Health Organization (WHO) classification system.

The hematological parameters included platelet count, fibrinogen, MPV, neutrophil count, monocyte count, and lymphocyte count. We used the following terms to express the correlation of hematological indices:

PF = platelet count*fibrinogen

MF = MPV*fibrinogen

PMF = platelet count*MPV*fibrinogen

FMR = fibrinogen/MPV ratio

PMR = platelet count/MPV ratio

NLR = neutrophil count/lymphocyte count ratio

MLR = monocyte count/lymphocyte count ratio

PLR = platelet count/lymphocyte count ratio

All patients signed an informed consent form upon admission. This study was approved by the Medical Ethics Committee of Shandong Cancer Hospital.

Definition of Recurrence and Patient Follow-Up

We defined LRR as local treatment failure (including ipsilateral chest wall and skin, surgical area, and ipsilateral breast recurrence) and ipsilateral area treatment failure (ipsilateral internal mammary, supraclavicular, and axillary lymph node recurrence). There were fewer patients who had distant recurrence in our center. Distant recurrence includes nonipsilateral local recurrence and other secondary cancers (20). In our center, the definition of resection margin is to use the upper, lower, inner, and outer four margins after extended resection to represent the margin of the whole residual cavity. Re-resection is defined as secondary resection to achieve a negative margin in patients with a positive margin of first resection. A positive margin was defined as <2 mm from the surgical margin (21). The types of axillary surgery are divided into axillary lymph node dissection (ALND) and sentinel lymph node biopsy (SLNB). The subjects were followed up until February 1, 2021. The median follow-up time was 66 months (range: 6–180 months). They were followed up every 3 months in the first 2 years and every 6 months after the 3rd year.

Treatments

For all BCT patients, we recommended radiotherapy for the whole breast at a median dose of 50 Gy, usually given in a fraction of 2 Gy/FX. Boost doses were given to the primary tumor site. The choice of chemotherapy was according to the St. Gallen consensus: patients with moderate recurrence risk received cyclophosphamide, doxorubicin (or epirubicin), and 5-Fu (CAF) regimen; patients with low risk received cyclophosphamide, methotrexate, and 5-Fu (CMF) regimen, or AC regimen; patients with high risk would receive taxane-containing regimens [AC followed by paclitaxel (P), or CAF followed by docetaxel (T), or TAC]. All the patients with positive hormone receptor status received tamoxifen (for both pre-menopausal and postmenopausal women) or aromatase inhibitors (only for postmenopausal women) for 5 years. The anti-HER2 targeted drug (Herceptin) had not officially entered the Chinese market during the study period (2006–2016) in this study group.

Statistical Analysis

The optimal cutoff levels of PF, MF, PMF, FMR, PMR, NLR, MLR, and PLR were identified by receiver operating characteristic (ROC) curve analysis. The chi-square (χ^2) test was used to test the difference between categorical variables. The Kaplan–Meier method was used to calculate the survival curve, and the log rank test was used for univariate analysis. The Cox risk ratio model was used for multivariate analysis, and the significant risk factors in univariate analysis were used for multivariate analysis. Then, binary logistic regression was used to establish the prediction model, in which the variables came from the significant factors in multivariate analysis. A nomogram for LRR was created based on the multivariable logistic regression ($p < 0.05$). Finally, ROC curves were drawn to assess the accuracy of the prediction model, with a reasonable range of 0.5 (random) to 1.0 (perfect). The y-axis of the calibration curve represents the actual observed survival rate, and the x-axis represents the survival rate predicted by the established nomogram in the training cohort and validation cohort. All statistical data were analyzed by SPSS version 26.0 (SPSS company, Chicago, Illinois, USA) and R 4.0.3 (The R

TABLE 1 | The basic information, tumor characteristics, and treatment methods of breast cancer patients receiving BCT.

Characteristic	Training cohort		Validation cohort	
	Total	LRR (%)	Total	LRR (%)
Age				
≤45	708	28 (3.9)	295	11 (3.7)
>45	752	16 (2.1)	330	8 (2.4)
Menopausal status				
Premenopausal	955	34 (3.6)	372	15 (4.0)
Postmenopausal	505	10 (2.0)	253 (33.4)	4 (1.6)
Nuclear grade				
I	220	2 (0.9)	112	3 (2.6)
II	731	17 (2.3)	292	10 (3.4)
III	443	23 (5.2)	177	6 (3.4)
Unknown	66	2 (3.0)	4	0 (0.0)
Pathologic T stage				
T1	1,041	34 (3.3)	388	11 (2.8)
T2	333	8 (2.4)	170	6 (3.5)
T3	10	0 (0.0)	3	0 (0.0)
Carcinoma *in situ*	76	2 (2.6)	64	2 (3.1)
Pathologic N stage				
N0	1,136	16 (1.4)	492	6 (1.2)
N1	276	16 (5.8)	117	8 (6.8)
N2	40	8 (20.0)	11	2 (18.2)
N3	8	4 (50.0)	5	3 (60.0)
Pathologic stage				
0	67	2 (3.0)	29	3 (10.3)
I	797	9 (1.1)	382	10 (2.6)
II	534	21 (3.9)	203	5 (2.5)
III	62	12 (19.4)	11	1 (9.1)
With carcinoma *in situ*				
Yes	318	21 (6.6)	189	10 (5.3)
No	1,142	23 (2.0)	436	9 (2.1)
Re-resection				
Yes	427	18 (4.2)	226	8 (3.5)
No	1,033	26 (2.5)	399	11 (2.8)
Molecular subtype				
Luminal A	707	7 (1.0)	298	4 (1.3)
Luminal B	114	3 (2.6)	48	1 (2.1)
HER-2 positive	504	20 (4.0)	226	7 (3.1)
TNBC	135	14 (10.4)	53	7 (13.2)
ER status				
Positive	1,140	19 (1.7)	403	12 (3.0)
Negative	320	25 (7.8)	222	7 (3.2)
PR status				
Positive	1,065	17 (1.6)	426	8 (1.9)
Negative	395	27 (6.8)	199	11 (5.5)
HER-2 status				
Positive	515	20 (3.9)	178	11 (6.2)
Negative	945	24 (2.5)	447	8 (1.8)
Receipt of chemotherapy				
Yes	1,076	34 (3.2)	452	14 (3.1)
No	384	10 (2.6)	173	5 (2.9)
Receipt of radiotherapy				
Yes	1,278	20 (1.6)	539	12 (2.2)
No	182	24 (13.3)	86	7 (8.1)
Receipt of endocrine therapy				
Yes	999	17 (1.7)	266	7 (2.6)
No	461	27 (5.9)	359	12 (3.3)

BCT, breast-conserving therapy; LRR, local–regional recurrence; ER, estrogen receptor; PR, progesterone receptor; TNBC, triple-negative breast cancer.

Project for Statistical Computing, www.r-project.org). $p < 0.05$ was considered as statistically significant.

RESULTS

Clinicopathological Characteristics and Hematological Parameters

According to the inclusion and exclusion criteria, 1,460 patients were included in the training cohort and 625 patients were included in the validation cohort. The baseline clinicopathological characteristics in the training cohort and the validation cohort are shown in **Table 1**. In the training cohort, the median age at diagnosis was 45 years (range, 20 to 85 years), and 955 (65.4%) patients were premenopausal. A total of 427 (29.2%) patients underwent re-resection after the first positive margin, and 182 (12.4%) patients did not receive radiotherapy. There were 318 (21.8%) patients who presented with carcinoma *in situ*, including 72 with pure DCIS and 246 with DCIS and invasive ductal carcinoma. Simple carcinoma *in situ* and T1, T2, and T3 tumors

were present in 76 (5.2%), 1,041 (71.3%), 333 (22.8%), and 10 (0.68%) patients respectively. A total of 1,136 (77.8%) patients were staged at N0, and the N1, N2, and N3 stages were present in 276 (18.9%), 40 (2.7%), and 8 (0.6%) patients, respectively. Among the molecular subtypes of all patients, luminal A accounted for the highest proportion (48.4%), and luminal B and triple-negative breast cancer (TNBC) accounted for relatively low proportions (7.8% and 9.2%, respectively). However, TNBC patients increased significantly in the recurrent population (31.8%). In the validation cohort, luminal A still accounted for the highest proportion (47.6%), and luminal B, HER-2 positive, and TNBC were showed in 48 (7.6%), 226 (36.1%), and 53 (8.4%) patients, respectively. A total of 492 (78.7%) patients were staged at N0, and the N1, N2, and N3 stages were present in 117 (18.7%), 11 (1.7%), and 5 (0.8%) patients, respectively.

Recurrence Outcomes

After the median follow-up of 66 months, 44 patients (3.01%) developed LRR in the training cohort. Among the 44 patients with LRR, 23 (52.3%) patients had recurrence in the ipsilateral breast, 18 (40.9%) patients had axillary lymph node involvement, and patients rarely had chest wall and skin recurrences. **Figure 2** and **Table 2** show the ROC and cutoff values of PF, MF, PMF, FMR, PMR, NLR, MLR, and PLR of patients with breast cancer before breast-conserving surgery. The optimal cutoff point could be used for the next survival analysis.

Univariate and Multivariate Survival Analysis

The results of the univariate analysis of LRR in the training cohort are shown in **Table 3** and **Figure 3**, which identified the following indicators associated with LRR among patients with BCT: clinicopathological variables (age of onset, pathological stage, molecular subtype, nuclear grade, re-resection, cancer *in situ*, pathological N stage, ER, PR, radiotherapy, and endocrine therapy) and hematological variables (including PF, MF, PMF, FMR, PMR, NLR, MLR, and PLR). Further multivariate Cox regression analysis demonstrated that the independent predictive factors for LRR were molecular subtype ($p < 0.001$, HR [95% CI] = 1.904 [1.392, 2.604]), pathological N stage ($p < 0.001$, HR [95% CI] = 2.330 [1.726, 3.145]), radiotherapy ($p < 0.001$, HR [95% CI] = 0.156 [0.084, 0.292]), re-resection ($p = 0.042$, HR [95% CI] = 2.210 [1.030, 4.742]), PMF ($p < 0.001$, HR [95% CI] = 1 [1, 1]), and NLR ($p < 0.001$, HR [95% CI] = 1.316 [1.187, 1.458]).

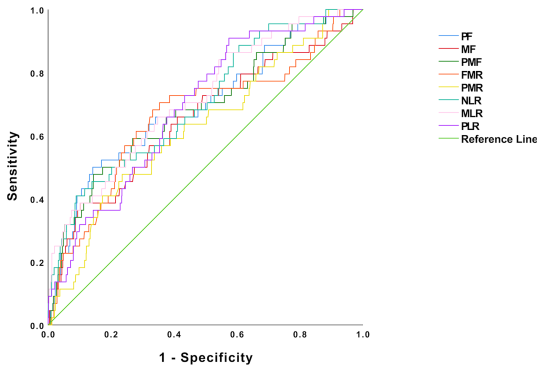


FIGURE 2 | Optimal cutoff points for hematologic parameters were on with ROC curves. PF, platelet count*fibrinogen; MF, mean platelet volume*fibrinogen; PMF, platelet count*mean platelet volume*fibrinogen; FMR, fibrinogen-to-mean platelet volume ratio; PMR, platelet count-to-mean platelet volume ratio; NLR, neutrophil count-to-lymphocyte count ratio; MLR, monocyte count-to-lymphocyte count ratio; PLR, platelet count-to-lymphocyte count ratio; ROC, receiver operating characteristic.

Prediction Model of the Local-regional Recurrence Nomogram

Through univariate and multivariate analysis, a predictive model was constructed based on the independent predictors, combined with meaningful clinicopathological features and hematological parameters in multivariate analysis. The dependent variable was the incidence of LRR. After entering binary logistic regression, it was determined that pathologic N stage was the best predictor. Re-resection did not show a significant difference ($p = 0.06$, HR [95% CI] = 2.61 [0.959, 7.103]). Molecular subtype, pathologic N stage, radiotherapy, PMF, and NLR were integrated and demonstrated using a visual nomogram (**Figure 4**). The nomogram scores were given based on the weights of the independent variables in the regression model. The scale length of the nomogram variables was positively correlated with their influence on the efficacy prediction. Among all factors,

TABLE 2 | The optimal cutoff point for local–regional recurrence.

Variables	AUC	Cutoff point	*p*-value
PF	0.694	910.60	<0.001
MF	0.652	35.24	0.001
PMF	0.687	9343.58	<0.001
FMR	0.665	0.29	<0.001
PMR	0.626	27.66	0.004
NLR	0.703	4.14	<0.001
MLR	0.715	0.28	<0.001
PLR	0.691	147.67	<0.001

*PF, platelet count*fibrinogen; MF, mean platelet volume*fibrinogen; PMF, platelet count*mean platelet volume*fibrinogen; FMR, fibrinogen-to-mean platelet volume ratio; PMR, platelet count-to-mean platelet volume ratio; NLR, neutrophil count-to-lymphocyte count ratio; MLR, monocyte count-to-lymphocyte count ratio; PLR, platelet count-to-lymphocyte count ratio; AUC, area under the curve.*

TABLE 3 | Univariate and multivariate analysis of factors for local–regional recurrence.

Factors	Univariate K-M	Multivariate Cox	
	p-value	HR (95% CI)	*p*-value
Age of onset	0.042	0.971 (0.939–1.004)	0.082
Pathologic stage	<0.001		0.942
Menstrual status	0.088		
Molecular subtype	<0.001	1.904 (1.392–2.604)	<0.001
Nuclear grade	0.008		0.352
Re-resection	<0.001	2.210 (1.030–4.742)	0.042
With carcinoma *in situ*	<0.001		0.390
Pathologic T stage	0.855		
DCIS only vs. pT1–3	0.896		
Pathologic N stage	<0.001	2.330 (1.726–3.145)	<0.001
Axillary surgery type	0.102		
ER	<0.001		0.161
PR	<0.001		0.663
HER-2	0.159		
Chemotherapy	0.600		
Radiotherapy	<0.001	0.156 (0.084–0.292)	<0.001
Endocrine therapy	<0.001		0.722
PF	<0.001		0.554
MF	<0.001		0.779
PMF	<0.001	1.658 (1.083–2.361)	<0.001
FMR	<0.001		0.484
PMR	0.001		0.774
NLR	<0.001	1.316 (1.187–1.458)	<0.001
MLR	<0.001		0.713
PLR	<0.001		0.762

*HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; PF, platelet count*fibrinogen; MF, mean platelet volume*fibrinogen; PMF, platelet count*mean platelet volume*fibrinogen; FMR, fibrinogen-to-mean platelet volume ratio; PMR, platelet count-to-mean platelet volume ratio; NLR, neutrophil count-to-lymphocyte count ratio; MLR, monocyte count-to-lymphocyte count ratio; PLR, platelet count-to-lymphocyte count ratio.*

pathologic N stage contributed the most to the prediction results. This was followed by molecular subtype, radiotherapy, PMF, and NLR. In pathological N stage, the high-risk segment corresponded to the high partition (scoring axis), and the low-risk segment corresponded to the low partition. The scores of all factors were added to obtain the total score perpendicular to the risk axis of LRR and the final risk of individual LRR. The nomogram of LRR showed ideal discrimination and prediction accuracy. Calibration curves for the prediction model in the training and validation cohort both demonstrated satisfactory consistency between the nomogram-predicted and actual LRR (**Figures 5A, B**). The area under the ROC curve (AUC) was 0.89 ($p < 0.001$, 95% CI = 0.83, 0.95) in the training cohort (**Figure 5C**) and 0.88 ($p < 0.001$, 95% CI = 0.8, 0.96) in the validation cohort (**Figure 5D**).

DISCUSSION

With the development of imaging examinations and systemic therapy, BCT has become the preferred surgical choice for patients with operable breast cancer. However, about 3% of patients still have LRR after BCT, which may be related to young age, tumor size, negative hormone receptor status, and pathologic N stage, as reported in a previous study (22–24). Moreover, the biological characteristics of breast cancer in Chinese women are different from those in Western women. The age of breast cancer patients in China is relatively young, and 50%–60% of breast cancer patients are premenopausal patients. Therefore, it is necessary to establish a practical nomogram to improve the prediction ability of LRR.

Univariate analysis showed that age of onset, pathological stage, molecular subtype, nuclear grade, re-resection, carcinoma *in situ*, pathologic N stage, ER, PR, radiotherapy, endocrine therapy, PF, MF, PMF, FMR, PMR, NLR, MLR, and PLR were related to LRR after BCT in the study. The multivariate analysis identified that independent factors for LRR included molecular subtype, pathologic N stage, re-resection, radiotherapy, PMF, and NLR. A predictive nomogram incorporating hematological parameters and clinicopathological characteristics showed ideal discrimination and consistency between the nomogram-predicted LRR and actual observation in both the training and validation cohorts.

Univariate analysis showed that TNBC had a higher recurrence rate than non-TNBC (including luminal A, luminal B, and HER-2 positivity). After multivariate adjustment, molecular subtype was still an independent factor for LRR. Our results were consistent with previous large sample studies, which proposed that IHC-based molecular subtype had significant prognostic effects. The IHC-based molecular subtype study proved that hormone receptor-negative subtypes were more likely to relapse. TNBC has strong tumor invasiveness, and hormone receptor and HER-2 receptor are negative. Due to the lack of endocrine and targeted therapeutic

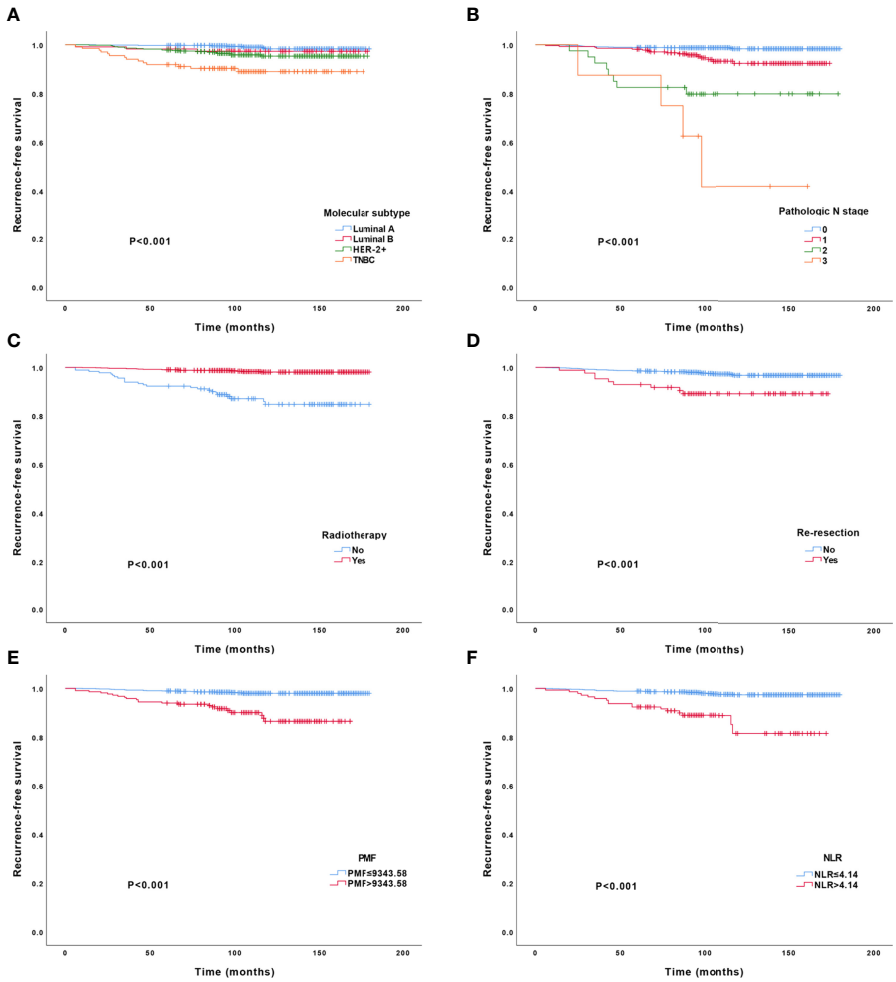


FIGURE 3 | Kaplan–Meier curves for local-regional recurrence. Kaplan–Meier curves for local–regional recurrence based on molecular subtype **(A)**, pathological N stage **(B)**, radiotherapy **(C)**, re-resection **(D)**, PMF **(E)**, and NLR **(F)**. TNBC, triple-negative breast cancer; PMF, platelet count*mean platelet volume*fibrinogen; NLR, neutrophil count-to-lymphocyte count ratio.

targets for TNBC, LRR and distant metastasis are more likely to occur in TNBC (25).

Pathologic N stage was also found to be an independent factor related to LRR. It represented axillary lymph node status. For patients with late axillary lymph node stage, on the one hand, the lymph node stage is relatively late, and there is still the risk of local residue after systematic treatment and local treatment. On the other hand, the tumors had the characteristics of near lymph node metastasis and local lymph node metastasis.

In our hospital, patients will receive re-resection due to positive surgical margins, and if the margin is positive again, mastectomy will be performed. Studies have shown that extensive intraductal carcinoma is a high-risk factor for positive margins (21). However, re-resection will destroy the integrity of the tumor. This may cause tumor cells to spread in the surgical cavity. In addition, re-resection will interfere with the definition of tumor margins, resulting in margins that are too close and even false negatives. Based on this, if only the first margin is positive, re-resection will increase the risk of local residue and recurrence. For specific types of tumors, it is more likely to need re-resection. For example, with extensive intraductal cancer, the risk of positive margins is higher, and some tumor types have the risk of false-negative margin, which is more likely to cause local recurrence. Therefore, the type of tumor requiring re-resection may also be a factor in LRR. Re-resection in multivariate analysis of Cox also showed the correlation with LRR.

Radiotherapy after BCT is the standard treatment for breast cancer in NCCN guidelines. Prospective randomized trials have shown that radiotherapy reduced the 10-year risk of any (i.e., local-regional or distant) first recurrence from 35.0% to 19.3% (absolute reduction 15.7%, 95% CI 13.7–17.7, $2p < 0.00001$) and reduced the 15-year risk of breast cancer death from 25.2% to 21.4% (absolute reduction 3.8%, 1.6–6.0, $2p = 0.00005$) (9, 26, 27). The overall results from these trials suggested that radiotherapy after BCT not only substantially reduced the risk

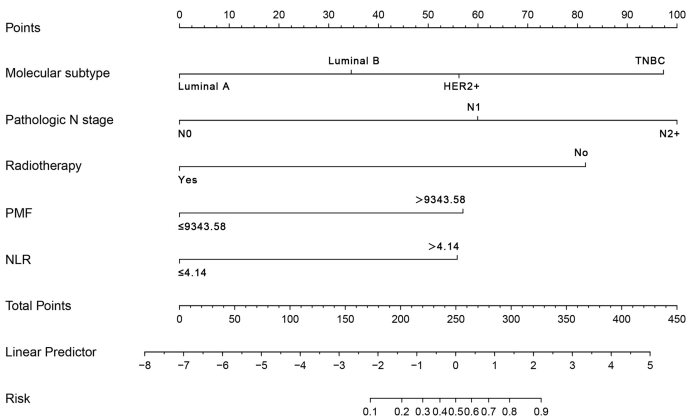


FIGURE 4 | Nomogram model predicts the probability of local–regional recurrence. Points refers to point for the individual risk factor and add together to the total points. Luminal A, HER-2 (–), ER (+), PR (+) and high expression, Ki67 low expression; Luminal B, HER-2 (–), ER (+), PR (–) or low expression, Ki67 high expression; HER2+, the breast cancer of HER2 positive; TNBC, triple-negative breast cancer; N0, No positive lymph nodes; N1, the number of positive lymph nodes is 1–3; N2 +, the number of positive lymph nodes is more than 3; PMF, platelet count*mean platelet volume*fibrinogen; NLR, neutrophil count-to-lymphocyte count ratio.

of recurrence, but also reduced the risk of breast cancer death. These results suggested that the use of radiotherapy to kill tiny tumor foci in the remaining breast could reduce the risk of LRR and distant metastasis. This study also showed that postoperative radiotherapy was an independent factor for LRR. Currently, with the development of research on circulating tumor cells, studies have found that hematological parameters are important intermediaries in the occurrence and development of breast

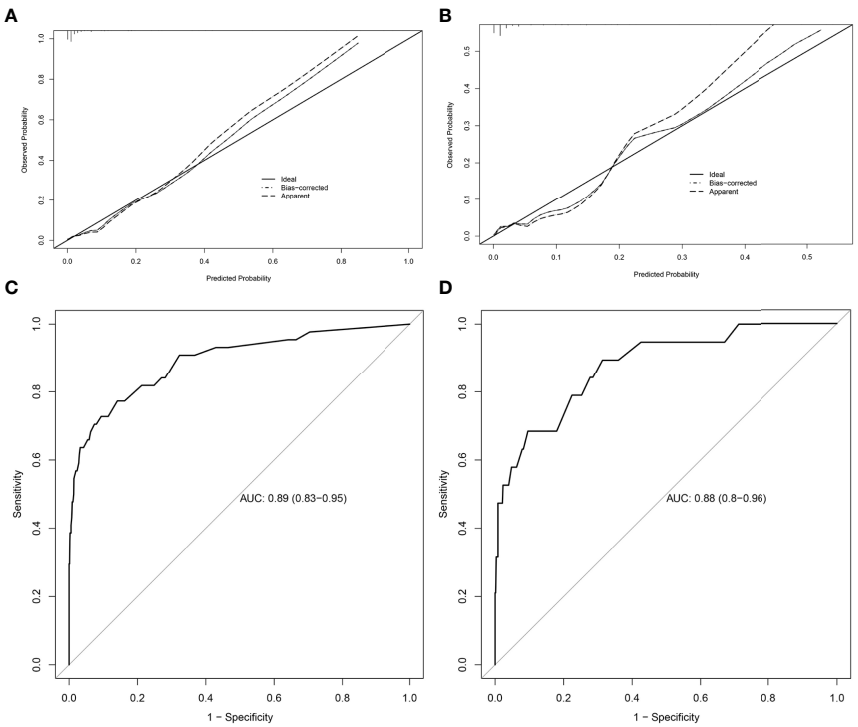


FIGURE 5 | Evaluation of the LRR nomogram **(A–D)**. Calibration curves for the nomogram in the training cohort **(A)** and validation cohort **(B)**. The *x*-axis shows the predicted probability of an LRR event. The *y*-axis shows the actual LRR outcome. The discrimination assessed by ROC curves for the nomogram in the training cohort **(C)** and validation cohort **(D)**. The AUCs for LRR prediction were 0.89 (95% CI = 0.83, 0.95) in the training cohort and 0.88 (95% CI = 0.8, 0.96) in the validation cohort. LRR, local–regional recurrence; ROC, receiver operating characteristic; AUC, area under the curve.

cancer. However, the detection of circulating tumor cells in clinical practice still needs more research. The literature has confirmed that common hematological parameters, such as platelets and coagulation factors, will change with the state of tumor. They are easy to obtain and can be used as an index to predict changes in tumor condition.

