Impact of Neoadjuvant Chemotherapy Combined With Modified Radical Mastectomy on Immune Function, Oxidative Stress, and Prognosis in Patients With Different Molecular Subtypes of Breast Cancer

Ann. Ital. Chir., 2025 96, 11: 1530–1540
https://doi.org/10.62713/aic.4263

Danxia Cao[1], Yi Sun[1], Hongying Pan[1]

[1]Department of Thyroid and Breast Surgery, The People's Hospital of Danyang, 212300 Danyang, Jiangsu, China

AIM: The objective of this study is to explore the impact of neoadjuvant chemotherapy (NACT) combined with modified radical mastectomy on immune function, oxidative stress and prognosis in patients with different molecular subtypes of breast cancer (BC).

METHODS: A total of 150 patients diagnosed with BC who received NACT and modified radical mastectomy from January 2020 to January 2024 were collected for retrospective analysis. All patients were divided into four groups according to the molecular subtype: luminal A group (n = 39), luminal B group (n = 44), human epidermal growth factor receptor 2 (HER-2) overexpression group (n = 34), and triple-negative group (n = 33). Data on pathological complete remission (pCR), recurrence, recurrence-free survival (RFS) and overall survival (OS) were collected. The serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α) were detected using assay kits.

RESULTS: The pCR rate of the entire sample cohort was 20.67% after NACT, with a recurrence rate during follow-up recorded at 22.67%. The pCR rate was significantly higher in the HER-2 overexpression group (13/34, 38.24%) than in the triple-negative (11/33, 33.33%), luminal A (1/39, 2.56%), and luminal B groups (6/44, 13.64%) ($p < 0.001$). Compared to the triple-negative (10/33, 30.30%), luminal A (5/39, 12.82%), and luminal B groups (6/44, 13.64%), the HER-2 overexpression group (13/34, 38.24%) had a significantly higher recurrence rate ($p = 0.019$). Both HER-2 overexpression and triple-negative groups also featured greater changes in IL-6, TNF-α, and SOD levels than the luminal A and luminal B groups ($p < 0.05$). Changes in MDA levels were the greatest in the HER-2 overexpression group among the tested group ($p < 0.05$). HER-2 overexpression was identified as the independent risk factor affecting RFS (hazard ratio [HR] = 3.883; 95% confidence interval [CI] = 1.371–11.004; $p = 0.011$). Clinical stage III (HR = 2.031; 95% CI = 1.023–4.030; $p = 0.043$), pCR (HR = 0.111; 95% CI = 0.015–0.809; $p = 0.030$) and recurrence (HR = 4.512; 95% CI = 2.412–8.441; $p < 0.001$) were the independent factors affecting OS in BC patients. The Kaplan–Meier curve analysis demonstrated significant differences in RFS among the four patient groups ($p = 0.005$), with no marked differences in OS ($p = 0.303$). The RFS of the HER-2 overexpression group was shorter than that of the other groups.

CONCLUSIONS: In BC patients undergoing NACT combined with modified radical mastectomy, the HER-2 overexpression subtype was associated with a shorter RFS. However, no significant differences in OS were observed among the four molecular subtypes during the follow-up period. Inflammatory and oxidative stress markers showed improvements three months postoperatively across all subtypes, with more pronounced changes observed in the HER-2 overexpression and triple-negative subtypes.

Keywords: breast cancer; neoadjuvant chemotherapy; modified radical mastectomy; molecular subtype; oxidative stress; inflammatory factor

Introduction

Breast cancer (BC) is one of the most common malignant tumours in women, posing challenges to the physical and mental health, as well as quality of life, in affected individuals. According to statistics, the year 2020 saw a BC incidence rate of more than 2.3 million new cases and a total BC-attributed death count of 685,000 worldwide. Unfortunately, these statistics are predicted to increase to more than 3 million new BC cases and 1 million deaths by 2040 [1]. The current clinical treatment programs include radiotherapy, chemotherapy, hormonal therapy, and surgical intervention. Modified radical mastectomy is one of the main surgical methods used to treat breast tumors [2]. Neoadjuvant chemotherapy (NACT), commonly administered prior to modified radical mastectomy, effectively reduces BC tumor burden, minimizes microscopic lesions, downstages the tumor preoperatively, and thus enhances the likelihood of surgical success [3,4]. Meanwhile, it has been reported that NACT improved the total remission rate and quality of life for patients undergoing modified radical mastectomy; NACT was associated with the progression-free survival time of BC patients [5].

Submitted: 15 July 2025 Revised: 18 September 2025 Accepted: 23 September 2025 Published: 10 November 2025
Correspondence to: Danxia Cao, Department of Thyroid and Breast Surgery, The People's Hospital of Danyang, 212300 Danyang, Jiangsu, China (e-mail: 13951406465@163.com).

BC is characterized by a high level of molecular heterogeneity. Even patients of the same stage and with comparable pathologic profiles exhibit significant variations in treatment sensitivity and prognosis [6,7]. According to the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2) and Ki-67, BC can be classified into four molecular subtypes: luminal A type, luminal B type, HER-2 overexpression type, and triple-negative type [8]. Molecular subtype is associated with metastasis and recurrence potential of BC cells. Patients of the luminal A type generally have a better prognosis than those of the HER-2 overexpression and triple-negative types [9]. Sensitivity to chemotherapy is higher in triple-negative patients than in those with luminal A and B types [10]. Previous investigations have attempted to examine the impact of NACT on the prognosis of BC patients across the different molecular subtypes [11,12]. After NACT treatment, triple-negative and HER-2-overexpressing patients have a higher pathological complete remission (pCR) rate than those with luminal A and luminal B types [11]. It has also been found that the 5-year recurrence-free survival (RFS) of triple-negative patients was decreased compared with those with luminal A and luminal B types [12]. However, most of these studies focused on NACT, without delving into distinguishing the efficacy of radical mastectomy and breast-conserving surgery following administration of NACT. At present, the practicality of adopting molecular typing to determine the efficacy of NACT combined with modified radical mastectomy in the context of BC is unknown. Despite limited data on the efficacy of the proposed treatment regimen, certain patients had voluntarily opted for receiving a modified radical mastectomy following NACT due to reasons such as failure to achieve the expected therapeutic effect, individual risk factors, and personal preferences [13]. Therefore, it is necessary to investigate the prognosis of patients receiving NACT combined with modified radical mastectomy across the four molecular subtypes of BC.

As an inflammatory disease, BC progresses with exacerbation of inflammation and oxidative stress. The inflammatory microenvironment promotes the development of BC. Pro-inflammatory cytokines can promote tumor growth and metastasis of BC cells by altering the biology of cells, and activating stromal cells in the tumor microenvironment (such as vascular endothelial cells, tumor-associated macrophages, and fibroblasts) [14]. Elevated oxidative stress promotes the malignant phenotype of BC cells by inducing DNA damage, enhancing angiogenesis, and triggering abnormal epigenetic modifications and uncontrolled cell proliferation [15]. Bioinformatics analyses revealed that high inflammation has a strong correlation with immunosuppressive microenvironments and a higher frequency of somatic mutations; the molecular subtype with negative ER and PR is associated with a high level of inflammation [16]. Compared with healthy female individuals, BC patients harbor elevated oxidative stress levels, with varying oxidative stress profiles observed across the four molecular subtypes [17]. However, no study has yet explored the effect of NACT combined with modified radical mastectomy on inflammation and oxidative stress across different molecular subtypes of BC. Therefore, the purpose of this study was to explore the influence of NACT combined with modified radical mastectomy on immune function, oxidative stress and prognosis in BC patients classified according to their molecular subtypes.

Materials and Methods

Patients

The clinical information of 150 patients diagnosed with BC at The People's Hospital of Danyang from January 2020 to January 2024 was collected for retrospective analysis. The inclusion criteria are as follows: (1) patients diagnosed with BC by means of histopathology, receiving NACT combined with modified radical mastectomy; (2) patients aged 18–75 years; (3) patients whose molecular types were determined through immunohistochemistry; (4) patients in clinical stage II–III; (5) patients having survived for longer than 3 months; and (6) patients with complete clinical medical record. Modified radical mastectomy is chosen through a shared decision-making process between patients and physicians, following a discussion of the surgery's risks and benefits regarding long-term survival, recurrence, aesthetic outcomes, and quality of life [18].

The exclusion criteria are as follow: (1) patients suffering from other severe, chronic diseases, such as failures of heart, liver, kidney and other critical organs; (2) patients suffering from psychiatric diseases or cognitive disorders, who are unable to cooperate during the treatment and follow-up processes; (3) patients with a previous history of malignant neoplasm; (4) pregnant or breastfeeding women; (5) patients who had received radiation therapy, chemotherapy, or antibiotic treatment within three months before the treatment of this study; and (6) patients who were lost of follow-up. Fig. 1 shows the selection process of patients. This study was approved by the ethics committee of The People's Hospital of Danyang (NO. 20241104). All procedures were conducted in compliance with the Declaration of Helsinki. Informed consents were obtained from all patients prior to follow-up to permit the use of their medical data for clinical research.

All patients were divided into four groups according to their molecular subtypes: luminal A group (n = 39), luminal B group (n = 44), HER-2 overexpression group (n = 34), and triple-negative group (n = 33). The classification criteria for BC molecular subtypes are as follows: (1) Luminal A: ER-positive, PR-positive ($\geq$20%), HER-2-negative, Ki-67 expression $<$14%; (2) Luminal B: This subtype include two categories: (i) ER-positive, HER-2-negative, Ki-67 expression $\geq$14%, and PR-negative or low ($<$20%); (ii) ER-positive, HER-2-positive, with no specific thresholds for

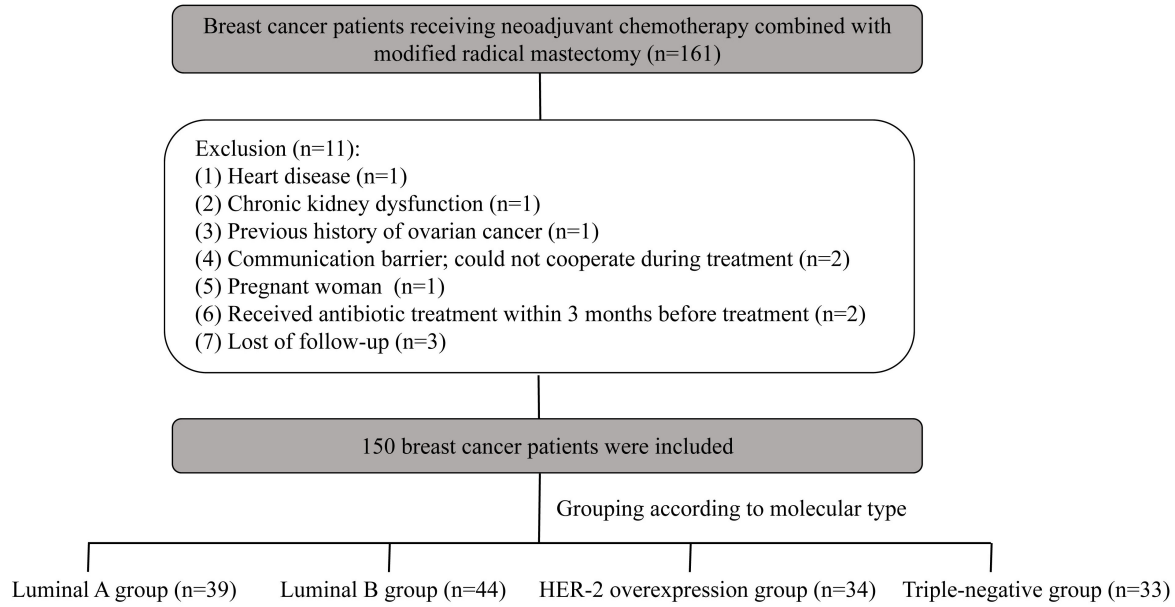


Fig. 1. The flowchart of patient selection and grouping. HER-2, human epidermal growth factor receptor 2.

PR or Ki-67; (3) HER-2 overexpression: HER-2-positive, ER- and PR-negative, with no limit on Ki-67 expression; (4) Triple-negative: ER-, PR- and HER-2-negative, with no limit on Ki-67 [19]. The threshold for ER and PR positivity is $\geq$1%. HER-2 positivity is defined as an immunohistochemistry (IHC) score of 3+, or 2+ with a positive result on *in situ* hybridization (ISH).

NACT

NACT was performed before a modified radical mastectomy. For BC patients with HER-2 negativity (luminal A, luminal B/HER-2 negative, and triple-negative), four to six NACT cycles were conducted based on the patient's condition, with each cycle lasting 21 days. On the first day of the NACT cycle, chemotherapy drugs were administered intravenously, including cyclophosphamide (600 mg/m^2; HJ20160467, Baxter Oncology GmbH, Halle, SA, Germany), epirubicin (75 mg/m^2; H20000496, Pfizer Pharmaceutical Co., Ltd., New York, NY, USA), and docetaxel (75 mg/m^2; H20030561, Jiangsu Hengrui Pharmaceutical Co., Ltd., Lianyungang, China). Ondansetron (H10970062, Qilu Pharmaceutical, Jinan, China) was administered to prevent gastrointestinal reactions during the NACT cycle and after treatment.