In the study, the PMF of the recurrence of breast cancer was obviously abnormal. Platelets have an important impact on the occurrence, development, and prognosis of tumors and can promote the direct interaction between aggrus/podoplanin and clec-2 to promote tumor growth and metastasis (13). As the main indicator of platelet activation status, MPV has also been reported to be associated with the prognosis of malignant diseases (28, 29).

In addition, some studies have found that fibrinogen levels will increase when malignant tumors or tumor-induced SIR occur, decrease after surgery, and increase again when tumor relapse occurred (30, 31). Hyperfibrinogenemia affects the prognosis of breast cancer. Tumor growth and local infiltration cause inflammation and elevate plasma fibrinogen levels, favoring stable adhesion of tumor cells and survival of metastatic embolism, which may be responsible for LRR of tumor and lymphatic metastasis (32). PMF is defined as platelet count*MPV*fibrinogen, which represents the combined effect of platelets, MPV, and fibrinogen. Some studies have shown that platelet count, MPV, and fibrinogen are changed in the recurrence and metastasis of thyroid and gallbladder cancer (33, 34). Our study also found that PMF is significantly associated with the LRR of breast cancer as an independent factor for LRR.

A prospective study conducted by the UK Biobank evaluated the correlation between prediagnostic markers of systemic inflammation and cancer risk in 440,000 participants. It proved that the ratio of inflammatory cells could be used as a biomarker of cancer risk, and it was possible to identify the disease early in the last year before clinical diagnosis (35). SIR is closely related to the prognosis of many tumors. Inflammation can promote the proliferation of cells in new plasma, stimulate angiogenesis, and reduce immunity, thereby promoting cancer recurrence and progression (19). Many studies have shown that the indicators of NLR, MLR, and PLR changed significantly in the recurrence or metastasis of breast cancer, liver cancer, and small cell lung cancer (36–38). In this study, NLR was significantly correlated with LRR as an independent factor, while MLR and PLR did not show significant correlation with LRR in multivariate analysis of LRR.

It is worth noting that age and tumor size were not independent factors for LRR in our results, which was inconsistent with previous studies. The reason may be that younger patients (less than or equal to 45 years old) are more inclined to BCT than older patients. In addition, for patients with tumors T2 or more, we can perform BCT with oncoplastic surgery, which can receive a larger margin and still keep the contour of breasts.

In this study, we established a nomogram to predict the LRR after BCT, and the AUC was 0.89, showing a satisfactory predictive effect. Additionally, despite the TNM staging system, several predictive models were explored according to inflammatory status, tumor markers, stromal tumor-infiltrating lymphocytes, gene signatures, and so on with C-indices from 0.69 to 0.77 (39, 40). Compared with these models, our predictive nomogram achieved comparative prognostic accuracy and was more economical and convenient.

However, it must be admitted that this study is a single-center retrospective study, and the number of recurrences is relatively small, so there are some uncertain biases. Therefore, the factors and prediction models of LRR need to be further verified.

In conclusion, molecular subtype, re-resection, pathological N stage, radiotherapy, PMF, and NLR are significantly related to LRR. Molecular subtype, pathological N stage, radiotherapy, PMF, and NLR can be combined to predict the LRR of patients with breast cancer after BCT. This will help clinicians to formulate individualized treatment strategies for patients after BCT according to the risk of LRR and provide patients with better treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Affiliated Cancer Hospital of Shandong First Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LS, ZY, and CL contributed to the conception and design of the study. LS organized the database. WZ performed the statistical analysis. LS wrote the first draft of the manuscript. FW, XS, and XW wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

The only funds used were those provided by the authors' institution.

ACKNOWLEDGMENTS

The authors would like to thank the patients, doctors, and nurses for their support to our study.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer Statistics. *CA Cancer J Clin* (2007) 57(1):43–66. doi: 10.3322/canjclin.57.1.43
2. van Dongen JA, Voogd AC, Fentiman IS, Legrand C, Sylvester RJ, Tong D, et al. Long-Term Results of a Randomized Trial Comparing Breast-Conserving Therapy With Mastectomy: European Organization for Research and Treatment of Cancer 10801 Trial. *J Natl Cancer Inst* (2000) 92(14):1143–50. doi: 10.1093/jnci/92.14.1143
3. Fisher B, Anderson S, Bryant J, Margolese RG, Deutsch M, Fisher ER, et al. Twenty-Year Follow-Up of a Randomized Trial Comparing Total Mastectomy, Lumpectomy, and Lumpectomy Plus Irradiation for the Treatment of Invasive Breast Cancer. *N Engl J Med* (2002) 347(16):1233–41. doi: 10.1056/NEJMoa022152
4. Litiere S, Werutsky G, Fentiman IS, Rutgers E, Christiaens MR, Van Limbergen E, et al. Breast Conserving Therapy Versus Mastectomy for Stage I-II Breast Cancer: 20 Year Follow-Up of the EORTC 10801 Phase 3 Randomised Trial. *Lancet Oncol* (2012) 13(4):412–9. doi: 10.1016/S1470-2045(12)70042-6
5. Elyasinia F, Keramati MR, Ahmadi F, Rezaei S, Ashouri M, Parsaei R, et al. Neutrophil-Lymphocyte Ratio in Different Stages of Breast Cancer. *Acta Med Iran* (2017) 55(4):228–32.
6. Ma JY, Hu G, Liu Q. Prognostic Significance of the Lymphocyte-to-Monocyte Ratio in Bladder Cancer Undergoing Radical Cystectomy: A Meta-Analysis of 5638 Individuals. *Dis Markers* (2019) 2019:7593560. doi: 10.1155/2019/7593560
7. Chen L, Hao Y, Cong X, Zou M, Li S, Zhu L, et al. Peripheral Venous Blood Platelet-to-Lymphocyte Ratio (PLR) for Predicting the Survival of Patients With Gastric Cancer Treated With SOX or XELOX Regimen Neoadjuvant Chemotherapy. *Technol Cancer Res Treat* (2019) 18:1533033819829485. doi: 10.1177/1533033819829485
8. Arvold ND, Taghian AG, Niemierko A, Abi Raad RF, Sreedhara M, Nguyen PL, et al. Age, Breast Cancer Subtype Approximation, and Local Recurrence After Breast-Conserving Therapy. *J Clin Oncol* (2011) 29(29):3885–91. doi: 10.1200/JCO.2011.36.1105
9. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Darby S, McGale P, Correa C, Taylor C, Arriagada R, et al. Effect of Radiotherapy After Breast-Conserving Surgery on 10-Year Recurrence and 15-Year Breast Cancer Death: Meta-Analysis of Individual Patient Data for 10801 Women in 17 Randomised Trials. *Lancet* (2011) 378(9804):1707–16. doi: 10.1016/s0140-6736(11)61629-2
10. Corso G, Maisonneuve P, Massari G, Invento A, Pravettoni G, De Scalzi A, et al. Validation of a Novel Nomogram for Prediction of Local Relapse After Surgery for Invasive Breast Carcinoma. *Ann Surg Oncol* (2020) 27(6):1864–74. doi: 10.1245/s10434-019-08160-7
11. Zheng S, Shen J, Jiao Y, Liu Y, Zhang C, Wei M, et al. Platelets and Fibrinogen Facilitate Each Other in Protecting Tumor Cells From Natural Killer Cytotoxicity. *Cancer Sci* (2009) 100:859–65. doi: 10.1111/j.1349-7006.2009.01115.x
12. Takagi S, Sato S, Oh-hara T, Takami M, Koike S, Mishima Y, et al. Platelets Promote Tumor Growth and Metastasis *via* Direct Interaction Between Aggrus/podoplanin and CLEC-2. *PLoS One* (2013) 8:e73609. doi: 10.1371/journal.pone.0073609
13. Asgiri A, Lesyk G, Poitras E, Govindasamy N, Terry K, To R, et al. Platelets Stimulate Programmed Death-Ligand 1 Expression by Cancer Cells: Inhibition by Anti-Platelet Drugs. *J Thromb Haemost* (2021) 19(11):2862–72. doi: 10.1111/jth.15478
14. Davi G, Patrono C. Platelet Activation and Atherothrombosis. *N Engl J Med* (2007) 357:2482–94. doi: 10.1056/NEJMra071014
15. Oge T, Yalcin OT, Ozalp SS, Isikci T. Platelet Volume as a Parameter for Platelet Activation in Patients With Endometrial Cancer. *J Obstet Gynaecol* (2013) 33:301–4. doi: 10.3109/01443615.2012.758089
16. Balkwill F, Mantovani A. Inflammation and Cancer: Back to Virchow? *Lancet* (2001) 357(9255):539–45. doi: 10.1016/s0140-6376(00)04046-0
17. Watteberg MM, Beatty GL. Overcoming Immunotherapeutic Resistance by Targeting the Cancer Inflammation Cycle. *Semin Cancer Biol* (2020) 65:38–50. doi: 10.1016/j
18. McMillan DC. Systemic Inflammation, Nutritional Status and Survival in Patients With Cancer. *Curr Opin Clin Nutr Metab Care* (2009) 12(3):223–6. doi: 10.1097/mco.0b013e32832a7902
19. Nøst TH, Alcala K, Urbarova I, Byrne KS, Guida F, Sandanger TM, et al. Systemic Inflammation Markers and Cancer Incidence in the UK Biobank. *Eur J Epidemiol* (2021) 36:841–8. doi: 10.1007/s10654-021-00752-6
20. Wapnir IL, Anderson SJ, Mamounas EP, Geyer CEJr, Jeong JH, Tan-Chiu E, et al. Prognosis After Ipsilateral Breast Tumor Recurrence and Locoregional Recurrences in Five National Surgical Adjuvant Breast and Bowel Project Node-Positive Adjuvant Breast Cancer Trials. *J Clin Oncol* (2006) 24(13):2028–37. doi: 10.1200/jco.2005.04.3273
21. Morrow M, Van Zee KJ, Solin LJ, Houssami N, Chavez-MacGregor M, Harris JR, et al. Society of Surgical Oncology-American Society for Radiation Oncology-American Society of Clinical Oncology Consensus Guidelines on Margins for Breast-Conserving Surgery With Whole Breast Irradiation in Ductal Carcinoma in Situ. *Ann Surg Oncol* (2016) 34:4040–6. doi: 10.1200/jco.2016.68.3573
22. Calle R, Vilcoq JR, Zafrani B, Vielh P, Fourquet A. Local Control and Survival of Breast Cancer Treated by Limited Surgery Followed by Irradiation. *Int J Radiat Oncol Biol Phys* (1986) 12(6):873–8. doi: 10.1016/0360-3016(86)90379-2
23. Arriagada R, Le MG, Contesso G, Guinebretiere JM, Rochard F, Spielmann M. Predictive Factors for Local Recurrence in 2006 Patients With Surgically Resected Small Breast Cancer. *Ann Oncol* (2002) 13(9):1404–13. doi: 10.1093/annonc/mdf227
24. Fowble BL, Schultz DJ, Overmoyer B, Solin LJ, Fox K, Jardines L, et al. The Influence of Young Age on Outcome in Early Stage Breast Cancer. *Int J Radiat Oncol Biol Phys* (1994) 30(1):23–33. doi: 10.1016/0360-3016(94)90515-0
25. Albert JM, Gonzalez-Angulo AM, Guray M, Sahin A, Strom EA, Tereffe W, et al. Estrogen/progesterone Receptor Negativity and HER2 Positivity Predict Locoregional Recurrence in Patients With T1a,bN0 Breast Cancer. *Int J Radiat Oncol Biol Phys* (2010) 77:1296–302. doi: 10.1016/j.ijrobp.2009.12.011
26. Early Breast Cancer Trialists Collaborative Group. Favourable and Unfavourable Effects on Long-Term Survival of Radiotherapy for Early Breast Cancer: An Overview of the Randomised Trials. *Lancet* (2000) 355:1757–70. doi: 10.1016/S0140-6736(00)02263-7
27. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. ESMO Guidelines Committee. Early Breast Cancer: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up. *Ann Oncol* (2019) 30(8):1194–220. doi: 10.1093/annonc/mdz173
28. Inagaki N, Kibata K, Tamaki T, Shimizu T, Nomura S. Prognostic Impact of the Mean Platelet Volume/Platelet Count Ratio in Terms of Survival in Advanced non-Small Cell Lung Cancer. *Lung Cancer* (2014) 83:97–101. doi: 10.1016/j.lungcan.2013.08.020
29. Kurt M, Onal IK, Sayilir AY, Beyazit Y, Oztas E, Kekilli M, et al. The Role of Mean Platelet Volume in the Diagnosis of Hepatocellular Carcinoma in Patients With Chronic Liver Disease. *Hepatogastroenterology* (2012) 59:1580–2. doi: 10.5754/hge10444
30. Suzuki T, Shimada H, Nanami T, Oshima Y, Yajima S, Ito M, et al. Hyperfibrinogenemia is Associated With Inflammatory Mediators and Poor Prognosis in Patients With Gastric Cancer. *Surg Today* (2016) 46:1394–401. doi: 10.1007/s00595-016-1339-z
31. Zhu LR, Li J, Chen P, Jiang Q, Tang XP. Clinical Significance of Plasma Fibrinogen and D-Dimer in Predicting the Chemotherapy Efficacy and Prognosis for Small Cell Lung Cancer Patients. *Clin Transl Oncol* (2016) 18:178–88. doi: 10.1007/s12094-015-1350-7
32. Mei Y, Zhao S, Lu X, Liu H, Li X, Ma R. Clinical and Prognostic Significance of Preoperative Plasma Fibrinogen Levels in Patients With Operable Breast Cancer. *PLoS One* (2016) 11:e0146233. doi: 10.1371/journal.pone.0146233.eCollection2016
33. Martin S, Mustata T, Enache O, Ion O, Chifulescu A, Sirbu A, et al. Platelet Activation and Inflflammation in Patients With Papillary Thyroid Cancer. *Diagnostics (Basel)* (2021) 11(11):1959. doi: 10.3390/diagnostics11111959
34. Cao P, Jiang L, Zhou LY, Chen YL. The Clinical Significance of Preoperative Serum Fibrinogen Levels and Platelet Counts in Patients With Gallbladder Carcinoma. *BMC Gastroenterol* (2021) 21:366. doi: 10.1186/s12876-021-01943-x
35. Wu Y, Zhou BP. Inflammation: A Driving Force Speeds Cancer Metastasis. *Cell Cycle* (2009) 8(20):3267–73. doi: 10.4161/cc.8.20.9699

36. Chen C, Yang H, Cai D, Xiang L, Fang W, Wang R. Preoperative Peripheral Blood Neutrophil-to-Lymphocyte Ratios (NLR) and Platelet-to-Lymphocyte Ratio (PLR) Related Nomograms Predict the Survival of Patients With Limited-Stage Small-Cell Lung Cancer. *Transl Lung Cancer Res* (2021) 10 (2):866–77. doi: 10.21037/tlcr-20-997
37. Wu W, Wang Q, Han D, Li J, Nie W, Guo D, et al. Prognostic Value of Preoperative Inflammatory Markers in Patients With Hepatocellular Carcinoma Who Underwent Curative Resection. *Cancer Cell Int* (2021) 21:500. doi: 10.1186/s12935-021-02204-3
38. Berckelaer CV, Geyt MV, Linders S, Rypens C, Trinh XB, Laere SV, et al. A High Neutrophil-Lymphocyte Ratio and Platelet-Lymphocyte Ratio are Associated With a Worse Outcome in Inflammatory Breast Cancer. *Breast* (2020) 53:212–20. doi: 10.1016/j.breast.2020.08.006
39. Yang Y, Wang Y, Deng H, Tan C, Li Q, He Z, et al. Development and Validation of Nomograms Predicting Survival in Chinese Patients With Triple Negative Breast Cancer. *BMC Cancer* (2019) 19(1):541. doi: 10.1186/s12885-019-5703-4
40. Zheng S, Zou Y, Liang J, Xiao W, Yang A, Meng T, et al. Identifification and Validation of a Combined Hypoxia and Immune Index for Triple-Negative Breast Cancer. *Mol Oncol* (2020) 14(11):2814–33. doi: 10.1002/1878-0261.12747

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 22 July 2022
DOI 10.3389/fonc.2022.845823

OPEN ACCESS

EDITED BY
Michael Gnant,
Medical University of Vienna, Austria

REVIEWED BY
Alexandra Resch,
Franziskus Spital, Austria
Wenjun Yi,
Central South University, China
Isabella Castellano,
University of Turin, Italy
Stephanie Wong,
McGill University, Canada

*CORRESPONDENCE
Xiaosong Chen
chenxiaosong0156@hotmail.com
Kunwei Shen
kwshen@medmail.com.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 30 December 2021
ACCEPTED 27 June 2022
PUBLISHED 22 July 2022

CITATION
Li Z, Tong Y, Chen X and Shen K (2022) Accuracy of ultrasonographic changes during neoadjuvant chemotherapy to predict axillary lymph node response in clinical node-positive breast cancer patients. *Front. Oncol.* 12:845823. doi: 10.3389/fonc.2022.845823

Accuracy of ultrasonographic changes during neoadjuvant chemotherapy to predict axillary lymph node response in clinical node-positive breast cancer patients

Zhuoxuan Li†, Yiwei Tong†, Xiaosong Chen* and Kunwei Shen*

Department of General Surgery, Comprehensive Breast Health Center, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Purpose: To evaluate whether changes in ultrasound features during neoadjuvant chemotherapy (NAC) could predict axillary node response in clinically node-positive breast cancer patients.

Methods: Patients with biopsy-proven node-positive disease receiving NAC between February 2009 and March 2021 were included. Ultrasound (US) images were obtained using a 5-12-MHz linear array transducer before NAC, after two cycles, and at the completion of NAC. Long and short diameter, cortical thickness, vascularity, and hilum status of the metastatic node were retrospectively reviewed according to breast imaging-reporting and data system (BI-RADS). The included population was randomly divided into a training set and a validation set at a 2:1 ratio using a simple random sampling method. Factors associated with node response were identified through univariate and multivariate analyses. A nomogram combining clinical and changes in ultrasonographic (US) features was developed and validated. The receiver operating characteristic (ROC) and calibration plots were applied to evaluate nomogram performance and discrimination.

Results: A total of 296 breast cancer patients were included, 108 (36.5%) of whom achieved axillary pathologic complete response (pCR) and 188 (63.5%) had residual nodal disease. Multivariate regression indicated that independent predictors of node pCR contain ultrasound features in addition to clinical features, clinical features including neoadjuvant HER2-targeted therapy and clinical response, ultrasound features after NAC including cortical thickness, hilum status, and reduction in short diameter ≥50%. The nomogram combining clinical features and US features showed better diagnostic performance compared to clinical-only model in the training cohort (AUC: 0.799 vs. 0.699, P=0.001) and the validation cohort (AUC: 0.764 vs. 0.638, P=0.027).

Conclusions: Ultrasound changes during NAC could improve the accuracy to predict node response after NAC in clinically node-positive breast cancer patients.

KEYWORDS

breast cancer, lymph nodes, ultrasound, nomogram, neoadjuvant chemotherapy

Introduction

According to the latest global cancer statistics, breast cancer has become the most commonly diagnosed cancer in women with 2.3 million new cases in 2020 (1). Axillary lymph node status is one of the most important factors for clinical staging and also an independent prognostic predictor for breast cancer patients (2). Neoadjuvant chemotherapy (NAC) has become an important treatment for inoperable locally advanced and large operable breast cancer patients (3). NAC aims to convert inoperable breast tumors to operable disease and to downstage the primary large breast tumor and metastatic axillary lymph node. According to previous reports, 40%-60% of node-positive patients could convert to node-negative after NAC. Predictive markers of candidates who could benefit from NAC such as molecular subtype, NAC regimens, and clinical response had been extensively confirmed (3–5). However, accurate evaluation of the response to NAC remains to be investigated.

For patients with primary node-positive disease, axillary lymph node dissection (ALND) remains the standard of care after completion of NAC (6). However, ALND is usually followed by increased risk of complications, including lymphedema and paresthesia, which leads to poor quality of life (7). To avoid such complications, the possibility of sentinel lymph node biopsy (SLNB) for patients achieving pCR after completion of NAC with primary node-positive disease was evaluated by the ACOSOG (American College of Surgeons Oncology Group) Z1071 and SENTINA (sentinel lymph-node biopsy in patients with breast cancer before and after neoadjuvant chemotherapy) trials and the false-negative rates (FNR) of SLNB in these two randomized trials were 12.6% and 14.2%, which were found to be above the acceptable 10% cut point. Although a reduced FNR of less than 10% could be achieved by using dual tracer or removing at least three sentinel lymph nodes (8, 9), SLNB remains debatable with relatively FNR for patients presenting clinically node-negative after completion of NAC according to the guideline (10). Predictive markers to select axillary pCR patients appropriate for SLNB is still a challenge.

Imaging modalities have been applied to increase the diagnostic accuracy for lymph node response evaluation. For instance, when axillary ultrasound (US) was added to assess the axillary response after NAC, the FNR decreased to 9.8% in the ACOSOG Z1071 population (11). A previous study has demonstrated that axillary lymphadenopathy in US after NAC had the strongest predictive capacity of residual axillary LN metastasis (OR=13.8), while other clinical predictive features including clinical N stage, Ki-67 negativity, hormone receptor positivity, and HER2 negativity showed an OR from 2.3 to 3.7 (12, 13). Moreover, breast pCR was also an independent positive predictor for nodal response in the Z1071 trial (14). Likewise, as demonstrated in our previous study, patients with breast pCR had a significantly lower ypN+ rate than those with residual tumor (23.9% vs. 62.5%, OR=0.14) (15). US features of lymph nodes observed after chemotherapy, including shorter short-axis, shorter long-axis, hilum preservation, and absence of cortical thickness, have been proven to be associated with axillary pCR (16, 17). In addition to the observation of the lymph node status at a certain point in time, US also has the advantages of convenient, dynamic, and continuous observation throughout the treatment (16, 18). In one study, axillary response was evaluated at separate time points before, during, and after NAC, and the results showed that only mid-NAC US features including breast tumor size and cortical thickness showed an average diagnostic performance with an AUC of 0.760 (19). Although different time points were included in this study, the imaging change of lymph nodes across treatment cycles, which might reflect treatment response, was not investigated. To further understand the association between the specific lymph node US features throughout the treatment and ALN response after NAC, more markers combining different time points of lymph node specific US indicators is needed to be explored.

Therefore, the aim of our study was to evaluate whether changes of ultrasound features during neoadjuvant chemotherapy (NAC) could predict axillary node response in clinically node-positive breast cancer patients, thus to develop a novel nomogram combining clinical and axillary US features to

predict the probability of axillary nodal pCR after NAC in primary node-positive patients, which may guide further ALN management after NAC.

Materials and methods

Data source and patients selection

We retrospectively reviewed consecutive female patients diagnosed with primary invasive breast cancer who received NAC from February 2009 to March 2021 in Comprehensive Breast Health Center, Shanghai Jiaotong University School of Medicine affiliated Ruijin Hospital. Eligible patients were women with node-positive disease confirmed by fine-needle aspiration biopsy/core needle biopsy before NAC initiation and with US monitoring of axilla performed at baseline, after two cycles, and after completion of NAC (Supplementary Figure S1). The exclusion criteria were as follows (1): without biopsy-proven nodal metastases (n=163) (2); absence of US images before, during, or after NAC (n=146) (3); treated with neoadjuvant endocrine therapy alone (n=9). The current study was approved by the independent Ethical Committees of Ruijin Hospital, Shanghai Jiaotong University School of Medicine.

Clinical and pathological evaluation

Patient clinical and pathological data were retrieved from Shanghai Jiaotong University Breast Cancer Database (SJTU-BCDB). Core needle biopsy and fine needle aspiration biopsy were performed for suspicious breast and lymph node lesions. Pathological evaluation was performed at the Department of Pathology, Ruijin Hospital by at least two independent pathologists. Histological type and pathological grade were referred to the World Health Organization classification (20). Clinical TNM staging was defined according to the Eighth edition of the American Joint Committee on Cancer staging system (21). ER, PR, HER2, and Ki-67 expression were assessed by immunohistochemistry (IHC) methods in core needle biopsy samples at baseline. Samples with HER2 IHC 2+ were further examined by fluorescence *in situ* hybridization (FISH). The positivity criteria accorded to the 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines (22). Molecular subtypes were classified as four types: Luminal A (ER+, PR high, HER2-, Ki67 low), Luminal B (ER+ or/and PR -/low or Ki67 high), HER2-enriched (ER-, PR-, HER2+), and TNBC (ER-, PR-, HER2-) (23). Patients were recommended with NAC after a multidisciplinary discussion (MDT) with surgical oncologist, medical oncologist, radiation oncologist, and other related clinicians. NAC regimes were classified based on anthracycline (A) and taxane (T). Patients were classified into A+T, such as EC-T (epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2 followed by docetaxel 100 mg/m^2 q3w), TEC (docetaxel 75 mg/m^2, epirubicin 75 mg/m^2 and cyclophosphamide 500 mg/m^2 q3w), A (anthracycline)-containing, such as EC (epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2 q3w), or T (taxane)-containing, such as PCb (weekly paclitaxel 80 mg/m^2 and carboplatin AUC 2). Neoadjuvant HER2-targeted therapy based on trastuzumab (8 mg/Kg at first cycle and followed by 6 mg/Kg q3w or 4 mg/Kg at first cycle and followed by 2 mg/Kg weekly) was also applied to patients according to the MDT decision.