For HER-2 positive patients (HER-2 overexpression and luminal B/HER-2 positive), HER-2 targeted therapy was conducted for 4–6 cycles. Each cycle lasts 21 days. Specifically, on the first day, the patients received intravenous injections of trastuzumab (8 mg loading dose, followed by 6 mg; SJ20181016, Roche, Mannheim, BW, Germany), pertuzumab (840 mg loading dose, followed by 420 mg; SJ20180029, Roche, Mannheim, BW, Germany), and docetaxel (75 mg/m^2, increasing to 100 mg/m^2 at cycle 2 if tolerated; HJ20140085, Sanofi, Frankfurt am Main, HE, Germany).

Modified Radical Mastectomy

Patients were placed in the dorsal position, with the upper affected limb extends abducted to 90°. The channel for intravenous administration of anesthesia was established. The general anesthesia procedure was performed by intravenously administering propofol (4–8 mg/kg/h; H20123138, Enhua Pharmaceutical, Xuzhou, China) and remifentanil (0.1–0.6 µg/kg/min; H20030197, Yichang Humanwell Pharmaceutical Co., Ltd., Yichang, China). Position and shape of incision were designed according to the location of the tumor and breast size. The skin was incised along the incision line, and the flaps were subsequently separated using an electrotome. Breast tissue, nipple and areola were removed. Partial skin was also excised depending on the extent of tumor involvement. Next, level I and II axillary lymph node clearance was performed. In the presence of bulky level II lymphadenopathy or any level III lymphadenopathy, level III lymph node clearance was carried out. The pectoralis major muscle was preserved during the procedure. After achieving complete hemostasis, a drainage tube was placed, followed by tissue suturing. Postoperative drainage was performed to prevent accumulation of subcutaneous fluid, and antibiotics were injected to prevent infection. Patient-controlled analgesia pump was applied to relieve pain. Postoperative functional exercises should be performed gradually in stages to promote limb function recovery.

Adjuvant therapy was performed after surgery. The HER-2-positive patients received HER-2 targeted therapy, which encompasses NACT and postoperative adjuvant therapy,

Table 1. Baseline characteristics of BC patients receiving NACT combined with modified radical mastectomy.

Characteristic	Luminal A group (n = 39)	Luminal B group (n = 44)	HER-2 overexpression group (n = 34)	Triple-negative group (n = 33)	p
Age (years)	62 (57, 67)	62 (55, 67)	64 (58, 69)	62 (56, 65)	0.354
BMI (kg/m^2)	23.93 (23.24, 24.65)	24.58 (23.15, 29.36)	26.93 (19.91, 27.82)	25.62 (22.77, 26.47)	0.218
Tumor diameter (cm)	4.80 ± 0.75	4.92 ± 0.77	4.72 ± 1.02	4.72 ± 1.37	0.364
Clinical stage (n, %)					0.281
II	22 (56.41%)	24 (54.55%)	15 (44.12%)	12 (36.36%)	
III	17 (43.59%)	20 (45.4%)	19 (55.88%)	21 (63.64%)	
Family history of breast cancer (n, %)					0.694
Yes	6 (15.38%)	5 (11.36%)	6 (17.65%)	7 (21.21%)	
No	33 (84.62%)	39 (88.64%)	28 (82.35%)	26 (78.79%)	
Menopause (n, %)					0.486
Yes	23 (58.97%)	23 (52.27%)	23 (67.65%)	17 (51.52%)	
No	16 (41.03%)	21 (47.73%)	11 (32.35%)	16 (48.48%)	
Tumor location (n, %)					0.691
Left	21 (53.85%)	20 (45.45%)	14 (41.18%)	14 (42.42%)	
Right	18 (46.15%)	24 (54.55%)	20 (58.82%)	19 (57.58%)	
Lymph node metastasis (n, %)					0.623
Yes	22 (56.41%)	29 (65.91%)	20 (58.82%)	23 (69.70%)	
No	17 (43.59%)	15 (34.09%)	14 (41.18%)	10 (30.30%)	

BC, breast cancer; BMI, body mass index; HER-2, human epidermal growth factor receptor 2; NACT, neoadjuvant chemotherapy.

according to the protocol of NACT (trastuzumab and pertuzumab) for a total duration of 1 year. Patients with hormone receptor positivity (luminal A and luminal B) were subject to endocrine therapy. The plan of endocrine therapy was determined by physicians based on menopausal status, preferences, and the economic level of the patients. The drugs used for endocrine therapy include tamoxifen (20 mg/day; H32021472, Yangtze River Pharmaceutical Group, Taizhou, China), anastrozole (1 mg/day; H20020194, Chongqing Huabang, Chongqing, China), letrozole (2.5 mg/day; H19991001, HengRui, Lianyungan, China), and exemestane (25 mg/day; HJ20160052, Pfizer, Ascoli Piceno, Italia). The patients with luminal B/HER-2 positivity received concurrent endocrine therapy and HER-2 targeted therapy. For triple-negative patients who failed to achieve pCR, 6–8 cycles of catecholamine treatment (H20133366; HengRui, Lianyungan, China) were administered after surgery. The dose of capecitabine was 1250 mg/m^2, twice daily. One cycle of the catecholamine treatment lasted 14 days, which was followed by a 7-day drug-free interval. Postoperative radiotherapy was administered to BC patients with a tumor diameter $\geq$5 cm, $\geq$4 axillary lymph node metastases, or 1–3 lymph node metastases accompanied by high-risk factors.

Data Collection

The clinical data of patients, including age, body mass index (BMI), tumor diameter, clinical stage, family history of breast cancer, menopausal status, tumor location, lymph node metastasis and NACT cycle, were collected. The clinical stage of cancer was determined according to the tumor node metastasis (TNM) system [20]. pCR was defined as the absence of invasive tumors in the breast and axilla after NACT based on the analysis of surgical specimens.

Follow-up

Patients were followed up every three months until May 2025. The methods of follow-up include phone calling, outpatient service, and mail correspondence. The median follow-up time was 34 months. Survival status and tumor recurrence in the patients were recorded. In this study, recurrence was more specifically regarded as locoregional or distant tumor recurrence. Recurrence-free survival (RFS) was defined as the period between the time point for NACT treatment data collection and any of these endpoints: tumor recurrence, the end of follow-up, or death. The overall survival (OS) was defined as the period between the time point for NACT treatment data collection and any of these endpoints: the end of follow-up or death.

Detection of Inflammatory Factors and Oxidative Stress Markers

The inflammatory factors and oxidative stress markers were detected using assay kits. These markers were detected in the patients' fasting venous blood specimens collected prior to NACT treatment and three months after surgery. Following the instructions of kits, the serum levels of malondialdehyde (MDA, A003-1-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), superoxide dismutase (SOD, A001-1-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), interleukin-6 (IL-6) (SP10234, Wuhan Saipei Biotechnology, Wuhan, China), interleukin-

Table 2. The pCR and recurrence of BC in patients receiving NACT combined with modified radical mastectomy.

	Luminal A group (n = 39)	Luminal B group (n = 44)	HER-2 overexpression group (n = 34)	Triple-negative group (n = 33)	p
Number of NACT cycles (n)	6 (6, 6)	6 (4, 6)	6 (6, 6)	6 (6, 6)	0.072
pCR (n, %)	1 (2.56%)	6 (13.64%)	13 (38.24%)	11 (33.33%)	<0.001
Recurrence (n, %)	5 (12.82%)	6 (13.64%)	13 (38.24%)	10 (30.30%)	0.019

pCR, pathological complete remission.

Table 3. Comparison of inflammatory and oxidative stress markers among the four groups.

Indicator	Luminal A group (n = 39)	Luminal B group (n = 44)	HER-2 overexpression group (n = 34)	Triple-negative group (n = 33)	p
IL-6 (pg/mL)					
Before	36.74 ± 5.31	38.27 ± 4.29	40.91 ± 5.22*	46.72 ± 5.58*#&	<0.001
After	20.33 ± 4.12	21.91 ± 4.33	20.35 ± 4.21	22.51 ± 4.12	0.063
Δpost-pre	–16.41 ± 6.52	–16.36 ± 5.99	–20.56 ± 6.20*#	–24.21 ± 7.53*#	<0.001
IL-8 (pg/mL)					
Before	49.78 ± 7.24	48.25 ± 7.58	50.12 ± 8.95	52.91 ± 8.27	0.094
After	30.00 ± 5.38	28.11 ± 5.62	30.58 ± 5.54	31.24 ± 6.27	0.088
Δpost-pre	–19.78 ± 9.45	–20.14 ± 9.58	–19.54 ± 11.71	–21.67 ± 10.53	0.830
TNF-α (pg/mL)					
Before	87.33 ± 15.14	90.57 ± 17.57	105.47 ± 18.11*#	107.35 ± 20.14*#	<0.001
After	42.57 ± 11.33	44.62 ± 10.91	43.58 ± 11.35	49.39 ± 12.68	0.074
Δpost-pre	–44.76 ± 17.67	–45.95 ± 20.19	–61.89 ± 22.90*#	–57.96 ± 25.66*#	<0.001
MDA (nmol/mL)					
Before	12.38 ± 3.58	13.39 ± 4.11	19.33 ± 5.64*#	18.35 ± 5.24*#	<0.001
After	10.33 ± 2.10	11.01 ± 1.87	14.22 ± 3.57*#	14.36 ± 2.58*#	<0.001
Δpost-pre	–1.41 (–3.51, 0.36)	–2.12 (4.52, 0.74)	–4.50 (–7.47, –1.44)*	–2.93 (–6.27, –0.75)	0.010
SOD (U/mL)					
Before	97.77 ± 20.63	95.35 ± 25.41	81.02 ± 15.36*#	82.35 ± 12.22*#	<0.001
After	121.91 ± 22.15	119.36 ± 26.38	120.83± 19.24	128.33 ± 20.35	0.355
Δpost-pre	24.14 ± 21.62	24.01 ± 21.46	39.81 ± 24.63*#	45.98 ± 21.04*#	<0.001

Notes: Δpost-pre = After – Before; *$p < 0.05$ compared with luminal A group, #$p < 0.05$ compared with luminal B group, &$p < 0.05$ compared with HER-2 overexpression group.

IL-6, interleukin-6; IL-8, interleukin-8; MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha.

8 (IL-8) (SP10233, Wuhan Saipei Biotechnology, Wuhan, China), and tumor necrosis factor alpha (TNF-α) (SP10205, Wuhan Saipei Biotechnology, Wuhan, China) were detected.

Statistical Analysis

SPSS software (version 27.0, IBM Corp., Armonk, NY, USA) was utilized for statistical analysis. Use the Shapiro–Wilk test to assess whether the data conform to a normal distribution. Continuous variables with a normal distribution are expressed as mean ± standard deviation, while their inter-group differences were tested using the one-way analysis of variance (ANOVA). Non-normally distributed data are presented as median (interquartile range), while their inter-group differences were measured using the Kruskal–Wallis test. Categorical data are expressed as counts and percentages, with the chi-square test being used for data comparison. The Cox proportional hazard regression model was utilized to analyze the influencing factors on the prognosis of BC patients. The Kaplan–Meier curve was used to analyze the OS and RFS, whereas log log-rank method was employed for the comparison among the four molecular subtypes. Differences with $p < 0.05$ were statistically significant.

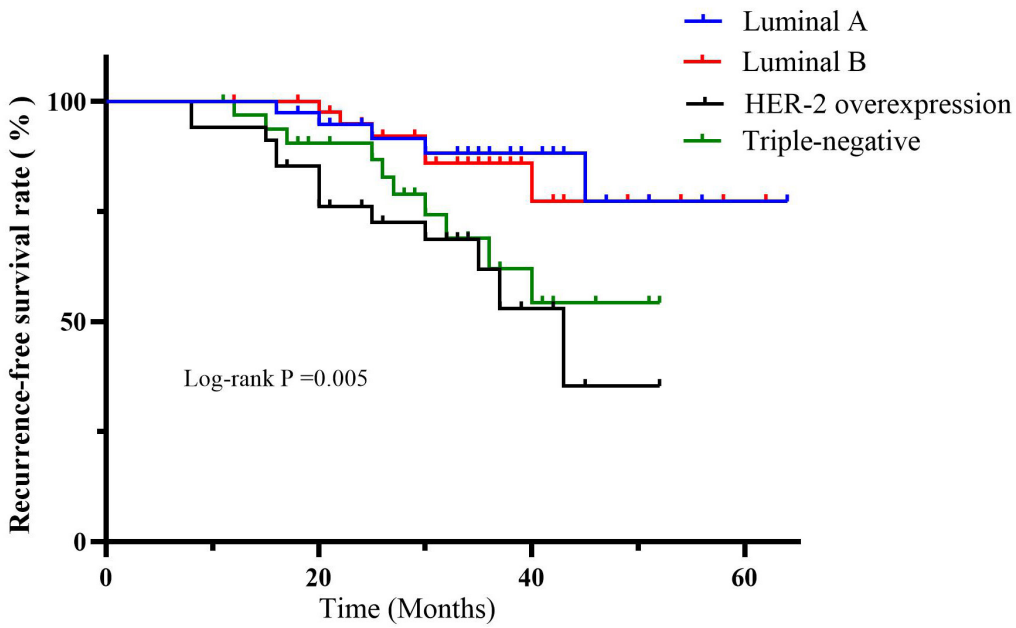
Results

Baseline Characteristics of the Study Population

The baseline characteristics are displayed in Table 1. There were no significant differences in baseline characteristics among the four groups ($p > 0.05$).

Pathological Complete Remission and Recurrence of BC in Patients Receiving NACT Combined With Modified Radical Mastectomy

There was no significant difference among the four groups in the number of NACT cycles (p = 0.072). Following NACT, 31 patients (20.67%) reached pCR. During follow-up, 34 patients experienced recurrence (22.67%). The



Number at risk

Luminal A	39	36	14	1
Luminal B	44	40	10	1
HER-2 overexpression	34	28	4	0
Triple-negative	33	26	8	0

Fig. 2. Kaplan–Meier curve analysis of the recurrence-free survival rate among the four groups. HER-2, human epidermal growth factor receptor 2.