After completion of NAC, clinical and pathological evaluations were repeated in the radical surgery specimen. Clinical response was judged according to RECIST 1.1 criteria as CR (complete response, disappearance of all target lesions), PR (partial response, the sum of diameters of target lesions decreased at least 30%), PD (progressive disease, the sum of diameters of target lesions increased at least 20%), and SD (stable disease, neither PD nor PR) (24). The primary endpoint of the current study was nodal complete response, which was defined as no metastatic carcinoma in the axillary lymph nodes (25). Isolated tumor cells or micrometastasis in the nodes were not considered complete response (26).

US evaluation procedure

Breast and axilla US examinations were performed before NAC (at baseline, before biopsy), after two cycles of NAC, and completion of NAC by experienced radiologists with more than 10 years of experience in breast imaging per individual in the Department of Ultrasonography, Ruijin Hospital. All patients were assessed with real-time US using a 5-12-MHz linear array transducer (Esaote MyLab 60, Esaote SpA, Genoa, Italy). The largest biopsy-confirmed positive node was viewed as the target lesion. US features for analysis included long diameter, short diameter, cortical thickness, vascularity (rare, minimal, or abundant), and hilum (preserved, partially preserved, or completely obliterated) according to the breast imaging-reporting and data system (BI-RADS) (27), as presented in Supplementary Figure S2. Imaging reports were retrospectively reviewed from SJTU-BCDB and analyzed in the current study. Changes of the US features were evaluated as the reduction in diameter compared to the baseline. A reduction of 30% in diameter at two cycles, as well as a reduction of 50% (1-0.7*0.7 = 0.51) at completion were applied as cut-offs according to the RECIST 1.1 criteria, where a reduction of 30% in diameter was considered PR (24).

Statistical analyses

The included population was randomly divided into a training set and a validation set at a 2:1 ratio using simple random sampling method. Categorical variables were analyzed by using Chi-square test or Fisher's exact test, if necessary. Continuous variables were analyzed by using independent *t*-test and Mann–Whitney *U*-test. Univariate and multivariate binary logistic regression analyses were used to identify the factors associated with axillary pCR in the training set.

Receiver operator characteristic (ROC) curve was used to assess the diagnostic performance of clinical and US imaging features. The area under the curve (AUC) was obtained at the cut-off value yielding the largest Youden index and compared using generalized estimating equations and the Delong test. Calibration was assessed by calibration plot with 1000 bootstrap resampling. P <0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using SPSS (version 24.0) and R software (version 4.0.5).

Results

Baseline patient characteristics

Baseline characteristics of the 296 participants in the training and validation set are described in Table 1. Among the 296 patients included, 108 (36.5%) achieved axillary pCR while 188 (63.5%) had residual axillary lymph nodes. No significant difference was observed at baseline between the training set and validation set, which justified their use as two independent sets. The average age of patients was 50 ± 11.9 years. In the training set, the ALN pCR group showed a higher proportion of ER negative (60.0%, P<0.001), PR negative (75.0%, P=0.002), and HER2 positive (52.3%, P=0.001) disease. Patients receiving NAC T-containing regimen (57.6%, P=0.006) and neoadjuvant HER2-targeted therapy (50.8%, P<0.001) were more likely to achieve ALN pCR. The rate of ALN pCR ranged from 71.4% to 9.1% among patients who had clinical CR and PD.

Ultrasound features

US features of the biopsy-confirmed metastatic axillary lymph node are shown in Table 2. At baseline, no significant difference in US features was observed between pCR and non-pCR groups (all P>0.050). After two cycles of NAC, medians of long diameter (15.6mm *vs*. 18.9mm, P=0.041), short diameter (7.3mm *vs*. 9.0mm, P=0.013), and cortical thickness (4.2mm *vs*. 5.4mm, P=0.011) were shorter in the pCR group compared with the non-pCR group, while vascularity (P=0.739) and hilum (P=0.270) remained similar. After completion of NAC, medians of long diameter (11.0mm *vs*. 15.6mm, P=0.006), short diameter (5.3mm *vs*. 7.1mm, P=0.001), and cortical thickness (3.0mm *vs*. 3.6mm, P=0.005) were significantly decreased in the pCR group, and hilum preservation was more common (65.4%) in the pCR group compared to the non-pCR group. Abundant vascularity tended to be more observed in the ypN+ population (11.1% vs. 1.9%, P=0.052).

The changes of US features for biopsy-confirmed metastatic axillary lymph node were evaluated in the training set (Table 3). Patients with ALN pCR tended to show more reduction in lymph node US quantitative features, reduction in cortical thickness ≥30% after two cycles of NAC (69.2%, P=0.034), reduction in short diameter ≥50% (69.2%, P<0.001), and cortical thickness ≥50% (76.9%, P<0.001) after completion of NAC were associated with axillary pCR.

Univariate and multivariate analysis of predictors for axillary pCR

In the univariate analysis, clinical features including ER status (P<0.001), PR status (P=0.002), HER2 status (P=0.001), molecular subtype (P=0.005), clinical response (P=0.010), NAC regimen (P=0.006), as well as neoadjuvant HER2-targeted therapy (P<0.001) were associated with axillary pCR rate in the training set (Figure S3). Among US features after two cycles of NAC, short diameter (P=0.022), cortical thickness (P=0.015), and reduction in cortical thickness (P=0.036) were associated with axillary pCR. Among US features after completion of NAC, short diameter (P=0.003), cortical thickness (P=0.004), hilum status (P=0.016), reduction in short diameter ≥50% (P<0.001), and reduction in cortical thickness (P<0.001) were associated with axillary pCR.

In further multivariate logistic regression analysis, neoadjuvant HER2-targeted therapy (P=0.009), clinical response (P=0.016), US features after completion of NAC including cortical thickness (P=0.001), hilum status (P=0.012), and reduction in short diameter ≥50% (P=0.006) were independent predictors for axillary pCR (Figure 1). Patients receiving neoadjuvant HER2-targeted therapy (OR=4.06, 95%CI 1.43-11.57, P=0.009) were more likely to achieve nodal pCR. Patients who had PR (OR=0.22, 95%CI 0.06-0.75, P=0.016), SD (OR=0.13, 95%CI 0.03-0.60, P=0.009), and PD (OR=0.03, 95% CI 0.00-0.37, P=0.005) were less likely to achieve nodal pCR than those who achieved CR. After completion of NAC, patients with lymph node reduction in short diameter ≥50% showed the highest possibility to achieve nodal pCR (OR=2.47, 95%CI 1.30-4.67, P=0.006), while patients with greater cortical thickness (OR=0.83, 95%CI 0.74-0.93, P=0.001) and hilum completely obliterated (OR=0.09, 95%CI 0.02-0.45, P=0.003) compared to hilum preservation were less likely to achieve nodal pCR.

10.3389/fonc.2022.845823

TABLE 1 Baseline characteristics for the training set and the validation set.

characteristics	Training set			Validation set		
	ypN0 n=65	ypN+ n=131	P	ypN0 n=4	ypN+ n=57	P
Age (mean ± SD)	49.63 ± 11.14	50.08 ± 11.73	0.796	51.70 ± 13.17	51.56 ± 12.28	0.958
Palpable node			0.917			0.891
No	12 (18.5%)	25 (19.1%)		8 (18.6%)	10 (17.5%)	
Yes	51 (81.5%)	106 (80.9%)		35 (81.4%)	47 (82.5%)	
cT			0.483			0.873
1	12 (18.8%)	26 (19.8%)		9 (21.4%)	10 (17.9%)	
2	44 (68.8%)	76 (58.0%)		25 (59.5%)	37 (66.1%)	
3	4 (6.3%)	19 (14.5%)		3 (7.1%)	5 (8.9%)	
4	3 (4.7%)	8 (6.1%)		3 (7.1%)	3 (5.4%)	
x	1 (1.6%)	2 (1.5%)		2 (4.8%)	1 (1.8%)	
cN			0.503			0.896
1	37 (56.9%)	65 (49.6%)		25 (58.2%)	31 (54.3%)	
2	24 (36.9%)	51 (38.9%)		17 (39.5%)	23 (40.4%)	
3	4 (6.2%)	15 (11.5%)		1 (2.3%)	3 (5.3%)	
Histology			0.793			0.076
IDC	63 (96.9%)	126 (96.2%)		40 (93.0%)	57 (100.0%)	
Others	2 (3.1%)	5 (3.8%)		3 (7.0%)	0 (0.0%)	
Grade			0.734			0.838
I-II	19 (29.2%)	50 (38.2%)		12 (27.9%)	22 (38.6%)	
III	27 (41.5%)	63 (48.1%)		18 (41.9%)	30 (52.6%)	
NA	19 (29.2%)	18 (13.7%)		13 (30.2%)	5 (8.8%)	
ER			<0.001			0.034
Negative	39 (60.0%)	42 (42.3%)		25 (58.1%)	21 (36.8%)	
Positive	26 (40.0%)	89 (57.7%)		18 (41.9%)	36 (63.2%)	
PR			0.002			0.049
Negative	49 (75.0%)	68 (51.9%)		33 (76.7%)	33 (57.9%)	
Positive	16 (25.0%)	63 (48.1%)		10 (23.3%)	24 (42.1%)	
HER2			0.001			0.013
Negative	31 (47.7%)	94 (71.8%)		18 (41.9%)	38 (66.7%)	
Positive	34 (52.3%)	37 (28.2%)		25 (58.1%)	19 (33.3%)	
Molecular subtype			0.004			0.021
Luminal A	2 (3.1%)	8 (6.1%)		0 (0.0%)	4 (7.0%)	
Luminal B	25 (38.5%)	81 (61.8%)		18 (41.9%)	32 (56.1%)	
HER2 enriched	22 (33.8%)	21 (16.0%)		12 (27.9%)	11 (19.3%)	
TNBC	16 (24.6%)	21 (16.0%)		13 (30.2%)	10 (17.5%)	
Ki-67			0.270			0.109
< 14%	5 (7.7%)	17 (13.0%)		2 (4.7%)	8 (14.3%)	
≥ 14%	60 (92.3%)	114 (87.0%)		41 (95.3%)	48 (85.7%)	
NAC regimen			0.006			0.028
A containing	4 (33.3%)	8 (66.7%)		1 (33.3%)	2 (66.7%)	
T containing	19 (57.6%)	14 (42.4%)		17 (65.4%)	9 (34.6%)	
A+T	42 (27.8%)	109 (72.2%)		25 (35.2%)	46 (64.8%)	
Neoadjuvant HER2-targeted therapy			<0.001			0.048
No	34 (25.2%)	101 (74.8%)		21 (35.0%)	39 (65.0%)	
Yes	31 (50.8%)	30 (49.2%)		22 (55.0%)	18 (45.0%)	

(Continued)

TABLE 1 Continued

characteristics	Training set			Validation set		
	ypN0 n=65	ypN+ n=131	P	ypN0 n=4	ypN+ n=57	P
Clinical response			0.003			0.195
CR	10 (15.4%)	4 (3.1%)		5 (11.6%)	2 (3.5%)	
PR	47 (72.3%)	93 (71.0%)		32 (74.4%)	39 (68.4%)	
SD	7 (10.8%)	23 (17.6%)		5 (11.6%)	14 (24.6%)	
PD	1 (1.5%)	11 (8.4%)		1 (2.3%)	2 (3.5%)	

ypN0, nodal pathological complete response; ypN+, residual nodal disease; SD, standard deviation; IDC, infiltrating ductal carcinoma; NA, not available; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TNBC: triple negative breast cancer; A, anthracycline; T, taxanes; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Nomogram development and validation

Clinical and US variables that were statistically significant from multivariate analysis were included to construct a nomogram predicting the probability of axillary pCR (Figure 2A). For each patient, scores of neoadjuvant HER2-targeted therapy, clinical response, hilum status after NAC, cortical thickness after NAC, and reduction in short diameter were added up for a total pCR score from 28 to 236. The greater the pCR score, the more probable nodal pCR would be achieved.

The predictive value of the pCR score was further tested using ROC in both the training and validation set. The combined clinical and US model showed the highest AUC of 0.799 (95% CI: 0.723-0.876) in the training set, indicating the promising predictive power for nodal pCR (Figure 2B). Compared to the clinical-only model (AUC=0.699, 95% CI: 0.626-0.779), adding changes of US features in the model could significantly improve the diagnosis performance (0.799 *vs.* 0.699, *P*=0.001). The improvement effect of US characteristics in the combined model (AUC=0.764, 95% CI: 0.659-0.869) also confirmed in the validation set of 100 patients (0.764 *vs.* 0.638, *P*=0.027) compared to the clinical-only model (AUC=0.638, 95% CI: 0.560-0.769) as shown in Figure 2C. The calibration curves of the nomograms are shown in Figure 2D for the training set and Figure 2E for the validation set with 1000 steps bootstrap resampling, illustrating good consistency between the predicted result and actual probability.

Discussion

In this study, we developed a predictive model to identify responders achieving axillary nodal pCR after NAC by combining the clinical features and changes of US features in a cohort of 296 primary node-positive breast cancer patients. Our

TABLE 2 Ultrasound features of biopsy-confirmed metastatic axillary lymph node(s)[a] of the training set.

	Baseline			2 cycles			Completion		
	yPN0 n=65	yPN+ n=131	P	ypN0 n=65	ypN+ n=13	P	ypN0 n=65	yPN+ n=131	p
Long diameter (mm)	25.0 (17.7-32.8)	25.0 (18.1-32.0)	0.951	15.6 (12.0-22.9)	18.9 (13.2-28.0)	0.041	11.0 (6.8-16.7)	15.6 (9.6-21.3)	0.006
Short diameter (mm)	13.0 (10.0-17.1)	13.0 (9.5-17.0)	0.831	7.3 (5.5-10.0)	9.0 (6.5-11.4)	0.013	5.3 (3.4-7.2)	7.1 (4.8-9.3)	0.001
Cortical thickness (mm)	7.9 (5.9-10.8)	8.5 (5.5-11.2)	0.835	4.2 (3.1-6.2)	5.4 (3.6-7.1)	0.011	3.0 (0.7-4.1)	3.6 (2.6-5.5)	0.005
Vascularity*			0.535			0.739			0.052
Rare	20 (30.8%)	44 (33.6%)		31 (48.4%)	65 (49.6%)		33 (63.5%)	60 (51.3%)	
Minimal	39 (60.0%)	69 (52.7%)		26 (40.6%)	48 (36.6%)		18 (34.6%)	44 (37.6%)	
Abundant	6 (9.2%)	18 (13.7%)		7 (11.0%)	18 (13.7%)		1 (1.9%)	13 (11.1%)	
Hilum*			0.486			0.270			0.005
Preserved	30 (46.2%)	56 (42.7%)		35 (54.7%)	56 (42.7%)		34 (65.4%)	54 (46.2%)	
Partially preserved	17 (26.2%)	45 (34.4%)		13 (20.3%)	37 (28.2%)		16 (30.8%)	35 (29.9%)	
Completely obliterated	18 (27.7%)	30 (22.9%)		16 (25.0%)	38 (29.0%)		2 (3.8%)	28 (23.9%)	

[a]The largest reported node on ultrasound was chosen as the target lesion.
*One patient achieved nodal pCR during NAC; twenty-seven patients achieved nodal pCR after NAC.

TABLE 3 Changes of ultrasound features for biopsy-confirmed metastatic axillary lymph node during NAC[a] in the training set.

Characteristics	All n=196	yPN0 n=65	yPN+ n=131	P
After 2 cycles of NAC				
Reduction in long diameter [b]				0.076
< 30%	134 (68.4%)	39 (60.0%)	95 (72.5%)	
≥ 30%	62 (31.6%)	26 (40.0%)	36 (27.5%)	
Reduction in short diameter				0.077
< 30%	102 (52.0%)	28 (43.1%)	74 (56.5%)	
≥ 30%	94 (48.0%)	47 (56.9%)	57 (43.5%)	
Reduction in cortical thickness				0.034
< 30%	81 (41.3%)	20 (30.8%)	61 (46.6%)	
≥ 30%	115 (58.7%)	45 (69.2%)	70 (53.4%)	
After completion of NAC				
Reduction in long diameter [c]				0.091
< 50%	116 (59.2%)	33 (50.8%)	83 (63.4%)	
≥ 50%	80 (40.8%)	32 (49.2%)	48 (36.6%)	
Reduction in short diameter				<0.001
< 50%	96 (49.0%)	20 (30.8%)	76 (58.0%)	
≥ 50%	100 (51.0%)	45 (69.2%)	55 (42.0%)	
Reduction in cortical thickness				<0.001
< 50%	82 (41.8%)	15 (23.1%)	67 (51.1%)	
≥ 50%	114 (58.2%)	50 (76.9%)	64 (48.9%)	

[a]The largest reported node on ultrasound was chosen as the target lesion.
[b]Change compared to baseline. The cut-off of 30% was set according to the RECIST 1.1 criteria, where a reduction of 30% in diameter was considered partial response.
[c]Change compared to baseline. The cut-off of 50% was set according to the RECIST 1.1 criteria, where a reduction of 30% in diameter was considered partial response, 50% referred to a reduction of 30% in diameter after two cycles of NAC, and another reduction of 30% in diameter compared to two-cycle after completion of NAC (1-0.7*0.7 = 0.51).

model including neoadjuvant HER2-targeted therapy, clinical response, cortical thickness after completion of NAC, hilum status, and reduction in short diameter ≥50% after completion of NAC showed better predictive capability compared to the clinical-alone model. To our knowledge, the current study is the first to combine clinical features and changes of axillary US imaging during NAC to predict the probability of axillary nodal pCR in primary node-positive patients.

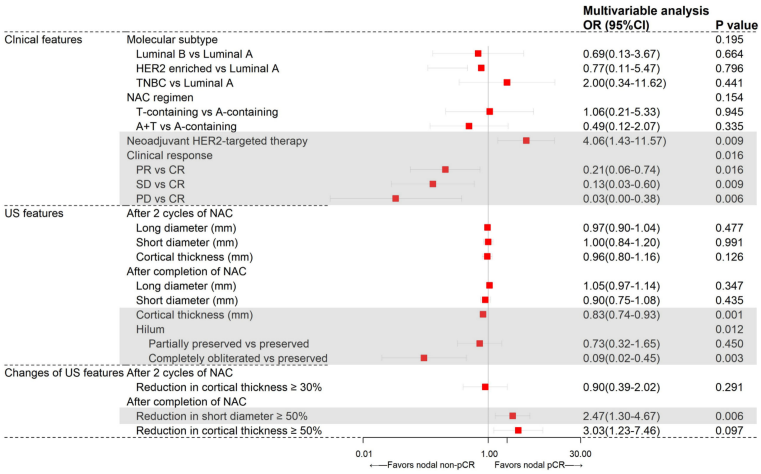


FIGURE 1
Results from multivariate logistic regression analysis of different variables predicting axillary pCR in the training set (N=196).

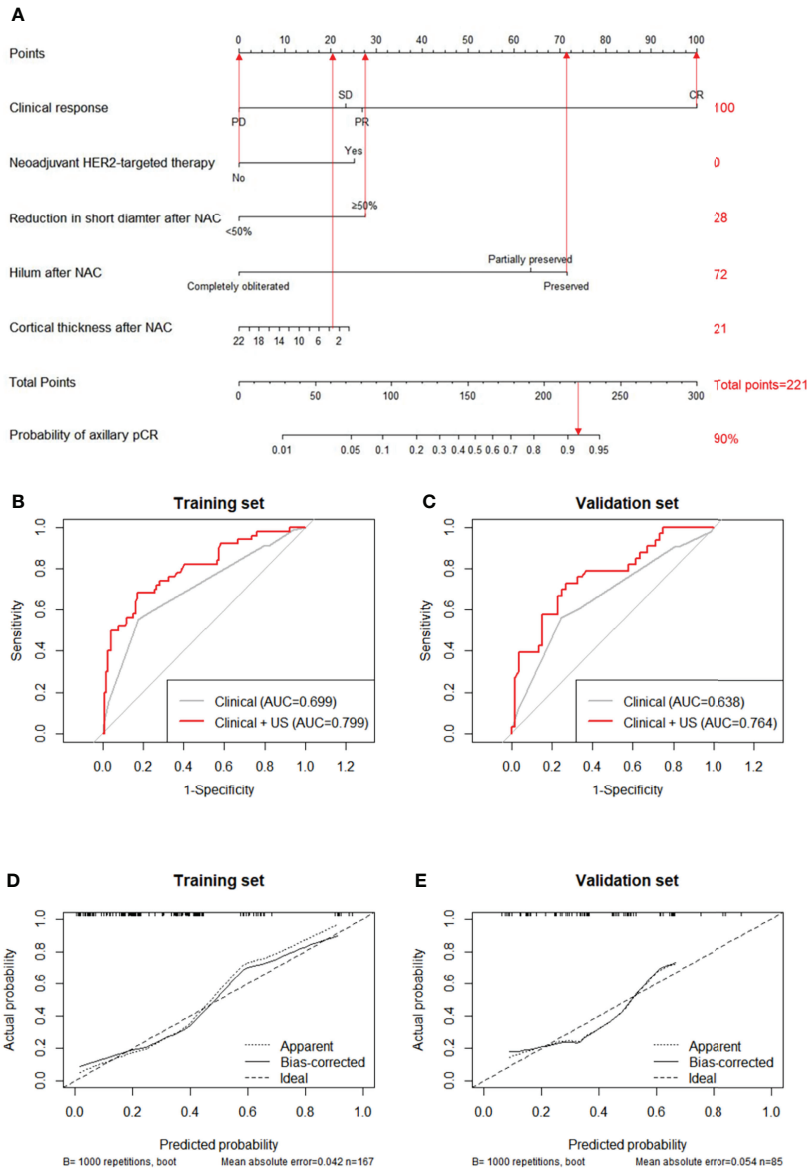


FIGURE 2
A nomogram for predicting the probability of axillary pCR **(A)**. Variables including neoadjuvant HER2-targeted therapy, clinical response, reduction in short diameter after NAC, hilum after NAC, and cortical thickness after NAC were assigned with points value. A total point added with these variables' points indicated the probability of axillary pCR. The vertical lines between five variables and the first row can be added as a total point, the probability of axillary pCR can be finally obtained by drawing a vertical line between total points and the final row. Receiver operating characteristic curves (ROCs) of the clinical features and both clinical and US features for the prediction model in the training set **(B)**, *P*=0.001) and in the validation set **(C)**, *P*=0.027). Calibration curve of the nomogram predicting axillary pCR after neoadjuvant chemotherapy of the training set **(D)** and the validation set **(E)**.

Clinical factors including neoadjuvant HER2-targeted therapy and clinical response have been commonly reported to be related to axillary nodal pCR. As expected, patients receiving neoadjuvant HER2-targeted therapy were more likely to achieve nodal pCR (28, 29). Among patients who achieved clinical CR, 60%-68% achieved nodal pCR (30, 31). Our results showed the 71% nodal pCR rate in clinical CR patients, which was consistent with the previous studies. The predicted accuracy using only clinical features of our study was in average with an AUC of 0.699, comparable with a previous study ranging from 0.649 to 0.835 (30–32).

Regarding US features, cortical thickness and complete obliteration of hilum after completion of NAC were found to be related to axillary pCR. High frequency linear array US could

evaluate LNs structure such as cortex, medulla, and hilum (33). Tumor cell infiltration in LNs could cause cortical thickening, finally efface the hilum, and obscure the visualization of the hilum (16, 33). Cortical thickness can be measured as an objective and quantitative variable while status of hilum always described as a qualitative variable. Previous studies have reported that cortical thickness >3 mm after NAC is the strongest independent predictor of axillary node metastasis with an OR of 46.754 (P=0.000) (34). *Akissue* used cortical thickness as a continuous variable and found that longer cortical thickness was more likely to have axillary node metastasis (OR=1.84, P=0.005) (35). Our study corroborated these findings; longer cortical thickness was more less likely to achieve nodal pCR (OR=0.83, P=0.001). The absence of hilum as a later change of cortical thicken also considered to be a marker for LN metastasis. The presence of hilum was proven to be significantly associated with nodal pCR (OR=2.94, P=0.001) by *Huong T* (16). *Won Hwa kim* also proved that the absence of hilum was a strong predictor for lymph node metastasis (OR=14.06, P=0.002) (17). This result was also verified in our study; complete obliteration of hilum had the lowest OR of 0.09 (P=0.003) for axillary nodal pCR.

Several studies have proven that primary tumor size or tumor size change after NAC as independent characteristics associated with lymph node metastasis, indicating lymph node status as an indicator of the tumor spreading ability (12, 13, 17, 35). However, in biopsy-proven node-positive patients receiving NAC, few studies focused on the response of the lymph node itself during treatment. According to the RECIST 1.1 guideline, tumor response to treatment requires the assessment of reduction in the long diameter of the target lesions, while in the lymph node, short diameter was considered more reproducible rather than long diameter (36). Therefore, we intended to investigate the changes of lymph node US features. Our results indicated that reduction in short diameter ≥50% after NAC had an OR of 2.47 (P=0.006) for axillary nodal pCR.

This study aimed to evaluate the changes of axillary lymph node in order to predict axillary nodal pCR in the clinically node-positive population. US monitoring of axilla before, after two cycles, and after completion of NAC was also obtained in this current study. With the help of US techniques, nodal features can be obtained before surgery in a non-invasive, low cost, and time-saving way. Moreover, US enables us to monitor the axilla continuously at different time points of NAC, providing dynamic observation of nodal response to treatment. This concise nomogram combining the clinical and changes of US features would provide an accurate and personalized evaluation to select potential candidates who may be exempt from ALND.

There are several limitations in our study. First, this was a retrospective study which enrolled patients in a single institution. Only a limited number of patients who completed three US examinations before, during, and after NAC were included, leading to possible selection bias. Therefore, further prospective multicenter validations in larger populations are needed to verify our conclusions. Second, the largest suspicious reported node on US was chosen as the target lesion. Without special marking, the observed lymph node may not necessarily be the same, which may lead to a decrease in accuracy of our research. In our future work, we would trace the US change of lymph nodes during treatment by using potential special marking technology before NAC initiation to loce the biopsy-proven positive lymph node.