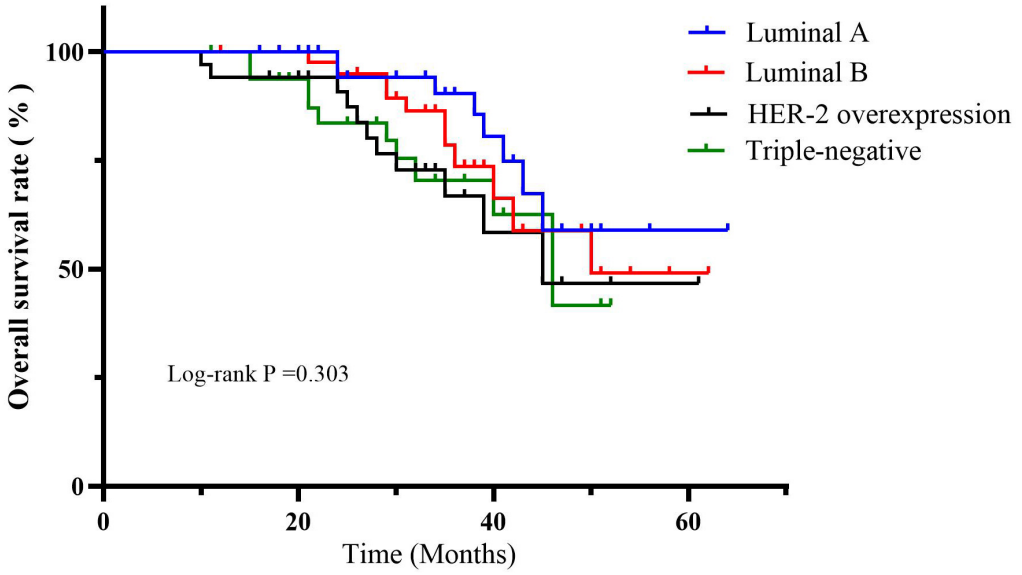
pCR rate in the HER-2 overexpression group was 38.24% (13/34), which was significantly higher than that in the triple-negative (11/33, 33.33%), luminal A (1/39, 2.56%), and luminal B groups (6/44, 13.64%) ($p < 0.001$). The recurrence rate in the HER-2 overexpression group (13/34, 38.24%) was higher than that of the triple-negative (10/33, 30.30%), luminal A (5/39, 12.82%), and luminal B groups (6/44, 13.64%) ($p = 0.019$) (Table 2).

Levels of Inflammatory Factors and Oxidative Stress Markers

Prior to NACT, the four groups of patients exhibited significant differences in IL-6, TNF-α, MDA and SOD ($p < 0.001$), but no significant difference in IL-8 level was observed ($p = 0.094$). Compared to the luminal A and luminal B groups, the IL-6, TNF-α, MDA in the HER-2 overexpression and triple-negative groups were increased, with a reduction in SOD level ($p < 0.05$), before the NACT administration. Three months after surgical intervention, the levels of IL-6, IL-8, TNF-α and MDA were decreased in all patients, accompanied by an increased level of SOD, when compared with the pre-surgical levels ($p < 0.05$). Three months after surgery, there were no significant differences in IL-6, IL-8, TNF-α, and SOD among the four groups of patients ($p > 0.05$). Both the HER-2 overexpression and triple-negative groups recorded greater changes in IL-6, TNF-α, and SOD levels than the luminal A and luminal B groups ($p < 0.05$), with the MDA level change being the greatest in the HER-2 overexpression group ($p < 0.05$) (Table 3).

Recurrence-Free Survival in BC Patients Receiving NACT Combined With Modified Radical Mastectomy

Cox regression analysis was conducted to identify the influencing factors of RFS in BC patients receiving NACT combined with modified radical mastectomy. The univariate analysis revealed HER-2 overexpression (hazard ratio [HR] = 4.197; 95% confidence interval [CI] = 1.485–11.859, $p = 0.007$), triple-negative status (HR = 3.073; 95% CI = 1.047–9.023; $p = 0.041$), and clinical stage III (HR = 2.196; 95% CI = 1.069–4.511; $p = 0.032$) as the influencing factors of RFS. Multivariate analysis was further conducted by including these influencing factors. According to the results, HER-2 overexpression (HR = 3.883; 95% CI = 1.371–11.004; $p = 0.011$) was found to be the independent risk factor affecting RFS (Table 4). Kaplan–Meier curve analysis demonstrated a significant difference in RFS among four molecular subtypes ($p = 0.005$). The RFS in HER-



Number at risk

Luminal A	39	37	14	1
Luminal B	44	40	10	1
HER-2 overexpression	34	31	7	1
Triple-negative	33	28	9	0

Fig. 3. Kaplan–Meier curve analysis of the overall survival rate among the four groups. HER-2, human epidermal growth factor receptor 2.

2-overexpressing patients was shorter compared to other molecular subtypes (Fig. 2).

Overall Survival in BC Patients Receiving NACT Combined With Modified Radical Mastectomy

According to the multivariate Cox analysis results, clinical stage III (HR = 2.031; 95% CI = 1.023–4.030; $p = 0.043$), pCR (HR = 0.111; 95% CI = 0.015–0.809; $p = 0.030$) and recurrence (HR = 4.512; 95% CI = 2.412–8.441; $p < 0.001$) were the independent factors affecting the OS in BC patients receiving NACT combined with modified radical mastectomy. The molecular subtypes did not affect the OS in this study population ($p > 0.05$) (Table 5). Kaplan–Meier curve analysis demonstrated no significant difference in OS among the four molecular subtypes ($p = 0.303$) (Fig. 3).

Discussion

Primarily caused by abnormal hyperplasia of the breast epithelial cells, BC has high incidence and mortality rates in the female population [1]. NACT combined with modified radical mastectomy can effectively reduce recurrence/metastasis rates and improve OS [5,21]. Notably, BC can be classified into different subtypes based on receptor expression and molecular biological characteristics. There are significant differences in the sensitivity of treatment and prognosis among different subtypes [22,23]. Among the 150 BC patients included in this study, luminal A type accounts for 26.0%, luminal B for 29.3%, HER-2 overexpression type for 22.7%, and triple-negative type for 22.0%. A multi-center clinical study conducted in China showed that luminal A type accounted for the highest proportion (41.1%) among all the primary BC cases, followed by luminal B and HER-2 overexpression types, with triple-negative type accounting for the lowest proportion [24]—a finding that is different from ours. This is mainly attributed to the type of patients included; our study included only the BC patients who underwent NACT combined with modified radical mastectomy, excluding those who received other treatment methods. In addition, the small sample size may have contributed to this disparity.

In this study, a total of 31 patients had achieved pCR (20.67%). The pCR rate in the HER-2 overexpression and triple-negative groups was higher than that in the luminal A and luminal B groups. Improving pCR rate is an important goal for NACT treatment, which is also associated with a high survival rate [25]. Previous study also reported that the pCR rate in the luminal A type was the lowest (0.3%), while the highest was noted in the HER-2 overexpression type (38.7%) [26], consistent with our results.

Tumor recurrence poses critical challenges to achieving a better quality of life and prognosis in BC patients. There are differences in recurrence characteristics among differ-

Table 4. Identification of influencing factors of RFS in BC patients receiving NACT combined with modified radical mastectomy via Cox regression analysis.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Molecular subtype				
Luminal A	1.000 (Reference)	/	1.000 (Reference)	/
Luminal B	1.200 (0.365–3.937)	0.764	1.202 (0.366–3.946)	0.762
HER-2 overexpression	4.197 (1.485–11.859)	0.007	3.883 (1.371–11.004)	0.011
Triple-negative	3.073 (1.047–9.023)	0.041	2.786 (0.944–8.222)	0.064
Age	1.032 (0.984–1.083)	0.198		
BMI	0.965 (0.864–1.077)	0.526		
Tumor diameter	0.996 (0.696–1.425)	0.982		
Clinical stage III	2.196 (1.069–4.511)	0.032	1.900 (0.919–3.928)	0.083
Family history of breast cancer	1.686 (0.763–3.729)	0.197		
Menopause	1.299 (0.650–2.597)	0.459		
Tumor location (left)	1.155 (0.589–2.264)	0.675		
Lymph node metastasis	2.012 (0.936–4.325)	0.073		
Number of NACT cycles	1.325 (0.785–2.236)	0.292		
pCR	0.864 (0.358–2.088)	0.746		
IL-6 (before treatment)	1.026 (0.974–1.080)	0.336		
IL-6 (after treatment)	1.043 (0.966–1.125)	0.284		
IL-8 (before treatment)	1.022 (0.979–1.066)	0.321		
IL-8 (after treatment)	0.955 (0.898–1.015)	0.136		
TNF-α (before treatment)	1.012 (0.995–1.030)	0.179		
TNF-α (after treatment)	1.022 (0.993–1.051)	0.137		
MDA (before treatment)	1.050 (0.987–1.116)	0.122		
MDA (after treatment)	1.085 (0.974–1.208)	0.138		
SOD (before treatment)	0.993 (0.976–1.011)	0.462		
SOD (after treatment)	1.001 (0.985–1.017)	0.925		

HR, hazard ratio; CI, confidence interval; RFS, recurrence-free survival.

ent molecular subtypes [27]. A study revealed that the median recurrence time in luminal A type was longer than that of HER-2 overexpression and triple-negative types; triple-negative and HER-2 overexpression types were associated with shorter median recurrence time [28]. In the current study, the total recurrence rate was 22.67%. HER-2 overexpression type has the highest recurrence rate, followed by the triple-negative type. Meanwhile, the HER-2 overexpression type was an independent risk factor for RFS. The Kaplan–Meier curve analysis showed the RFS in the HER-2 overexpression group was lower than other groups. A previous study has reported that the recurrence rate was 20.1% in BC patients receiving NACT, with the HER-2 overexpression type recording the highest recurrence rate, followed by the luminal B, luminal A and triple-negative types [29]. In addition, a study reported that pCR was a predictive factor for the recurrence of BC patients receiving NACT and total mastectomy [30]. In this study, we did not observe a significant impact of pCR on cancer recurrence, which is probably due to the varying clinical characteristics among the BC patients. Meanwhile, the small sample size may also be a plausible reason.

It has been reported that the OS in BC patients could be influenced by the molecular subtypes [31]. In this study, we found that molecular subtypes did not affect the prognosis of BC patients receiving NACT combined with modified radical mastectomy. According to the Kaplan–Meier curve analysis, there was no difference in OS across the patient groups with different molecular subtypes. Concordant with our findings, a previous study reported that there was no significant difference in 3-year OS among the four molecular subtypes after the patients received modified radical mastectomy [32]. The HER-2-overexpressing patients with BC face an increased risk of recurrence, although their OS remains unaffected by the molecular subtype. Our study revealed that NACT combined with modified radical mastectomy improves the prognosis of patients with HER-2 overexpression type, despite the inherently high recurrence risk and lack of OS benefit. The high recurrence risk faced by the HER-2-overexpressing patients is precipitated by the high proliferative activity, strong invasiveness, and less controlled metastasis of the HER-2-overexpressing BC cells [33]. The lack of OS benefit is probably caused by the insufficient follow-up time.

Table 5. Identification of factors affecting OS in BC patients receiving NACT combined with modified radical mastectomy via Cox regression analysis.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Molecular subtype				
Luminal A	1.000 (Reference)	/		
Luminal B	1.409 (0.566–3.511)	0.462		
HER-2 overexpression	2.136 (0.857–5.326)	0.104		
Triple-negative	2.064 (0.812–5.247)	0.128		
Age	1.012 (0.969–1.105)	0.597		
BMI	1.001 (0.907–1.105)	0.980		
Tumor diameter	1.223 (0.881–1.698)	0.230		
Clinical stage III	2.503 (1.270–4.932)	0.008	2.031 (1.023–4.030)	0.043
Family history of breast cancer	1.392 (0.660–2.933)	0.385		
Menopause	1.337 (0.704–2.539)	0.375		
Tumor location (left)	0.869 (0.463–1.629)	0.661		
Lymph node metastasis	1.822 (0.922–3.600)	0.084		
NACT cycle number	1.745 (0.681–4.466)	0.246		
pCR	0.109 (0.015–0.794)	0.028	0.111 (0.015–0.809)	0.030
Recurrence	4.624 (2.479–8.626)	<0.001	4.512 (2.412–8.441)	<0.001
IL-6 (before treatment)	1.041 (0.993–1.092)	0.095		
IL-6 (after treatment)	1.036 (0.965–1.111)	0.332		
IL-8 (before treatment)	1.027 (0.988–1.068)	0.182		
IL-8 (after treatment)	0.952 (0.897–1.009)	0.098		
TNF-α (before treatment)	0.999 (0.983–1.016)	0.940		
TNF-α (after treatment)	1.017 (0.991–1.044)	0.195		
MDA (before treatment)	1.049 (0.991–1.111)	0.102		
MDA (after treatment)	0.997 (0.897–1.108)	0.952		
SOD (before treatment)	0.993 (0.976–1.009)	0.392		
SOD (after treatment)	1.013 (0.998–1.027)	0.085		

OS, overall survival.