In conclusion, ultrasound feature changes during NAC could improve the accuracy of predicting node response after NAC in clinically node-positive breast cancer patients, indicating continuous US monitoring of tumor response as well as axillary lymph node feature changes would help us identify candidate patients to receive potential axilla de-escalation treatment after completion of NAC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by independent Ethical Committees of Ruijin Hospital, Shanghai Jiaotong University School of Medicine. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

ZL analyzed and interpreted the patient data, and was a major contributor in writing the manuscript; YT analyzed and interpreted the patient data; XC made substantial contributions to the conception of the work and substantively revised the manuscript; KS substantively revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The authors received financial supported from the National Natural Science Foundation of China (Grant Number: 81772797, 82072937), Shanghai Municipal Education Commission - Gaofeng Clinical Medicine Grant Support (20172007); Science and Technology Commission of Shanghai

Municipality Shanghai Sailing Program (21YF1427400). All these financial sponsors had no role in the study design, information collection, data analysis or interpretation.

Acknowledgments

The authors would like to thank the assistance of Ms. Yidong Du in inputting SJTU-BCDB.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.845823/full#supplementary-material

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Stankowski-Drengler TJ, Neuman HB. Management of the axilla after neoadjuvant systemic therapy. *Curr Treat Options Oncol* (2020) 21(7):54. doi: 10.1007/s11864-020-00755-7

3. Wang H, Mao X. Evaluation of the efficacy of neoadjuvant chemotherapy for breast cancer. *Drug Des Devel Ther* (2020) 14:2423–33. doi: 10.2147/DDDT.S253961

4. Hennessy BT, Hortobagyi GN, Rouzier R, Kuerer H, Sneige N, Buzdar AU, et al. Outcome after pathologic complete eradication of cytologically proven breast cancer axillary node metastases following primary chemotherapy. *J Clin Oncol* (2005) 23(36):9304–11. doi: 10.1200/JCO.2005.02.5023

5. Kuerer HM, Sahin AA, Hunt KK, Newman LA, Breslin TM, Ames FC, et al. Incidence and impact of documented eradication of breast cancer axillary lymph node metastases before surgery in patients treated with neoadjuvant chemotherapy. *Ann Surg* (1999) 230(1):72–8. doi: 10.1097/00000658-199907000-00011

6. King TA, Morrow M. Surgical issues in patients with breast cancer receiving neoadjuvant chemotherapy. *Nat Rev Clin Oncol* (2015) 12(6):335–43. doi: 10.1038/nrclinonc.2015.63

7. Lucci A, McCall LM, Beitsch PD, Whitworth PW, Reintgen DS, Blumencranz PW, et al. Surgical complications associated with sentinel lymph node dissection (SLND) plus axillary lymph node dissection compared with SLND alone in the American college of surgeons oncology group trial Z0011. *J Clin Oncol* (2007) 25 (24):3657–63. doi: 10.1200/JCO.2006.07.4062

8. Boughey JC, Suman VJ, Mittendorf EA, Ahrendt GM, Wilke LG, Taback B, et al. Sentinel lymph node surgery after neoadjuvant chemotherapy in patients with node-positive breast cancer: the ACOSOG Z1071 (Alliance) clinical trial. *JAMA* (2013) 310(14):1455–61. doi: 10.1001/jama.2013.278932

9. Kuehn T, Bauerfeind I, Fehm T, Fleige B, Hausschild M, Helms G, et al. Sentinel-lymph-node biopsy in patients with breast cancer before and after neoadjuvant chemotherapy (SENTINA): a prospective, multicentre cohort study. *Lancet Oncol* (2013) 14(7):609–18. doi: 10.1016/S1470-2045(13)70166-9

10. Gradishar WJ, Anderson BO, Abraham J, Aft R, Agnese D, Allison KH, et al. Breast cancer, version 3.2020, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* (2020) 18(4):452–78. doi: 10.6004/jnccn.2020.0016

11. Boughey JC, Ballman KV, Hunt KK, McCall LM, Mittendorf EA, Ahrendt GM, et al. Axillary ultrasound after neoadjuvant chemotherapy and its impact on sentinel lymph node surgery: Results from the American college of surgeons oncology group Z1071 trial (Alliance). *J Clin Oncol* (2015) 33(30):3386–93. doi: 10.1200/JCO.2014.57.8401

12. Kim R, Chang JM, Lee HB, Lee SH, Kim SY, Kim ES, et al. Predicting axillary response to neoadjuvant chemotherapy: Breast MRI and US in patients with node-positive breast cancer. *Radiology* (2019) 293(1):49–57. doi: 10.1148/radiol.2019190014

13. Luo Y, Zhao C, Gao Y, Xiao M, Li W, Zhang J, et al. Predicting axillary lymph node status with a nomogram based on breast lesion ultrasound features: Performance in N1 breast cancer patients. *Front Oncol* (2020) 10:581321. doi: 10.3389/fonc.2020.581321

14. Boughey JC, Ballman KV, McCall LM, Mittendorf EA, Symmans WF, Julian TB, et al. Tumor biology and response to chemotherapy impact breast cancer-specific survival in node-positive breast cancer patients treated with neoadjuvant chemotherapy: long-term follow-up from ACOSOG Z1071 (Alliance). *Ann Surg* (2017) 266(4):667–76. doi: 10.1097/SLA.0000000000002373

15. Hong J, Tong Y, He J, Chen X, Shen K. Association between tumor molecular subtype, clinical stage and axillary pathological response in breast cancer patients undergoing complete pathological remission after neoadjuvant chemotherapy: potential implications for de-escalation of axillary surgery. *Ther Adv Med Oncol* (2021) 13:17588359219966673. doi: 10.1177/17588359219966673

16. Le-Petross HT, McCall LM, Hunt KK, Mittendorf EA, Ahrendt GM, Wilke LG, et al. Axillary ultrasound identifies residual nodal disease after chemotherapy: Results from the American college of surgeons oncology group Z1071 trial (Alliance). *AJR Am J Roentgenol* (2018) 210(3):669–76. doi: 10.2214/AJR.17.18295

17. Kim WH, Kim HJ, Park HY, Park JY, Chae YS, Lee SM, et al. Axillary pathologic complete response to neoadjuvant chemotherapy in clinically node-positive breast cancer patients: A predictive model integrating the imaging characteristics of ultrasound restaging with known clinicopathologic characteristics. *Ultrasound Med Biol* (2019) 45(3):702–9. doi: 10.1016/j.ultrasmedbio.2018.10.026

18. Hieken TJ, Boughey JC, Jones KN, Shah SS, Glazebrook KN. Imaging response and residual metastatic axillary lymph node disease after neoadjuvant chemotherapy for primary breast cancer. *Ann Surg Oncol* (2013) 20(10):3199–204. doi: 10.1245/s10434-013-3118-z

19. Eun NL, Son EJ, Gweon HM, Kim JA, Youk JH. Prediction of axillary response by monitoring with ultrasound and MRI during and after neoadjuvant chemotherapy in breast cancer patients. *Eur Radiol* (2020) 30(3):1460–9. doi: 10.1007/s00330-019-06539-4

20. Tan PH, Ellis I, Allison K, Brogi E, Fox SB, Lakhani S, et al. The 2019 world health organization classification of tumours of the breast. *Histopathology* (2020) 77(2):181–5. doi: 10.1111/his.14091

21. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The eighth edition AJCC cancer staging manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin* (2017) 67(2):93–9. doi: 10.3322/caac.21388

22. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of clinical Oncology/College of American pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol* (2010) 28(16):2784–95. doi: 10.1200/JCO.2009.25.6529

23. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St gallen international expert consensus on the primary therapy of early breast cancer 2013. *Ann Oncol* (2013) 24(9):2206–23. doi: 10.1093/annonc/mdt303

24. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* (2009) 45(2):228–47. doi: 10.1016/j.ejca.2008.10.026

25. Fumagalli D, Bedard PL, Nahleh Z, Michiels S, Sotiriou C, Loi S, et al. A common language in neoadjuvant breast cancer clinical trials: proposals for standard definitions and endpoints. *Lancet Oncol* (2012) 13(6):e240–8. doi: 10.1016/S1470-2045(11)70378-3

26. Provenzano E, Bossuyt V, Viale G, Cameron D, Badve S, Denkert C, et al. Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group. *Mod Pathol* (2015) 28(9):1185–201. doi: 10.1038/modpathol.2015.74

27. Magny SJ, Shikhman R, Keppke AL. *Breast imaging reporting and data system*. Treasure Island (FL: StatPearls (2022).

28. Hong J, Wu J, Huang O, He J, Zhu L, Chen W, et al. Early response and pathological complete remission in breast cancer with different molecular subtypes: a retrospective single center analysis. *J Cancer* (2020) 11(23):6916–24. doi: 10.7150/jca.46805

29. Kim JY, Park HS, Kim S, Ryu J, Park S, Kim SI. Prognostic nomogram for prediction of axillary pathologic complete response after neoadjuvant chemotherapy in cytologically proven node-positive breast cancer. *Med (Baltimore)* (2015) 94(43):e1720. doi: 10.1097/MD.0000000000001720

30. Guo R, Su Y, Si J, Xue J, Yang B, Zhang Q, et al. A nomogram for predicting axillary pathologic complete response in hormone receptor-positive breast cancer with cytologically proven axillary lymph node metastases. *Cancer* (2020) 126 Suppl 16:3819–29. doi: 10.1002/cncr.32830

31. Kim HS, Shin MS, Kim CJ, Yoo SH, Yoo TK, Eom YH, et al. Improved model for predicting axillary response to neoadjuvant chemotherapy in patients with clinically node-positive breast cancer. *J Breast Cancer* (2017) 20(4):378–85. doi: 10.4048/jbc.2017.20.4.378

32. Vila J, Mittendorf EA, Farante G, Bassett RL, Veronesi P, Galimberti V, et al. Nomograms for predicting axillary response to neoadjuvant chemotherapy in clinically node-positive patients with breast cancer. *Ann Surg Oncol* (2016) 23 (11):3501–9. doi: 10.1245/s10434-016-5277-1

33. Mao Y, Hedgire S, Harisinghani M. Radiologic assessment of lymph nodes in oncologic patients. *Curr Radiol Rep* (2014) 2(2):2. doi: 10.1007/s40134-013-0036-6

34. Yu X, Hao X, Wan J, Wang Y, Yu L, Liu B. Correlation between ultrasound appearance of small breast cancer and axillary lymph node metastasis. *Ultrasound Med Biol* (2018) 44(2):342–9. doi: 10.1016/j.ultrasmedbio.2017.09.020

35. Akissue de Camargo Teixeira P, Chala LF, Shimizu C, Filassi JR, Maesaka JY, de Barros N. Axillary lymph node sonographic features and breast tumor characteristics as predictors of malignancy: a nomogram to predict risk. *Ultrasound Med Biol* (2017) 43(9):1837–45. doi: 10.1016/j.ultrasmedbio.2017.05.003

36. van Persijn van Meerten EL, Gelderblom H, Bloem JL. RECIST revised: implications for the radiologist. a review article on the modified RECIST guideline. *Eur Radiol* (2010) 20(6):1456–67. doi: 10.1007/s00330-009-1685-y

frontiers | Frontiers in Oncology

REVIEW
published: 22 July 2022
doi: 10.3389/fonc.2022.859838

How Dual-Energy Contrast-Enhanced Spectral Mammography Can Provide Useful Clinical Information About Prognostic Factors in Breast Cancer Patients: A Systematic Review of Literature

Federica Vasselli[1], Alessandra Fabi[2], Francesca Romana Ferranti[1], Maddalena Barba[3], Claudio Botti[4], Antonello Vidiri[1] and Silvia Tommasin[5,6]*

[1] Radiology and Diagnostic Imaging, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Regina Elena National Cancer Institute, Rome, Italy, [2] Precision Medicine in Breast Cancer Unit, Fondazione Policlinico Universitario A. Gemelli, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy, [3] Division of Medical Oncology 2, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Regina Elena National Cancer Institute, Rome, Italy, [4] Division of Breast Surgery, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Regina Elena National Cancer Institute, Rome, Italy, [5] Human Neuroscience Department, Sapienza University of Rome, Rome, Italy, [6] Neuroimmunology Unit, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Fondazione Santa Lucia, Rome, Italy

OPEN ACCESS

Edited by:
Xiaosong Chen,
Shanghai Jiao Tong University, China

Reviewed by:
Guolin Ma,
China-Japan Friendship Hospital, China
Raffaella Massafra,
National Cancer Institute Foundation (IRCCS), Italy

***Correspondence:**
Antonello Vidiri
antonello.vidiri@ifo.it

Specialty section:
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

Received: 21 January 2022
Accepted: 27 May 2022
Published: 22 July 2022

Citation:
Vasselli F, Fabi A, Ferranti FR, Barba M, Botti C, Vidiri A and Tommasin S (2022) How Dual-Energy Contrast-Enhanced Spectral Mammography Can Provide Useful Clinical Information About Prognostic Factors in Breast Cancer Patients: A Systematic Review of Literature. Front. Oncol. 12:859838. doi: 10.3389/fonc.2022.859838

Introduction: In the past decade, a new technique derived from full-field digital mammography has been developed, named contrast-enhanced spectral mammography (CESM). The aim of this study was to define the association between CESM findings and usual prognostic factors, such as estrogen receptors, progesterone receptors, HER2, and Ki67, in order to offer an updated overview of the state of the art for the early differential diagnosis of breast cancer and following personalized treatments.

Materials and Methods: According to the PRISMA guidelines, two electronic databases (PubMed and Scopus) were investigated, using the following keywords: breast cancer AND (CESM OR contrast enhanced spectral mammography OR contrast enhanced dual energy mammography) AND (receptors OR prognostic factors OR HER2 OR progesterone OR estrogen OR Ki67). The search was concluded in August 2021. No restriction was applied to publication dates.

Results: We obtained 28 articles from the research in PubMed and 114 articles from Scopus. After the removal of six replicas that were counted only once, out of 136 articles, 37 articles were reviews. Eight articles alone have tackled the relation between CESM imaging and ER, PR, HER2, and Ki67. When comparing radiological characterization of the lesions obtained by either CESM or contrast-enhanced MRI, they have a similar association with the proliferation of tumoral cells, as expressed by Ki-67. In CESM-enhanced lesions, the expression was found to be 100% for ER and 77.4% for PR, while moderate or high HER2 positivity was found in lesions with non-mass enhancement and with mass closely associated with a non-mass enhancement component. Conversely, the

non-enhancing breast cancer lesions were not associated with any prognostic factor, such as ER, PR, HER2, and Ki67, which may be associated with the probability of showing enhancement. Radiomics on CESM images has the potential for non-invasive characterization of potentially heterogeneous tumors with different hormone receptor status.

Conclusions: CESM enhancement is associated with the proliferation of tumoral cells, as well as to the expression of estrogen and progesterone receptors. As CESM is a relatively young imaging technique, a few related works were found; this may be due to the "off-label" modality. In the next few years, the role of CESM in breast cancer diagnostics will be more thoroughly investigated.

Keywords: breast cancer: mammography, contrast-enhanced spectral mammography, HER2, progesterone, estrogen, Ki67

INTRODUCTION

Breast cancer is the first cause of death in the female population in western countries (1). Early diagnosis and treatment have led to an increase in survival rate and better clinical outcome of women affected by breast cancer. However, up to 50% of patients may experience the relapse. Therefore, early identification of women at high risk of recurrence or who may benefit from treatment adjuvant setting is needed (2). Prognostic factors are essential to estimating individual patient risk of developing clinically silent micro-metastatic diseases and to determining patient eligibility for postsurgical systemic adjuvant therapy (3). The immunohistochemical prognostic factors that are assessed in order to plan a surgical and medical treatment for breast cancer are estrogen receptors (ER), progesterone receptors (PR), and epidermal growth factor (HER-2) (4). These factors, assessed on biopsy or surgical specimens, have permitted a classification in subtypes of breast cancer and a fine personalization of the treatment, thus tailoring the treatment in single cases. In addition to the abovementioned factors, also nuclear protein Ki-67 may influence the prognosis of the disease (5). Lastly, the histological grade is assessed in the diagnostic process (6) and used in the prognosis evaluation.

In mammography, breast cancer may not be identified due to the low difference between tumoral and background tissue x-ray attenuation (7), and to overcome this limit, during the past years, several studies have aimed at providing aid to physicians in the imaging analysis process, resulting in automated software able to improve sensitivity and specificity of diagnostic performances (8–10). Moreover, artificial intelligence (AI) has been applied to mammography and other imaging methodologies in cancer diagnosis, characterization, prognosis, and prediction of therapy outcome (11).

A recent diagnostic tool, with an improved background subtraction procedure, is the contrast-enhanced spectral mammography (CESM), a new technique derived from full-field digital mammography. CESM includes the administration of an iodine-based contrast material and the performance of low- (28–32 kV) and high-energy (45–49 kV) consecutive exposures to reveal areas of increased blood supply within the breast. In post-processing, these exposures are mutually subtracted in order to create a contrast-enhanced image and detect tumor vascularity (7). An image is acquired before contrast injection, and two more images are acquired about 2 min after contrast injection, one at low and the other at high energy. Postinjection images are combined in a single image that minimizes the appearance of breast tissue and increases the signal of an iodinated contrast agent (enhancement) (12). Recently, CESM has been becoming a valuable tool in the diagnosis and staging of primary breast cancer. It improves the diagnostic accuracy of mammography, providing a more accurate tumor sizing and the identification of multifocal diseases (13). Indeed, CESM improves the sensitivity for breast cancer detection without decreasing specificity, since it provides higher contrast and better lesion delineation as well as a better evaluation of lesion size and detects more multifocal breast cancers, than mammography alone or combined with ultrasonography (14–17). Similarly to breast magnetic resonance imaging (MRI), which is considered the gold standard in the assessment of tumor, the findings obtained with CESM examination suggest that it should be considered a useful tool in the evaluation of disease extension. As a matter of fact, both CESM and MRI may also evaluate tumor response during neoadjuvant chemotherapy (NAC), which, reducing tumor volume and metastasis occurrence, increases the probability of a positive response to breast-conserving surgery, to be used instead of mastectomy, and of a high survival rate in advanced breast cancer (18).

The aim of this study was to define the association between CESM findings and prognostic factors, such as ER, PR, HER2, and Ki67, with the aim to offer an updated overview of state of the art for the early differential diagnosis of breast cancer and the following personalized treatments. In this framework, we performed a systematic review of the literature.

MATERIALS AND METHODS

According to the PRISMA guidelines (19), two electronic databases (PubMed and Scopus) were used to perform the literature

investigation, using the following keywords: breast cancer AND (CESM OR contrast enhanced spectral mammography OR contrast enhanced dual energy mammography) AND (receptors OR prognostic factors OR her2 OR progesterone OR estrogen OR Ki67). The search was concluded in August 2021. No restriction was applied to publication dates.

First, we identified all documents in both databases. After identifying existing studies, we cross-checked all the collected articles to avoid duplicates. Abstracts were examined carefully, and the following exclusion criteria were applied: not a research article (e.g., review, book chapter, conference report, case report, meta-analysis), articles written in languages other than English, and articles investigating diagnostic methodologies other than CESM or not investigating prognostic factors. The flowchart of article selection is shown in **Figure 1**.

To assess the scientific quality of the studies included in our review and any possible source of bias, we prepared a checklist of questions in accordance with QUADAS guidelines (20). The overall procedure was carried out by two investigators (FV, ST).

RESULTS

We obtained 28 articles from the research in PubMed and 114 articles from Scopus. After the removal of six replicas that were counted only once, out of 136 articles, 37 articles were reviews and were removed. The abstracts of the remaining 99 articles were inspected to verify conformity to exclusion criteria: 2 articles were case reports, 3 were written in a non-English language, 14 did not include prognostic factors, 44 did not include CESM, 12 used prognostic factors as diagnostic characterization, 13 were book chapters, 1 was a conference report, 1 was a note, and 1 was a meta-analysis. Finally, eight articles tackled the relation between CESM imaging and ER, PR, HER2, and Ki67. Among the eight articles admissible for the following analysis, the relation between CESM and prognostic factors was investigated with CESM-MRI comparison (two articles), with CESM enhancement (three articles), and with radiomic analysis of CESM enhancement (three articles).

Since CESM is a recent diagnostic technique, articles investigating how CESM may provide clinical information on biological prognostic factors date back to the last 2 years.

CESM MRI Comparison

CESM is often compared to MRI to test its utility in tumor diagnosis, and indeed, enhancement patterns were moderately in agreement between the two techniques (21). CESM may produce an enhancement intensity weaker in the ER-positive group than in the ER-negative group, as well as weaker in the PR-positive group than in the PR-negative group, and stronger in the HER-2-positive group than in the HER-2-negative group (21). Further, when comparing radiological characterization of the lesions obtained by either CESM or contrast-enhanced MRI, they have a similar association with the proliferation of tumoral cells, as expressed by Ki-67 (22). However, the authors do not describe if there are any differences between CESM and MRI in differentiating hormonal receptor status.

CESM Enhancement

In CESM-enhanced lesions, the expression was found to be 100% for ER and 77.4% for PR, while moderate or high HER2 positivity was found in lesions with non-mass enhancement and with mass closely associated with a non-mass enhancement component (23). Further, *via* CESM enhancement, neoplasms larger than 5 mm, with a high proliferative index and frequently HER2-positive, are recognized (24). Conversely, the non-enhancing breast cancer lesions were not associated with any prognostic factor, such as status of ER and/or PR, HER2 expression and/or amplification, and percentage of Ki67, which might be associated with the probability of showing enhancement (25).

Radiomic Analysis

Nowadays, one of the cutting-edge methods for image analysis is based on radiomics. For non-invasively assessing the hormone receptor status, other than tumor invasiveness and grade, radiomic features were derived from the first-order histogram of primary breast cancer lesions contoured on both CESM and MRI images and the two techniques resulted to be alternative in the assessment of hormone receptor status (26). Further, radiomics on CESM images showed the potential for the non-invasive characterization of heterogeneous tumors with different hormone receptor statuses (27). Lastly, radiomic features may predict histological outcomes and molecular subtypes *via* discriminating lesions with a positive or negative expression of hormonal receptors, and being associated with HER2. In particular, in an immunohistochemical study, the performances for discriminating positive versus negative expressions were 90.87% for HER2 positive versus HER2 negative, 83.79% for ER positive versus ER negative, and 84.80% for Ki67 positive versus Ki67 negative (28). The list of the final articles and their relationship with biologic prognostic factors is summarized in **Table 1**.

Quality Assessment

The Quadas-2 survey showed that the articles considered in the analysis were at risk of bias, especially for what concerns the study tests conducted in each research. Indeed, at the time of the radiological evaluation the investigators, i.e., the radiologists, were aware of the results of the histological test, in all studies with the exception of one study, who performed a blinded histological analysis (22). However, all articles referred to a proper reference test, i.e., definitive histology or diagnostic biopsy. Further, four articles were biased in patient selection, because they removed either patients with a tumor not easily identifiable, such as that with suspicious but not contrast-enhancing lesions (27), or patients with post-histology edema or not willing to undergo CESM (25), or because they did not clarify whether patients have mono- or multifocal diseases (21, 22). Lastly, one article alone was at risk of flow bias, because of using both definitive histology and diagnostic biopsy as standard reference (21). The Quadas-2 survey is shown in **Table 2**.

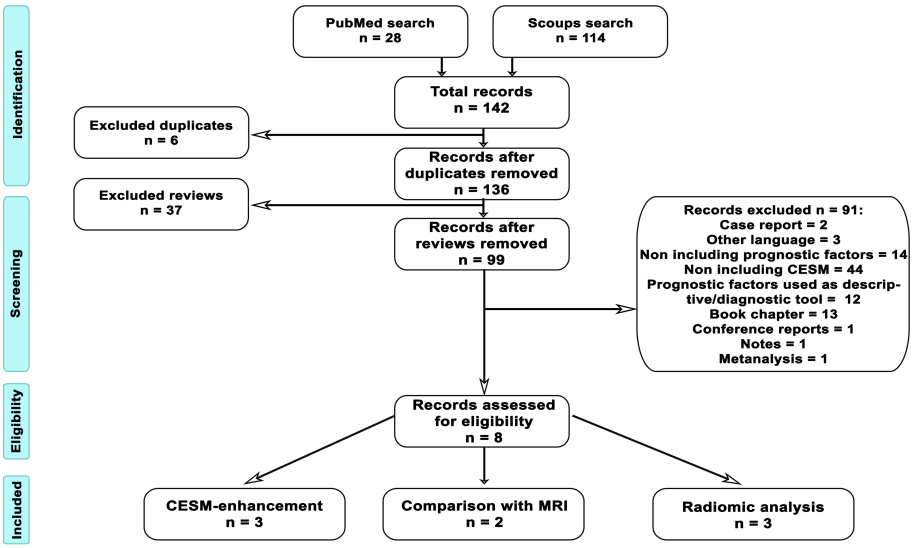


FIGURE 1 | Flowchart of article selection. The procedure to identify suitable articles to be included in the systematic review was performed following PRISMA guidelines and schematized in the figure. From database search, we identified 28 articles in PubMed and 114 in Scopus, with a total of 142 records. Six of the retrieved articles were duplicated across the two databases; therefore, we further investigated 136 articles. Out of those, 37 articles were reviews and, after abstract examination, 99 more articl es were excluded, because they were case reports (2), were written in no English language (3), did not include prognostic factors (14), did not include CESM (44), used prognostic factors as diagnostic characterization (12), were book chapters (13), were conference reports (1), were notes (1), and were meta-analyses (1). Finally, eight records were assessed to be eligible. Specifically, three articles addressed CESM enhancement, two articles compared CESM and MRI, and three articles investigated radiomic analysis of CESM images.

DISCUSSION

CESM is a very young modality recently introduced in the breast imaging scenario; therefore, the eligible articles found on our research were not older than 2 years. CESM shows considerable promise as the primary imaging test in symptomatic patients, providing improved diagnostic and staging information at the first evaluation.

Prognostic Factors

Prognostic factors are correlated with patient prognosis and allow important information about the efficacy of antitumoral treatment. Literature demonstrated that proliferative activity indicator (Ki67), HER-2, and hormonal receptor, such as ER and PR, statuses are important in treatment choice and that they have prognostic value in predicting pathological response and clinical outcome (29). As a matter of fact, HER-2 status represents a solid prognostic factor that predicts the response to trastuzumab alone or associated with pertuzumab treatment in locally advanced or early disease therapy (30). Also, determination of ER and PR status is crucial as their expression on the tumor cellular surface is related to a good response to endocrine therapy in both neoadjuvant and adjuvant therapy (30).