Inflammatory reaction and oxidative stress are important pathological mechanisms of BC. Levels of inflammation and oxidative stress vary across the molecular subtypes of BC. A study conducted in Iran showed that the female individuals with the highest intake of proinflammatory diets had a threefold increased likelihood of developing triple-negative cancer compared to luminal A type [34]. Triple-negative and HER-2 overexpression types exhibit high levels of inflammation and oxidative stress, and are associated with poorer efficacy and prognosis [17,35]. The present study also demonstrated significant differences in IL-6, TNF-α, MDA, and SOD levels among the four groups before treatment. Compared to luminal A and luminal B types, HER-2 overexpression and triple-negative types exhibited higher levels of inflammation and oxidative stress. After three months of NACT and modified radical mastectomy, the levels of inflammation and oxidative stress in all groups were attenuated, with decreased IL-6, IL-8, TNF-α and MDA, and increased SOD. Meanwhile, there were no significant differences in the levels of IL-6, IL-8, TNF-α, and SOD among the four groups. It was suggested that HER-2 overexpression and triple-negative types gain greater benefits from NACT combined with modified radical mastectomy, demonstrating greater reductions in oxidative stress and inflammation levels than luminal A and luminal B types.

Several shortcomings of this study should be acknowledged. Firstly, this study was a retrospective investigation with a relatively small sample size, which raises the possibility of yielding biased results. Thus, a large-scale prospective study should be conducted to validate these findings. Secondly, our study included exclusively the BC patients undergoing NACT combined with modified radical mastectomy. It is unclear whether our findings apply to BC patients receiving other treatment schemes. Thirdly, high BMI (>26 kg/m^2) in certain BC patients of the current cohort may obfuscate the actual results, since increased BMI, waist circumference and body fat percentage have been found to associate with high risk of BC recurrence [36], and obesity could exacerbate oxidative stress and promote chronic inflammation [37]. Also, we found that the median BMI value of the HER-2 overexpression group was slightly higher than that of the other three groups, although it is unclear whether the BMI of the HER-2 overexpres-

sion type is associated with a higher recurrence rate. Future study could attempt to explore the impact of BMI on the prognosis of patients with this molecular subtype and delineate the potential mechanisms through stratification of patients by their BMI values. Finally, we did not analyze the influence of molecular subtypes on Miller–Payne grading due to missing data. Subsequent research can supplement the Miller–Payne grading information.

Conclusions

In summary, among the BC patients receiving NACT combined with modified radical mastectomy, the individuals with the HER-2 overexpression type exhibited higher pCR and recurrence rates compared to those of other molecular subtypes. Patients with the HER-2 overexpression type also faced a shorter RFS, but there was no difference in OS among the four molecular subtypes during the follow-up period. Significant amelioration in oxidative stress and inflammation was detected in all subtypes three months after the surgery, with greater changes of related markers noted in the HER-2 overexpression and triple-negative types.

Availability of Data and Materials

All data included in this study are available from the corresponding author upon reasonable request.

Author Contributions

DC designed the research study and drafted the manuscript. YS performed the research. DC and HP analyzed the data. All authors contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the ethics committee of The People's Hospital of Danyang (NO. 20241104). All procedures were conducted in compliance with the Declaration of Helsinki. Informed consents were obtained from all patients prior to follow-up to permit the use of their medical data for clinical research.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Zhang Y, Ji Y, Liu S, Li J, Wu J, Jin Q, *et al*. Global burden of female breast cancer: new estimates in 2022, temporal trend and future projections up to 2050 based on the latest release from GLOBOCAN. Journal of the National Cancer Center. 2025; 5: 287–296. https://doi.org/10.1016/j.jncc.2025.02.002.

[2] Bryan AF, Castillo-Angeles M, Minami C, Laws A, Dominici L, Broyles J, *et al*. Value of Ambulatory Modified Radical Mastectomy. Annals of Surgical Oncology. 2023; 30: 4637–4643. https://doi.org/10.1245/s10434-023-13588-z.

[3] Gentile D, Sagona A, De Carlo C, Fernandes B, Barbieri E, Di Maria Grimaldi S, *et al*. Pathologic response and residual tumor cellularity after neo-adjuvant chemotherapy predict prognosis in breast cancer patients. Breast. 2023; 69: 323–329. https://doi.org/10.1016/j.breast.2023.03.016.

[4] Lv FY, Mo Z, Chen B, Huang Z, Mo Q, Tan Q. Locoregional recurrence and survival of breast-conserving surgery compared to mastectomy following neoadjuvant chemotherapy in operable breast cancer. Frontiers in Oncology. 2024; 14: 1308343. https://doi.org/10.3389/fonc.2024.1308343.

[5] Xie X, Li H, Wang C, Li W, Xie D, Li M, *et al*. Effect of modified radical mastectomy combined with neo-adjuvant chemotherapy on postoperative recurrence rate, negative emotion, and life quality of patients with breast cancer. American Journal of Translational Research. 2022; 14: 460–467.

[6] Rakha EA, Tse GM, Quinn CM. An update on the pathological classification of breast cancer. Histopathology. 2023; 82: 5–16. https://doi.org/10.1111/his.14786.

[7] Guo L, Kong D, Liu J, Zhan L, Luo L, Zheng W, *et al*. Breast cancer heterogeneity and its implication in personalized precision therapy. Experimental Hematology & Oncology. 2023; 12: 3. https://doi.org/10.1186/s40164-022-00363-1.

[8] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann 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. Annals of Oncology. 2011; 22: 1736–1747. https://doi.org/10.1093/annonc/mdr304.

[9] Zarean Shahraki S, Azizmohammad Looha M, Mohammadi Kazaj P, Aria M, Akbari A, Emami H, *et al*. Time-related survival prediction in molecular subtypes of breast cancer using time-to-event deep-learning-based models. Frontiers in Oncology. 2023; 13: 1147604. https://doi.org/10.3389/fonc.2023.1147604.

[10] Bando Y, Kobayashi T, Miyakami Y, Sumida S, Kakimoto T, Saijo Y, *et al*. Triple-negative breast cancer and basal-like subtype : Pathology and targeted therapy. The Journal of Medical Investigation. 2021; 68: 213–219. https://doi.org/10.2152/jmi.68.213.

[11] Myers SP, Ahrendt GM, Lee JS, Steiman JG, Soran A, Johnson RR, *et al*. Association of Tumor Molecular Subtype and Stage with Breast and Axillary Pathologic Complete Response After Neoadjuvant Chemotherapy for Breast Cancer. Annals of Surgical Oncology. 2021; 28: 8636–8642. https://doi.org/10.1245/s10434-021-10195-8.

[12] Chen D, Wang Q, Dong M, Chen F, Huang A, Chen C, *et al*. Analysis of neoadjuvant chemotherapy for breast cancer: a 20-year retrospective analysis of patients of a single institution. BMC Cancer. 2023; 23: 984. https://doi.org/10.1186/s12885-023-11505-x.