Among the biomarkers used to define tumor aggressiveness, Ki67, HER-2, ER, and PR are quantitative values. On the contrary, grading, which is used as well to define tumor aggressiveness, is a qualitative biomarker; therefore, we rather avoided to include it as an investigated prognostic factor in this study.

Comparison Between CESM and MRI and the Association With Prognostic Factors

CESM is a recent tool for diagnostic imaging that, although it uses ionizing radiations thus presenting some limitations in terms of radioprotection (7), may overtake the use of MRI in breast cancer monitoring, since it is more accessible, cheaper, faster, and more tolerated by patients (31), while maintaining performance equivalent to MRI and improving specificity (7, 17). As a matter of fact, the promising results of diagnostic performance could suggest CESM to be a valid alternative for patients who are not eligible for MRI. As a matter of fact, CESM and breast MRI similarly detect physiological, benign background parenchymal enhancement, which may be significantly associated with menopausal status, radiation therapy, hormonal treatment, and breast density and that rarely causes diagnostic issues if showing a bilateral, symmetrical appearance (32, 33). At the same time, the background parenchymal enhancement on MRI is considered a biomarker for increased risk of breast malignancy, while it is not known if the same holds true for CESM (34, 35).

Indeed, CESM and MRI show similar enhancement patterns (21) and a similar association with the proliferation of tumoral cells (22). The equivalence of CESM and MRI might rise from tumor vascularization, which is a crucial feature observed by both diagnostic modalities and is influenced by Ki67.

TABLE 1 | Characteristics of articles investigating the relationship between dual-energy contrast enhanced spectral mammography (CESM) and biologic prognostic factors.

Study	Participants	Standard references	Hormonal prognostic factor	Analysis technique	Radiological feature	Results
(23)	31 women (mean age 57.1 years; range 41–78)	Definitive histology	ER PR HER2 Ki67	CESM-histology agreement in lesion size measurement	Focus, ME and NME, and diameter	The totality of the lesions had a receptor positivity to estrogens. NME is associated with HER2 positivity:
(25)	348 women (mean age 60.1 years; 11.93 years; range 37–88)	Definitive histology	ER PR HER2 Ki67	CESM enhancement at lesion site	CESM enhancement	HER2 negative molecular subtype associated with higher probability of enhancement. False negative lesions are not associated with hormonal status
(24)	34 women (median age 53.9 years, 8.5 years)	Definitive histology	ER PR HER2 Ki67	Manually contoured lesions	CESM enhancement at calcification site	Association between enhancement and expression of Ki-67, HER-2; ER, PG
(28)	52 women (median age 50 years; 1st quartile 45.75, 3rd 60.25 years; range 37–80)	Diagnostic biopsy	ER PR HER2 Ki67	Radiomics of manually outlined ROIs	Mean, VC, difference between max and min gray level, SK, EN, RS and kurtosis.	Multivariate analysis of the histogram features can discriminate lesions with positive ER, PG, and Ki67 from lesions with negative ER, PG, and Ki67
(21)	131 women (mean age 42 years; range 18–77)	Diagnostic biopsy or definitive histology	ER PR HER2	CNR and relative signal difference	CESM enhancements	Enhancement of ER positive lesions < ER negative lesions. Enhancement of PR positive lesions < PR negative lesions. Enhancement of HER 2 positive lesions > HER2 negative lesions.
(26)	48 women (mean age 50.7 ± 8 years; range 38–74)	Diagnostic biopsy	ER PR HER2	Radiomics of manually contoured lesions	COM, RLM, GRA, ARM, WAV, GEO.	HR positivity and HR negativity differentiation accuracy observed.
(27)	100 women (mean age 51.5 years; 12 years; range 25-79)	Definitive histology	ER PR HER2	Radiomics of manually contoured lesions	HIS, COM, RLM, WAV.	HR positivity and HR negativity differentiation accuracy. HER2 positivity/HR negativity and HER2 negativity/HR positivity differentiation accuracy. Triple-negative and triple-positive differentiation accuracy.
(22)	100 women (range 42–80; median 58; 10.2)	Diagnostic biopsy for benign lesions Definitive histology for malignant lesions	ER PR HER2 Ki6	CESM enhancement	BI-RADS classification	Ki-67 correlation with CESM BIRADS.

ER, estrogen receptors; PR, progesterone receptors; HER2, epidermal growth factor; CESM, contrast-enhanced spectral mammography; ME, mass enhancement; NME, non-mass enhancement; ROI, region of interest; CNR, contrast noise ratio; COM, co-occurrence matrix; RLM, run-length matrix; GRA, absolute gradient; ARM, autoregressive model; WAV, discrete Haar wavelet transform; GEO, lesion geometry; MI, mutual information; VC, variation coefficient; SK, skewness; EN, entropy; RS, relative smoothness; BI-RADS, Breast Imaging Report and Data System.

A necessary step to include CESM in everyday clinical practice will be the standardization of diagnostic criteria. Given the similarity of the basic principles of lesion blood supply of the two modalities, MRI morphology descriptors have been already investigated and used to characterize lesions on CESM (36); however, more studies are needed to finalize the use of these descriptors in CESM image evaluation.

As in any imaging modality, patient motion may affect image quality. Due to the simultaneous acquisition of low-energy and high-energy images, the length of each exposure with CESM is longer than a standard full-field digital mammography, increasing the possibility of motion. However, the examination time of CESM is still shorter than the second-level examination MRI, reducing the risk of motion artifacts. Moreover, to instruct well the patient to hold as still as possible during the exposure is fundamental to reducing the possibility of motion (37).

CESM Enhancement and Prognostic Factors

CESM combines an iodinated contrast agent with the standard mammographic technique to improve lesion detectability. Since the growth of tumors is accompanied by angiogenesis, CESM

TABLE 2 | QUADAS2.

	Bias risk				Applicability issue		
	Patient selection	**Study test**	**Standard reference**	**Timing and flow**	**Patient selection**	**Study test**	**Standard reference**
(23)	YES	NO	YES	YES	YES	YES	YES
(25)	NO	NO	YES	YES	NO	YES	YES
(24)	YES	NO	YES	YES	YES	YES	YES
(26)	YES	NO	YES	YES	YES	YES	YES
(27)	NO	NO	YES	YES	YES	YES	YES
(21)	NO	NO	YES	NO	YES	YES	YES
(28)	YES	NO	YES	YES	YES	YES	YES
(22)	NO	YES	YES	YES	YES	YES	YES

Articles fulfilling (YES) or not fulfilling (NO) QUADAS2 criteria to assess the study quality.

permits to assess the enhancement related to the neovascularity of breast cancers, allowing a functional characterization in addition to the morphological features provided by structural images (16).

In literature, CESM-enhancing lesions have been associated with higher levels of prognostic factors, such as ER, PR, and HER2 (23, 24). On the other hand, non-enhancing lesions have been found not to relate to prognostic factors (25). Indeed, tumors have a higher enhancement compared to normal tissue due to the increase in vascularization, which in turn is associated with different tumor characteristics and therefore different expressions of prognostic factors.

CESM-Based Radiomic Analysis and Prognostic Factors

Feature extraction in radiomics is typically realized by means of pattern recognition algorithms and provides, as a result, a set of numbers, each one representing a quantitative description of a specific either geometric or physical property of the image portion under consideration. In oncological applications, examples of features are tumor size, shape, intensity, and texture, collectively providing a comprehensive tumor characterization, called the radiomics signature of the tumor (38). From an epistemological perspective, radiomics is based on the hypothesis that the extracted features reflect mechanisms occurring at genetic and molecular levels (39) and may reveal the relationship of tumor lesion surfaces with prognostic factor expression. The potential of radiomics applied on breast imaging has been investigated recently, and studies have already demonstrated the additive value of radiomics on MRI in breast cancer evaluation and prognosis (40, 41). Indeed, radiomics on CESM images might assess hormone receptor status (26) and characterize the related heterogeneous tumors (27), as well as predict histological outcomes and molecular subtypes associated with hormone receptors' expression (28). As a matter of fact, radiomics arises from the analysis of cell morphology, which may be influenced by the expression of the different receptors on the cell surface of the different tumors, thus permitting to differentiate the receptor status starting from imaging.

Radiomics could also contribute to differentiating benign from malignant enhancement in complicated cases, as in patients with high background parenchymal enhancement or low vascularized lesions, that may have a high risk for underestimation or even overestimation of the lesion (42), and to predicting response to NAC (43).

Literature

The articles included are all published in the last 2 years, and none of them was blinded, except that of Petrillo et al. (22). Nevertheless, this bias did not invalidate the articles, as the goal of these studies was to find a relationship between imaging features and prognostic factors, not just detecting a tumor. Indeed, knowing the histological subtypes was part of patients' preliminary information needed to obtain a sample of patients with heterogeneous radiologic patterns. Conversely, homogeneous histologic analysis was crucial in order to obtain consistent results among patients, and in one article the authors did not grant this consistency.

Only eight articles investigated the association between CESM imaging and prognostic factors, suggesting that the use of this technique in cancer prognosis and monitoring is still to be deeply investigated. Indeed, the modality is relatively young and large data pools are required to get strong results on this topic.

CONCLUSION

CESM is a relatively young diagnostic tool, and our review showed its potential on finding a precise imaging semeiotic, thanks to its association with prognostic factors, in order to provide patients with the most accurate pre-therapy and surgery evaluation. In this review, CESM enhancement showed an association with the proliferation of tumoral cells, as well as the expression of estrogen and progesterone receptors, although there is not a certain correlation between specific patterns of enhancement and prognostic factor outlines. Future studies might investigate CESM's ability in identifying ER/PR positivity and HER2 positivity/amplification, as, so far, they have not been investigated. Moreover, even if recent studies have investigated the radiomic application on CESM (26, 28), more results are requested to enforce these promising applications.

As CESM is a relatively young imaging technique, literature shows a few related works, often suffering from bias risk, and this is certainly due to the "off-label" use in clinical practice. The role

of CESM in breast cancer diagnostics will be further investigated, and radiomics studies will provide further predictive and prognostic information on the clinical impact of this technique.

AUTHOR CONTRIBUTIONS

Study concept and design, analysis and interpretation of data: FV and ST; statistical analysis: MB and CB; drafting of the manuscript: AF, FF, and AV; critical revision and final approval of the manuscript: all authors. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Funds Ricerca Corrente 2022 from Italian Ministry of Health.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer J Clin* (2018) 68:394–424. doi: 10.3322/caac.21492
2. Pisano ED, Baum JK, Bassett L. Diagnostic Performance of Digital Versus Film Mammography for Breast-Cancer Screening. *N Engl J Med* (2005) 353:1773–83. doi: 10.1056/NEJMoa052911
3. Cianfrocca M, Goldstein LJ. Prognostic and Predictive Factors in Early-Stage Breast Cancer. *Oncologist* (2004) 9:606–16. doi: 10.1634/theoncologist.9-6-606
4. Kwa M, Makris A, Esteva FJ. Clinical Utility of Gene-Expression Signatures in Early Stage Breast Cancer. *Nat Rev Clin Oncol* (2017) 14:595–610. doi: 10.1038/nrclinonc.2017.74
5. Penault-Llorca F, Radosevic-Robin N. Ki67 Assessment in Breast Cancer: An Update. *Pathology* (2017) 49:166–71. doi: 10.1016/j.pathol.2016.11.006
6. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast Cancer Prognostic Classification in the Molecular Era: The Role of Histological Grade. *Breast Cancer Res* (2010) 12:207. doi: 10.1186/bcr2607
7. Sogani J, Mango VL, Keating D, Sung JS, Jochelson MS. Contrast-Enhanced Mammography: Past, Present, and Future. *Clin Imaging* (2021) 69:269–79. doi: 10.1016/j.clinimag.2020.09.003
8. De Mitri IMAGIC-5 Collaboration. The MAGIC-5 Project: Medical Applications on a Grid Infrastructure Connection. *Stud Health Technol Inform* (2005) 112:157–66.
9. Salama WM, Aly MH. Deep Learning in Mammography Images Segmentation and Classification: Automated CNN Approach. *Alexandria Eng J* (2021) 60:4701–9. doi: 10.1016/j.aej.2021.03.048
10. Massafra R, Bove S, Lorusso V, Biafora A, Comes MC, Didonna V, et al. Radiomic Feature Reduction Approach to Predict Breast Cancer by Contrast-Enhanced Spectral Mammography Images. *Diagnostics (Basel)* (2021) 11:684. doi: 10.3390/diagnostics11040684
11. Avanzo M, Porzio M, Lorenzon L, Milan L, Sghedoni R, Russo G, et al. Artificial Intelligence Applications in Medical Imaging: A Review of the Medical Physics Research in Italy. *Phys Med* (2021) 83:221–41. doi: 10.1016/j.ejmp.2021.04.010
12. Patel BK, Lobbes MBI, Lewin J. Contrast Enhanced Spectral Mammography: A Review. *Semin Ultrasound CT MRI* (2018) 39:70–9. doi: 10.1053/j.sult.2017.08.005
13. Helal MH, Mansour SM, Zaglol M, Salaleldin LA, Nada OM, Haggag MA. Staging of Breast Cancer and the Advanced Applications of Digital Mammogram: What the Physician Needs to Know? *BJR* (2017) 90:20160717. doi: 10.1259/bjr.20160717
14. Dromain C, Balleyguier C, Muller S, Mathieu M-C, Rochard F, Opolon P, et al. Evaluation of Tumor Angiogenesis of Breast Carcinoma Using Contrast-Enhanced Digital Mammography. *Am J Roentgenology* (2006) 187:W528–37. doi: 10.2214/AJR.05.1944
15. Dromain C, Thibault F, Muller S, Rimareix F, Delaloge S, Tardivon A, et al. Dual-Energy Contrast-Enhanced Digital Mammography: Initial Clinical Results. *Eur Radiol* (2011) 21:565–74. doi: 10.1007/s00330-010-1944-y
16. Dromain C, Thibault F, Diekmann F, Fallenberg EM, Jong RA, Koomen M, et al. Dual-Energy Contrast-Enhanced Digital Mammography: Initial Clinical Results of a Multireader, Multicase Study. *Breast Cancer Res* (2012) 14:R94. doi: 10.1186/bcr3210
17. Fallenberg EM, Dromain C, Diekmann F, Engelken F, Krohn M, Singh JM, et al. Contrast-Enhanced Spectral Mammography Versus MRI: Initial Results in the Detection of Breast Cancer and Assessment of Tumour Size. *Eur Radiol* (2014) 24:256–64. doi: 10.1007/s00330-013-3007-7
18. Gradishar WJ, Anderson BO, Balassanian R, Blair SL, Burstein HJ, Cyr A, et al. Breast Cancer, Version 4.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* (2018) 16:310–20. doi: 10.6004/jnccn.2018.0012
19. Moher D, Liberati A, Tetzlaff J, Altman DGfor the PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *BMJ* (2009) 339:b2535–5. doi: 10.1136/bmj.b2535
20. Whiting PF. QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. *Ann Intern Med* (2011) 155:529. doi: 10.7326/0003-4819-155-8-201110180-00009
21. Liu Y, Zhao S, Huang J, Zhang X, Qin Y, Zhong H, et al. Quantitative Analysis of Enhancement Intensity and Patterns on Contrast-Enhanced Spectral Mammography. *Sci Rep* (2020) 10:9807. doi: 10.1038/s41598-020-66501-z
22. Petrillo A, Fusco R, Vallone P, Filice S, Granata V, Petrosino T, et al. Digital Breast Tomosynthesis and Contrast-Enhanced Dual-Energy Digital Mammography Alone and in Combination Compared to 2D Digital Synthetized Mammography and MR Imaging in Breast Cancer Detection and Classification. *Breast J* (2020) 26:860–72. doi: 10.1111/tbj.13739
23. Amato F, Bicchierai G, Cirone D, Depretto C, Di Naro F, Vanzi E, et al. Preoperative Loco-Regional Staging of Invasive Lobular Carcinoma With Contrast-Enhanced Digital Mammography (CEDM). *Radiol Med* (2019) 124:1229–37. doi: 10.1007/s11547-019-01116-7
24. Depretto C, Borelli A, Liguori A, Presti G, Vingiani A, Cartia F, et al. Contrast-Enhanced Mammography in the Evaluation of Breast Calcifications: Preliminary Experience. *Tumori* (2020) 106:491–6. doi: 10.1177/0300891620919170
25. Bicchierai G, Amato F, Vanzi B, De Benedetto D, Boeri C, Vanzi E, et al. Which Clinical, Radiological, Histological, and Molecular Parameters Are Associated With the Absence of Enhancement of Known Breast Cancers With Contrast Enhanced Digital Mammography (CEDM)? *Breast* (2020) 54:15–24. doi: 10.1016/j.breast.2020.08.009
26. Marino MA, Leithner D, Sung J, Avendano D, Morris EA, Pinker K, et al. Radiomics for Tumor Characterization in Breast Cancer Patients: A Feasibility Study Comparing Contrast-Enhanced Mammography and Magnetic Resonance Imaging. *Diagnostics* (2020) 10:492. doi: 10.3390/diagnostics10070492
27. Marino MA, Pinker K, Leithner D, Sung J, Avendano D, Morris EA, et al. Contrast-Enhanced Mammography and Radiomics Analysis for Noninvasive Breast Cancer Characterization: Initial Results. *Mol Imaging Biol* (2020) 22:780–7. doi: 10.1007/s11307-019-01423-5
28. La Forgia D, Fanizzi A, Campobasso F, Bellotti R, Didonna V, Lorusso V, et al. Radiomic Analysis in Contrast-Enhanced Spectral Mammography for Predicting Breast Cancer Histological Outcome. *Diagnostics* (2020) 10:708. doi: 10.3390/diagnostics10090708
29. Dowsett M, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J, et al. Meta-Analysis of Breast Cancer Outcomes in Adjuvant Trials of Aromatase Inhibitors Versus Tamoxifen. *JCO* (2010) 28:509–18. doi: 10.1200/JCO.2009.23.1274
30. Placido SD, Laurentiis MD, Carlomagno C, Gallo C, Perrone F, Pepe S, et al. Twenty-Year Results of the Naples GUN Randomized Trial: Predictive Factors of Adjuvant Tamoxifen Efficacy in Early Breast Cancer. *Clin Cancer Res* (2003) 9(3):1039–46.

31. Hobbs MM, Taylor DB, Buzynski S, Peake RE. Contrast-Enhanced Spectral Mammography (CESM) and Contrast Enhanced MRI (CEMRI): Patient Preferences and Tolerance: CESM and CEMRI Preferences and Tolerance. *J Med Imaging Radiat Oncol* (2015) 59:300–5. doi: 10.1111/1754-9485.12296
32. Sogani J, Morris EA, Kaplan JB, D'Alessio D, Goldman D, Moskowitz CS, et al. Comparison of Background Parenchymal Enhancement at Contrast-Enhanced Spectral Mammography and Breast MR Imaging. *Radiology* (2017) 282:63–73. doi: 10.1148/radiol.2016160284
33. Savaridas SL, Taylor DB, Gunawardana D, Phillips M. Could Parenchymal Enhancement on Contrast-Enhanced Spectral Mammography (CESM) Represent a New Breast Cancer Risk Factor? Correlation With Known Radiology Risk Factors. *Clin Radiol* (2017) 72:1085.e1–1085.e9. doi: 10.1016/j.crad.2017.07.017
34. Dontchos BN, Rahbar H, Partridge SC, Korde LA, Lam DL, Scheel JR, et al. Are Qualitative Assessments of Background Parenchymal Enhancement, Amount of Fibroglandular Tissue on MR Images, and Mammographic Density Associated With Breast Cancer Risk? *Radiology* (2015) 276:371–80. doi: 10.1148/radiol.2015142304
35. Sorin V, Yagil Y, Shalmon A, Gotlieb M, Faermann R, Halshtok-Neiman O, et al. Background Parenchymal Enhancement at Contrast-Enhanced Spectral Mammography (CESM) as a Breast Cancer Risk Factor. *Acad Radiol* (2020) 27:1234–40. doi: 10.1016/j.acra.2019.10.034
36. Kamal RM, Helal MH, Mansour SM, Haggag MA, Nada OM, Farahat IG, et al. Can We Apply the MRI BI-RADS Lexicon Morphology Descriptors on Contrast-Enhanced Spectral Mammography? *BJR* (2016) 89:20160157. doi: 10.1259/bjr.20160157
37. Bhimani C, Li L, Liao L, Roth RG, Tinney E, Germaine P. Contrast-Enhanced Spectral Mammography: Modality-Specific Artifacts and Other Factors Which May Interfere With Image Quality. *Acad Radiol* (2017) 24:89–94. doi: 10.1016/j.acra.2016.08.024
38. Crivelli P, Ledda RE, Parascandolo N, Fara A, Soro D, Conti M. A New Challenge for Radiologists: Radiomics in Breast Cancer. *BioMed Res Int* (2018) 2018:1–10. doi: 10.1155/2018/6120703
39. Lambin P, Leijenaar RTH, Deist TM, Peerlings J, de Jong EEC, van Timmeren J, et al. Radiomics: The Bridge Between Medical Imaging and Personalized Medicine. *Nat Rev Clin Oncol* (2017) 14:749–62. doi: 10.1038/nrclinonc.2017.141
40. Parekh VS, Jacobs MA. Integrated Radiomic Framework for Breast Cancer and Tumor Biology Using Advanced Machine Learning and Multiparametric MRI. *NPJ Breast Cancer* (2017) 3:43. doi: 10.1038/s41523-017-0045-3
41. Whitney HM, Taylor NS, Drukker K, Edwards AV, Papaioannou J, Schacht D, et al. Additive Benefit of Radiomics Over Size Alone in the Distinction Between Benign Lesions and Luminal A Cancers on a Large Clinical Breast MRI Dataset. *Acad Radiol* (2019) 26:202–9. doi: 10.1016/j.acra.2018.04.019
42. Boca (Bene) I, Ciurea AI, Ciortea CA, Ştefan PA, Lisencu LA, Dudea SM. Differentiating Breast Tumors From Background Parenchymal Enhancement at Contrast-Enhanced Mammography: The Role of Radiomics—A Pilot Reader Study. *Diagnostics* (2021) 11:1248. doi: 10.3390/diagnostics11071248
43. Wang Z, Lin F, Ma H, Shi Y, Dong J, Yang P, et al. Contrast-Enhanced Spectral Mammography-Based Radiomics Nomogram for the Prediction of Neoadjuvant Chemotherapy-Insensitive Breast Cancers. *Front Oncol* (2021) 11:605230. doi: 10.3389/fonc.2021.605230

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 02 August 2022
DOI 10.3389/fonc.2022.861324

OPEN ACCESS

EDITED BY
Kunwei Shen,
Shanghai Jiao Tong University, China

REVIEWED BY
Gaia Griguolo,
Universitàdi Padova,
Italy
Francesco Pepe,
University of Naples Federico II, Italy

*CORRESPONDENCE
Sander Ellegård
sander.ellegard@regionostergotland.se

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 24 January 2022
ACCEPTED 11 July 2022
PUBLISHED 02 August 2022

CITATION
Ellegård S, Engvall K, Asowed M, Hallbeck A-L, Elander N and Stål O (2022) Long-term follow-up of early stage HER2-positive breast cancer patients treated with trastuzumab: A population-based real world multicenter cohort study. *Front. Oncol.* 12:861324. doi: 10.3389/fonc.2022.861324

Long-term follow-up of early stage HER2-positive breast cancer patients treated with trastuzumab: A population-based real world multicenter cohort study

Sander Ellegård[1,2]*, Kristina Engvall[2,3], Mustafa Asowed[1], Anna-Lotta Hallbeck[2,4], Nils Elander[1,2] and Olle Stål[2]

[1]Department of Oncology, Linköping University, Linköping, Sweden, [2]Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden, [3]Department of Oncology, Jönköping, Sweden, [4]Department of Clinical Genetics, Linköping University, Linköping, Sweden

Introduction: Since its introduction in standard of care, trastuzumab has revolutionized the treatment of patients with early and late stages of HER2-positive breast cancer. While the initial clinical trials were convincing and lead to major changes in practice, more knowledge on the long-term outcome and tolerability is needed. The present study was designed to assess the survival, prognostic factors and relapse patterns after the implementation of trastuzumab in a real-world cohort.

Methods: All cases of HER2-positive breast cancer diagnosed between 2006 and 2014 in the Southeast Healthcare Region of Sweden were retrospectively identified. Medical records were thoroughly reviewed with regard to clinicopathological parameters, treatments, relapse pattern and adverse events.

Results: 643 patients were identified and 599 were eligible for analysis. Breast cancer specific survival, distant recurrence free survival and local recurrence free survival were 93.4%, 89.7% and 98.0% for trastuzumab treated patients and 87.4%, 81.6% and 87.4% in patients not treated with trastuzumab, respectively. ER status, nodal status and trastuzumab treatment were all independent prognostic factors in multivariable analysis. No new safety concerns were discovered.

Conclusion: The real-world outcome of trastuzumab-treated patients with early HER2-positive breast cancer is similar to what has been previously reported in long-term follow up of prospective clinical trials. ER status, nodal

status and trastuzumab treatment are independent prognostic factors for breast cancer specific mortality rate, distant recurrence rate and locoregional recurrence rate in HER2-positive patients in the trastuzumab era.