[13] Golara A, Kozłowski M, Lubikowski J, Cymbaluk-Płoska A. Types of Breast Cancer Surgery and Breast Reconstruction. Cancers. 2024; 16: 3212. https://doi.org/10.3390/cancers16183212.

[14] Olivier DW, Eksteen C, Plessis MD, de Jager L, Engelbrecht L, McGregor NW, *et al*. Inflammation and Tumor Progression: The Differential Impact of SAA in Breast Cancer Models. Biology. 2024; 13: 654. https://doi.org/10.3390/biology13090654.

[15] Bel'skaya LV, Dyachenko EI. Oxidative Stress in Breast Cancer: A Biochemical Map of Reactive Oxygen Species Production. Current Issues in Molecular Biology. 2024; 46: 4646–4687. https://doi.org/10.3390/cimb46050282.

[16] Yu K, Xu C, Wang F, Wang H. Identification of the new molecular subtypes related to inflammation in breast cancer. Medicine. 2024; 103: e38146. https://doi.org/10.1097/MD.0000000000038146.

[17] Herrera ACSA, Panis C, Victorino VJ, Campos FC, Colado-Simão AN, Cecchini AL, *et al*. Molecular subtype is determinant on inflammatory status and immunological profile from invasive breast cancer patients. Cancer Immunology, Immunotherapy. 2012; 61: 2193–2201. https://doi.org/10.1007/s00262-012-1283-8.

[18] Gradishar WJ, Moran MS, Abraham J, Aft R, Agnese D, Allison KH, *et al*. Breast Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network. 2022; 20: 691–722. https://doi.org/10.6004/jnccn.2022.0030.

[19] 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. Annals of Oncology. 2013; 24: 2206–2223. https://doi.org/10.1093/annonc/mdt303.

[20] Cserni G, Chmielik E, Cserni B, Tot T. The new TNM-based staging of breast cancer. Virchows Archiv. 2018; 472: 697–703. https://doi.org/10.1007/s00428-018-2301-9.

[21] Chen C, Zhu J, Zhang C, Wang L, Li Y, Du M. Pathological Complete Response with Neoadjuvant Trastuzumab, Pertuzumab, and Chemotherapy Followed by Modified Radical Mastectomy in a Patient with HER2-Positive Occult Breast Cancer. The American Journal of Case Reports. 2024; 25: e943936. https://doi.org/10.12659/AJCR.943936.

[22] Shan R, Dai LJ, Shao ZM, Jiang YZ. Evolving molecular subtyping of breast cancer advances precision treatment. Cancer Biology & Medicine. 2024; 21: 731–739. https://doi.org/10.20892/j.issn.2095-3941.2024.0222.

[23] Yıldırım E, Bektas S, Yetıs F, Yıldız EO, Ozdemir M, Komut N, *et al*. Correlation between breast and axillary pathologic complete response after neoadjuvant chemotherapy in breast cancer. Annali Italiani di Chirurgia. 2023; 94: 336–345.

[24] Zeng H, Wu S, Ma F, Ji JS, Lu L, Ran X, *et al*. Disparities in stage at diagnosis among breast cancer molecular subtypes in China. Cancer Medicine. 2023; 12: 10865–10876. https://doi.org/10.1002/cam4.5792.

[25] 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. Clinical Cancer Research. 2020; 26: 2838–2848. https://doi.org/10.1158/1078-0432.CCR-19-3492.

[26] Haque W, Verma V, Hatch S, Suzanne Klimberg V, Brian Butler E, Teh BS. Response rates and pathologic complete response by breast cancer molecular subtype following neoadjuvant chemotherapy. Breast Cancer Research and Treatment. 2018; 170: 559–567. https://doi.org/10.1007/s10549-018-4801-3.

[27] Cheun JH, Kim HK, Moon HG, Han W, Lee HB. Locoregional Recurrence Patterns in Patients With Different Molecular Subtypes of Breast Cancer. JAMA Surgery. 2023; 158: 841–852. https://doi.org/10.1001/jamasurg.2023.2150.

[28] Courtney D, Davey MG, Moloney BM, Barry MK, Sweeney K, McLaughlin RP, *et al*. Breast cancer recurrence: factors impacting occurrence and survival. Irish Journal of Medical Science. 2022; 191: 2501–2510. https://doi.org/10.1007/s11845-022-02926-x.

[29] Orsaria P, Grasso A, Ippolito E, Pantano F, Sammarra M, Altomare C, *et al*. Clinical Outcomes Among Major Breast Cancer Subtypes After Neoadjuvant Chemotherapy: Impact on Breast Cancer Recurrence and Survival. Anticancer Research. 2021; 41: 2697–2709. https://doi.org/10.21873/anticanres.15051.

[30] Zhang J, Sun M, Chang E, Lu CY, Chen HM, Wu SY. Pathologic response as predictor of recurrence, metastasis, and survival in breast cancer patients receiving neoadjuvant chemotherapy and total mastectomy. American Journal of Cancer Research. 2020; 10: 3415–3427.

[31] Fallahpour S, Navaneelan T, De P, Borgo A. Breast cancer survival by molecular subtype: a population-based analysis of cancer registry data. CMAJ Open. 2017; 5: E734–E739. https://doi.org/10.9778/cmajo.20170030.

[32] Dong B, Yin X, Xu H, Zhou K, Li L, Tian B, *et al*. Application value of modified radical mastectomy in female patients with breast cancer of different molecular types: a prognosis study. American Journal of Translational Research. 2022; 14: 2490–2496.

[33] Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions. Nature Reviews. Drug Discovery. 2023; 22: 101–126. https://doi.org/10.1038/s41573-022-00579-0.

[34] Hayati Z, Montazeri V, Shivappa N, Hebert JR, Pirouzpanah S. The association between the inflammatory potential of diet and the risk of histopathological and molecular subtypes of breast cancer in northwestern Iran: Results from the Breast Cancer Risk and Lifestyle study. Cancer. 2022; 128: 2298–2312. https://doi.org/10.1002/cncr.34183.

[35] Autenshlyus A, Davletova K, Varaksin N, Marinkin I, Lyakhovich V. Cytokines in various molecular subtypes of breast cancer. International Journal of Immunopathology and Pharmacology. 2021; 35: 20587384211034089. https://doi.org/10.1177/20587384211034089.

[36] Harborg S, Feldt M, Cronin-Fenton D, Klintman M, Dalton SO, Rosendahl AH, *et al*. Obesity and breast cancer prognosis: prediagnostic anthropometric measures in relation to patient, tumor, and treatment characteristics. Cancer & Metabolism. 2023; 11: 8. https://doi.org/10.1186/s40170-023-00308-0.

[37] Devericks EN, Carson MS, McCullough LE, Coleman MF, Hursting SD. The obesity-breast cancer link: a multidisciplinary perspective. Cancer Metastasis Reviews. 2022; 41: 607–625. https://doi.org/10.1007/s10555-022-10043-5.