KEYWORDS

HER2, breast cancer, real-world evidence, adjuvant, trastuzumab (Herceptin), prognostic factors

Introduction

Breast cancer is the most common malignancy in women, each year affecting more than two million new individuals around the world (1). Between 15 and 25 percent of all breast cancers are classified as Human epidermal growth factor receptor 2 (HER2) positive (2, 3). Reports of the incidence of HER2-positive disease have varied and 2009, a Swedish study showed a prevalence of 14.3% (4). HER2-is a trans membrane tyrosine kinase receptor without any known ligands, that through homo- and hetero-dimerization with other receptors of the HER family initiate a signaling cascade resulting in tumor cell proliferation and survival (5). HER2 has been identified both as a negative prognostic factor and a treatment predictive target for receptor specific drugs such as the antibody trastuzumab. Since its introduction in standard of care, trastuzumab has significantly improved the outcome of patients with early and late stages of HER2-positive breast cancer (6, 7). Several publications have reported similar outcomes in real-world and randomized controlled trial populations (8, 9). The optimistic long term prognosis for stage I patients has led to de-escalation attempts in terms of the adjuvant systemic therapy offered, including the trial by Tolaney et al. where excellent outcome following paclitaxel weekly in combination with trastuzumab was observed (10). Nevertheless, a substantial proportion of patients with HER2-positive disease will eventually experience locoregional or systemic relapse. In order to potentiate the HER2 targeting treatment, novel drugs and/or combinations of trastuzumab and novel compounds have therefore been explored. Pertuzumab, a monoclonal antibody binding to the HER2 subdomain II, thus inhibiting the heterodimerization of HER2 and other receptors of the HER family, has proven clinically beneficial when combined with trastuzumab (11–13). While the combination of pertuzumab and trastuzumab has been demonstrated to improve the pathological complete response (pCR) rate in the neoadjuvant setting, the role of the combination in adjuvant settings is still under debate (11, 13). On the other hand, the KATHERINE trial demonstrated an additional value of the antibody-drug conjugate compound trastuzumab emtansine for patients with residual disease following neo-adjuvant chemotherapy (14). Furthermore, the tyrosine kinase inhibitor neratinib, which inhibits HER1, HER2 and HER4, were shown to improve long term prognosis for patients with HER2-positive disease, in particular for patients who had estrogen receptor (ER) positive tumors (15).

Despite many treatment options available for patients with HER2-positive breast cancer, there is still a need to learn how to identify patients who benefit from additional treatment. This is particularly important when results from randomized controlled trials are implemented in standard of care, where patients might be different in terms of age, performance status and comorbidities to those who were included in the clinical trials. Real-world follow ups are important in this context, to help us evaluate current treatment strategies and to identify groups of patients where additional studies are necessary.

This study was designed in order to assess the real-world treatment coverage and long-term outcome, including prognostic parameters and recurrence patterns, of patients treated with adjuvant trastuzumab in early stages of HER2-positive breast cancer. All HER2-positive patients since the introduction of adjuvant trastuzumab in the Southeast Health Care Region of Sweden were retrospectively identified and formed the study cohort. The cohort represents a true real-world perspective, as the Scandinavian health care system with public funded free of charge treatment ensures that all citizens regardless of socio-economic status are offered equal care.

Materials and methods

Study design and patients

A population based retrospective multicenter cohort study in the Southeast Health Care Region of Sweden was designed. In this region, systemic cancer therapy such as trastuzumab is available at three public funded oncology departments located

at the three major hospitals (Linköping, Kalmar, and Jönköping). Approximately 1.1 million citizens live in the region. Patients were identified *via* the national Swedish cancer registry and by using local pathology department databases to ensure the inclusion of all HER2-positive patients.

All female patients diagnosed with HER2-positive breast cancer between 2006-01-01 and 2014-03-13 were included. Exclusion criteria were male sex, stage IV disease, other malignancy affecting treatment and follow up, and incomplete data (e.g. missing medical records). HER2 status was determined according to clinical routine using immunohistochemistry (IHC) and/or fluorescent/chromogenic *in situ* hybridization (FISH/ISH). HER2-positive tumors were defined as either IHC score of 3+ or IHC score of 2+ in combination with confirmed amplification of the HER2 encoding gene with FISH/ISH analysis. A majority of patients received chemotherapy in addition to trastuzumab. The predominant chemotherapy schedule included three cycles of epirubicin, cyclophosphamide and fluorouracil every three weeks followed by either three cycles of docetaxel every three weeks or twelve cycles of weekly paclitaxel. Endocrine therapy was given to ER-positive patients per clinical routine with tamoxifen or aromatase inhibitor with or without GNRH-analogue depending on the menopausal status. ER positivity was defined as per Swedish clinical guidelines as ≥10% positive cells, measured with immunohistochemistry. Local and locoregional radiotherapy were given according to regional- and national guidelines.

Trastuzumab in combination with chemotherapy was initially recommended only for lymph node positive patients. However, this was changed early during the studied interval to the current indication, which includes all patients with tumor size larger than 5 mm with or without lymph node involvement.

Follow-up time was defined as the time from diagnosis until death or loss to follow up. Loss to follow up was defined either as the cutoff date of medical record review or the last date when data of the patient were available in the medical records (e.g. due to emigration).

Endpoints

Key endpoints were breast cancer-specific survival (BCSS), distant recurrence-free survival (DRFS), and local recurrence-free survival (LRFS). BCSS was defined as survival time from diagnosis until death caused by breast cancer. BCSS rather than overall survival was utilized in order to better reflect trastuzumab's anti-tumoral effect. This assumption was made due to the retrospective nature of this study where patients not treated with trastuzumab were expected to be older and have more comorbidities, which could lead to an overestimation of the effect of trastuzumab on survival. Censoring was made at loss to follow up or death of other cause. DRFS was defined as the time from diagnosis until first evidence of breast cancer distant metastasis diagnosed either with radiology or cytology/histology. LRFS was defined as the time to local recurrence as diagnosed with biopsy or clinical examination. Secondary endpoints were clinicopathological prognostic factors, safety in terms of adverse events and complications and relapse pattern.

Statistical analysis

Hazard ratios were calculated using univariate and multivariable Cox regression. P-values <0.05 were considered statistically significant. Survival was calculated using the Kaplan-Meier method and significance was determined using the log-rank test. In the Cox regression analysis age was used as a continuous variable.

Pearsons Chi-square test was used to detect statistical differences in distribution of clinical characteristics between trastuzumab treated and untreated patients.

Fisher's exact test, using a 2-sided alpha, was used to detect statistical differences between metastatic sites due to the small number of events.

For analysis of hazard ratio and survival related to nodal status, subjects with four to nine positive nodes were combined with those who had more than nine positive nodes due to few patients in both groups. Similarly, and as the frequency of NHG (Nottingham Histological Grade) I tumors was low, patients with NHG I and II were merged and compared with NHG III in all subgroup analyses. SPSS Statistics version 25 (IBM) was used for Cox proportional hazard, Chi-square tests and Fisher's exact test. The R package in survminer was used to make Kaplan-Meier curves with life tables and calculating log rank statistics.

Ethical consideration

This study was conducted according to the Helsinki declaration and was approved by the Regional Ethical Review Board in Linköping (original approval DNR M140-06, approved amendments DNR 2014/163-32 and DNR 2020-05501). Based on the retrospective non-interventional design, the Ethical Review Board waived the need for informed consent.

Results

Study population and treatment data

During the study interval of 2006 – 2014, 643 patients were diagnosed with HER2-positive breast cancer in the Southeast health care region of Sweden. Following the exclusion of 44 patients who did not meet the inclusion criteria, 599 subjects remained and formed the study population (Figure 1).

Median follow-up time in the total cohort was 6.8 years (Range: 0.5-13.1 and 95% CI: 6.5-7.1).

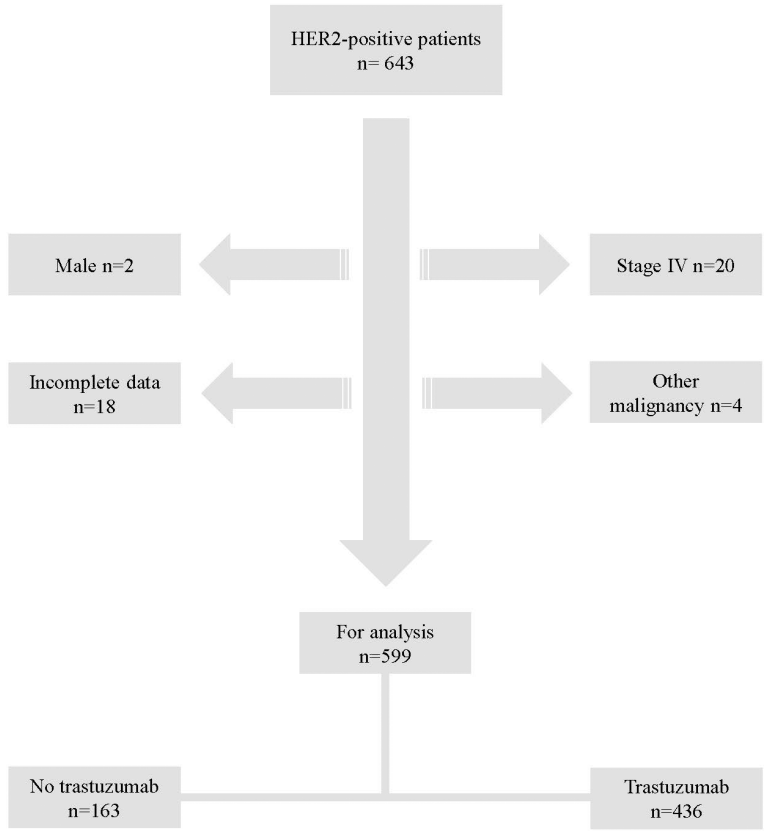


FIGURE 1
Flow chart of study population.

The proportion of patients receiving trastuzumab was initially 52-57% in 2006-2008 but increased over time, reaching a maximum of 82-88% in 2011-2013. Patients that did not receive trastuzumab were significantly older, had significantly less nodal involvement, significantly smaller primary tumors, significantly lower tumor grade and did, almost exclusively, not receive chemotherapy (Table 1).

During the studied interval, 94% (95% CI: 91.7-96.1%) of the patients that received chemotherapy also received trastuzumab. In 2006, 79.3% of the patients that received chemotherapy were treated with trastuzumab. This proportion increased to 96% in 2009 and then remained above 95% for the rest of the studied interval.

Trastuzumab was prematurely discontinued in 41 (9.4%) of cases. The most common cause for treatment discontinuation was heart toxicity (n=21, 4.8%). Other specified causes that led to discontinuation of trastuzumab were allergic reactions (n=1, 0.2%), pain (n=2, 0.5%), pancreatitis (n=1, 0.2%), infection (n=1, 0.2%) and progressive disease (n=4, 0.9%) although in many cases the cause was not clearly stated (n=11, 2.5%).

Prognostic factors and events

5-year BCSS was 87.4% (95% CI; 82.3-92.0) in the cohort not treated with trastuzumab and 93.4% (95% CI; 91.2-95.6) in the trastuzumab-treated cohort. The 5-year DRFS was 81.6% (95% CI; 76.5-86.7) in the cohort not treated with trastuzumab and 89.7% (95% CI; 87.1-92.3) in the trastuzumab-treated cohort. The 5-year LRFS was 87.4% (95% CI; 82.7-92.0) in the cohort not treated with trastuzumab and 98.0% (95% CI; 96.7-99.3) in the trastuzumab-treated cohort. Kaplan-meier curves of survival with regard to ER and nodal status are displayed in Figures 2, 3 for patients not treated with trastuzumab and Figures 4, 5 for patients treated with trastuzumab. The five-year BCSS, DRFS and LRFS are shown in Table 2.

Cox proportional hazard analysis revealed that trastuzumab treatment, nodal status and ER-status were significant prognostic factors for breast cancer specific mortality rate (BCSMR), distance recurrance rate (DRR) and local recurrence rate (LRR) when adjusted for age, grade and tumor size (Table 3). The adjusted HR for presence vs absence of

TABLE 1 Clinicopathological parameters.

		No trastuzumab	Trastuzumab	
		N (%)	N (%)	p-value
All patients		163 (100)	436 (100)	
Age	<30	1 (1)	5 (1)	**<0.0005**
	30–39	2 (1)	38 (9)	
	40–49	19 (12)	89 (20)	
	50–59	23 (14)	107 (25)	
	60–69	35 (22)	139 (32)	
	70–79	40 (25)	54 (12)	
	80+	43 (26)	4 (1)	
Neoadjuvant treatment	No	157 (96)	385 (88)	**0.003**
	Yes	6 (4)	51 (12)	
Type of surgery	Breast conserving	52 (32)	164 (38)	0.35
	Mastectomy	111 (68)	271 (62)	
Tumor size	<21mm	85 (52)	203 (47)	0.67
	21-50mm	60 (37)	167 (38)	
	>50mm	6 (4)	19 (4)	
	Missing	12 (7)	47 (11)	
ER-status	ER-negative	59 (36)	165 (38)	0.71
	ER-positive	104 (64)	271 (62)	
NHG	NHG I	12 (7)	10 (2)	**0.005**
	NHG II	64 (39)	139 (32)	
	NHG III	86 (53)	261 (60)	
	Missing	1 (1)	26 (6)	
Nodal status	0	111 (68)	184 (42)	**<0.0005**
	1–3	27 (17)	113 (26)	
	≥4	17 (10)	83 (19)	
	Missing	8 (5)	56 (13)	
Histological subtype	Ductal	145 (95)	395 (93)	0.56
	Lobular	3 (2)	16 (4)	
	Other	5 (3)	14 (3)	
Chemotherapy	Yes	26 (16)	420 (96)	**<0.0005**
	No	137 (84)	16 (4)	
Radiotherapy	Yes	81 (51)	348 (80)	**<0.0005**
	No	78 (49)	85 (20)	
	Missing	4	3	

Bold is used when numbers are statistically significant ($p<0.05$).

trastuzumab was improved with regard to all endpoints reflecting the increased rate of unfavorable tumor characteristics in the trastuzumab-treated cohort. Individual Cox proportional hazard models were made for the trastuzumab treated cohort and the cohort not treated with trastuzumab respectively, where nodal status and ER status were the only significant prognostic factors in both cohorts for BCSMR and DRR although not for LRR where no single factor retained its prognostic value (data not shown).

Metastatic sites and metastatic pattern

In total, 87 (15%) patients experienced distant recurrence during the studied interval, 33 of which were in the cohort not treated with trastuzumab and 54 in the trastuzumab treated cohort. The most common metastatic sites were lung (n=41, 47%), liver (n=38, 44%) and bone (n=36, 41%) followed by brain (n=29, 33%) and skin (n=5, 6%).

There were few differences regarding metastatic pattern between the trastuzumab treated and untreated subgroups.

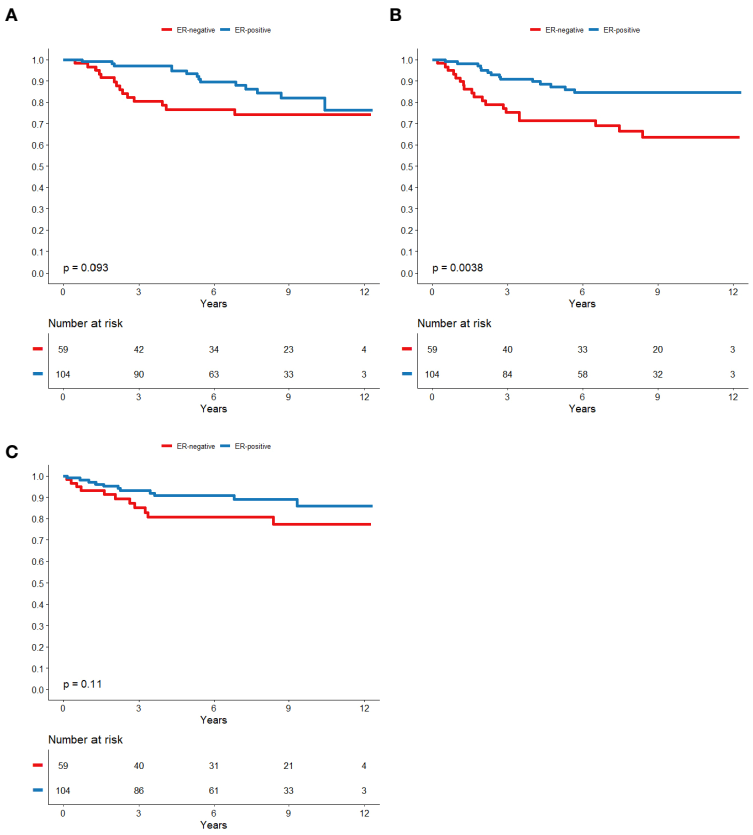


FIGURE 2
ER-status in relation to BCSS **(A)**, DRFS **(B)** and LRFS **(C)** respectively in patients that did not receive trastuzumab.

Overall, 29 (4.8% (95% CI; 3.3-6.8)) patients were diagnosed with brain metastasis during the studied interval, with no significant difference between trastuzumab treated and untreated patients. These 29 cases corresponded to 33.3% (95% CI; 24.1-43.7) of all patients who experienced distant metastasis, still with no significant difference between trastuzumab treated and untreated subjects (39% (95% CI 27-52) vs. 28% (95% CI 12-40). However, at the time of the first distant recurrence, brain metastases were significantly more common in patients treated with trastuzumab (n=14, 26% (95% CI 16-50)) as compared to the patients not treated with trastuzumab (n=2, 6% (95% CI 1-18)), p=0.023. No other significant differences in metastatic pattern were observed (Table 4).

Forty six of 50 (92%) biopsy confirmed recurrent/metastatic lesions (all locations combined) were HER2-positive. HER2-negative recurrence occurred in three trastuzumab-treated patients and in one patient not treated with trastuzumab.

Discussion

This population-based cohort study describes the long-term outcome and relapse pattern in a real-world cohort covering all patients with early stages of HER2-positive breast cancer, both those treated with trastuzumab in the adjuvant setting and those who did not undergo such treatment, under a period of eight years. The results demonstrate that the long-term prognosis for patients treated with trastuzumab is similar to what has been seen in early clinical trials (16) as well as more recent studies with similarly distributed patients such as the PERSEPHONE trial (17). The main prognostic factors for breast cancer survival, distant recurrence and locoregional recurrence were lymph node status, ER-status, and trastuzumab treatment.

The proportion of patients with early stage HER2-positive breast cancer undergoing adjuvant treatment with trastuzumab was relatively low during the first years after the introduction, only reaching 57% in 2006, which is somewhat lower than corresponding data from the Netherlands (18) but slightly higher than 50% that was reported from New Zeeland and Australia (19). The proportion of patients receiving trastuzumab increased over time, to a maximum of 88% (2011) and then remained over 80% for the rest of the studied interval in this study. The optimal percentage is difficult to determine due to the fact that some patients with HER2-positive disease will not actually

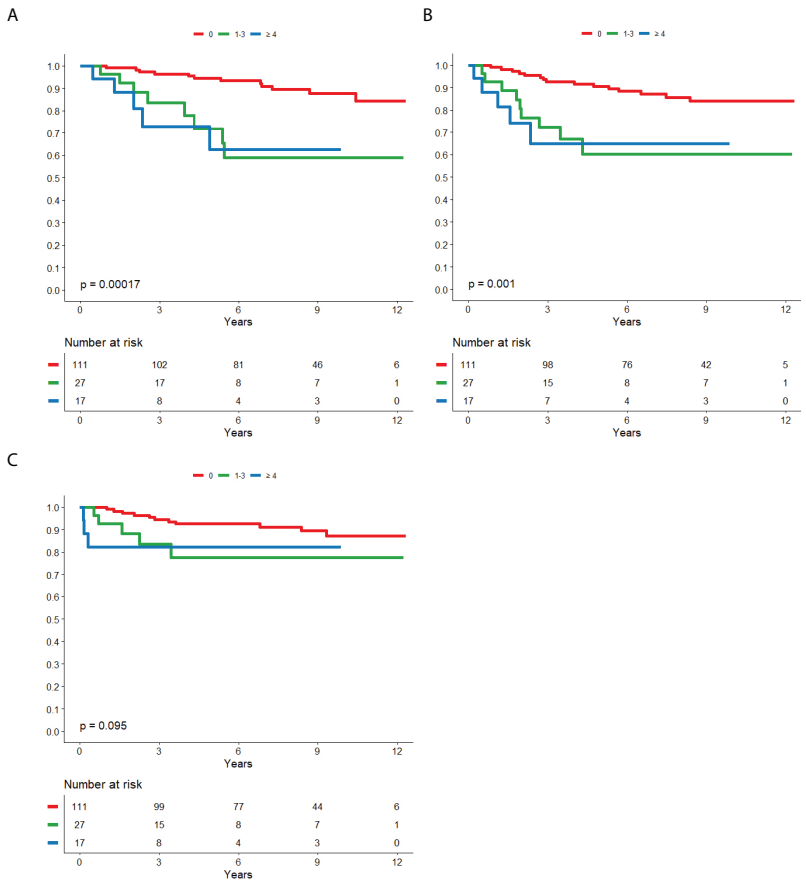


FIGURE 3
Nodal status in relation to BCSS **(A)**, DRFS **(B)** and LRFS **(C)** respectively in patients that did not receive trastuzumab.

benefit from the treatment or be suitable for it due to their health status in general or various contraindications. It is likely to believe that part of this change was attributable to changes in national and international practice guidelines (20, 21), and to some extent it might also reflect accumulated knowledge and clinical experience. In addition, the prescription of adjuvant trastuzumab is closely linked to the prescription of adjuvant chemotherapy, the latter now being recommended and offered to a larger proportion of patients due to more data regarding chemotherapy in elderly patients (22). Notably, since 2009 more than 95% of the patients undergoing adjuvant chemotherapy also underwent trastuzumab treatment in parallel which is in line with current Swedish national guidelines.

In accordance with early trials on the topic, brain metastases were notably common as first metastatic lesion in patients experiencing relapse under follow up (6). In the present cohort, 25% of the patients who experienced relapse after trastuzumab treatment presented with brain metastases, which is in the upper range of what was previously reported (23). However, brain metastases were common regardless of trastuzumab treatment and overall brain recurrences did not significantly differ between trastuzumab treated and untreated patients. The high prevalence of brain metastases in patients with metastatic disease is in line with previous data from our group (24). These results further strengthen the current theory that the increased number of brain metastases noted in early trastuzumab trials is due to trastuzumab´s proportionally higher effect on extracranial disease. We do believe that these data suggest that metastatic screening of the brain is warranted at the time of distant recurrence for patients with HER2-positive breast cancer. Furthermore, the incidence of local recurrences in patients treated with trastuzumab was low (n=12, 3%) which is noteworthy as HER2-positive breast cancer was associated with an increased risk of local recurrence in the pre-trastuzumab era (25). These data are in line with more recent publications regarding trastuzumab-treated patients where local recurrences are reported in 2-4% of the patients (26, 27).

Adverse events attributed to trastuzumab were rare, and we could not identify any new safety concerns with regard to

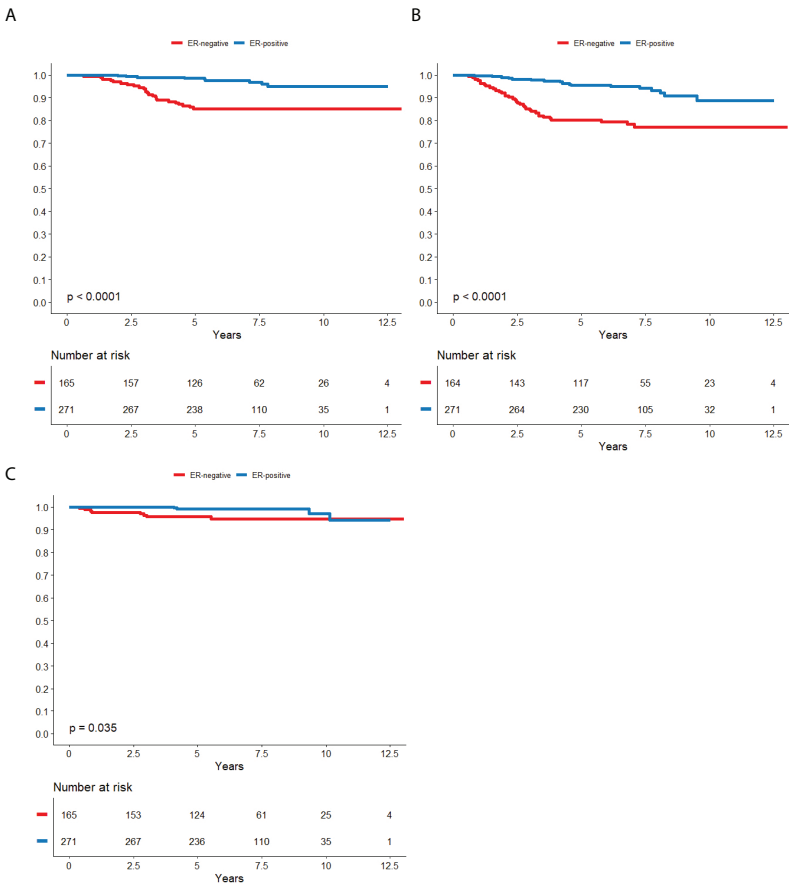


FIGURE 4
ER-status in relation to BCSS **(A)**, DRFS **(B)** and LRFS **(C)** respectively in patients that was treated with trastuzumab.

trastuzumab. Heart toxicity is one of the most common adverse events that lead to discontinuation, however only 4.9% discontinued trastuzumab treatment due to heart toxicity in our study, a low figure compared to early trials and other studies (6, 28). The low occurrence of heart toxicity could be due to the use of epirubicin instead of doxorubicin or improved clinical awareness and understanding of how to handle trastuzumab´s heart toxicity over time. The retrospective nature and real-world focus of the present study could result in an underestimation of less serious adverse events, not leading to treatment discontinuation, as data collection exclusively relied on what was evident from the medical records and no standardized CTCAE scorings or similar were available.

The main strengths of the present study include the truly real-world perspective, as all HER2-positive patients in a large geographical region diagnosed under a period of eight years, regardless socioeconomic status, performance status, and comorbidity, were included. The long follow up ensures that mature survival data, rather than surrogate endpoints, were available.

Self-evidently, long term follow-up of patients who received trastuzumab and other anti-HER2 targeting treatments in the last few years is not yet possible, and the results here presented do not necessarily fully reflect the predicted outcome of today's state of the art treatment strategies. Another limitation is that important prognostic variables such as node status and tumor size could not reliably be extracted from the medical records for the neoadjuvant patients in this study, hence extrapolation to this growing subgroup of patients should be done with caution. Due to the retrospective nature of the study, no additional examinations or sampling have been made and no reliable comorbidity score was deemed possible to calculate from the medical records. Importantly, trastuzumab and chemotherapy were almost exclusively administered together in this study, hence we could not evaluate the survival benefit by trastuzumab as compared to chemotherapy alone.

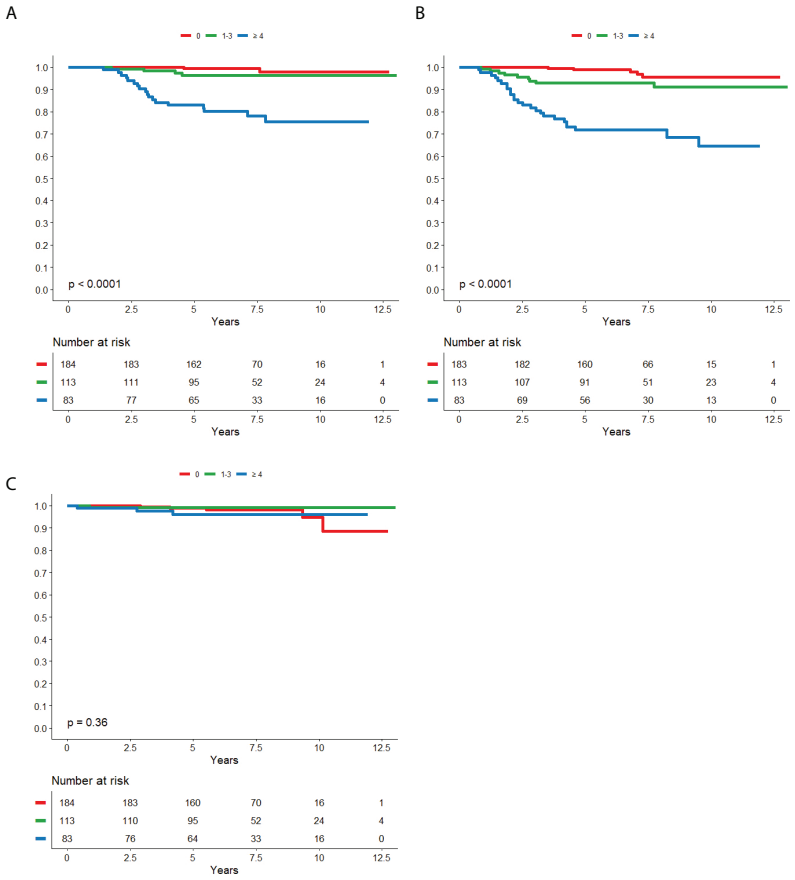


FIGURE 5
Nodal status in relation to BCSS **(A)**, DRFS **(B)** and LRFS **(C)** respectively in patients that was treated with trastuzumab.

10.3389/fonc.2022.861324

TABLE 2 Subgroup dependent 5-year survival rates.

	5-year BCSS % (95% CI)		5-year DRFS % (95% CI)		5-year LRFS % (95% CI)	
	No trastuzumab	Trastuzumab	No traztuzumab	Traztuzumab	No traztuzumab	Traztuzumab
ER-positive	84.4 (76.0-92.8)	97.6 (95.6-99.6)	84.3 (76.7-91.9)	95.5 (93.0-98.0)	89.0 (82.3-95.7)	99.2 (98.2-100)
ER negative	74.1 (62.3-85.9)	84.5 (78.8-90.2)	63.4 (49.9-76.9)	80.2 (74.1-86.3)	77.1 (64.8-89.4)	94.7 (91.2-98.2)
Number of metastatic lymph nodes = 0	87.7 (80.6-94.8)	98.3 (96.5-100)	83.9 (76.3-91.5)	98.9 (97.3-100)	89.4 (82.9-95.9)	98.2 (96.2-100)
Number of metastatic lymph nodes = 1-3	58.7 (35.8-81.6)	96.4 (92.9-99.9)	60.0 (38.8-81.2)	92.9 (88.2-97.6)	77.3 (59.3-95.3)	99.1 (97.3-100)
Number of metastatic lymph nodes = >4	62.4 (35.0-89.8))	75.5 (65.1-85.9)	64.9 (39.2-90.6)	71.9 (62.1-81.7)/	82.4 (64.4-100)	96.0 (91.5-100)

TABLE 3 Multivariable cox regression analysis.

Variable in equation	Univariate HR	Univariate p-value	Multivariable HR	Multivariable p-value
BREAST CANCER SPECIFIC MORTALITY RATE				
Trastuzumab	**0.42**	**<0.0005**	**0.19**	**<0.0005**
Nodal status	**2.33**	**<0.0005**	**3.32**	**<0.0005**
ER-status	**0.34**	**<0.0005**	**0.39**	**0.003**
Tumor size	**2.82**	**<0.0005**	1.42	NS
NHG-grade	1.75	0.065	1.85	0.097
Age (continuous)	**1.02**	**0.022**	1.02	NS
DISTANT RECURRENCE RATE				
Trastuzumab	**0.56**	**0.008**	**0.31**	**<0.0005**
Nodal status	**2.33**	**<0.0005**	**2.63**	**<0.0005**
ER-status	**0.32**	**<0.0005**	**0.31**	**<0.0005**
Tumor size	**2.74**	**<0.0005**	1.44	0.091
NHG-grade	1.43	NS	1.32	NS
Age (continuous)	1.01	NS	1.01	NS
LOCOREGIONAL RECURRENCE RATE				
Trastuzumab	**0.19**	**<0.0005**	**0.13**	**<0.0005**
Nodal status	1.06	NS	**1.65**	**0.048**
ER-status	**0.45**	**0.021**	**0.43**	**0.034**
Tumor size	1.31	NS	1.05	NS
NHG-grade	1.04	NS	1.61	NS
Age (continuous)	1.02	NS	0.98	NS

Bold is used when numbers are statistically significant (p<0.05). NS, Not significant.

In conclusion, adjuvant treatment with trastuzumab is a tolerable and effective treatment when prescribed according to current clinical guidelines and praxis and confer a favorable prognosis for patients with HER2-postive breast cancer. However, using prognostic variables identified in this study patients with unfavorable prognosis can be identified, e.g., patients with ER negative disease and four or more nodal metastases treated with trastuzumab only had a 65.6% (95% CI; 54.8-76.4) 5-year BCSS, a survival rate much lower than for the overall study-population emphasizing the need for increased efforts to improve the treatment for these patients.

Conclusion

This study provides real world evidence supporting the data from early clinical trials regarding the excellent long-term outcome of adjuvant trastuzumab in patients with early HER2-positive breast cancer. ER status, nodal status and trastuzumab treatment were the only individual factors significantly associated with the long-term prognosis in this cohort. Among the minority of patients who experienced distant recurrence brain metastases were common and brain

TABLE 4 Metastatic site for patients with distant recurrences.

		No trastuzumab	Trastuzumab	
Metastatic site		**N (%)**	**N (%)**	**p-value**
Lymph node	No	20 (61)	41 (76)	NS
	Yes	13 (39)	13 (24)	
Cutaneous	No	31 (94)	51 (94)	NS
	Yes	2 (6)	3 (6)	
Bone	No	16 (49)	35 (65)	NS
	Yes	17 (52)	19 (35)	
Lung	No	17 (52)	29 (54)	NS
	Yes	16 (49)	25 (46)	
Liver	No	19 (58)	30 (56)	NS
	Yes	14 (42)	24 (44)	
Brain	No	25 (76)	33 (61)	NS
	Yes	8 (24)	21 (39)	
Brain metastasis as first distant recurrence	No	31 (94)	40 (74)	**0.023**
	Yes	2 (6)	14 (26)	
Other metastatic site	No	23 (70)	45 (83)	NS
	Yes	10 (30)	9 (17)	

Bold is used when numbers are statistically significant ($p<0.05$). NS, Not significant.

metastasis diagnosed as first metastatic relapse was more common in patients treated with trastuzumab.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Regional Ethical Review Board in Linköping. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SE, KE, A-LH, NE, and OS designed the study. SE, KE, and MA collected and organized the data. SE and OS analyzed the data. All authors interpreted and discussed the data, were major contributors to the manuscript, and read and approved the final manuscript.

Funding

This study was supported by grants from the Swedish Cancer Society (grant number 17-0479), Medical research council of South-Eastern Sweden (FORSS, grant number RÖ-966444), ALF Grants Region Östergötland (Grant number LIO-795201) and Stiftelsen Onkologiska Klinikernas Forskningsfond i Linköping (2018–12–19).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. *Breast cancer* . Available at: https://www.who.int/news-room/fact-sheets/detail/breast-cancer (Accessed May 27, 2021).

2. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. *Oncologist* (2009) 14:320–68. doi: 10.1634/theoncologist.2008-0230

3. Farshid G, Walters D. Molecular subtypes of screen-detected breast cancer. *Breast Cancer Res Treat* (2018) 172:191–9. doi: 10.1007/s10549-018-4899-3

4. Rydén L, Haglund M, Bendahl P-O, Hatschek T, Kolaric A, Kovács A, et al. Reproducibility of human epidermal growth factor receptor 2 analysis in primary breast cancer: a national survey performed at pathology departments in Sweden. *Acta Oncol* (2009) 48:860–6. doi: 10.1080/02841860902862511

5. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer* (2005) 5:341–54. doi: 10.1038/nrc1609

6. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE, Davidson NE, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* (2005) 353:1673–84. doi: 10.1056/NEJMoa052122

7. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* (2001) 344:783–92. doi: 10.1056/nejm200103153441101

8. Vici P, Pizzuti L, Natoli C, Moscetti L, Mentuccia L, Vaccaro A, et al. Outcomes of HER2-positive early breast cancer patients in the pre-trastuzumab and trastuzumab eras: a real-world multicenter observational analysis. the RETROHER study. *Breast Cancer Res Treat* (2014), 147:599–607. doi: 10.1007/s10549-014-3133-1

9. Seferina SC, Lobbezoo DJA, Boer M, Dercksen MW, Berkmortel F, Kampen RJW, et al. Real-life use and effectiveness of adjuvant trastuzumab in early breast cancer patients: A study of the southeast Netherlands breast cancer consortium. *Oncologist* (2015) 20:856–63. doi: 10.1634/theoncologist.2015-0006

10. Tolaney SM, Barry WT, Dang CT, Yardley DA, Moy B, Marcom PK, et al. Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer. *N Engl J Med* (2015) 372:134–41. doi: 10.1056/NEJMoa1406281

11. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): A randomised multicentre, open-label, phase 2 trial. *Lancet Oncol* (2012) 13:25–32. doi: 10.1016/S1470-2045(11)70336-9

12. Baselga J, Cortés J, Kim S-B, Im S-A, Hegg R, Im Y-H, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *n Engl J Med* (2012) 366:109–28. doi: 10.1056/NEJMoa1113216

13. von Minckwitz G, Procter M, de Azambuja E, Zardavas D, Benyunes M, Viale G, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. *N Engl J Med* (2017) 377:122–31. doi: 10.1056/NEJMoa1703643

14. von Minckwitz G, Huang C-S, Mano MS, Loibl S, Mamounas EP, Untch M, et al. Trastuzumab emtansine for residual invasive HER2-positive breast cancer. *N Engl J Med* (2019) 380:617–28. doi: 10.1056/NEJMoa1814017

15. Martin M, Holmes FA, Ejlertsen B, Delaloge S, Moy B, Iwata H, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* (2017) 18:1688–700. doi: 10.1016/S1470-2045(17)30717-9

16. Perez EA, Romond EH, Suman VJ, Jeong J, Davidson NE, Geyer CE, et al. Updated results of the combined analysis of NCCTG N9831 and NSABP b-31 adjuvant chemotherapy with/without trastuzumab in patients with HER2-positive breast cancer. *J Clin Oncol* (2007) 25:512. doi: 10.1200/jco.2007.25.18_suppl.512

17. Earl HM, Hiller L, Vallier A, Loi S, Mcadam K, Hughes-davies L, et al. 6 versus 12 months of adjuvant trastuzumab for HER2-positive early breast cancer (PERSEPHONE): 4-year disease-free survival results of a randomised phase 3 non-inferiority trial. *The Lancet* (2019) 393:2599–612. doi: 10.1016/S0140-6736(19)30650-6

18. De Munck L, Schaapveld M, Siesling S, Wesseling J, Voogd AC, Tjan-Heijnen VCG, et al. Implementation of trastuzumab in conjunction with adjuvant chemotherapy in the treatment of non-metastatic breast cancer in the Netherlands. *Breast Cancer Res Treat* (2011) 129:229–33. doi: 10.1007/s10549-011-1451-0

19. Whitfield R, Kollias J, De Silva P, Zorbas H, Maddern G. Use of trastuzumab in Australia and new Zealand: Results from the national breast cancer audit. *ANZ J Surg* (2012) 82:234–9. doi: 10.1111/j.1445-2197.2011.05998.x

20. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thürlimann B, Senn HJ. Progress and promise: Highlights of the international expert consensus on the primary therapy of early breast cancer 2007. *Ann Oncol* (2007) 18:1133–44. doi: 10.1093/annonc/mdm271

21. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: Highlights of the st gallen international expert consensus on the primary therapy of early breast cancer 2013. *Ann Oncol* (2013) 24:2206–23. doi: 10.1093/annonc/mdt303

22. Sun J, Chia S. Adjuvant chemotherapy and HER-2-directed therapy for early-stage breast cancer in the elderly. *Br J Cancer* (2017) 116:4–9. doi: 10.1038/bjc.2016.360

23. Moja L, Brambilla C, Compagnoni A, Pistotti V. Trastuzumab containing regimens for early breast cancer. *Cochrane Database Syst Rev* (2006), 3371–81. doi: 10.1002/14651858.CD006243

24. Ellegård S, Veenstra C, Pérez-Tenorio G, Fagerström V, Gårsjö JON, Gert K, et al. ERBB2 and PTPN2 gene copy numbers as prognostic factors in HER2-positive metastatic breast cancer treated with trastuzumab. *Oncol Lett* (2019) 17. doi: 10.3892/ol.2019.9998

25. Ignatov A, Eggemann H, Burger E, Ignatov T. Patterns of breast cancer relapse in accordance to biological subtype. *J Cancer Res Clin Oncol* (2018) 144:1347–55. doi: 10.1007/s00432-018-2644-2

26. Cao L, Cai G, Xu F, Yang Z-Z, Yu X-L, Ma J-L, et al. Trastuzumab improves locoregional control in HER2-positive breast cancer patients following adjuvant radiotherapy. *Med (Baltimore)* (2016) 95:e4230. doi: 10.1097/MD.0000000000004230

27. McGuire A, Lowery AJ, Kell MR, Kerin MJ, Sweeney KJ. Locoregional recurrence following breast cancer surgery in the trastuzumab era: A systematic review by subtype. *Ann Surg Oncol* (2017) 24:3124–32. doi: 10.1245/s10434-017-6021-1

28. Farolfi A, Melegari E, Aquilina M, Scarpi E, Ibrahim T, Maltoni R, et al. Trastuzumab-induced cardiotoxicity in early breast cancer patients: a retrospective study of possible risk and protective factors. *Heart* (2013) 303151:1–6. doi: 10.1136/heartjnl-2012-303151

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 05 August 2022
DOI 10.3389/fonc.2022.800813

OPEN ACCESS

EDITED BY
Xiaosong Chen,
Shanghai Jiao Tong University, China

REVIEWED BY
Serdar Ozbas,
Private Practitioner, Ankara, Turkey
Can Zhou,
The First Affiliated Hospital of Xi'an Jiaotong University, China

*CORRESPONDENCE
Pin Zhang
zppumc@163.com

SPECIALTY SECTION
This article was submitted to Breast Cancer, a section of the journal Frontiers in Oncology

RECEIVED 24 October 2021
ACCEPTED 07 July 2022
PUBLISHED 05 August 2022

CITATION
Xiao M and Zhang P (2022) Conditional cause-specific survival after chemotherapy and local treatment for primary stage IV breast cancer: A population-based study. *Front. Oncol.* 12:800813. doi: 10.3389/fonc.2022.800813

Conditional cause-specific survival after chemotherapy and local treatment for primary stage IV breast cancer: A population-based study

Min Xiao[1,2] and Pin Zhang[1*]

[1]Department of Medical Oncology, National Cancer Center/ Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2]Department of Intensive Care Unit, Fujian Medical University Cancer Hospital, Fujian Cancer Hospital, Fuzhou, China

Background: Conditional survival (CS) represents the probability of surviving for additional years after the patient has survived for several years, dynamically describing the survival rate of the patient with the varying time of survival. The aim of this study was to evaluate the conditional cause-specific survival (CCSS) after chemotherapy and local treatment for metastatic breast cancer, and to identify the prognostic factors affecting the CCSS.

Methods: Patients diagnosed with primary stage IV breast cancer in the Surveillance, Epidemiology, and End Results (SEER) database from 2010 to 2015 were included. CS is defined as the probability of additional survival for *y* years after the patient had survived *x* years with the calculation formula CCSS ($x \mid y$) = CSS ($x + y$)/CSS (x), where CSS(x) indicates the patient's cause-specific survival rate at the time of *x* years. Cox proportional hazard models were used to evaluate predictors of CCSS.

Results: A total of 3,194 patients were included. The 5-year CSS was 39%, whereas the 5-year CCSS increased to 46%, 57%, 71%, and 85% after the diagnosis of 1, 2, 3, and 4 years. For patients with adverse clinical pathological features, CCSS had more pronounced increase with survival time and is more different from the CSS at diagnosis. No matter at the time of diagnosis or 1 year or 3 years after diagnosis, HER2 status, local treatment, and multisite metastasis were independent prognostic factors that affect the long-term survival of patients (all $P < 0.05$).

Conclusion: The 5-year CCSS of patients with stage IV breast cancer was extended as the survival years increased. HER2 status, multisite metastasis, and local treatment were independent prognostic factors even 3 years after diagnosis.

KEYWORDS

Breast cancer, conditional survival, prognosis, therapy, SEER program

Introduction

Breast cancer is the most common malignant tumor and is also the most frequent cause of death from cancer in women (1). Globally, over two million patients are diagnosed annually and over 600,000 die from the disease (2). About 5–10% patients at diagnosis have metastases (3). Despite the use of various traditional systemic treatments such as chemotherapy, endocrine treatment, and targeted therapy, the overall survival (OS) of patients with metastatic breast cancer is still not so satisfactory. Recently, some studies showed that chemotherapy combined with local treatment including primary tumor site surgery or radiotherapy or both may improve the prognosis of advanced breast cancer (4–6).

Most survival rates reported in the literature are static, being calculated from the day of diagnosis or surgery (7–10). This statistical method could only reflect the continuous hazard ratio and survival rate of patients from the beginning of follow-up. Since the survival rate, death risk, and risk ratio of patients will change with the extension of survival time, this approach has limitations, especially for long-term survival. Conditional survival (CS) represents the probability of surviving a certain number of years after diagnosis treatment based on the time the patient has already survived (11). Compared with the traditional survival evaluation, CS can provide more accurate information for long-term prognosis and is more meaningful in the process of follow-up. Thus, it has been used in many kinds of malignant tumors, such as gastrointestinal, liver, pancreatic, and urinary tract cancer (12–15).

As we know, there is no report on the conditional cause-specific survival (CCSS) in patients with metastatic breast cancer who underwent chemotherapy combined with local treatment. Our study aims to evaluate the dynamic cause-specific survival (CSS) of this type of population and prognostic factors that change with time.

Material and methods

Data source and study population

A retrospective cohort study was performed with data extracted from the Surveillance, Epidemiology, and End Results (SEER) database. The SEER program collects and publishes cancer incidence and survival data from population-based cancer registries covering approximately 34.6% of the U.S. population. The inclusion criteria were as follows: (1) patients histologically diagnosed as stage IV breast cancer according to the 7th edition of the American Joint Committee on Cancer (AJCC) TNM classification between 2010 and 2015, and (2) chemotherapy combined with local surgery and/or radiotherapy were performed. The exclusion criteria were as follows: (1) male, (2) more than 84 years old, (3) T0 local disease, (4) not the only primary tumor, (5) lack of information on distant metastatic lesion, (6) incomplete follow-up data, (7) 0 survival month, and (8) incomplete baseline data. A total of 3,194 cases entered the final analysis (Figure 1). All data obtained included age at diagnosis, race, tumor grade, human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), progesterone receptor (PR) status, AJCC TNM stage, metastatic organ, treatment, and follow-up information. The SEER program identifies only the first course of therapy, defined as those recorded in the treatment plan at diagnosis and administered before disease progression or recurrence. Surgery in the current research refers to the primary lesion (16). SEER data are publicly available, and a signed Research Data Agreement form was required to access the database. No institutional review board approval was required for this study.

Statistical analysis

CSS was measured by the time between diagnosis and breast cancer–related death. Survival curves were constructed according to the Kaplan–Meier (K–M) method, and difference curves were analyzed using the log-rank test.

CS is defined as the possibility of surviving an additional number of y years given that a patient has already survived for x years. The CCSS formula is CCSS $(x \mid y)$ = CSS $(x + y)$/CSS (x), where CSS (x) represents the cause-specific survival at x year calculated by the K–M curve. For example, CS for surviving another year among patients who had already survived 4 years, CCSS (1|4), was calculated by dividing the 5-year K–M survival estimate CSS (5) by the 4-year survival estimate CSS (4).

Multivariate Cox proportional-hazards regression was performed to evaluate the hazard of CSS at the time of diagnosis and CCSS for multiple survival periods (1 and 3 years after diagnosis). For instance, to compute the CCSS at 1 year after diagnosis, 1-year survivors were selected. After subtraction of 12 months from their survival time, a multivariate analysis was performed. Only the variables that were prognostic with P-value less than 0.1 in the analysis of the previous period were selected and incorporated in the next period's multivariate analysis sequentially. Differences were statistically significant when $P < 0.05$. Statistical analyses were performed using the SPSS 22.0 statistical software.

Results

Clinicopathological characteristics

This study included 3,194 breast cancer patients who met the criteria in the SEER database (Table 1). Most of the patients were

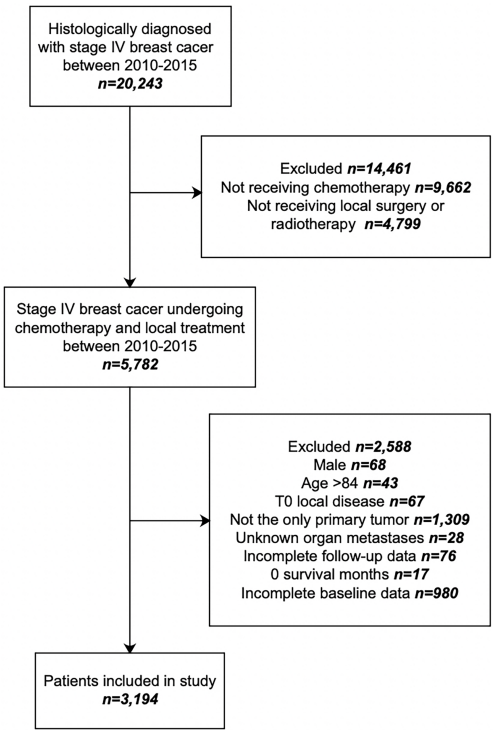


FIGURE 1
Study flow chart.

younger than 65 years old (78.4%). Majority of the patients were White (71.1%) followed by Black (19.6%). The most frequent histopathological grade was poorly differentiated (60.7%). Bone metastasis (35.6%) was the most common site of metastasis, followed by lung metastasis (10.9%) and brain metastasis (1.6%). In terms of treatment, more than 70% of the patients received chemotherapy combined with surgery, of which 1,222 (38.3%) patients received chemotherapy combined with surgery and radiotherapy.

Comparison of CSS and CCSS

With a median follow-up time of 26 (1–83) months until 2018, the CSS of patients at 1, 3, and 5 years was 84%, 55%, and 39%, respectively. The CCSS related to the number of years are shown in Table 2, and the K–M survival curves are shown in Figure 2. The 5-year CCSS increased from 39% directly after diagnosis to 46% (Δ 7%), 57% (Δ 18%), 71% (Δ 32%), and 85% (Δ 46%), given 1, 2, 3, and 4 years already survived, respectively. The longer the patients have survived, the more likely they are to survive for additional years. This growth leveled off after many years.

Factors associated with CSS and CCSS rates

Multivariate analysis showed that age, race, AJCC T, N categories, tumor grade, HER2, ER, PR status, metastatic organ, and treatment were independent prognostic factors for CSS of metastatic breast cancer (all $P < 0.05$, Table 3) at diagnosis. For patients surviving for 1 year after diagnosis, multivariate analysis identified that T4, poorly grade, HER2 positive, brain, and multisite metastasis were independent risk factors (all $P < 0.05$), whereas ER positive, PR positive, and surgery or surgery combined with radiotherapy were independent protective factors (all $P < 0.05$). After 3 years of diagnosis, only HER2 positive (HR = 0.598, $P < 0.001$), multisite metastasis (HR = 1.621, $P = 0.002$), and surgery (HR = 0.507, $P < 0.001$) or surgery combined with radiotherapy (HR = 0.521, $P < 0.001$) were still independent prognostic factors.

Subgroup analysis of CSS and CCSS rates

All patients were divided into subgroups according to the independent prognostic factors to evaluate their effects on CSS

TABLE 1 Baseline and treatment characteristics.

	No. of patients (%, n=3194)
Age	
<65 years	2503 (78.4)
≥65 years	691 (21.6)
Race	
White	2270 (71.1)
Black	625 (19.6)
Other	299 (9.3)
AJCC 7th, T Stage	
T1	326 (10.2)
T2	1135 (35.5)
T3	590 (18.5)
T4	1143 (35.8)
AJCC 7th, N Stage	
N0	487 (15.2)
N1	1369 (42.9)
N2	572 (17.9)
N3	766 (24.0)
Grade	
Well	145 (4.5)
Moderate	1078 (33.8)
Poorly	1939 (60.7)
Anaplastic	32 (1.0)
HER2 Status	
Negative	2079 (65.1)
Positive	1115 (34.9)
Breast type	
HR+/HER2+	690 (21.6)
HR+/HER2-	1415 (44.3)
HR-/HER2+	425 (13.3)
HR-/HER2-	664 (20.8)
ER Status	
Negative	1148 (35.9)
Positive	2046 (64.1)
PR Status	
Negative	1594 (49.9)
Positive	1600 (50.1)
Metastatic organ	
Bone	1136 (35.6)
Brain	52 (1.6)
Liver	281 (8.8)
Lung	349 (10.9)
Multisites	843 (26.4)
Other	533 (16.7)
Treatment	
Chemo+radio	792 (24.8)
Chemo+surgery	1180 (36.9)
Chemo+surgery+radio	1222 (38.3)

AJCC, the American Joint Committee on Cancer; ER, Estrogen receptor; PR, Progesterone receptor; HER2, Human epidermal growth factor receptor 2; chemo, chemotherapy; radio, radiotherapy.

and CCSS. Figure 3 shows that the 5-year CSS of HER2 positive patients was significantly better than that of HER2 negative patients (52% vs. 31%, $P < 0.001$, Figure 3A). In the subgroup analysis according to the metastatic site, the 5-year CSS of patients with brain metastasis (9%) was significantly worse than that of patients with bone (47%), liver (44%), and other sites (49%) ($P < 0.001$, Figure 3C). The 5-year CCSS of patients with bone, liver, and other site metastasis who have survived 4 years after diagnosis increased to 84%, 88%, and 92%, respectively, whereas the 5-year CCSS of patients with brain metastasis was only 53% (Figure 3D), which indicated that patients with brain metastasis disease at diagnosis still experience disease progression despite surviving 4 years. The subgroup analysis according to the treatment methods showed that the prognosis of patients who underwent surgery with or without radiotherapy was significantly better than that of patients who only underwent radiotherapy (5-year CSS of radiotherapy = 16%, 5-year CSS of surgery = 43%, 5-year CSS of surgery combined with radiotherapy = 47%, $P < 0.001$) (Figure 3E).

In each subgroup, CSS showed a downward trend, whereas the 5-year CCSS gradually went up with the passage of survival time. In each subgroup, the 5-year CCSS was better than the 5-year CSS. Moreover, the difference between the CSS and the 5-year CCSS was more significant in patients with poor clinicopathological factors at baseline. In contrast, this difference was relatively small in patients with good initial clinicopathological factors at baseline. For example, the 5-year CSS (baseline) of patients with HER2 positive was 52%, whereas the 5-year CCSS of 4 years after diagnosis was 87% (Δ 35%). For patients with HER2 negative, the 5-year CCSS was 31% at diagnosis and the 5-year CCSS increased to 79% (Δ 47%) at 4 years after diagnosis (Figure 3B).

Discussion

To the best of our knowledge, this is the first study evaluating the CS of metastatic breast cancer. More than 3,000 cases of metastatic breast cancer with chemotherapy and local treatment in the SEER database were included in this study. It has been found that although the population has poor prognosis with the 5-year CSS only 39%, the 5-year CCSS increased with the extension of survival time. For patients who have survived for 4 years, the 5-year CCSS is as high as 85%, especially for patients with adverse prognostic factors. Furthermore, HER2 status, multisite metastasis, and treatment were independent prognostic factors at the time of diagnosis, and their prognostic effects persisted until 3 years after diagnosis.

CS represents the possibility that a patient can survive a certain number of years after diagnosis or treatment based on the time the patient has already survived. It can dynamically

TABLE 2 Conditional cause-specific survival estimates.

Total years of survival after diagnosis	Probability of survival (%)					
	Years already survived by patient					
	0	1	2	3	4	5
1	84					
2	68	81				
3	55	65	81			
4	46	55	68	84		
5	39	46	57	71	85	
6	34	40	50	62	74	87

The probability of survival after diagnosis is shown in relation to the number of years already survived. For example, if a patient has survived 2 years after diagnosis, the probability of achieving 3-year survival after diagnosis is 81 percent and of achieving 5-year survival after diagnosis is 57 percent.

describe the survival rate of patients as time progresses (17). In this study, the 5-year CCSS of metastatic breast cancer increased year by year with the increase in survival years. For example, the probability of survival at 5 years after diagnosis went from 39% at 0 years to 71% at 3 years. In the subgroup analysis, this increasing trend was more obvious in patients with poor clinicopathological factors. The prognosis of surviving patients with high risk factors will be close to those of patients with some low risk factors as time goes on, which can reduce anxiety and improve the quality of life, especially for high-risk patients. For instance, the 5-year CSS of HER2 positive and HER2 negative patients at diagnosis were 52% and 31% (difference of 21%), and the 5-year CCSS of 4 years after diagnosis were 87% and 79% (difference of 8%). This may be due to the rapid death of high-risk patients after diagnosis. In the traditional survival analysis, patients with risk factors tend to have worse CSS. Therefore, cumulative survival analysis is somewhat crude for accurately assessing long-term survival, especially for patients who have survived for a period of time (18).

Currently, the treatment of metastatic breast cancer is still controversial. Three prospective randomized trials (the MF07-01, an Indian study, and the recent ECOG-ACRIN 2108 Trial) have shown different effects of the local treatments (19–22). The 3-year OS was similar between systemic therapy and primary surgery arms in all of them. However, the MF07-01 trial showed a better 5-year and 10-year OS in patients who underwent local treatment followed by system therapy compared with those who received only system therapy. There are some pitfalls in the above studies. The imbalance of baseline variables, insufficiency of system therapy, and high tumor burden are thought to lead to bias. Thus, it is very difficult to conduct a perfect random trial about the local therapy for primary stage IV breast cancer in a real world. Of particular note is oligometastatic disease, which can achieve long-term remission and even be cured through different treatment strategies (23). The BOMET MF14-01 study showed that bone metastasis only (especially oligometastatic bone and solitary bone) may take more advantage from local surgery (24). The subgroup analysis of the MF07-01 trial also favored the fact that the solitary bone metastasis was the proper candidate for local therapy (19, 20).

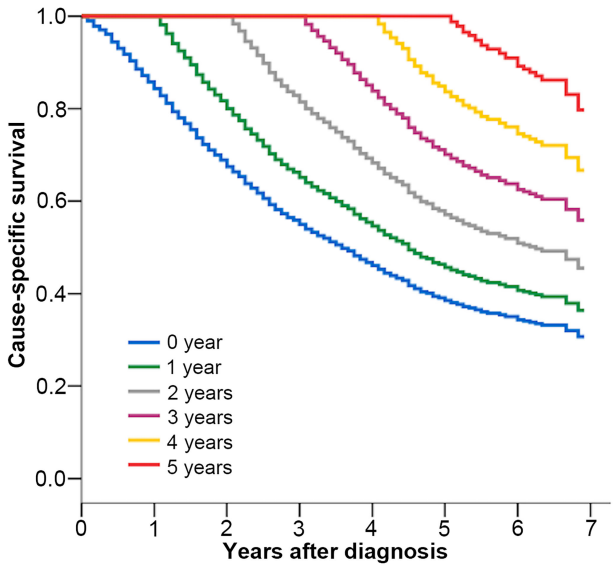


FIGURE 2
Kaplan–Meier estimates of cancer-specific survival after diagnosis (0 year) and conditional cancer-specific survival, according to years already survived after diagnosis (1–5 years).

TABLE 3 Multivariable Cox proportional hazards analysis of risk factors associated with cause-specific survival.

	At diagnosis (n=3194)		1 year after diagnosis (n=2585)		3 years after diagnosis (n=1071)	
	Hazard ratio	P	Hazard ratio	P	Hazard ratio	P
Age		0.001		0.542		NA
<65 years	Reference		Reference			
≥65 years	1.223 (1.082-1.381)	0.001	1.050 (0.898-1.228)	0.542		
Race		0.041		0.253		NA
White	Reference		Reference			
Black	1.102 (0.973-1.248)	0.127	1.029 (0.880-1.203)	0.723		
Other	0.846 (0.702-1.021)	0.082	0.839 (0.671-1.049)	0.123		
AJCC 7th, T Stage		0.002		0.011		0.132
T1	Reference		Reference		Reference	
T2	1.136 (0.934-1.382)	0.202	1.219 (0.960-1.548)	0.105	1.475 (0.926-2.351)	0.102
T3	1.091 (0.882-1.349)	0.442	1.146 (0.884-1.486)	0.303	1.668 (1.014-2.742)	0.044
T4	1.342 (1.104-1.632)	0.003	1.415 (1.112-1.800)	0.005	1.724 (1.077-2.761)	0.023
AJCC 7th, N Stage		0.085		0.147		NA
N0	Reference		Reference			
N1	0.937 (0.807-1.089)	0.396	0.992 (0.823-1.196)	0.934		
N2	0.995 (0.833-1.1887)	0.952	1.038 (0.835-1.289)	0.739		
N3	1.113 (0.942-1.314)	0.209	1.189 (0.967-1.461)	0.100		
Grade		<0.001		<0.001		0.610
Well	Reference		Reference		Reference	
Moderate	1.462 (1.085-1.970)	0.013	1.322 (0.948-1.844)	0.100	1.470 (0.838-2.577)	0.179
Poorly	1.952 (1.453-2.622)	<0.001	1.711 (1.230-2.381)	0.001	1.431 (0.811-2.523)	0.216
Anaplastic	1.847 (1.100-3.103)	0.020	1.517 (0.804-2.862)	0.198	1.334 (0.376-4.730)	0.655
HER2 Status		<0.001		<0.001		<0.001
Negative	Reference		Reference		Reference	
Positive	0.386 (0.342-0.435)	<0.001	0.380 (0.330-0.439)	<0.001	0.598 (0.457-0.783)	<0.001
ER Status		<0.001		0.001		0.566
Negative	Reference		Reference		Reference	
Positive	0.674 (0.585-0.777)	<0.001	0.739 (0.621-0.880)	0.001	0.893 (0.608-1.312)	0.566
PR Status		<0.001		<0.001		0.271
Negative	Reference		Reference		Reference	
Positive	0.525 (0.456-0.606)	<0.001	0.497 (0.420-0.588)	<0.001	0.829 (0.594-1.157)	0.271
Metastatic organ		<0.001		<0.001		<0.001
Bone	Reference		Reference		Reference	
Brain	3.295 (2.392-4.538)	<0.001	2.367 (1.445-3.877)	0.001	1.470 (0.360-6.002)	0.591
Liver	1.332 (1.083-1.639)	0.007	1.261 (0.986-1.611)	0.064	0.783 (0.459-1.336)	0.369
Lung	1.065 (0.884-1.282)	0.508	1.128 (0.908-1.401)	0.277	0.936 (0.598-1.464)	0.771
Multisites	1.877 (1.639-2.149)	<0.001	1.645 (1.394-1.942)	<0.001	1.621 (1.190-2.208)	0.002
Other	0.846 (0.712-1.005)	0.057	0.805 (0.660-0.983)	0.033	0.677 (0.455-0.978)	0.038
Treatment		<0.001		<0.001		<0.001
Chemo+radio	Reference		Reference		Reference	
Chemo+surgery	0.503 (0.439-0.576)	<0.001	0.559 (0.468-0.667)	<0.001	0.507 (0.357-0.720)	<0.001
Chemo+surgery+radio	0.392 (0.341-0.450)	<0.001	0.494 (0.415-0.588)	<0.001	0.521 (0.372-0.729)	<0.001

AJCC, the American Joint Committee on Cancer; ER, Estrogen receptor; PR, Progesterone receptor; HER2, Human epidermal growth factor receptor 2; chemo, chemotherapy; radio, radiotherapy, NA, Not Available.

So, it is very important to recognize those patients who can really benefit from the local treatment, and CS may be a better predictor of continued survival for people with long-term survival benefits.

Our study found that surgery combined with radiotherapy as the local treatment was more efficient compared with surgery or radiotherapy alone. The 5-year CSS increased from 16% to 43% (Δ 27%, $P < 0.001$), and it further increased to 47% (Δ 31%, $P <$

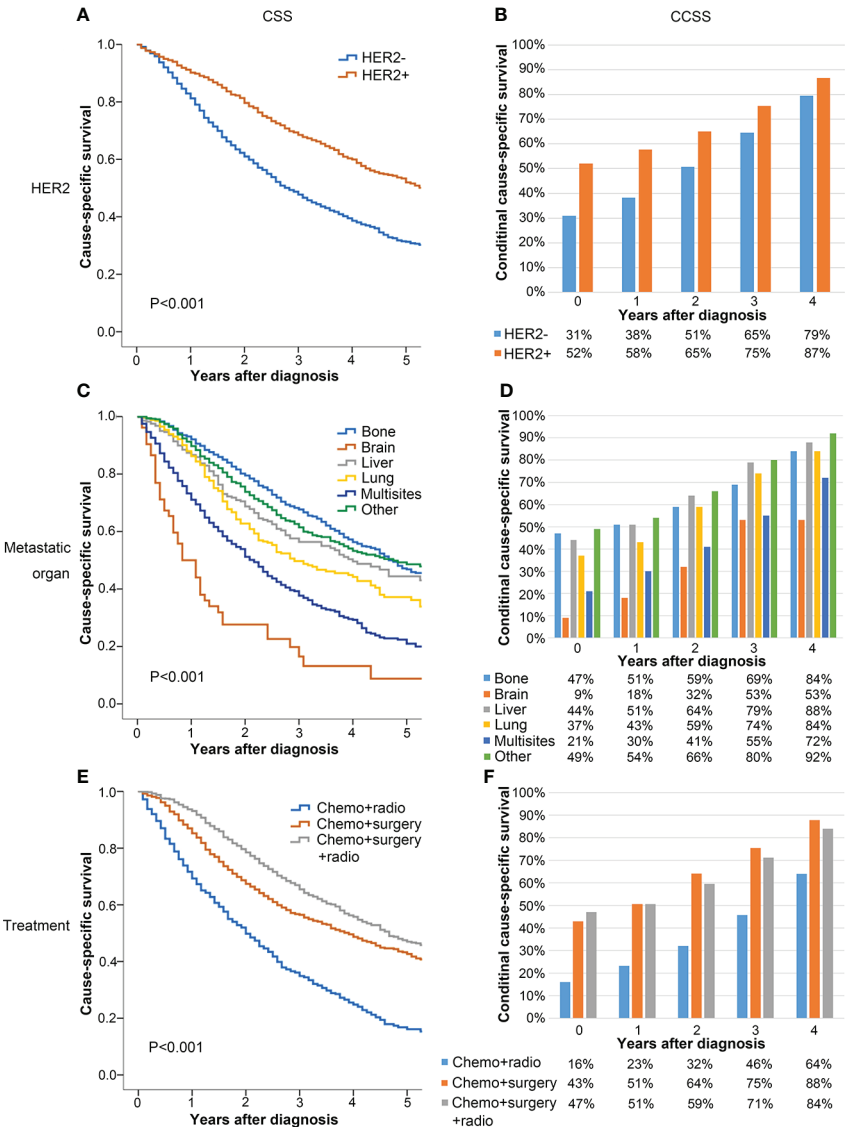


FIGURE 3
Comparison between CSS **(A, C, E)** and CCSS **(B, D, F)** according to HER2 **(A, B)**, metastatic organ **(C, D)**, and treatment **(E, F)**.

0.001) in patients accepting surgery combined with radiotherapy. Lian et al. collected data from SEER between 2004 and 2012 and also drew a similar conclusion (25). The 3-year CSS were 35.9%, 57.1%, and 63.9% in patients who underwent radiotherapy alone, surgery alone, and surgery combined with radiotherapy. Our study suggests that the local treatment can affect the prognosis for a long time. Due to the inability to obtain metastatic tumor load from the SEER database, we were unable to perform further analysis. In addition, the patients who have a good initial prognosis (low tumor burden, metastatic clearance with system therapy, fewer complications, and younger age), as evaluated subjectively by the physician, were more likely to opt for surgery, leading to bias.

Previous studies have shown that age, HER2 status, hormone receptor state, metastatic sites, and treatment were important factors affecting the prognosis of metastatic breast cancer (8, 26, 27), but there is no study on the prognostic factors for patients with metastatic breast cancer who have survived for several years. In this study, we found that age, race, grade, HER2, ER, PR status, metastatic organ, and local treatment were independent prognostic factors for CSS, which is consistent with the previous studies (8). However, at 1 year and 3 years after diagnosis, only

HER2 status, metastatic organ, and local treatment continued to affect the prognosis. With HER2-targeted therapy, the prognosis of HER2 positive metastatic breast cancer has been improved (28). Our study also showed that the prognosis of HER2 positive patients was significantly better than HER2 negative, and this factor continued to influence the long-term survival during follow-up, which verified that the targeted treatment of HER2 had long-term survival benefits to the metastatic breast cancer. The common metastatic sites were bone, lung, brain, and liver, of which the prognosis of brain and multisite metastasis was the worst (29, 30). In this study, the 5-year CSS of brain metastasis and multisite metastasis patients were only 9% and 21%, and the latter remained an independent risk factor for prognosis as years of survival increased. Obviously, the more the metastases, the higher the tumor burden. As a result, these patients have a poor prognosis.

This study has some limitations. First of all, this is a retrospective study and inevitably leads to selection bias. Second, information such as the treatment of targeted and endocrine, the sequence of chemotherapy and surgery, and the therapeutic effect evaluation cannot be obtained from the SEER database. However, this is the first study to assess the 5-year CCSS of metastatic breast cancer and to analyze the potential factors that continue to influence the prognosis. The results of this study can be used as an important basis for improving treatment options as well as the prognosis of patients with metastatic breast cancer in the future.

Conclusions

CCSS of metastatic breast cancer was dynamic and increases with each additional year survived. Compared with CSS, CCSS provided a more individualized prognosis. Furthermore, HER2 status, multisite metastasis, and local treatment were independent prognostic factors that continued to influence the survival of metastatic breast cancer. These patients seemed to benefit more from surgery combined with radiotherapy.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

PZ and MX designed and performed the research. MX performed the statistical analyses, interpreted the data, and wrote the manuscript. Both authors critically reviewed and approved the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* (2018) 144(8):1941–53. doi: 10.1002/ijc.31937

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer J Clin* (2018) 68 (6):394–424. doi: 10.3322/caac.21492

3. Heller DR, Chiu AS, Farrell K, Killelea BK, Lannin DR. Why has breast cancer screening failed to decrease the incidence of *de novo* stage IV disease? *Cancers* (2019) 11(4):500. doi: 10.3390/cancers11040500

4. Lane WO, Thomas SM, Blitzblau RC, Plichta JK, Rosenberger LH, Fayanju OM, et al. Surgical resection of the primary tumor in women with *de novo* stage IV breast cancer. *Ann Surg* (2019) 269(3):537–44. doi: 10.1097/SLA.0000000000002621

5. Kim Y-J, Jung S-Y, Kim K. Survival benefit of radiotherapy after surgery in de novo stage IV breast cancer: A population-based propensity-score matched analysis. *Sci Rep* (2019) 9(1):8527. doi: 10.1038/s41598-019-45016-2

6. Nguyen DHA, Truong PT, Alexander C, Walter CV, Hayashi E, Christie J, et al. Can locoregional treatment of the primary tumor improve outcomes for women with stage IV breast cancer at diagnosis? *Int J Radiat Oncol BiologyPhysics* (2012) 84(1):39–45. doi: 10.1016/j.ijrobp.2011.11.046

7. Chia SK, Speers CH, D'Yachkova Y, Kang A, Malfair-Taylor S, Barnett J, et al. The impact of new chemotherapeutic and hormone agents on survival in a population-based cohort of women with metastatic breast cancer. *Cancer* (2007) 110(5):973–9. doi: 10.1002/cncr.22867

8. Kim BH, Kim S, Kim YI, Chang JH, Hwang K-T, Kim S, et al. Development of an individualized prediction calculator for the benefit of postoperative radiotherapy in patients with surgically resected *de novo* stage IV breast cancer. *Cancers* (2020) 12(8):2103. doi: 10.3390/cancers12082103

9. Short NJ, Jabbour E, Sasaki K, Patel K, O'Brien SM, Cortes JE, et al. Impact of complete molecular response on survival in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood* (2016) 128(4):504–7. doi: 10.1182/blood-2016-03-707562

10. Lin JP, Zhao YJ, He QL, Hao HK, Tian YT, Zou BB, et al. Adjuvant chemotherapy for patients with gastric neuroendocrine carcinomas or mixed

adenoneuroendocrine carcinomas. *Br J Surg* (2020) 107(9):1163–70. doi: 10.1002/bjs.11608

11. Bischof DA, Kim Y, Dodson R, Jimenez MC, Behman R, Cocieru A, et al. Conditional disease-free survival after surgical resection of gastrointestinal stromal tumors. *JAMA Surg* (2015) 150(4):299. doi: 10.1001/jamasurg.2014.2881

12. Wang P, Sun Z, Wang W, Deng J, Wang Z, Liang H, et al. Conditional survival of patients with gastric cancer who undergo curative resection: A multi-institutional analysis in China. *Cancer* (2018) 124(5):916–24. doi: 10.1002/cncr.31160

13. Shah MM, Meyer BI, Rhee K, NeMoyer RE, Lin Y, Tzeng CWD, et al. Conditional survival analysis of hepatocellular carcinoma. *J Surg Oncol* (2020) 122 (4):684–90. doi: 10.1002/jso.26049

14. Rosiello G, Palumbo C, Knipper S, Pecoraro A, Luzzago S, Deuker M, et al. Contemporary conditional cancer-specific survival after radical nephroureterectomy in patients with nonmetastatic urothelial carcinoma of upper urinary tract. *J Surg Oncol* (2020) 121(7):1154–61. doi: 10.1002/jso.25877

15. Latenstein AEJ, van Roessel S, van der Geest LGM, Bonsing BA, Dejong CHC, Groot Koerkamp B, et al. Conditional survival after resection for pancreatic cancer: A population-based study and prediction model. *Ann Surg Oncol* (2020) 27 (7):2516–24. doi: 10.1245/s10434-020-08235-w

16. Wang Z, Cheng Y, Chen S, Shao H, Chen X, Wang Z, et al. Novel prognostic nomograms for female patients with breast cancer and bone metastasis at presentation. *Ann Trans Med* (2020) 8(5):197–7. doi: 10.21037/atm.2020.01.37

17. Moriwaki Y, Kunisaki C, Kobayashi S, Harada H, Imai S, Kido Y, et al. Progressive improvement of prognosis for patients with gastric cancer (dynamic stage grouping) with increasing survival interval from initial staging: How much longer can a given survivor expect to live? *Surgery* (2003) 133(2):135–40. doi: 10.1067/msy.2003.95

18. Ai B, Wang X, Kong X, Wang Z, Fang Y, Wang J. Conditional survival of female patients with operable invasive breast cancer in US: A population-based study. *J Cancer* (2020) 11(19):5782–91. doi: 10.7150/jca.46183

19. Soran A, Ozmen V, Ozbas S, Karanlik H, Muslumanoglu M, Igci A, et al. Randomized trial comparing resection of primary tumor with no surgery in stage IV breast cancer at presentation: Protocol MF07-01. *Ann Surg Oncol* (2018) 25 (11):3141–9. doi: 10.1245/s10434-018-6494-6

20. Soran A, Ozmen V, Ozbas S, Karanlik H, Muslumanoglu M, Igci A, et al. Primary surgery with systemic therapy in patients with de Novo stage IV breast cancer: 10-year follow-up; protocol MF07-01 randomized clinical trial. *J Am Coll Surg* (2021) 233(6):742–51.e5. doi: 10.1016/j.jamcollsurg.2021.08.686

21. Badwe R, Hawaldar R, Nair N, Kaushik R, Parmar V, Siddique S, et al. Locoregional treatment versus no treatment of the primary tumour in metastatic breast cancer: an open-label randomised controlled trial. *Lancet Oncol* (2015) 16 (13):1380–8. doi: 10.1016/S1470-2045(15)00135-7

22. Khan SA, Zhao F, Goldstein LJ, Cella D, Basik M, Golshan M, et al. Early local therapy for the primary site in *de novo* stage IV breast cancer: Results of a randomized clinical trial (EA2108). *J Clin Oncol* (2022) 40(9):978–87. doi: 10.1200/JCO.21.02006

23. Barberi V, Pietragalla A, Franceschini G, Marazzi F, Paris I, Cognetti F, et al. Oligometastatic breast cancer: How to manage it? *J Pers Med* (2021) 11(6):532. doi: 10.3390/jpm11060532

24. Soran A, Dogan L, Isik A, Ozbas S, Trabulus DC, Demirci U, et al. The effect of primary surgery in patients with *de novo* stage IV breast cancer with bone metastasis only (Protocol BOMET MF 14-01): A multi-center, prospective registry study. *Ann Surg Oncol* (2021) 28(9):5048–57. doi: 10.1245/s10434-021-09621-8

25. Lian C-L, Guo L-Y, Zhang L, Wang J, Lei J, Hua L, et al. Aggressive local treatment improves survival in stage IV breast cancer with synchronous metastasis. *Front Oncol* (2020) 10:522580. doi: 10.3389/fonc.2020.522580

26. Li S, Zhao J, Zhu L, Su F, Chen K. Development and validation of a nomogram predicting the overall survival of stage IV breast cancer patients. *Cancer Med* (2017) 6(11):2586–94. doi: 10.1002/cam4.1224

27. Leone BA, Vallejo CT, Romero AO, Machiavelli MR, Pérez JE, Leone J, et al. Prognostic impact of metastatic pattern in stage IV breast cancer at initial diagnosis. *Breast Cancer Res Treat* (2016) 161(3):537–48. doi: 10.1007/s10549-016-4066-7

28. Kunte S, Abraham J, Montero AJ. Novel HER2-targeted therapies for HER2-positive metastatic breast cancer. *Cancer* (2020) 126(19):4278–88. doi: 10.1002/cncr.33102

29. Diéras V, Miles D, Verma S, Pegram M, Welslau M, Baselga J, et al. Trastuzumab emtansine versus capecitabine plus lapatinib in patients with previously treated HER-2-positive advanced breast cancer (EMILIA): A descriptive analysis of final overall survival results from a randomised, open-label, phase 3 trial. *Lancet Oncol* (2017) 18(6):732–42. doi: 10.1016/S1470-2045(17)30312-1

30. Guo Y, Arciero C, Jiang R, Behera M, Peng L, Li X. Different breast cancer subtypes show different metastatic patterns: A study from a Large public database. *Asian Pac J Cancer Prev APJCP* (2020) 21(12):3587–93. doi: 10.31557/APJCP.2020.21.12.3587

Frontiers in Oncology

Advances knowledge of carcinogenesis and tumor progression for better treatment and management

The third most-cited oncology journal, which highlights research in carcinogenesis and tumor progression, bridging the gap between basic research and applications to imrpove diagnosis, therapeutics and management strategies.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact