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Heterogeneity in breast cancer: Clinical and therapeutic implications

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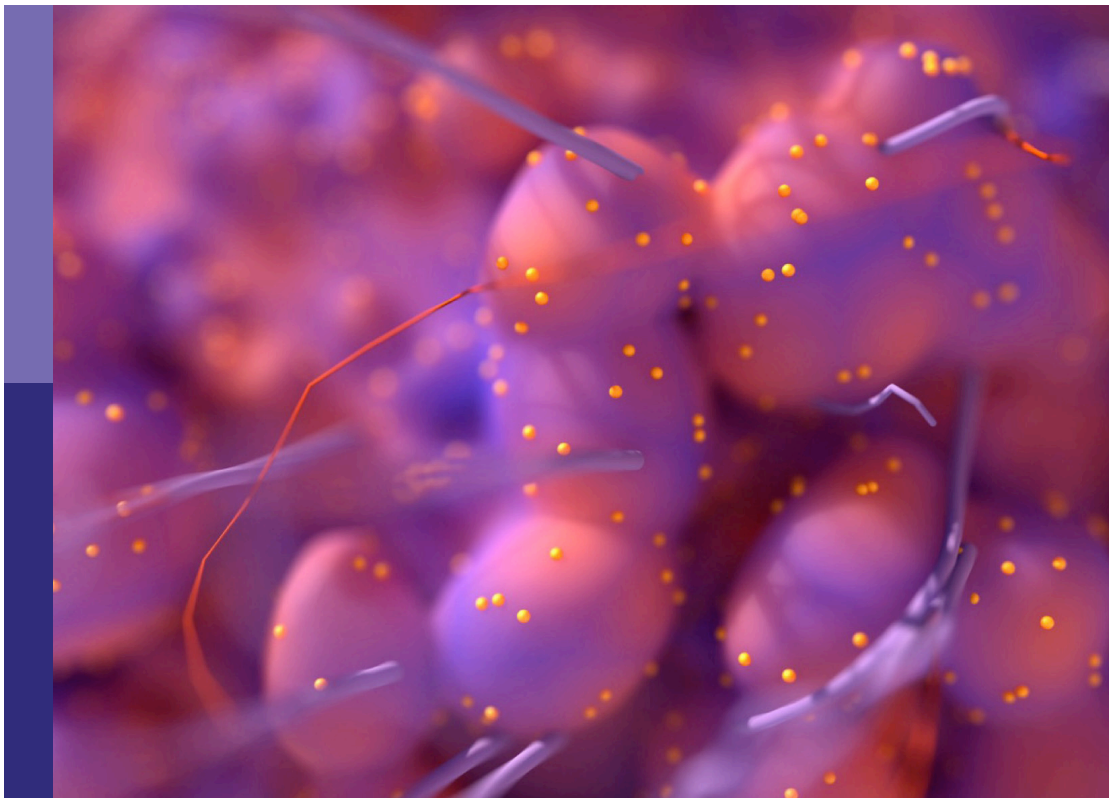

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Heterogeneity in breast cancer: Clinical and therapeutic implications

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Editorial: Heterogeneity in breast cancer: clinical and therapeutic implications

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Editorial on the Research Topic
Heterogeneity in breast cancer: clinical and therapeutic implications

Breast cancer (BC) is a complex disease with high intratumoral and intertumoral heterogeneity. Such heterogeneity plays a critical role in treatment response, therapeutic failure, and disease outcome (1). Despite significant advances in early detection and therapy, BC remains the leading cause of cancer-related death in women worldwide (2). While clinicians and researchers are actively engaged in identifying the optimal treatment strategy, the limited understanding of the molecular mechanism of BC heterogeneity in the context of drug resistance and disease recurrence represents one of the major challenges in current BC research. To address this issue, there is a growing interest in developing innovative methods to better understand the mechanisms underlying BC heterogeneity in order to facilitate effective diagnosis and provide tailored treatment.

The Research Topic entitled "*Heterogeneity in Breast Cancer: Clinical and Therapeutic Implications*" includes 16 research articles, 1 review, 1 network meta-analysis, and 1 case report that address various aspects of heterogeneity in BC disease: histologic and immunohistochemical characteristics, clinical manifestations, radiomic features, surgical and medical approaches, treatment responses, implications of DNA repair gene alterations and treatment adherence. Below are the main topics covered in the various articles.

Conventional imaging techniques, such as mammography, ultrasound, and magnetic resonance imaging (MRI) are effective tools for measuring heterogeneity in BC patients. Several studies have demonstrated that specific imaging-related features such as mass lesion shape, margin characteristics, T2 signal intensity, and contrast enhancement dynamics, reflect the distinct molecular subtypes of breast tumors.

Moreover, in order to improve current prognostic models and treatment planning, radiomics, a non-invasive approach that combines quantitative features extracted from

medical imaging with genomic biosignatures, has emerged in recent years as a strategy to study BC heterogeneity (3).

Phyllodes tumors are uncommon neoplasms that exhibit both epithelial and mesenchymal characteristics, resembling fibroadenomas in terms of their histological appearance. These tumors can range in morphological presentation from benign to malignant. When assessed by conventional MRI, it can be challenging to distinguish between the features of benign, borderline, and malignant phyllodes tumors due to their overlapping characteristics (4). In their retrospective study, Fang et al. demonstrated that the apparent diffusion coefficient (ADC) value, a parameter derived from diffusion-weighted imaging (DWI), offers quantitative information with the ability to differentiate between phyllodes tumors, fibroadenomas, and breast neoplasms and to provide a classification of phyllodes tumors.

The integration of histological, clinicopathological, and molecular information, in addition to individual patient characteristics and preferences, is essential to establishing the optimal therapeutic pathway for a patient.

Surrogate classification of BC subtypes based on biological markers such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki67 expression levels accurately predict clinical characteristics of recurrence patterns and disease-free survival. Several studies on BC have revealed that single Progesterone Receptor (sPR) expression is associated with more aggressive behavior in early-stage BC, resembling the characteristics of triple-negative breast cancer (TNBC) (5). Luo et al. conducted a retrospective analysis involving a large cohort of 10,877 metastatic BC patients to understand the behavior and prognosis of sPR-positive and TNBC patients with advanced disease. The study results suggest that, as in the early stage, even in the advanced or metastatic setting, sPR-positive and TNBC patients show similar biological behavior supporting chemotherapy as the preferred treatment option for these subtypes.

Triple-positive breast cancer (TPBC), characterized by positivity for HER2, ER, and PR, is a rare subtype displaying features linked to a less favorable prognosis compared to other Luminal B-like BC (6).

To improve risk assessment, Geng et al. conducted a retrospective analysis of data from the Fourth Military Medical University Affiliated Xijing Hospital and the SEER database. The study identified several independent risk factors affecting the prognosis of TPBC patients, including age, chemotherapy, radiotherapy, TNM stage, and the type of surgery. These prognostic variables were then utilized to construct a nomogram designed to predict the 3-year and 5-year overall survival rates of TPBC patients. Nomograms are statistical prognostic models that are particularly useful for individualizing the clinical decision-making process, especially in the case of rare tumor types, and provide an easier estimation of the probability of a specific event than that with traditional evaluation methods (7). In particular, this nomogram serves as a valuable tool for clinicians to estimate and communicate the likelihood of survival outcomes based on individual patient characteristics and treatment modalities.

Approximately half of breast cancers, traditionally classified as HER2 negative exhibit low levels of HER2 expression, identified by an immunohistochemical (IHC) score of 1+ or 2+ with negative *in situ* hybridization. Retrospective data suggests that HER2-low BC does not represent a distinct subtype in terms of biological characteristics. Nevertheless, the prognostic impact of HER2-low expression BC remains controversial (8).

In a meta-analysis of 14 studies involving 52106 patients Wei et al. found that among early-stage, HER2-low-expressing BC patients, OS was better in the overall population and the hormone receptor-positive and TNBC subgroups. Notably, favorable DFS and RFS were observed in both the overall population and the hormone receptor-positive subgroup.

Since HER2-low breast cancer is highly unstable during disease progression, Shang et al. explored the evolution of HER2 expression in primary breast cancer and residual tumors after neoadjuvant therapy in 775 patients with pathological non-pCR breast cancer after preoperative therapy. HER2-low-expressing breast cancers accounted for just over half (59.61%) of the total HER2-negative cohort, with the proportion of HER2-low cases in breast cancer samples with residual tumors after neoadjuvant therapy being lower than in BC primaries. This discrepancy was primarily attributed to the phenomenon of HER2-low cases switching to HER2-zero status. Specifically, approximately 17% of patients with HER2-low primary BC experienced a transition to HER2-zero status following neoadjuvant therapy. In contrast, approximately 38% of patients initially identified as HER2-zero in the primary tumor shifted to HER2-low, providing additional evidence of the instability associated with HER2-low expression. This study confirmed the correlation between HER2-low and HR status but also demonstrated a correlation with AR status. These findings underscore the importance of re-evaluating HER2 status in BC patients following neoadjuvant therapy. This approach expands the range of treatment options available to patients. However, whether HER2-low BC can be definitively classified as a new subtype requires further confirmation through additional studies.

The metaplastic tumor is another extremely rare BC defined by the histological presence of at least two cell types, typically epithelial and mesenchymal components. This variant shows a TNBC phenotype with more aggressive behavior, less chemosensitivity, and a worse prognosis in comparison to other BC types (9). Based on the Surveillance, Epidemiology, and End Results (SEER) database and cases from the Union Hospital of Fujian Medical University, Zheng et al. analyzed prognostic factors (age, T stage, N stage, M stage, surgery, and radiotherapy) and constructed a nomogram to provide more accurate individualized survival analyses for patients with this rare histotype. Male BC is a seldom-occurring condition, accounting for less than 1% of all malignancies in men and less than 1% of malignant breast tumors. Due to the absence of established treatment guidelines, patients with BC are currently managed similarly to the female population. Nevertheless, male BC exhibits different characteristics and clinical behavior compared to its female counterpart, highlighting the need for a unique predictive model to develop a personalized therapeutic

approach (10). To this end, Wen et al. developed a prediction model based on univariate and multivariate logistic regression analyses. By extrapolating data from the SEER registry between 2010 and 2015 and cases from Fujian Medical University Union Hospital, the authors showed that the type of surgery, age, T and M status, histologic grade, expression of ER and HER2, and use of chemotherapy were predictors of male BC prognosis and used them to construct a nomogram that outperformed the AJCC staging system.

Improved survival rates following cancer diagnosis have resulted in an increase in the occurrence of second primary cancers. While extensive research has been conducted on the risks of second primary malignancies in female BC patients over several decades, there is a notable lack of knowledge when it comes to second primary tumors in men (11). Huang et al. performed an analysis of data from 1,843 male patients with BC collected from the SEER database. They employed competing risk models and nomograms to create tools for predicting the probability of cancer-specific mortality and the development of second primary malignancies. According to their predictive model, factors such as older age at diagnosis, advanced TNM stage, lack of surgery and radiotherapy, a waiting time of more than one month before treatment initiation, and positive hormone receptor and HER2 status were associated with a less favorable prognosis in male BC patients. Furthermore, they developed an additional prediction model to assess the risk of second primary malignancies in male BC survivors. This model aims to facilitate risk-based follow-up and counseling.

Nearly 10% of breast cancers are related to the inheritance of damaged genes. The most common inherited gene mutations that increase the risk of BC are involved in the DNA repair pathway. In particular, genetic variants in Homologous Recombination Repair (HRR) genes, including BRCA1 and BRCA2, ATM, PALB2, and RAD51, play a critical role in BC inheritance and susceptibility (12). Yu and Wang's meta-analysis focused on the relationship between polymorphisms in the HRR RAD51, G172T XRCC2, and XRCC3 genes and BC risk, showing an increased cancer risk associated with polymorphisms in the RAD51 genes which was significantly higher in the Arab population.

Moreover, homologous recombination deficiency confers increased sensitivity to PARPi and platinum (13). In order to assess the efficacy and safety of various pharmacotherapies for patients with metastatic, locally advanced, or recurrent BC carrying pathogenic BRCA1/BRCA2 variants, Zhu et al. conducted a network meta-analysis including nine randomized controlled trials (RCTs) with 1,912 participants. They demonstrated that, despite the increased occurrence of side effects, the most effective treatment combination for patients with advanced BC harboring germline BRCA variants was the use of PARP inhibitors alongside platinum-based chemotherapy.

Furthermore, the complex crosstalk between tumor cells and other cells in the microenvironment contributes to defining the tumor's profile and behavior. Among these, tumor-infiltrating immune cells play two contrasting roles: they can protect against tumor progression by killing immunogenic neoplastic cells but, at the same time, they can also contribute to tumor escape and drug resistance by shaping tumor immunogenicity. Reactivation of the immune system using immune checkpoint inhibitors (ICIs) has emerged as a promising therapeutic strategy for many solid tumors and, more recently, for BC patients. BC has traditionally been considered an immunologically "cold" tumor with a low tumor mutational burden. However, among BC, TNBC and HER2+ subtypes exhibit certain indicators of immunogenicity, including Tumor Mutational Burden (TMB), high Tumor Infiltrating Lymphocytes (TILs), and expression of immunoinhibitory molecules. Preclinical studies demonstrating the enhanced immune-mediated effects of anti-HER2 monoclonal antibody therapy when combined with PD-1 antibodies, strongly support the addition of ICIs in HER2+ BC. Various immunotherapeutic strategies, including combinations of anti-HER2 therapy with ICIs and novel vaccines, are currently under investigation for the management of HER2+ BC (14). Nevertheless, none of these approaches has received regulatory approval to date. Padmanabhan et al. developed a mathematical model-based study demonstrating that the combination therapy of trastuzumab (anti-HER2 monoclonal antibody) and BMS-202 (anti-PD-1/PD-L1 small molecule inhibitor) significantly inhibits the growth of HER2+ BC cell lines, surpassing the efficacy of monotherapies, even in an immune cell-depleted environment. Results from *in vitro* monoculture experiments suggest that BMS-202 may suppress tumor growth not only by modulating the immune response but also by interfering with HER2+ BC growth signaling pathways. However, further studies are needed to demonstrate the potential interaction between PD-1/PD-L1 inhibitors and HER2 growth signaling pathways in BC.

In addition to genetic aberrations and the tumor microenvironment, environmental conditions, which are known to vary with changes in altitude, are relevant modulators of disease development and outcome (15). Chen et al. focused on BC patients at high altitudes who showed distinct characteristics in patient delay, BMI, tumor size, lymph node metastasis, and subtype distribution. This study highlights the complexity of factors influencing BC heterogeneity and suggests the need for a personalized therapeutic approach for patients living at high altitudes.

The prognosis of BC is influenced not only by the intrinsic characteristics of the tumor and its interactions with the microenvironment but also, particularly in the early stages, by the impact of surgical and radiotherapy (RT) treatments, along with patient adherence to medical therapy.

In the early stages, breast-conserving treatment or mastectomy are the surgical options. Given the increasing incidence of BC in young women and the limited evidence available regarding its management in this population (16), Pu et al. explored whether young patients (≤35 years old) might derive greater survival benefit from either breast-conserving surgery (BCS) or mastectomy. They performed a univariate and multivariate logistic regression analysis to identify independent factors influencing the benefit of BCS in young BC patients. According to the nomogram, among patients aged ≤35 years, those with older age, with lower T and N stages, and treated with postoperative RT without chemotherapy were more

likely to benefit from BCS. These findings provide clinicians with guidance for decision-making.

Adjuvant RT after BCS for early-stage BC is considered the standard treatment because it improves the survival rate and reduces the risk of recurrence. The supine position has been widely used for radiotherapy in BC, but some evidence suggests better cosmetic outcomes and lower rates of late toxicity in the prone position (17). Gao et al. compared the prone and supine positions to assess differences in dose distribution and normal organ sparing when using VMAT in these two positions. In addition, they aimed to identify the biotype that derives the greatest benefit from RT administered in the prone position. The greatest benefit of the prone position was reported in patients with right-sided BC, those characterized by a drooping breast shape, a larger breast and cup size, and, in particular, a larger chest height dimension.

Adjuvant endocrine therapy (AET) is a mainstay of treatment in the management of women with HR+ tumors. However, the side effects of AET pose a significant challenge for BC survivors, leading to irregular adherence and treatment interruptions, which may have detrimental effects on their overall survival (18). The review by Huifang et al. focuses on the mechanism of poor adherence to endocrine therapy in BC patients. Clinical data show that the neuro-immuno-endocrine mechanisms play a decisive role in the occurrence of adverse reactions leading to poor compliance. The rapid decrease in estrogen levels triggered by AIs within a short timeframe intensifies sympathetic activity, thereby modulating the release of inflammatory factors by diverse immune cells. Therefore, gaining a deeper understanding of the potential mechanisms underlying poor adherence during treatment could reveal pharmacological targets and guide early clinical intervention, aiming to improve adherence and maximize the benefits for BC patients.

In *de novo* metastatic disease, which accounts for approximately 6% of metastatic BC, locoregional therapy (LRT) is controversial with inconsistent results from randomized control trials (RCTs) (19). In their review, Merloni et al. examine all available data and aim to identify a specific patient subgroup that may derive the greatest benefit from LRT for the primary tumor. Even if the majority of RCTs did not support LRT of the primary tumor, this conclusion should be interpreted with caution in view of the limitations identified including small sample sizes and the utilization of outdated systemic therapies. Conversely, the results of some retrospective studies and one Turkish randomized trial suggest that patients with oligometastatic, bone-only disease, and HR-positive disease may be the best candidates for LRT. In this context, biomarkers such as circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) may be useful to better predict the metastatic disease course. Therefore, considering the advances in systemic therapies and radiotherapeutic/surgical methods the authors suggest designing further randomized trials, in which a properly selected population, and new biomarkers are strongly encouraged.

In conclusion, our Research Topic offers a comprehensive overview of various aspects of BC heterogeneity to unravel the complexity of BC. These efforts aim to lay the foundation for more effective and personalized diagnostic and therapeutic approaches. Continued research in this area is crucial, as it has the potential to guide future cancer therapy and ultimately improve outcomes.

Author contributions

FC: Writing – original draft. CS: Writing – review & editing. MO: Writing – review & editing. BP: Writing – review & editing. AD: Writing – review & editing. MB: Writing – review & editing.

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.

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References

1. Guo L, Kong D, Liu J, Zhan L, Luo L, Zheng W, et al. Breast cancer heterogeneity and its implication in personalized precision therapy. *Exp Hematol Oncol*. (2023) 12:3. doi: 10.1186/s40164-022-00363-1

2. 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) 71:209–49. doi: 10.3322/caac.21660

3. Orsini A, Diquigiovanni C, Bonora E. Omics technologies improving breast cancer research and diagnostics. *Int J Mol Sci*. (2023) 24:12690. doi: 10.3390/ijms241612690

4. Kalambo M, Adrada BE, Adeyefa MM, Krishnamurthy S, Hess K, Carkaci S, et al. Phyllodes tumor of the breast: ultrasound-pathology correlation. *AJR Am J Roentgenol*. (2018) 210:W173–9. doi: 10.2214/AJR.17.18554

5. Li Y, Yang D, Yin X, Zhang X, Huang J, Wu Y, et al. Clinicopathological characteristics and breast cancer-specific survival of patients with single hormone receptor-positive breast cancer. *JAMA Netw Open*. (2020) 3:e1918160. doi: 10.1001/jamanetworkopen.2019.18160

6. Dieci MV, Guarneri V. Should triple-positive breast cancer be recognized as a distinct subtype? *Expert Rev Anticancer Ther*. (2020) 20:1011–4. doi: 10.1080/14737140.2020.1829484

7. Iasonos A, Schrag D, Raj GV, Panageas KS. How to build and interpret a nomogram for cancer prognosis. *J Clin Oncol*. (2008) 26:1364–70. doi: 10.1200/JCO.2007.12.9791

8. Tarantino P, Hamilton E, Tolaney SM, Cortes J, Morganti S, Ferraro E, et al. HER2-low breast cancer: pathological and clinical landscape. *J Clin Oncol*. (2020) 38:1951–62. doi: 10.1200/JCO.19.02488

9. Thapa B, Arobelidze S, Clark BA, Xuefei J, Daw H, Cheng YC, et al. Metaplastic breast cancer: characteristics and survival outcomes. *Cureus*. (2022) 14:e28551. doi: 10.7759/cureus.28551

10. Gucalp A, Traina TA, Eisner JR, Parker JS, Selitsky SR, Park BH, et al. Male breast cancer: a disease distinct from female breast cancer. *Breast Cancer Res Treat*. (2019) 173:37–48. doi: 10.1007/s10549-018-4921-9

11. Hemminki K, Scélo G, Boffetta P, Mellemkjaer L, Tracey E, Andersen A, et al. Second primary Malignancies in patients with male breast cancer. *Br J Cancer*. (2005) 92:1288–92. doi: 10.1038/sj.bjc.6602505

12. Apostolou P, Fostira F. Hereditary breast cancer: the era of new susceptibility genes. *BioMed Res Int*. (2013) 2013:747318. doi: 10.1155/2013/747318

13. Stewart MD, Merino Vega D, Arend RC, Baden JF, Barbash O, Beaubier N, et al. Homologous recombination deficiency: concepts, definitions, and assays. *Oncologist*. (2022) 27:167–74. doi: 10.1093/oncolo/oyab053

14. Agostinetto E, Montemurro F, Puglisi F, Criscitiello C, Bianchini G, Del Mastro L, et al. Immunotherapy for HER2-positive breast cancer: clinical evidence and future perspectives. *Cancers (Basel)*. (2022) 14:2136. doi: 10.3390/cancers14092136

15. Coyle YM. The effect of environment on breast cancer risk. *Breast Cancer Res Treat*. (2004) 84:273–88. doi: 10.1023/B:BREA.0000019964.33963.09

16. Zhu JW, Charkhchi P, Adekunte S, Akbari MR. What is known about breast cancer in young women? *Cancers (Basel)*. (2023) 15:1917. doi: 10.3390/cancers15061917

17. Shah C, Al-Hilli Z, Vicini F. Advances in breast cancer radiotherapy: implications for current and future practice. *JCO Oncol Pract*. (2021) 17:697–706. doi: 10.1200/OP.21.00635

18. Rosso R, D'Alonzo M, Bounous VE, Actis S, Cipullo I, Salerno E, et al. Adherence to adjuvant endocrine therapy in breast cancer patients. *Curr Oncol*. (2023) 30:1461–72. doi: 10.3390/curroncol30020112

19. Reinhorn D, Mutai R, Yerushalmi R, Moore A, Amir E, Goldvaser H. Locoregional therapy in *de novo* metastatic breast cancer: Systemic review and meta-analysis. *Breast*. (2021) 58:173–81. doi: 10.1016/j.breast.2021.05.003

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Correlation of dynamic contrast-enhanced MRI and diffusion-weighted MR imaging with prognostic factors and subtypes of breast cancers

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Objective: To determine the preoperative magnetic resonance imaging (MRI) findings of breast cancer on dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and diffusion-weighted magnetic resonance imaging (DWI) in different molecular subtypes.

Materials and methods: A retrospective study was conducted on 116 breast cancer subjects who underwent preoperative MRI and surgery or biopsy. Three radiologists retrospectively assessed the morphological and kinetic characteristics on DCE-MRI and tumor detectability on DWI, by using apparent diffusion coefficient (ADC) values of lesions. The clinicopathologic and MRI features of four subtypes were compared. The correlation between clinical and MRI findings with molecular subtypes was evaluated using the chi-square and ANOVA tests, while the Mann–Whitney test was used to analyze the relationship between ADC and prognostic factors.

Results: One hundred and sixteen women diagnosed with breast cancer confirmed by surgery or biopsy had the following subtypes of breast cancer: luminal A (27, 23.3%), luminal B (56, 48.2%), HER2 positive (14, 12.1%), and triple-negative breast cancer (TNBC) (19, 16.4%), respectively. Among the subtypes, significant differences were found in axillary node metastasis, histological grade, tumor shape, rim enhancement, margin, lesion type, intratumoral T2 signal intensity, Ki-67 index, and paratumoral enhancement ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, and $p = 0.02$, respectively). On DWI, the mean ADC value of TNBC (0.910×10^{-3} mm^2/s) was the lowest compared to luminal A (1.477×10^{-3} mm^2/s), luminal B (0.955×10^{-3} mm^2/s), and HER2 positive (0.996×10^{-3} mm^2/s) ($p < 0.001$). Analysis of the correlation between different prognostic factors and ADC value showed that only axillary lymph node status and ADC value had a statistically significant difference ($p = 0.009$).

Conclusion: The morphologic features of MRI can be used as imaging biomarkers to identify the molecular subtypes of breast cancer. In addition,

quantitative assessments of ADC values on DWI may also provide biological clues about molecular subtypes.

KEYWORDS

ADC value, dynamic contrast-enhanced MR imaging, diffusion-weighted MR imaging, breast cancer, molecular subtypes

Introduction

Breast cancer is a group of heterogeneous diseases with different molecular subtypes, morphological features, clinical behaviors, and treatment responses. For a better patient-based approach, one of the most important indicators to evaluate disease and its prognosis is the molecular subtype, together with tumor size, histological grade, and the presence of metastatic axillary lymph nodes (1, 2). In addition to these, other standard histological factors are useful to determine different prognoses and management of the disease, including histological grade, the Ki-67 proliferation index, and the expression of the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) (3). By immunohistochemistry and fluorescence *in-situ* hybridization, the current commonly accepted molecular subtypes include luminal A (ER+/PR+/HER2–, Ki-67 < 15%), luminal B (ER +/PR+ or –/HER2 positive or negative, Ki-67 ≥ 15%), HER2-enriched (EP–/PR–, HER2 positive), and triple-negative breast cancer (TNBC) (ER–/RP–, HER2 negative). Several studies have confirmed that distinct molecular subtypes respond differently to therapy and are related to different prognoses: luminal A is usually the most common molecular subtype and typically confers the best prognosis, luminal B shows a good response to radiation therapy and has intermediate survival, and HER2-enriched and triple-negative breast cancer have a good response to chemotherapy but the worst overall survival (4, 5).

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is the most accurate and the highest sensitivity diagnostic imaging technique for detecting breast cancer, which might not be identified with mammography or ultrasound (6, 7). In the case of breast cancer, the ability to predict tumor molecular subtypes with imaging may provide an important contribution to clinical practice of early treatment planning and understanding of prognosis. Until now, very little is known about the diffusion-weighted MRI (DWI) characteristics of different subtypes of breast cancer (8). By studying the underlying biological and functional characteristics, DWI is expected to eventually improve our understanding of the subtypes of breast cancer, especially prognosis and treatment plans (9–13). The aim of our study was to investigate the MRI features of the molecular subtypes of cancer in patients using DCE-MRI and DWI.

Materials and methods

Patient selection

The local institutional review board approved this retrospective study, and the informed consent requirement was waived. A retrospective analysis was performed on 116 women aged 26–74 years who underwent breast magnetic resonance examination and have been submitted to biopsy or surgery with the diagnosis of breast cancer in our hospital from September 2017 to March 2022. The following exclusion criteria were applied: 1) patients treated with neoadjuvant chemotherapy; 2) patients with incomplete information on ER, PR, and HER2 status; and 3) those who dropped treatment or did not receive follow-up treatment in our hospital.

Histopathologic assessment

Serial slices of specimens from breast-conserving surgery or from mastectomy were analyzed by one pathologist who evaluated the size of the tumor, axillary node invasion, and histopathologic grade according to the Elston–Ellis classification and then classified the histotype according to the World Health Organization system. The tissue specimens were fixed with 10% formaldehyde, embedded in paraffin, sliced into 5-μm-thick sections, and stained with hematoxylin and eosin (HE). The receptor status was considered positive if the expression of each receptor was 10% or greater. In HER2 immunohistochemical staining, a score of 0 or 1+ was negative, 3+ was positive, and 2+ was equivocal, and the status of patients was verified using fluorescence *in-situ* hybridization (FISH), where FISH results were either positive or negative. Breast cancer was classified into four types according to the expression of ER/PR/HER2 in immunohistochemistry.

Imaging protocol

All breast MR examinations were performed using a 3.0-T MRI system (Signa Pioneer, GE Healthcare (Boston, USA)) in a

prone position using dedicated bilateral breast surface coils. Each study included a precontrast non-fat-saturated T1-weighted sequence, a precontrast fat-saturated T2-weighted sequence, and DWI (with two *b*-values, 0 and 1,000 s/mm^2). Gadolinium with meglumine Magenwijan (Guangzhou, China) was administered intravenously at 0.2 mmol/kg. The images were collected once before the contrast scan with 3D Vibrant technology (California, USA), and then eight images within 6 min should be collected after contrast injection. All the 3D Vibrant images used the ReadyView dynamic enhancement curve post-processing.

Image interpretation

Magnetic resonance imaging including DWI was independently reviewed by three radiologists (with 15, 9, and 6 years of experience in breast MRI, respectively), using the American College of Radiology BI-RADS (Breast Imaging Reporting and Data System) MR lexicon (14). All of them were blinded to clinical and pathologic information. The conclusions of the three radiologists were compared and discordances were resolved by consensus. The MR imaging findings were evaluated for lymph node involvement, morphological characteristics (margin, shape, T2 intensity), rim enhancement, and contrast enhancement kinetics, while kinetic analysis was evaluated with a time–intensity curve (TIC). TIC is based on a region of interest (ROI) that is plotted on the brightest enhancement region to avoid bleeding and necrosis. In the end, morphological manifestations, enhancement types, and TIC types of lesions were analyzed and recorded.

Statistical analysis

The chi-square test or Fisher's exact test was used to compare the clinicopathological features among the four tumor subtypes for categorical variables and the ANOVA test for continuous variables. Categorical data were presented as frequency and percentage, whereas continuous data were presented as mean and standard deviation. To evaluate the normality of the quantitative variable distributions, the Mann–Whitney test and the Kruskal–Wallis test were carried out. All analyses were performed using SPSS version 23 (SPSS Inc., SPSS®, Chicago, IL, USA), with p <0.05 considered to indicate a significant difference.

Results

Clinicopathological features

The clinicopathological features of the patients are summarized in Table 1. Of the 116 invasive breast cancers, 27 (23.3%) were classified as luminal A, 56 (48.2%) as luminal B, 14 (12.1%) as HER2-enriched, and 19 (16.4%) as TNBC. The mean age of the patients was 51.90 ± 10.68 years (range 26 to 74). In our study, invasive ductal carcinoma was the main pathologic type (107 cases, 92.3%), and there were 9 cases only (accounting for 7.7%) of invasive lobular carcinoma and other types of breast cancer. The highest histological grade (grade 3) was associated with HER2-enriched and TNBC compared to the luminal subtypes. Tumor histological grade was significantly different among the four subtypes (p < 0.001), as well as the mean Ki-67 index (p < 0.001) and the presence of axillary nodal status (p < 0.001). However, there were no differences in age and tumor sizes.

MR imaging features

In our study, mass lesions were the most commonly detected in MRI (94%). MR imaging features stratified by molecular subtypes are summarized in Table 2 and two cases are shown in Figures 1, Figure 2. On DCE-MRI, the differences in tumor shape, internal enhancement mode, tumor margin, tumor type, and intratumoral T2 signal intensity among the molecular types were statistically significant between groups (p < 0.001, p < 0.001,

TABLE 1 Clinicopathological features stratified by molecular subtypes.

Tumor subtype	Luminal A	Luminal B	HER2-enriched	TNBC	*p*-value
Patient age (years)	50.74 ± 11.19	52.33 ± 10.92	51.07 ± 6.70	52.84 ± 12.13	0.889
Ki-67 index	7.78 ± 2.53	46.88 ± 21.52	48.93 ± 19.13	53.42 ± 25.44	<0.001
Histological grade					<0.001
Grade 1	17 (63%)	3 (5.3%)	1 (7.1%)	2 (10.5%)	
Grade 2	9 (33.3%)	30 (53.6%)	4 (28.6%)	3 (15.8%)	
Grade 3	1 (3.7%)	23 (41.1%)	9 (64.3%)	14 (73.7%)	
Axillary lymph node					<0.001
Positive	5 (18.5%)	36 (64.3%)	11 (78.6%)	10 (52.6%)	
Negative	22 (81.5%)	20 (35.7%)	3 (21.4%)	9 (47.4%)	

TABLE 2 MR imaging features stratified by molecular subtypes.

Tumor subtype(case)	Luminal A(27)	Luminal B(56)	HER2-enriched(14)	TNBC(19)	P value
Shape					<0.001
Regular	6 (22.2%)	9 (16.1%)	5 (35.7%)	14 (73.7%)	
Irregular	21 (77.8%)	47 (83.9%)	9 (64.3%)	5 (26.3%)	
Internal **enhancement**					<0.001
Rim	5 (18.5%)	36 (64.3%)	11 (78.6%)	14 (73.7%)	
Heterogeneous	22 (81.5%)	20 (35.7%)	3 (21.4%)	5 (26.3%)	
Tumor **number**					0.284
Unifocal	8 (29.6%)	24 (42.9%)	5 (35.7%)	15 (78.9%)	
Multifocal	19 (70.4%)	32 (57.1%)	9 (64.3%)	4 (21.1%)	
Margin					<0.001
Smooth	7 (25.9%)	7 (12.5%)	3 (21.4%)	17 (89.5%)	
Irregular	20 (74.1%)	49 (87.5%)	11 (78.6%)	2 (10.5%)	
Lesion **type**					<0.001
Mass	24 (88.9%)	52 (92.9%)	14 (100%)	19 (100%)	
Non-mass	3 (11.1%)	4 (7.1%)	0 (0)	0 (0)	
Kinetic **curve pattern**					0.46
Persistent	0 (0)	0 (0)	0 (0)	0 (0)	
Plateau	12 (44.4%)	17 (30.3%)	5 (35.7%)	9 (47.4%)	
Washout	15 (55.6%)	39 (69.7%)	9 (64.3%)	10 (52.6%)	
Tumor size (cm)					0.755
<2	7 (25.9%)	12 (21.4%)	3 (21.4%)	6 (31.6%)	
≥2,<5	17 (63.0%)	31 (55.4%)	8 (57.2%)	8 (42.1%)	
≥5	3 (11.1%)	13 (23.2%)	3 (21.4%)	5 (26.3%)	
Paratumoral enhancement					0.02
Yes	10 (37.0%)	40 (71.4%)	10 (71.4%)	13 (68.4%)	
No	17 (63.0%)	16 (28.6%)	4 (28.6%)	6 (31.6%)	
Intratumoral SI on T2WI					<0.001
Low	4 (14.8%)	7 (12.5%)	3 (21.4%)	0 (0)	
Equal	19 (70.4%)	45 (80.4%)	10 (71.4%)	2 (10.5%)	
High/Very high	4 (14.8%)	4 (7.1%)	1 (7.2%)	17 (89.5%)	
ADC value ($\times 10^{-3}$ mm^2/s)	1.477 ± 0.380	0.955 ± 0.190	0.996 ± 0.116	0.910 ± 0.184	<0.001
	(0.649-2.204)	(0.575-1.464)	(0.830-1.262)	(0.654-1.347)	

$p < 0.001$, $p < 0.001$, respectively). Compared to other molecular types, TNBC was more likely to present a regular shape (73.7%), rim enhancement (73.7%), unifocal tumor (78.9%), smooth margin (89.5%), and higher intratumor enhancement of T2 by Bonferroni-adjusted multiple comparisons (89.5%). Moreover, we found that all TNBC patients presented with medium/high T2 signal. Although TNBC in the study was more frequently detected as unifocal lesions than other subtypes (78.9%), the difference was not statistically significant. A detailed analysis of the kinetic curves has shown that all cases have a similar behavior, reaching a plateau before washing out. After dividing the cases into three groups with respect to the tumor diameter (<2, ≥2, <5, ≥5 cm), it was found that there was no significant difference in the distribution of each curve among subgroups as well as tumor size. In addition, the comparative analysis of paratumor signal intensity showed statistically significant differences among subtypes ($p = 0.02$), which could be better used for molecular typing identification.

Correlation between the prognostic factors and apparent diffusion coefficient values

On DWI, the mean apparent diffusion coefficient (ADC) value of TNBC (0.910×10^{-3} mm^2/s) was lower compared to the mean ADC values for luminal A, luminal B, and HER2+ (1.477, 0.955, and 0.996; $p < 0.001$) (Table 2). The correlation between the prognostic factors and ADC values is summarized in Table 3. The average ADC values of the ER-positive (84, 72.4%) and PR-positive (72, 62.1%) groups were greater than those of the ER- and PR-negative ones (0.993×10^{-3} vs. 0.941×10^{-3} mm^2/s,

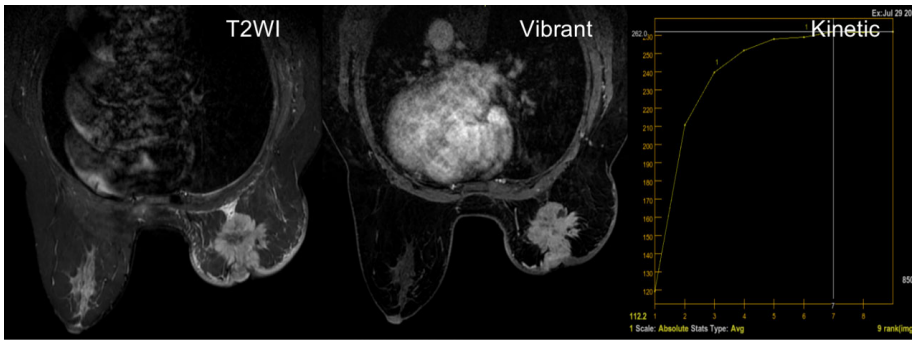

FIGURE 1
A 53-year-old woman diagnosed with invasive ductal carcinoma of the luminal B subtype. The T2-weighted image shows a strong hyperintense signal inside the mass without enhancement on subtracted images, representing necrosis. The Vibrant technology shows an irregular mass with an irregular margin and a heterogeneous enhancement. Kinetic curves generated from two regions of the enhanced ring demonstrate a plateau appearance (type II curve).

1.002×10^{-3} vs. 0.940×10^{-3} mm^2/s). However, the difference between the ADC values of the HER2 and axillary lymph nodes under different states was higher in the negative group (1.001×10^{-3} vs. 0.923×10^{-3} mm^2/s, 1.078×10^{-3} vs. 0.892×10^{-3} mm^2/s). The difference was statistically significant only in axillary lymph node status ($p = 0.009$).

Discussion

Knowing the molecular subtypes of breast cancer is key to defining a correct, patient-oriented plan. The different molecular subtypes of breast cancer could have different initial symptoms and metastatic spread and respond differently to radiotherapy and chemotherapy (15). These findings suggest that diagnostic tests, treatment strategies, and surveillance may better guide the collection of information from each patient's specific molecular subtype of breast cancer. Our study provides an additional step in that direction by identifying clinical findings between different molecular subtypes, which may help guide the preoperative use of breast MR imaging.

The cancer subtype has been shown to be a key condition to determine the correct treatment. As of today, though, the existence of axillary lymph node metastasis still determines the treatment sequence (preoperative vs. postoperative), the type of therapy (endocrine, chemotherapy, and/or targeted therapy), and the drugs and cycle used (16, 17). Lymph node status is also helpful to estimate the prognosis and the consequent benefits of systemic therapies. The clinical approaches to the assessment and treatment of axillary breast cancer in the early stage are evolving and are guided by studies supporting less aggressive surgery (18) and more aggressive radiotherapy for lymph node-positive disease (19). However, the relationship between tumor subtypes and axillary lymph node status is

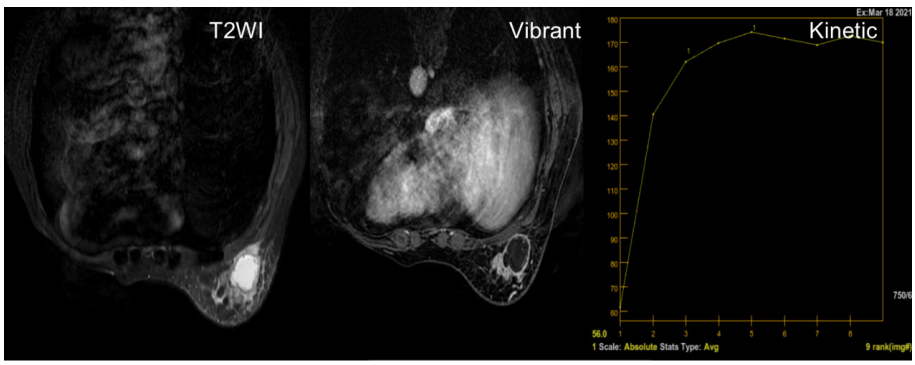

FIGURE 2
A 50-year-old woman with a solitary hyperintensity in T2 lesion, with rim enhancement in Vibrant and a type II kinetic curve (plateau).

TABLE 3 The correlation between the prognostic factors and ADC values.

Prognostic factors	Case	ADC value ($\times 10^{-3}$ mm^2/s)	*p*-value
ER			0.956
Positive	84 (72.4%)	0.993 ± 0.352 (0.429–2.204)	
Negative	32 (27.6%)	0.941 ± 0.179 (0.654–1.347)	
PR			0.959
Positive	72 (62.1%)	1.002 ± 0.377 (0.429–2.204)	
Negative	44 (37.9%)	0.940 ± 0.162 (0.654–1.347)	
HER2			0.553
Positive	33 (28.4%)	0.923 ± 0.171 (0.575–1.283)	
Negative	83 (71.6%)	1.001 ± 0.354 (0.429–2.204)	
Axillary lymph node			0.009
Positive	62 (53.4%)	0.892 ± 0.209 (0.429–1.361)	
Negative	54 (46.6%)	1.078 ± 0.381 (0.456–2.204)	

currently unclear. Previous studies have had conflicting results on the incidence of lymph node metastasis in TNBC, with no clear evidence of increase in axillary lymph node metastasis in more aggressive tumors (20, 21). Our study found that pathological analysis confirmed the presence of metastatic lymph node metastasis in luminal B and HER2-positive breast cancer, which was consistent with Grimm et al. Because HER2 and luminal B subtypes are easier to diagnose, the clinical use of MRI to help guide treatment plans such as axillary management and systemic therapy may be more effective for HER2 and luminal B subtypes and may influence clinical outcomes (22).

Breast cancer subtypes have some specific imaging features. From the literature, we know that some particular characteristics of TNBC can be found on breast MRI, such as regular shape, smooth edge, rim enhancement, unifocal lesion, higher histological grade, and high intratumoral signal intensity on T2-weighted images (23–27). In contrast, the luminal type of breast cancer showed more irregular-shaped masses on MRI (24, 27), which was consistent with our findings. In our study, 73.7% of TNBC showed rim enhancement. Navarro Vilar et al. (27) confirmed that 68.7% of TNBC tumors had rim enhancement. Based on this conclusion, the authors pointed out that rim enhancement of the mass is the most useful finding for predicting TNBC. According to relevant literature, the incidence of rim enhancement in TNBC varies from 41% to 80% (7, 24, 27), and our findings are also within this range. Meanwhile, we found irregular margin features, homogeneous enhancement, and medium/low T2 signal intensity within the tumor associated with luminal subtypes. These findings are similar to other studies in the literature (23, 27). Due to the different intensities of tissue hyperplasia response, high-grade and fast-growing masses have a well-defined margin, while low-grade and slow-growing masses have a poorly defined margin and are spiculated, which may be explained by the desmoplastic reaction in adjacent breast tissues. This is the main reason for the detection of different morphological characteristics in different subtypes. What is striking is that morphological features such as round shape, circumscribed margin, and increased T2 signal intensity are also indicators of benign breast lesions (28). It should be kept in mind when evaluating breast MRI that these features are common in invasive breast cancer subtypes.

DCE-MRI has high sensitivity in assessing breast cancer, but there are differences in specificity. DWI can improve the diagnostic accuracy of DCE-MRI, and it is usually used as a component of multiparameter imaging to evaluate breast cancer (29–33). Some studies have reported the relationship between ADC values and prognostic factors in other subtypes of breast cancer, except TNBC (34, 35). Studies have reported that in luminal breast cancer, the average ADC value in the high proliferation group was significantly lower than that in the low proliferation group (36). However, few studies have reported the relationship between ADC value and the prognostic factors of breast cancer. In our study, the mean value of ADC was lower in the positive axillary lymph node, HER2-positive, ER-negative, and PR-negative groups. Although such difference was statistically significant only in the axillary lymph node status group, we assumed that the difference between ADC value and other prognostic factors may also be meaningful within a larger sample. A low ADC value is known as a hallmark of malignancy (28, 37). On this premise, we boldly hypothesized that ADC value might be a prognostic indicator of breast cancer.

As we all know, breast cancer is a heterogeneous disease, so early detection may be more helpful in clinical practice, such as early treatment planning and follow-up strategies. Unfortunately, the molecular typing of breast cancer can only be determined by the histopathological assessment of receptor status. Studies have shown that different molecular subtypes can be predicted by imaging findings, such as the shape of mass lesions, rim features, T2 signal intensity, and contrast enhancement features (23–26, 28–31). However, to our knowledge, there is no formal diagnostic method based on MRI.

Different from past research, we not only compared tumor lesions on MRI but also observed specific differences in the signal performance of surrounding tissues. The comparative analysis of paratumor signal intensity showed statistically significant differences among subtypes, which could help us better conduct molecular typing. Furthermore, all patients in our study received 3.0 T MRI. Compared to 1.5 T, our image resolution and quality were better, and these greatly enhanced the credibility of our study.

However, our study also had limitations. The biggest limitation was that the sample size was relatively small, with a limited number of some tumor subtypes. Secondly, this was a retrospective study and all the data were from a single institution, which may lead to selection bias. Finally, our study design did not collect patient prognostic data, which will be an important next step in evaluating the relationship between molecular subtypes and preoperative MRI.

Conclusions

In summary, breast cancer subtypes, especially TNBC, exhibit multiple characteristic MRI features on DCE-MRI. With advances in imaging technology, the morphologic features of MRI can be used as imaging biomarkers to identify the molecular subtypes of breast cancer in the future. In addition, quantitative assessments of ADC values on DWI may also provide biological clues about molecular subtypes. Of course, a multicenter study with a larger sample size is needed to investigate this issue.

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

CW: conceptualization, methodology, and visualization. WL: supervision. JZ: data curation, writing—original draft preparation, and investigation. HC: writing—review and editing. All authors contributed to the article and approved the submitted version.

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. Yamamoto Y, Iwase H. Clinicopathological features and treatment strategy for triple-negative breast cancer. *Int J Clin Oncol* (2010) 15:341–51. doi: 10.1007/s10147-010-0106-1

2. De Ronde JJ, Hannemann J, Halfwerk H, Mulder L, Straver ME, Vrancken Peeters MJ, et al. Concordance of clinical and molecular breast cancer subtyping in the context of preoperative chemotherapy response. *Breast Cancer Res Treat* (2010) 119(1):119–26. doi: 10.1007/s10549-009-0499-6

3. Montagna E, Bagnardi V, Rotmensz N, Viale G, Cancello G, Mazza M, et al. Immunohistochemically defined subtypes and outcome in occult breast carcinoma with axillary presentation. *Breast Cancer Res Treat* (2011) 129(3):867–75. doi: 10.1007/s10549-011-1697-6

4. Tran B, Bedard PL. Luminal-b breast cancer and novel therapeutic targets. *Breast Cancer Res* (2011) 13(6):221. doi: 10.1186/bcr2904

5. Lam SW, Jimenez CR, Boven E. Breast cancer classification by proteomic technologies: current state of knowledge. *Cancer Treat Rev* (2014) 40(1):129–38. doi: 10.1016/j.ctrv.2013.06.006

6. Li SP, Padhani AR, Taylor NJ, Beresford MJ, Ah-See ML, Stirling JJ, et al. Vascular characterisation of triple negative breast carcinomas using dynamic MRI. *Eur Radiol* (2011) 21(7):1364–73. doi: 10.1007/s00330-011-2061-2

7. Dogan BE, Gonzalez-Angulo AM, Gilcrease M, Dryden MJ, Yang WT. Multimodality imaging of triple receptor-negative tumors with mammography, ultrasound, and MRI. *AJR Am J Roentgenol* (2010) 194(4):1160–6. doi: 10.2214/AJR.09.2355

8. Mao C, Jiang W, Huang J, Wang M, Yan X, Yang Z, et al. Quantitative parameters of diffusion spectrum imaging: HER2 status prediction in patients with breast cancer. *Front Oncol* (2022) 12:817070. doi: 10.3389/fonc.2022.817070

9. Leithner D, Bernard-Davila B, Martinez DF, Horvat JV, Jochelson MS, Marino MA, et al. Radiomic signatures derived from diffusion-weighted imaging for the assessment of breast cancer receptor status and molecular subtypes. *Mol Imaging Biol* (2020) 22(2):453–61. doi: 10.1007/s11307-019-01383-w

10. Podo F, Buydens LM, Degani H, Hilhorst R, Klipp E, Gribbestad IS, et al. Triple-negative breast cancer: present challenges and new perspectives. *Mol Oncol* (2010) 4(3):209–29. doi: 10.1016/j.molonc.2010.04.006

11. Youk JH, Son EJ, Chung J, Kim JA, Kim EK. Triple-negative invasive breast cancer on dynamic contrast-enhanced and diffusion-weighted MR imaging: comparison with other breast cancer subtypes. *Eur Radiol* (2012) 22(8):1724–34. doi: 10.1007/s00330-012-2425-2

12. Horvat JV, Bernard-Davila B, Helbich TH, Zhang M, Morris EA, Thakur SB, et al. Diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) mapping as a quantitative imaging biomarker for prediction of immunohistochemical receptor status, proliferation rate, and molecular subtypes of breast cancer. *J Magn Reson Imaging* (2019) 50(3):836–46. doi: 10.1002/jmri.26697

13. Martincich L, Deantoni V, Bertotto I, Redana S, Kubatzki F, Sarotto I, et al. Correlations between diffusion-weighted imaging and breast cancer biomarkers. *Eur Radiol* (2012) 22(7):1519–28. doi: 10.1007/s00330-012-2403-8

14. American College of Radiology. *Breast Imaging Reporting and Data System (BI-RADS). 5rd* ed. Va Reston: American College of Radiology (2013).

15. Li J, Chen Z, Su K, Zeng J. Clinicopathological classification and traditional prognostic indicators of breast cancer. *Int J Clin Exp Pathol* (2015) 8(7):8500–5.

16. 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(9):2206–23. doi: 10.1093/annonc/mdt303

17. Theriault RL, Carlson RW, Allred C, Anderson BO, Burstein HJ, Edge SB, et al. National comprehensive cancer network. breast cancer, version 3.2013: featured updates to the NCCN guidelines. *J Natl Compr Canc Netw* (2013) 11 (7):753–60. doi: 10.6004/jnccn.2013.0098

18. 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) 305(6):569–75. doi: 10.1001/jama.2011.90

19. Li Y, Moran MS, Huo Q, Yang Q, Haffty BG. Post-mastectomy radiotherapy for breast cancer patients with t1-t2 and 1-3 positive lymph nodes: a meta-analysis. *PLoS One* (2013) 8(12):e81765. doi: 10.1371/journal.pone.0081765

20. Kim JJ, Kim JY, Suh HB, Hwangbo L, Lee NK, Kim S, et al. Characterization of breast cancer subtypes based on quantitative assessment of intratumoral heterogeneity using dynamic contrast-enhanced and diffusion-weighted magnetic resonance imaging. *Eur Radiol* (2022) 32(2):822–33. doi: 10.1007/s00330-021-08166-4

21. 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

22. Grimm LJ, Johnson KS, Marcom PK, Baker JA, Soo MS. Can breast cancer molecular subtype help to select patients for preoperative MR imaging? *Radiology* (2015) 274(2):352–8. doi: 10.1148/radiol.14140594

23. Sung JS, Jochelson MS, Brennan S, Joo S, Wen YH, Moskowitz C, et al. MR imaging features of triple-negative breast cancers. *Breast J* (2013) 19(6):643–9. doi: 10.1111/tbj.12182

24. Costantini M, Belli P, Distefano D, Bufi E, Matteo MD, Rinaldi P, et al. Magnetic resonance imaging features in triple-negative breast cancer: comparison with luminal and HER2-overexpressing tumors. *Clin Breast Cancer* (2012) 12 (5):331–9. doi: 10.1016/j.clbc.2012.07.002

25. Angelini G, Marini C, Iacconi C, Mazzotta D, Moretti M, Picano E, et al. Magnetic resonance (MR) features in triple negative breast cancer (TNBC) vs receptor positive cancer (nTNBC). *Clin Imaging* (2018) 49:12–6. doi: 10.1016/j.clinimag.2017.10.016

26. Öztürk VS, Polat YD, Soyder A, Tanyeri A, Karaman CZ, Taşkın F. The relationship between MRI findings and molecular subtypes in women with breast cancer. *Curr Probl Diagn Radiol* (2020) 49(6):417–21. doi: 10.1067/j.cpradiol.2019.07.003

27. Navarro Vilar L, Alandete Germán SP, Medina García R, Blanc García E, Camarasa Lillo N, Vilar Samper J. MR imaging findings in molecular subtypes of breast cancer according to BIRADS system. *Breast J* (2017) 23(4):421–8. doi: 10.1111/tbj.12756

28. Tezcan S, Ozturk FU, Uslu N, Akcay EY. The role of combined diffusion-weighted imaging and dynamic contrast-enhanced MRI for differentiating malignant from benign breast lesions presenting washout curve. *Can Assoc Radiol J* (2021) 72(3):460–9. doi: 10.1177/0846537120907098

29. Xie T, Zhao Q, Fu C, Bai Q, Zhou X, Li L, et al. Differentiation of triple-negative breast cancer from other subtypes through whole-tumor histogram analysis on multiparametric MR imaging. *Eur Radiol* (2019) 29(5):2535–44. doi: 10.1007/s00330-018-5804-5

30. Montemezzi S, Camera L, Giri MG, Pozzetto A, Caliò A, Meliadò G, et al. Is there a correlation between 3T multiparametric MRI and molecular subtypes of breast cancer? *Eur J Radiol* (2018) 108:120–7. doi: 10.1016/j.ejrad.2018.09.024

31. Suo S, Cheng F, Cao M, Kang J, Wang M, Hua J, et al. Multiparametric diffusion-weighted imaging in breast lesions: Association with pathologic diagnosis and prognostic factors. *J Magn Reson Imaging* (2017) 46(3):740–50. doi: 10.1002/jmri.25612

32. Horvat JV, Iyer A, Morris EA, Apte A, Bernard-Davila B, Martinez DF, et al. Histogram analysis and visual heterogeneity of diffusion-weighted imaging with apparent diffusion coefficient mapping in the prediction of molecular subtypes of invasive breast cancers. *Contrast Media Mol Imaging* (2019) 2019:2972189. doi: 10.1155/2019/2972189

33. Park SH, Choi HY, Hahn SY. Correlations between apparent diffusion coefficient values of invasive ductal carcinoma and pathologic factors on diffusion-weighted MRI at 3.0 Tesla. *J Magn Reson Imaging* (2015) 41(1):175–82. doi: 10.1002/jmri.24519

34. Choi SY, Chang YW, Park HJ, Kim HJ, Hong SS, Seo DY. Correlation of the apparent diffusion coefficiency values on diffusion-weighted imaging with prognostic factors for breast cancer. *Br J Radiol* (2012) 85(1016):e474–9. doi: 10.1259/bjr/79381464

35. Kim SY, Kim EK, Moon HJ, Yoon JH, Koo JS, Kim SG, et al. Association among T2 signal intensity, necrosis, ADC and ki-67 in estrogen receptor-positive and HER2-negative invasive ductal carcinoma. *Magn Reson Imaging* (2018) 54:176–82. doi: 10.1016/j.mri.2018.08.017

36. Mori N, Ota H, Mugikura S, Takasawa C, Ishida T, Watanabe G, et al. Luminal-type breast cancer: correlation of apparent diffusion coefficients with the ki-67 labeling index. *Radiology* (2015) 274(1):66–73. doi: 10.1148/radiol.14140283

37. Che SN, Li J, Xue M, Song Y, Zhao LY, Guo N, et al. The value of synthetic MRI in differential diagnosis of benign and malignant breast lesions. *Zhonghua Zhong Liu Za Zhi* (2021) 43(8):872–7. doi: 10.3760/cma.j.cn112152-20210322-00254

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A nomogram to identify appropriate candidates for breast-conserving surgery among young women with breast cancer: A large cohort study

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Background: There is a gradual increase of female breast cancer under 35 years old, who was characterized as poor prognosis. Whether young patients could obtain greater survival benefits from breast-conserving surgery (BCS) than mastectomy remains controversial.

Methods: Breast cancer patients (≤35 years old) were selected from the Surveillance, Epidemiology, and End Results (SEER) database and divided into BCS and mastectomy group. Propensity score matching (PSM) was used to eliminate the distributional imbalance of variables among two groups. The influence of BCS on overall survival (OS) and breast cancer-specific survival (BCSS) was evaluated by Cox regression. Logistic regression was used to identify factors related to the benefit of BCS and to construct a nomogram. The nomogram was validated by the First Affiliated Hospital of Xi'an Jiaotong University cohort.

Results: Totally, 15,317 cases in the SEER database and 149 cases of external validation cohort were included. BCS was an independent protective factor for OS (P = 0.028) and BCSS (P = 0.042). A nomogram was established, and the AUC values both in the internal and external validation set were 0.780. The applicability of the model was verified in the PSM cohort and indicated that the survival advantage in the BCS-Benefit group was higher than that in the BCS-Nonbenefit and mastectomy group (P <0.001).

Conclusions: For young breast cancer patients, BCS may bring better OS and BCSS than mastectomy, but not all benefit from it. We constructed a model for

young patients (≤35 years old) that could identify appropriate candidates who benefit from BCS.

KEYWORDS

young breast cancer, breast-conserving surgery, mastectomy, survival, nomogram

Introduction

The incidence of breast cancer in young women has been increasing since the mid-1990s and has become a leading cause of cancer death in them (1).There is no consensus on a cutoff age value for defining young women with breast cancer by Eastern and Western scholars. The European Society for Medical Oncology (ESMO) used <40 years old as cutoff age (2), while Chinese researchers regard 35 years old as a reasonable cutoff age. In addition, considering that there is a significant incidence age difference of breast cancer in the worldwide: the average age of breast cancer diagnosis is 45–55 years in China (3), which is 10 years younger than that in Western countries. Therefore, we choose the patients <=35 years old for analysis. Many studies have shown that age <=35 years old was an independent risk factor for local recurrence of breast cancer (4, 5). A previous cohort study reported that the overall survival (OS) and the breast cancer-specific survival (BCSS) rates of patients aged 30 and 30-39 years old were significantly lower than those who were 40-49 or 50-59 (6). The reasons for the poor prognosis in young women with breast cancer are complex, the most important being the more aggressive nature of it, including a high proportion of triple-negative, human epidermal growth factor receptor 2 (HER2) overexpression, grade 3, lymphovascular invasion, and lymphocytic infiltration (7).

The option for local surgical treatment has a significant impact on the prognosis of breast cancer patients. The NSABP B-06 demonstrated that survival outcomes after breast-conserving surgery (BCS) combined with radiotherapy (BCT) were equivalent to those after mastectomy for those with early breast cancer (8). Moreover, A large cohort study found that BCT improved 10-year OS compared with mastectomy (9). However, whether young patients obtain a greater survival benefit from BCS than mastectomy remains controversial. Some analyses of outcomes in young patients who underwent BCS versus mastectomy showed no significant differences in the risk of mortality (10–13). Moreover, some studies have reported that those younger than 35 had an independent risk factor for local recurrence after undergoing BCS (5, 8, 14–17). More recently, several studies have found that patients who underwent BCT have a survival benefit compared to those receiving a mastectomy (9, 18–20).To our knowledge, there are no studies to determine who is more likely to benefit from BCS.

This study aimed to determine who benefits from BCS by extracting breast cancer patients under the age of 35 from the Surveillance, Epidemiology, and End Results (SEER) database for retrospective analysis. Logistic regression was used to screen out factors related to the benefit of BCS and constructed a nomogram. In addition, a cohort from the First Affiliated Hospital of Xi'an Jiaotong University was used for confirmation of the findings. Finally, suitable candidates for BCS were identified and referred for clinical treatment.

Materials and methods

Study population and data collection

Figure 1 shows the process of case screening and analysis. We obtained the data of young female patients with stage T1-3 breast cancer from 1995 to 2016 in the SEER database. The included data were demographic characteristics (age, race, and marital status), tumor-related characteristics (laterality of tumor, grade, histological type, TNM stage, surgical approach, radiation, chemotherapy, and molecular subtype), and follow-up information (survival time and status). Cases with the following characteristics were excluded (1): age > 35 years old (2); male breast cancer (3); bilateral breast cancer (4); distant metastasis (5); follow-up time less than one month (6); incomplete case information. We included 15,317 cases from the SEER database for retrospective analysis. In addition, we also extracted 149 young breast cancer cases who met the inclusion criteria from the First Affiliated Hospital of Xi'an Jiaotong University from 2015 to 2020 as an external validation cohort. The endpoints of this study were OS and BCSS. No intervention or treatment is conducted to patients and the data from SEER database is publicly available, so informed consent is waived in this study.

Evaluation of the independent protective effect of BCS on prognosis

We divided the samples into two groups according to the surgical approach: the BCS group and the mastectomy group. To adjust for unbalanced variable distributions between the two groups, we performed propensity score matching (PSM) (21) for

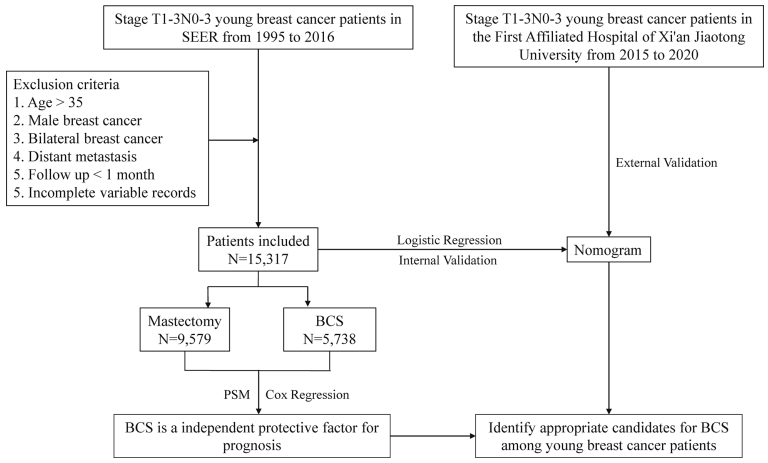

FIGURE 1
Flow diagram of data analysis.

age, race, marital status, laterality, grade, histology type, AJCC T stage, N stage, radiation, chemotherapy, and subtype. Patients who received a BCS were matched 1:1 on propensity scores with those who received a mastectomy. The standardized mean difference (SMD) was used to evaluate the difference in distribution between the groups for each variable (22). SMD <10% indicated no significant difference. We next observed the differences in OS and BCSS between the two groups before and after PSM using the Kaplan-Meier (KM) survival analysis. Among the PSM cohort, we performed univariate and multivariate Cox regression analyses to evaluate the independent protective effect of BCS on OS and BCSS.

Construction and validation of a screening nomogram

Entire cohort were randomly divided into training set and validation set in a ratio of 7:3. We performed univariate and multivariate Logistic regression analysis to screen independent predictors of the benefit of BCS in the training set, with a threshold of P <0.05. *A* nomogram was then constructed based on the results to quantify the likelihood of a benefit from BCS in young patients and to screen possible candidates for receiving it. Next, we validated the predictive performance of the model on the validation set and external cohort. The discrimination and calibration of the model were evaluated by the time-dependent area under the receiver operating characteristic (ROC) curve (AUC) and calibration curve, respectively. Concurrently, we generated decision curve analysis (DCA) to assess the clinical utility of the model (23). In addition, using the 50% likelihood of benefit based on the score of each patient calculated by the nomogram, we divided the patients in the BCS cohort into two groups: the BCS-Benefit group (benefit possibility>50%) and the BCS-Nonbenefit one (benefit possibility <=50%). The KM survival analysis was performed to compare the OS of patients in the BCS-Benefit, BCS-Nonbenefit, and mastectomy groups to determine if the model could quantify the benefit probability of BCS and identify candidates for receiving it.

Statistical analysis

The demographic and clinicopathological characteristics were compared using Pearson's chi-square test. BCSS and OS were observed by Kaplan-Meier analysis and Cox regression analysis, and the survival outcomes were compared using the log-rank test. Logistic regression analysis was used to screen out independent predictors of the benefit of BCS. All statistical analyses were performed with R software (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria). A two-tailed P <0.05 was considered statistically significant.

Results

Demographic and clinicopathological features of the patients

We included 15,317 cases from the SEER database (Table 1) and 149 cases from the First Affiliated Hospital of Xi'an Jiaotong University (Supplementary Table 1) for this analysis. As shown in Table 1, 5,738 (37.5%) patients received BCS and 9,579 (62.5%) patients received mastectomy. Most patients were white with IDC histology. Patients with low T stage, N stage,

and that had received radiation and chemotherapy accounted for the majority. Most variables before PSM were distributed differently between the two groups (SMD >10%). The unbalance distribution was adjusted for all covariates after PSM, and the 3,625 patients that had BCS were matched with 3625 who had a mastectomy. As shown in Figure 2, all demographic and clinicopathological characteristics, including age, race, marital status, laterality, histology type, grade, T stage, N stage, radiation, chemotherapy, and molecular subtype, were all balanced between the two groups (SMD <10%).

Influence of BCS on the prognosis among PSM cohort

The OS and BCSS before and after PSM of young breast cancer patients are shown in Figure 3. The results revealed that those receiving BCS had a better OS (Figures 3A, B). Similarly, those receiving BCS also produced beneficial outcomes for BCSS (Figures 3C, D). The detailed 3-year, 5-year, and 10-year OS and BCSS rates are shown in Table 2. We also determined the effect of receiving BCS on the prognosis of young breast cancer patients and performed univariate and multivariate Cox regression analysis on OS (Table 3) and BCSS (Supplementary Table 2). The regression analyses indicated that receiving BCS was a significantly protective factor for OS (mastectomy vs. BCS; HR = 1.127, 95% confidence interval (CI): 1.013–1.254, P = 0.028) and BCSS (mastectomy vs. BCS, HR = 1.126; 95% CI: 1.004–1.263, P = 0.042). Moreover, other variables such as age, race, grade, T stage, N stage, and molecular subtype were also independent prognostic factors in young breast cancer patients. However, radiation and chemotherapy were not independent factors for OS and BCSS.

A nomogram to quantify the benefits of BCS

We conducted univariate and multivariate Logistic regression analysis to identify independent factors influencing the benefit of BCS in young breast cancer patients. The age (P = 0.002), marital status (P < 0.001), T stage (P < 0.001), N stage (P < 0.001), radiation (P < 0.001), and chemotherapy (P < 0.001) were screened out as independent influencing factors (Table 4). Based on these variables, we established a nomogram to identify candidates for BCS in young patients with T1-3 and N0-3 breast cancer (Figure 4). The probability of benefit from BCS was calculated according to the total points in the nomogram (Supplementary Tables 3 and 4). ROC and calibration curves were generated to evaluate the discrimination and calibration. The AUC values in the training and validation sets were 0.790 (Figure 5A) and 0.780 (Figure 6A), respectively. In the external validation cohort, the model also achieved an AUC value of 0.780 (Figure 7A). The calibration curves in the three cohorts indicated that the nomogram has a good prediction ability (Figures 5B, 6B, 7B), with the predicted probability being highly consistent with the actual observed probability. In addition, the DCA curve confirmed the clinical utility of the nomogram (Figures 5C, 6C, 7C).

Finally, we verified the use of the model in the PSM cohort. Based on the risk score in the nomogram, 1,259 patients were classified in the BCS-Benefit group, and 2,366 patients were classified in the BCS-Nonbenefit group. The KM survival curves were generated to observe the difference in survival benefits between groups (Figure 8). The results showed that the survival advantage of patients in the BCS-Benefit group was higher than that in the BCS-Nonbenefit or mastectomy ones (P <0.001). Moreover, there was no significant difference in OS between the BCS-non-benefit group and mastectomy one (P =0.700). These results indicated that not all young breast cancer patients benefit from BCS, and some have an equal benefit to a mastectomy.

Discussion

In recent years, the incidence of breast cancer in women under the age of 40 and even 30 has continued to increase, while the prognosis for them is poor. In addition, there are more challenges and demands in the treatment of young breast cancer patients, as well as more socioeconomic implications. There is a lack of reliable evidence for the treatment decisions due to the small proportion of young breast cancer patients in clinical trials (11, 24). Therefore, it is necessary to determine the best way to manage the treatment of young breast cancer patients.

Sun et al. compared prognosis between BCT and mastectomy for early breast cancer in young patients under 40 years old, they found that there was no significant survival difference for 18-35 years old group (25). Quan et al. draw a similar conclusion (26). But two retrospective studies found that BCS could significantly improved prognosis in young breast cancer patients under the age of 40 (27, 28).However, none of the previous studies above have comprehensively establish a model to screen young breast cancer patients who are suitable for BCS. Our study is the first to quantify the benefit of BCS in young breast cancer patients by Logistic regression and to construct a nomogram. In our analysis, the cohort was divided into BCS group and mastectomy group. PSM was performed to eliminate demographic or pathological baseline imbalances between the two groups. We next observed the clinicopathological features between the two groups and identified BCS as an independent factor for OS and BCSS in young patients by univariate and multivariate Cox regression analyses. Finally, we screened out the factors affecting the benefit of BCS by univariate and multivariate Logistic regression and constructed a nomogram. The nomogram was validated that its predictive performance was favorable both in internal and external cohort. Our research

TABLE 1 Clinical and pathological characteristics for breast cancer patients before and after PSM.

Variables	Unmatched			PSM		
	Non-BCS (%)	BCS (%)	*SMD*	Non-BCS (%)	BCS (%)	*SMD*
	N=9579 (62.5)	N=5738 (37.5)		N=3625 (50.0)	N=3625 (50.0)	
Age (Mean (SD))	31.72 (3.12)	31.84 (3.01)	0.041	31.75 (3.11)	31.75 (3.03)	<0.001
Race (%)			0.080			0.050
Black	1397 (14.6)	955 (16.6)		607 (16.7)	547 (15.1)	
Other	1108 (11.6)	751 (13.1)		443 (12.2)	477 (13.2)	
White	7074 (73.8)	4032 (70.3)		2575 (71)	2601 (71.8)	
Marital (%)			0.095			0.017
No	3720 (38.8)	2496 (43.5)		1450 (40)	1480 (40.8)	
Yes	5859 (61.2)	3242 (56.5)		2175 (60)	2145 (59.2)	
Laterality (%)			0.020			0.005
Left	4805 (50.2)	2821 (49.2)		1781 (49.1)	1790 (49.4)	
Right	4774 (49.8)	2917 (50.8)		1844 (50.9)	1835 (50.6)	
Grade (%)			0.126			0.035
I	528 (5.5)	480 (8.4)		220 (6.1)	213 (5.9)	
II	3029 (31.6)	1646 (28.7)		1027 (28.3)	1084 (29.9)	
III	5860 (61.2)	3487 (60.8)		2308 (63.7)	2259 (62.3)	
IV	162 (1.7)	125 (2.2)		70 (1.9)	69 (1.9)	
Histology (%)			0.092			0.034
IDC	8550 (89.3)	5139 (89.6)		3267 (90.1)	3267 (90.1)	
ILC	181 (1.9)	49 (0.9)		54 (1.5)	41 (1.1)	
Other	848 (8.9)	550 (9.6)		304 (8.4)	317 (8.7)	
T stage (%)			0.433			0.061
T1	3739 (39.0)	2996 (52.2)		1605 (44.3)	1590 (43.9)	
T2	4373 (45.7)	2527 (44.0)		1754 (48.4)	1820 (50.2)	
T3	1467 (15.3)	215 (3.7)		266 (7.3)	215 (5.9)	
N stage (%)			0.386			0.018
N0	4260 (44.5)	3533 (61.6)		1649 (45.5)	1678 (46.3)	
N1	3506 (36.6)	1692 (29.5)		1461 (40.3)	1434 (39.6)	
N2	1160 (12.1)	358 (6.2)		356 (9.8)	358 (9.9)	
N3	653 (6.8)	155 (2.7)		159 (4.4)	155 (4.3)	
Chemotherapy (%)			0.081			0.030
No	1628 (17.0)	1156 (20.1)		628 (17.3)	670 (18.5)	
Yes	7951 (83.0)	4582 (79.9)		2997 (82.7)	2955 (81.5)	
Radiation (%)			0.726			0.026
No	5946 (62.1)	1613 (28.1)		1567 (43.2)	1613 (44.5)	
Yes	3633 (37.9)	4125 (71.9)		2058 (56.8)	2012 (55.5)	
Subtype (%)			0.341			0.032
HR-/HER2-	879 (9.2)	352 (6.1)		281 (7.8)	288 (7.9)	
HR-/HER2+	283 (3.0)	90 (1.6)		90 (2.5)	78 (2.2)	
HR+HER2-	2222 (23.2)	861 (15.0)		664 (18.3)	635 (17.5)	
HR+/HER2+	803 (8.4)	286 (5.0)		241 (6.6)	239 (6.6)	
Not 2010+	5392 (56.3)	4149 (72.3)		2349 (64.8)	2385 (65.8)	

BCS, Breast conserving surgery; HR, Hormone receptor; IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; HER2, Human epidermal growth factor receptor 2; PSM, propensity score matched; SMD, Standardized mean differences.

showed that receiving BCS could improve the OS and BCSS of young patients, but not all of them benefited from it.

We found that most young patients should receive BCS and benefit from it. This conclusion is consistent with the recommendations of many breast cancer conference guidelines. The St. Gallen Consensus Group in 2013 stated that young age is not an absolute contraindication to BCS (29). The European Association of Breast Cancer Specialists

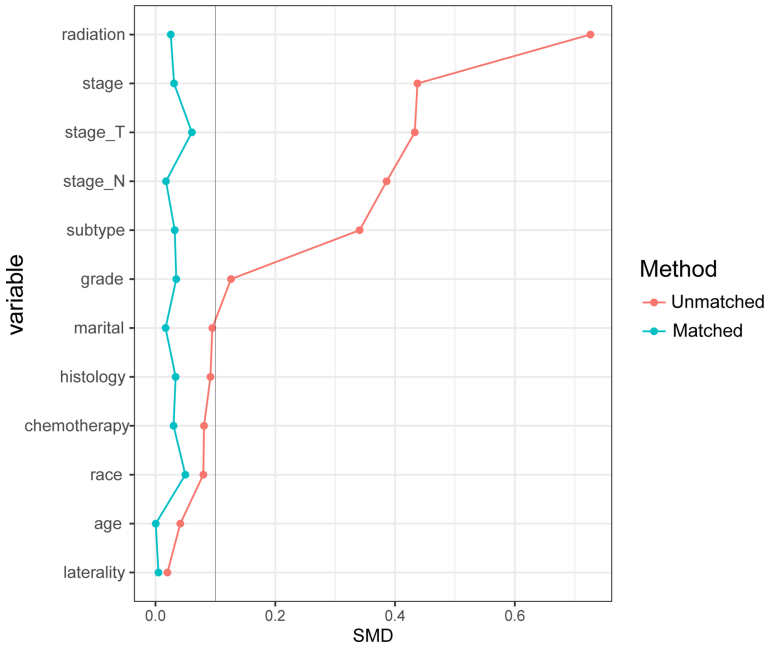

FIGURE 2
The matching effects of the propensity score matching (PSM).

(EUSOMA) working group suggested that BCT is the first choice for suitable young breast cancer patients (30). Moreover, the first International Consensus Conference on Breast Cancer in Young Women proposed the same recommendation (31). For young breast cancer patients, it is emphasized that there be a balance between tumor treatment efficacy, postoperative aesthetics, and long-term complications to protect their physical and mental health. In addition, young patients have a high risk of recurrence after BCS, and follow-up management should be strengthened.

Our analysis indicated that among patients under 35 years old, those who are older age, have lower T and N stage, radiation, as well as no chemotherapy, were associated with a benefit from BCS. Studies have shown that age is an independent risk factor for tumor recurrence after BCS (7). According to our nomogram, in patients <= 35 years old, younger age was associated with less benefit after receiving BCS. In addition, the key to BCS in clinical practice is to ensure that there is no residual tumor at the resection margin when removing the tumor. Therefore, BCS can be performed on patients with T1-3 stage who have an appropriate breast volume and a ratio of tumor to breast volume. Plastic repair techniques for tumors may improve breast shape and symmetry after BCS in young breast cancer patients. For N stage, preoperative confirmation of lymph node metastasis is not an absolute contraindication to BCS, and even for some N1 patients, BCS and postoperative radiotherapy can avoid further axillary lymph node dissection (32). However, patients with N1-3 breast cancer have higher local and regional recurrence risks compared with node-negative patients after BCS (33). Therefore, how to select T and N stages that are more suitable for BCS is crucial. Our model confirms that patients with lower T and N stages are more likely to benefit from BCS, which also provides a reference for clinicians to make decisions. In terms of systemic treatment, BCS followed by radiation is a widely accepted standard approach that allows for organ preservation in most early-stage breast cancers (8, 14). Our study also confirms that patients with postoperative radiation are more likely to benefit from BCS.

The greater benefit of BCS without chemotherapy than with chemotherapy is seen in Logistic regression, and the following aspects should be considered. The cohort study in 127 hospitals in the UK (POSH) and the breast cancer study in young women in Europe (HOHO) showed that young breast cancer patients had a higher proportion of HR+ tumor compared with older women (34, 35). Similar results were found in our findings, with the highest proportion of HR +HER2-type in our study cohort. ESO-ESMO 5th International Consensus Guidelines for Breast Cancer in Young Women (BCY5) confirmed that young breast cancer patients with luminal-like tumors have poorer outcome (2), which may explained by different tumor or host biological behavior, less chemotherapy-induced amenorrhea, poor endocrine therapy response, and poor adherence to adjuvant

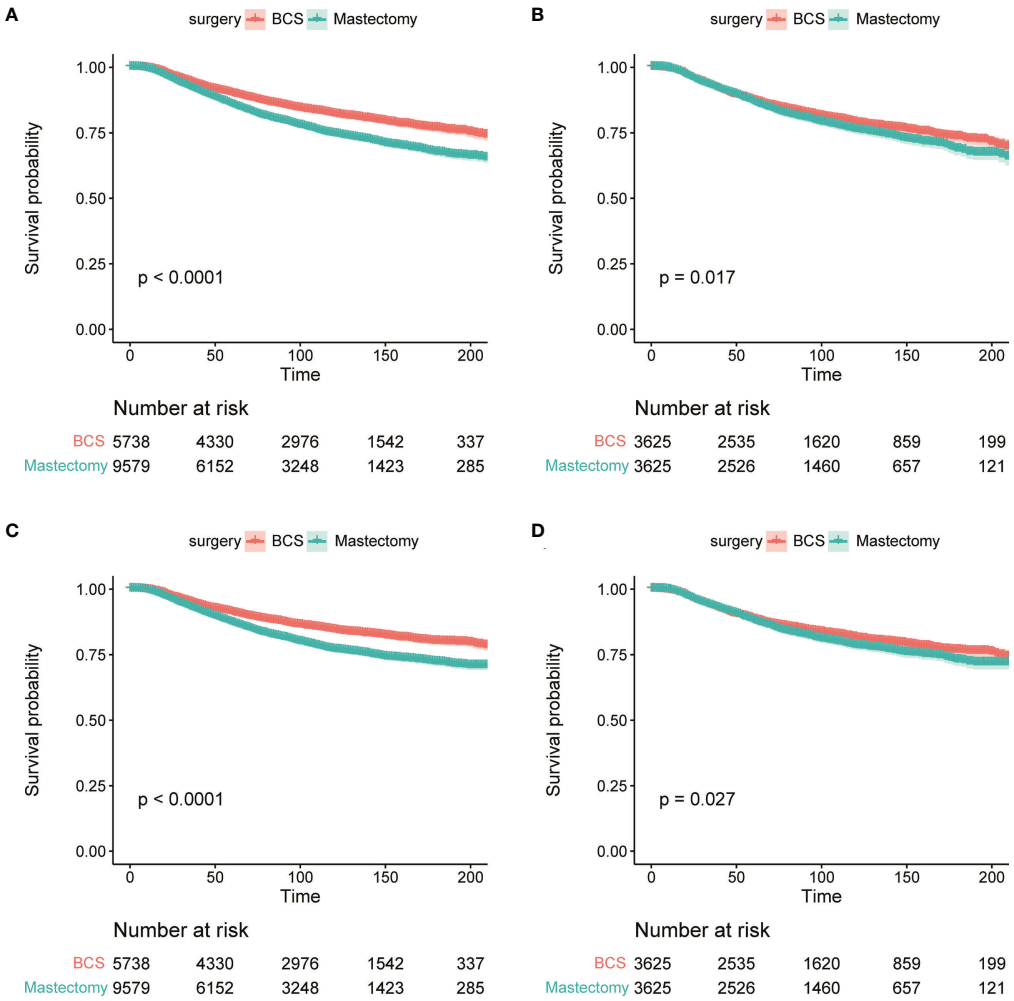

FIGURE 3
The KM survival analysis of OS **(A, B)** and BCSS **(C, D)** between BCS group and mastectomy group before **(A, C)** and after PSM **(B, D)**.

endocrine therapy etc. According to our analysis, beneficial was diminished after BCS for those received chemotherapy, which was generally associated with poor tumor features of patients received chemotherapy rather than treatment failure. However, considering that our study was a large retrospective study and the under-representation of young breast cancer patients, the results still need to be treated with caution, and more prospective studies are needed to be further verified in the future.

TABLE 2 Comparison of patient survival rates between the two surgery groups before and after PSM.

	Before PSM	After PSM
	BCS vs. Mastectomy (95% CI)	BCS vs. Mastectomy (95% CI)
3-year OS rate	0.944 (0.938-0.950) vs.0.921 (0.916-0.927)	0.929 (0.920-0.938) vs. 0.928 (0.919-0.937)
5-year OS rate	0.899 (0.891-0.908) vs. 0.857 (0.849-0.865)	0.873 (0.861-0.885) vs. 0.867 (0.855-0.879)
10-year OS rate	0.821 (0.810-0.833) vs. 0.745 (0.734-0.757)	0.788 (0.772-0.804) vs. 0.762 (0.745-0.779)
3-year BCSS rate	0.950 (0.944-0.956) vs. 0.929 (0.924-0.935)	0.935 (0.927-0.944) vs. 0.936 (0.928-0.944)
5-year BCSS rate	0.911 (0.903-0.919) vs. 0.871 (0.864-0.879)	0.885 (0.874-0.897) vs. 0.879 (0.868-0.891)
10-year BCSS rate	0.844 (0.833-0.855) vs. 0.769 (0.758-0.780)	0.813 (0.798-0.829) vs. 0.784 (0.768-0.801)

BCS, Breast conserving surgery; PSM, propensity score matched; OS, overall survival; BCSS, breast cancer specific survival.

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TABLE 3 Univariate and multivariable analysis of overall survival (OS) predictors in breast cancer patients after PSM.

	Univariate analysis			Multivariate analysis		
Variables	**HR***	**95%CI**	***P*-value**	**HR***	**95%CI**	***P*-value**
Age	0.984	(0.967, 1.001)	0.058	0.986	(0.969, 1.003)	0.111
Race						
Black	Reference			Reference		
Other	0.593	(0.486, 0.723)	0.000	0.720	(0.589, 0.881)	0.001
White	0.624	(0.548, 0.711)	0.000	0.693	(0.605, 0.792)	0.000
Laterality						
Left	Reference					
Right	1.031	(0.927, 1.146)	0.575			
Marital						
No	Reference			Reference		
Yes	0.876	(0.787, 0.976)	0.016	0.931	(0.832, 1.042)	0.215
Grade						
I	Reference			Reference		
II	2.351	(1.617, 3.417)	0.000	1.958	(1.345, 2.851)	0.000
III	3.373	(2.346, 4.851)	0.000	2.472	(1.711, 3.570)	0.000
IV	3.110	(1.903, 5.082)	0.000	2.546	(1.554, 4.171)	0.000
Histology						
IDC	Reference					
ILC	1.036	(0.659, 1.63)	0.879			
Other	0.775	(0.633, 0.95)	0.014			
T stage						
T1	Reference			Reference		
T2	1.635	(1.458, 1.834)	0.000	1.294	(1.149, 1.458)	0.000
T3	2.339	(1.925, 2.843)	0.000	1.761	(1.441, 2.154)	0.000
N stage						
N0	Reference			Reference		
N1	1.727	(1.519, 1.962)	0.000	1.701	(1.485, 1.948)	0.000
N2	2.750	(2.339, 3.233)	0.000	2.564	(2.157, 3.049)	0.000
N3	4.905	(4.073, 5.908)	0.000	4.342	(3.566, 5.287)	0.000
Chemotherapy						
No	Reference			Reference		
Yes	1.392	(1.197, 1.619)	0.000	0.955	(0.809, 1.127)	0.584
Radiation						
No	Reference			Reference		
Yes	1.380	(1.236, 1.54)	0.000	0.978	(0.862, 1.110)	0.728
Surgery						
BCS	Reference			Reference		
Mastectomy	1.138	(1.023, 1.265)	0.018	1.127	(1.013, 1.254)	0.028
Subtype						
HR-/HER2-	Reference			Reference		
HR-/HER2+	0.524	(0.285, 0.964)	0.038	0.545	(0.296, 1.003)	0.051
HR+/HER2-	0.418	(0.305, 0.574)	0.000	0.475	(0.345, 0.654)	0.000
HR+/HER2+	0.279	(0.169, 0.461)	0.000	0.301	(0.182, 0.498)	0.000
Not 2010+	0.69	(0.544, 0.874)	0.002	0.704	(0.553, 0.897)	0.004

BCS, Breast conserving surgery; IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; HR, Hormone receptor; HER2, Human epidermal growth factor receptor 2; HR*, hazard ratio; CI, confidence interval.

TABLE 4 Univariate and multivariable Logistic analysis of BCS benefit for young breast cancer patients.

	Univariate analysis			Multivariate analysis		
Variables	**HR***	**95%CI**	***P*-value**	**HR***	**95%CI**	***P*-value**
Age						
	1.004	(1.001, 1.007)	0.009	1.004	(1.002, 1.007)	0.002
Race						
Black	Reference					
Other	1.006	(0.972, 1.042)	0.724			
White	0.964	(0.939, 0.989)	0.005			
Laterality						
Left	Reference					
Right	1.011	(0.993, 1.03)	0.229			
Marital						
No	Reference			Reference		
Yes	0.959	(0.941, 0.977)	0.000	0.946	(0.93, 0.962)	0.000
Grade						
I	Reference			Reference		
II	0.868	(0.835, 0.902)	0.000	0.968	(0.935, 1.002)	0.067
III	0.889	(0.857, 0.923)	0.000	1.002	(0.968, 1.037)	0.900
IV	0.951	(0.882, 1.026)	0.196	1.018	(0.952, 1.089)	0.595
Histology						
IDC	Reference					
ILC	0.871	(0.806, 0.941)	0.001			
Other	1.023	(0.991, 1.056)	0.166			
T stage						
T1	Reference			Reference		
T2	0.926	(0.909, 0.944)	0.000	0.945	(0.928,0.962)	0.000
T3	0.730	(0.708, 0.753)	0.000	0.743	(0.722,0.765)	0.000
N stage						
N0	Reference			Reference		
N1	0.872	(0.854, 0.889)	0.000	0.884	(0.868, 0.900)	0.000
N2	0.808	(0.783, 0.834)	0.000	0.785	(0.762, 0.808)	0.000
N3	0.770	(0.739, 0.803)	0.000	0.775	(0.746, 0.805)	0.000
Chemotherapy						
No	Reference			Reference		
Yes	0.946	(0.924, 0.969)	0.000	0.928	(0.907, 0.950)	0.000
Radiation						
No	Reference			Reference		
Yes	1.382	(1.358, 1.406)	0.000	1.459	(1.435, 1.484)	0.000
Subtype						
HR-/HER2-	Reference			Reference		
HR-/HER2+	0.92	(0.861, 0.983)	0.014	0.972	(0.917, 1.031)	0.350
HR+/HER2-	0.992	(0.956, 1.030)	0.680	0.973	(0.940, 1.008)	0.126
HR+/HER2+	0.969	(0.925, 1.015)	0.185	0.966	(0.927, 1.008)	0.109
Not 2010+	1.152	(1.114, 1.191)	0.000	1.127	(1.093, 1.162)	0.000

BCS, Breast conserving surgery; IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; HR, Hormone receptor; HER2, Human epidermal growth factor receptor 2; HR*, hazard ratio; CI, confidence interval.

Despite our model having a promising predictive value in identifying appropriate candidates for BCS among young women with breast cancer, several limitations remain. First, some information is missing from the SEER database, such as BRCA1/2 mutation, Ki67, HER2 status before 2010, and tumor progression, which may affect the performance of the model.

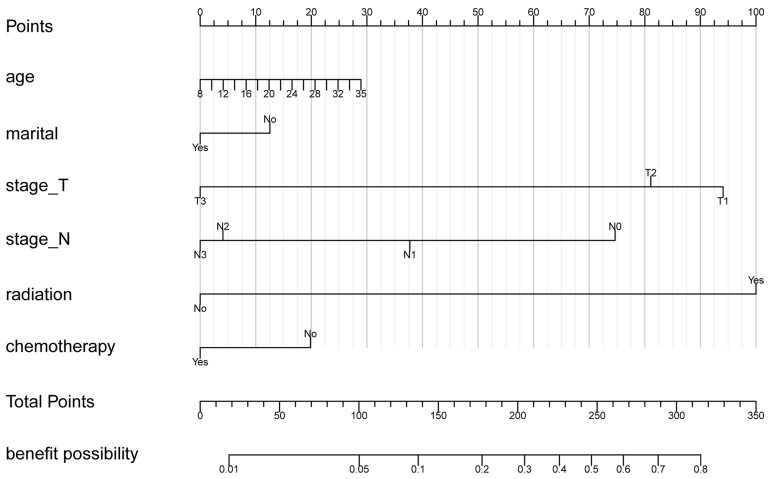

FIGURE 4
The nomogram to predict the benefit from breast conserving surgery (BCS).

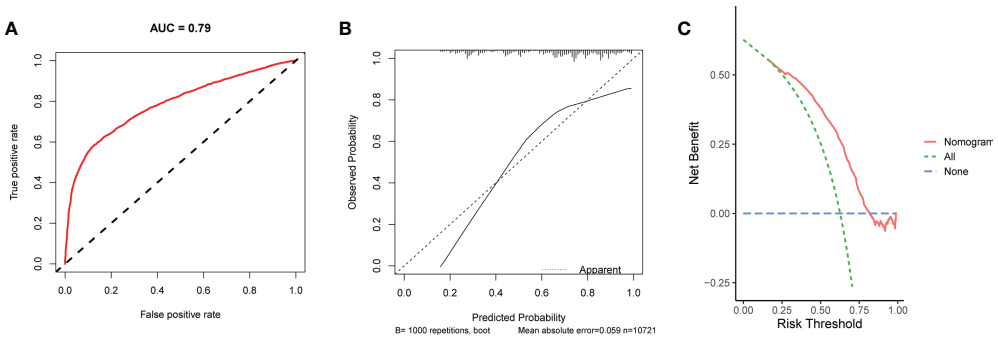

FIGURE 5
The ROC curve **(A)**, calibration curve **(B)**, and DCA curve **(C)** of the nomogram in the training set.

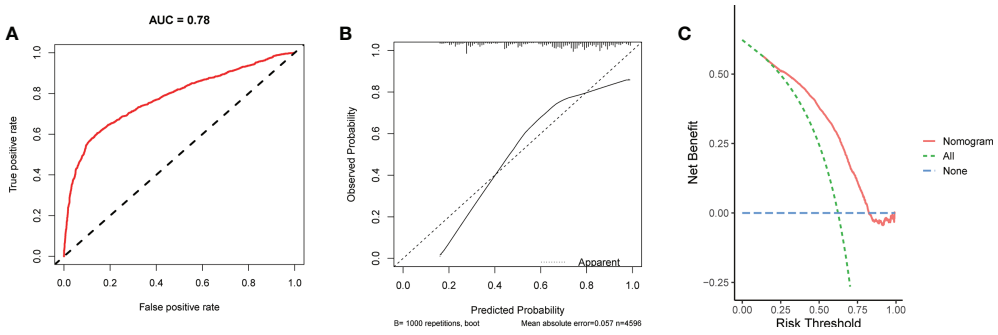

FIGURE 6
The ROC curve **(A)**, calibration curve **(B)**, and DCA curve **(C)** of the nomogram in the internal validation set.

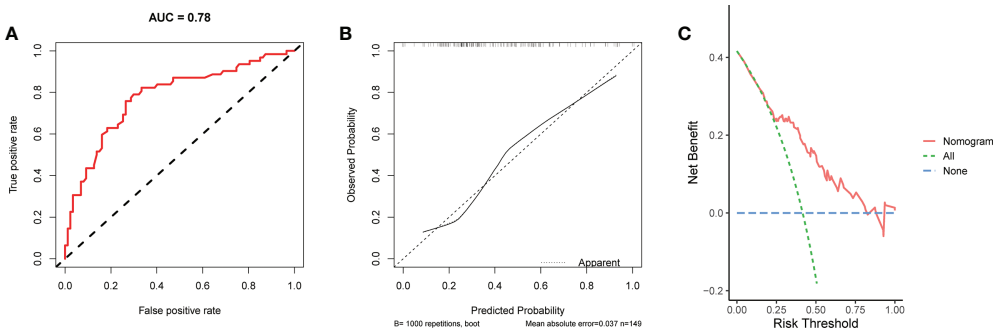

FIGURE 7
The ROC curve **(A)**, calibration curve **(B)**, and DCA curve **(C)** of the nomogram in the external validation set.

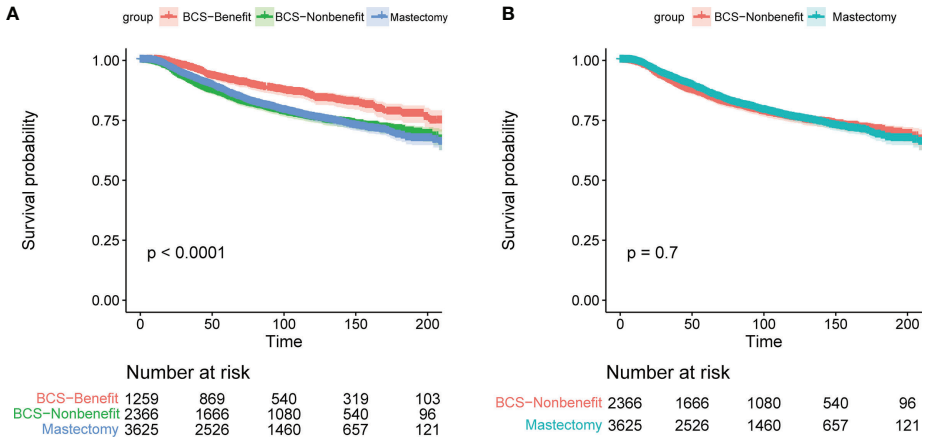

FIGURE 8
The KM survival analysis of patients in the BCS-Benefit group, BCS-Nonbenefit group, and mastectomy group.

Second, the impact of systemic therapy on prognosis cannot be analyzed comprehensively, such as endocrine therapy, targeted therapy, and immune therapy. Third, PSM requires a large sample size to achieve high-quality matching, and may lose more data and cause the remaining samples to be unrepresentative. Finally, this is a retrospective study, which may have selection bias, and our findings need to be supplemented and validated with prospective studies.

In conclusion, our findings suggest that BCS can bring better OS and BCSS than mastectomy for young breast cancer patients, but not all benefit from it. Herein we constructed a model for young breast cancer patients (≤35 years old) which could identify appropriate candidates who may benefit from BCS. For patients assigned to the BCS-Nonbenefit group, their OS did not differ from those who received a mastectomy. These findings could provide a reference for clinicians in therapy decisions.

Data availability statement

The dataset for this study can be found in the SEER database [https://seer.cancer.gov/]. 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

SP: Conceptualization, Methodology, Validation. Writing - Original Draft. SS, HC: Formal analysis, Investigation. CZ, HZ: Software, Visualization. KW: Data Curation, Resources. JH: Writing- Reviewing and Editing, Supervision. JZ: Conceptualization, Writing - Review and Editing. All authors

read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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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.2022.1012689/full#supplementary-material

References

1. Paluch-Shimon S, Cardoso F, Partridge AH, Abulkhair O, Azim HAJr., Bianchi-Micheli G, et al. ESO-ESMO 4th international consensus guidelines for breast cancer in young women (BCY4). *Ann Oncol* (2020) 31(6):674–96. doi: 10.1016/j.annonc.2020.03.284

2. Paluch-Shimon S, Cardoso F, Partridge AH, Abulkhair O, Azim HA, Bianchi-Micheli G, et al. ESO-ESMO fifth international consensus guidelines for breast cancer in young women (BCY5). *Ann Oncol* (2022) S0923-7534(22):01858-0. doi: 10.1016/j.annonc.2022.07.007

3. Braunstein LZ, Taghian AG, Niemierko A, Salama L, Capuco A, Bellon JR, et al. Breast-cancer subtype, age, and lymph node status as predictors of local recurrence following breast-conserving therapy. *Breast Cancer Res Treat* (2017) 161(1):173–9. doi: 10.1007/s10549-016-4031-5

4. Han W, Kang SY, Korean Breast Cancer S. Relationship between age at diagnosis and outcome of premenopausal breast cancer: age less than 35 years is a reasonable cut-off for defining young age-onset breast cancer. *Breast Cancer Res Treat* (2010) 119(1):193–200. doi: 10.1007/s10549-009-0388-z

5. Voogd AC, Nielsen M, Peterse JL, Blichert-Toft M, Bartelink H, Overgaard M, et al. Differences in risk factors for local and distant recurrence after breast-conserving therapy or mastectomy for stage I and II breast cancer: pooled results of two large European randomized trials. *J Clin Oncol* (2001) 19(6):1688–97. doi: 10.1200/JCO.2001.19.6.1688

6. Chen HL, Zhou MQ, Tian W, Meng KX, He HF. Effect of age on breast cancer patient prognoses: A population-based study using the SEER 18 database. *PloS One* (2016) 11(10):e0165409. doi: 10.1371/journal.pone.0165409

7. Azim HAJr., Partridge AH. Biology of breast cancer in young women. *Breast Cancer Res* (2014) 16(4):427. doi: 10.1186/s13058-014-0427-5

8. 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

9. van Maaren MC, de Munck L, de Bock GH, Jobsen JJ, van Dalen T, Linn SC, et al. 10 year survival after breast-conserving surgery plus radiotherapy compared with mastectomy in early breast cancer in the Netherlands: a population-based study. *Lancet Oncol* (2016) 17(8):1158–70. doi: 10.1016/S1470-2045(16)30067-5

10. Veronesi U, Cascinelli N, Mariani L, Greco M, Saccozzi R, Luini A, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. *N Engl J Med* (2002) 347(16):1227–32. doi: 10.1056/NEJMoa020989

11. Kroman N, Holtveg H, Wohlfahrt J, Jensen MB, Mouridsen HT, Blichert-Toft M, et al. Effect of breast-conserving therapy versus radical mastectomy on prognosis for young women with breast carcinoma. *Cancer* (2004) 100(4):688–93. doi: 10.1002/cncr.20022

12. 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

13. Vila J, Gandini S, Gentilini O. Overall survival according to type of surgery in young (</=40 years) early breast cancer patients: A systematic meta-analysis comparing breast-conserving surgery versus mastectomy. *Breast* (2015) 24(3):175–81. doi: 10.1016/j.breast.2015.02.002

14. 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

15. Miles RC, Gullerud RE, Lohse CM, Jakub JW, Degnim AC, Boughey JC. Local recurrence after breast-conserving surgery: multivariable analysis of risk factors and the impact of young age. *Ann Surg Oncol* (2012) 19(4):1153–9. doi: 10.1245/s10434-011-2084-6

16. Botteri E, Bagnardi V, Rotmensz N, Gentilini O, Disalvatore D, Bazolli B, et al. Analysis of local and regional recurrences in breast cancer after conservative surgery. *Ann Oncol* (2010) 21(4):723–8. doi: 10.1093/annonc/mdp386

17. Bantema-Joppe EJ, de Munck L, Visser O, Willemse PH, Langendijk JA, Siesling S, et al. Early-stage young breast cancer patients: impact of local treatment on survival. *Int J Radiat Oncol Biol Phys* (2011) 81(4):e553–9. doi: 10.1016/j.ijrobp.2011.02.060

18. Hwang ES, Lichtensztajn DY, Gomez SL, Fowble B, Clarke CA. Survival after lumpectomy and mastectomy for early stage invasive breast cancer: the effect of age and hormone receptor status. *Cancer* (2013) 119(7):1402–11. doi: 10.1002/cncr.27795

19. Christiansen P, Carstensen SL, Ejlertsen B, Kroman N, Offersen B, Bodilsen A, et al. Breast conserving surgery versus mastectomy: overall and relative survival-a population based study by the Danish breast cancer cooperative group (DBCG). *Acta Oncol* (2018) 57(1):19–25. doi: 10.1080/0284186X.2017.1403042

20. Agarwal S, Pappas L, Neumayer L, Kokeny K, Agarwal J. Effect of breast conservation therapy vs mastectomy on disease-specific survival for early-stage breast cancer. *JAMA Surg* (2014) 149(3):267–74. doi: 10.1001/jamasurg.2013.3049

21. Desai RJ, Franklin JM. Alternative approaches for confounding adjustment in observational studies using weighting based on the propensity score: a primer for practitioners. *BMJ* (2019) 367:l5657. doi: 10.1136/bmj.l5657

22. Andrade C. Mean difference, standardized mean difference (SMD), and their use in meta-analysis: As simple as it gets. *J Clin Psychiatry* (2020) 81(5):20f13681. doi: 10.4088/JCP.20f13681

23. Van Calster B, Wynants L, Verbeek JFM, Verbakel JY, Christodoulou E, Vickers AJ, et al. Reporting and interpreting decision curve analysis: A guide for investigators. *Eur Urol* (2018) 74(6):796–804. doi: 10.1016/j.eururo.2018.08.038

24. de Bock GH, van der Hage JA, Putter H, Bonnema J, Bartelink H, van de Velde CJ. Isolated loco-regional recurrence of breast cancer is more common in young patients and following breast conserving therapy: long-term results of European organisation for research and treatment of cancer studies. *Eur J Cancer* (2006) 42(3):351–6. doi: 10.1016/j.ejca.2005.10.006

25. Sun ZH, Chen C, Kuang XW, Song JL, Sun SR, Wang WX. Breast surgery for young women with early-stage breast cancer: Mastectomy or breast-conserving

therapy? *Med (Baltimore)* (2021) 100(18):e25880. doi: 10.1097/MD.0000000000025880

26. Quan ML, Paszat LF, Fernandes KA, Sutradhar R, McCready DR, Rakovitch E, et al. The effect of surgery type on survival and recurrence in very young women with breast cancer. *J Surg Oncol* (2017) 115(2):122–30. doi: 10.1002/jso.24489

27. Lazow SP, Riba L, Alapati A, James TA. Comparison of breast-conserving therapy vs mastectomy in women under age 40: National trends and potential survival implications. *Breast J* (2019) 25(4):578–84. doi: 10.1111/tbj.13293

28. Yu P, Tang H, Zou Y, Liu P, Tian W, Zhang K, et al. Breast-conserving therapy versus mastectomy in young breast cancer patients concerning molecular subtypes: A SEER population-based study. *Cancer Control* (2020) 27 (1):1073274820976667. doi: 10.1177/1073274820976667

29. Harbeck N, Thomssen C, Gnant M. St. gallen 2013: brief preliminary summary of the consensus discussion. *Breast Care (Basel)* (2013) 8(2):102–9. doi: 10.1159/000351193

30. Cardoso F, Loibl S, Pagani O, Graziottin A, Panizza P, Martincich L, et al. The European society of breast cancer specialists recommendations for the management of young women with breast cancer. *Eur J Cancer* (2012) 48 (18):3355–77. doi: 10.1016/j.ejca.2012.10.004

31. Partridge AH, Pagani O, Abulkhair O, Aebi S, Amant F, Azim HAJr., et al. First international consensus guidelines for breast cancer in young women (BCY1). *Breast* (2014) 23(3):209–20. doi: 10.1016/j.breast.2014.03.011

32. Vane MLG, Hunter-Squires J, Kim S, Smidt ML, Giuliano AE. Women could avoid axillary lymph node dissection by choosing breast-conserving therapy instead of mastectomy. *Ann Surg Oncol* (2021) 28(5):2522–8. doi: 10.1245/s10434-021-09674-9

33. Truong PT, Jones SO, Kader HA, Wai ES, Speers CH, Alexander AS, et al. Patients with t1 to t2 breast cancer with one to three positive nodes have higher local and regional recurrence risks compared with node-negative patients after breast-conserving surgery and whole-breast radiotherapy. *Int J Radiat Oncol Biol Phys* (2009) 73(2):357–64. doi: 10.1016/j.ijrobp.2008.04.034

34. Copson ER, Maishman TC, Tapper WJ, Cutress RI, Greville-Heygate S, Altman DG, et al. Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. *Lancet Oncol* (2018) 19(2):169–80. doi: 10.1016/S1470-2045(17)30891-4

35. Ruggeri M, Pagan E, Bagnardi V, Bianco N, Gallerani E, Buser K, et al. Fertility concerns, preservation strategies and quality of life in young women with breast cancer: Baseline results from an ongoing prospective cohort study in selected European centers. *Breast* (2019) 47:85–92. doi: 10.1016/j.breast.2019.07.001

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Comparative analysis of dosimetry and predictive somatotype parameters of prone and supine whole-breast irradiation among Chinese women after breast-conserving surgery

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Purpose: Finding a better treatment position (prone or supine) for whole-breast irradiation for Chinese female patients diagnosed with breast cancer by identify the associations between predictive somatotype parameters and dosimetric gains.

Materials and methods: Two volumetric-modulated arc therapy (VMAT) plans were deployed for whole-breast irradiation in supine and prone position with a total dose of 50 Gy in 25 fractions. Dose-volume parameters were compared and analysed both in the target volume and organs at risk, and equivalent uniform dose-based figure-of-merit (fEUD) models were further used to quantitatively evaluate the overall merits of the two plans. Body shape parameters, including body mass index (BMI), body surface area (BSA), breast shape, cup size, bust size and chest size, were collected. Anatomic features such as the central heart distance (CHD) were measured on supine CT. Spearman's correlation analysis, receiver operating characteristic (ROC) curve analysis, and the linear regression models were conducted.

Results: Doses to the heart and left anterior descending coronary artery (LADCA) are greater in left-sided breast cancer (BC) patients in the prone position than in the supine position, and the opposite was true for right-sided BC patients ($p<0.001$). 19 of 63 patients (5 left-sided and 14 right-sided BC) achieved greater benefit from the prone position according to the fEUD score. Right-sided BC patients with a bust size ≥92.25 cm, drop-type breasts and cup size ≥B are very likely to benefit from prone-position radiotherapy. The CHD is significantly positively associated with △fEUD among right-sided BC patients (rho=0.506, $p=0.004$). Using a cut-off point of 2.215, the CHD had 71.4% sensitivity and 81.2% specificity in predicting a successful prone plan.

Conclusions: Right-sided BC patients had better dosimetric gain in the prone position than left-sided BC patients. The CHD is an especially good and novel predictor that could help to select prone-benefitting right-sided BC patients.

KEYWORDS

breast neoplasms, radiotherapy, prone position, somatotype, Chinese women

Introduction

Breast cancer (BC) is the most commonly diagnosed cancer both in China and the whole world (1, 2). Given the increased prevalence of cancer screening, the proportion of early BC diagnoses has significantly increased. Breast-conserving surgery (BCS) is the standard surgical treatment for operable, early-stage BC. The percentage of patients undergoing BCS increased from 10.83% to 30.83% between 2006 and 2015 in China (3). Adjuvant radiotherapy (RT) after BCS for early-stage BC can effectively improve the survival rate and reduce the risk of recurrence (4, 5) while providing satisfactory cosmetic results as well as psychological support. As such, postoperative RT is considered the standard treatment for early-stage BC.

Generally, the supine position has been widely used for clinical RT in BC, as it is more comfortable and reproducible for patients than the prone position. However, irradiation for BC patients in the prone position could achieve better dose distributions and spare more normal tissue than the supine position (6–8), especially those with large breasts. Two randomized trials focused on the 2-year and 5-year whole breast irradiation outcomes in the prone versus supine positions among large-breasted women (9, 10) demonstrated better cosmetic outcomes and lower rates of late toxicity in the prone position.

Consistent criteria have yet to be established for selecting patients who would benefit most from prone RT. Studies on prone positioning for BC treatment have mainly been conducted in American and European countries. One South Korean study (11) suggested that patients with small breast volumes (such as those with a clinical target volume (CTV) of approximately 100 cm^3) could also benefit from the prone position.

Therefore, we conducted this study comparing the prone position with the supine position for delivering volumetric-modulated arc therapy (VMAT) to Chinese BC patients. The purpose was to assess the effects of the prone position on the dose distribution and determine differences in normal organ sparing between VMAT in the two positions. We further attempted to identify that body shape characteristics associated with prone position-benefitting breast RT among Chinese women to provide a reference basis for the rational, clinical use of the radiotherapy position.

Materials and methods

Patients and treatment simulations

The inclusion criteria were as follows: age between 18 and 70 years, pathologically confirmed stage 0-II BC (Tis-T2) after BCS, and Eastern Cooperative Oncology Group performance status 0 or 1. Patients were excluded if they needed irradiation of the locoregional lymph node area and had prosthetic implants, supraclavicular/internal mammary nodes, bilateral BC, previous irradiation or other malignancies. All patients were asked to provide their written informed consent before being registered in the study, and the present study was approved by the ethics committee of Tumor Hospital of Yunnan Province (approval number of Institutional Review Board: KYLX2022025).

Enrolled patients underwent two computed tomography (CT) simulations in the supine and prone positions. First, patients were imaged on a conventional supine breast board (R610-DCF, Klarity Medical & Equipment Co. Ltd. Guangzhou, China) with arms above the head to adequately expose the breast (Figure 1A). Then, they were repositioned on a prone board (R62-BCF4, Klarity Medical & Equipment Co. Ltd. Guangzhou, China) with a removable right and left aperture to allow the index breast tissue to hang away from the chest wall (Figure 1B). The borders of the breast tissue and midline of the chest were marked for each patient with radio-opaque wires before CT acquisition. For both setups, free-breathing CT scans were performed using a large-aperture CT system (SOMATOM Sensation Open 24, Siemens, Germany) without contrast, starting below the mandible and caudally ending below the lower edge of the liver with a slice thickness of 3.0 mm. The CT scan images were transferred to the Treatment Planning System (Monaco version 5.11, Elekta AB, Stockholm, Sweden) of the department.

Radiotherapy planning and evaluation

CTVs and organs at risk (OARs) were contoured manually according to the Radiation Therapy Oncology Group (RTOG) breast cancer atlas (12) (Figures 2A, B). The breast CTV was

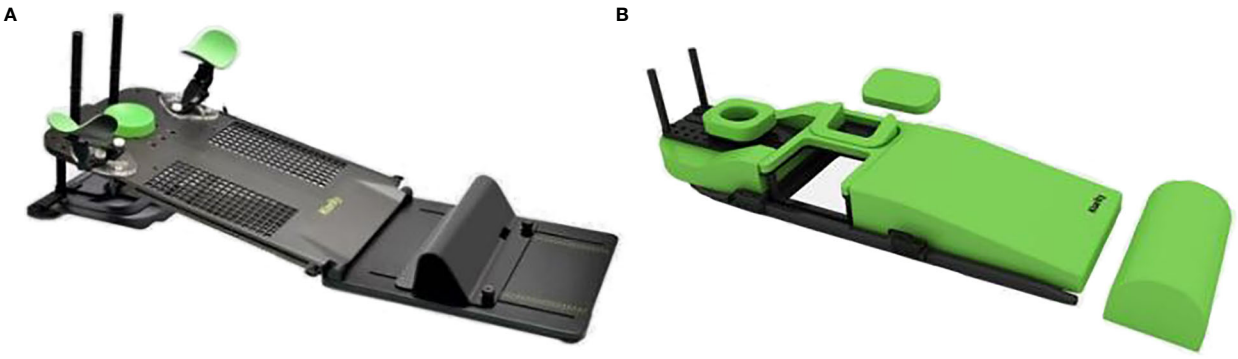

FIGURE 1
Supine/Prone breast board. **(A)** Supine breast board(Klarity, R610-DCF).**(B)** Prone breast board(Klarity, R62-BCF4).

contoured up to the inferior margin of the clavicular heads (cranially), to the farthest visible breast contour, at approximately the level of apex disappearance (caudally), to the perforating mammary vessels or to the edge of the sternum (medially), to the anterior edge of the latissimus dorsi (laterally), to the junction of the breast tissue and the pectoralis muscles (posteriorly), and up to 5 mm under the skin surface (anteriorly). The CTV was delineated based on the glandular breast tissue visible on the CT images. Planning target volumes (PTVs) were generated by the addition of three-dimensional, 5-mm margins to the CTV up to 5 mm from the skin. The whole heart was delineated in accordance with the guidelines proposed by Feng et al. (13). The left-anterior descending coronary artery (LADCA) does not include the left main trunk, which was delineated down to the apical level. Considering the planned volume of the heart while beating, the uniform diameter of the LADCA is 1 cm. OARs such as lungs, spinal cord, esophagus and liver were delineated according to the RTOG 1106 atlas (14). In detail, all inflated and collapsed, fibrotic, and emphysematic lungs were contoured with inclusion of small vessels extending beyond the hilar regions, excluding the proximal bronchial tree. The contralateral breast was delineated up to 5 mm under the skin surface. The spinal cord was delineated starting at the same cranial level as the esophagus to the bottom of L2 or at the level in which the cord ended. The oesophagus was delineated starting cranially from the inferior margin of the cricoid and ending inferiorly at the gastroesophageal junction. The whole liver was delineated along the outer edge of the liver, excluding the gallbladder. The CTV and OARs were delineated on CT slices by one radiation oncologist and verified by two other senior experienced radiation oncologists.

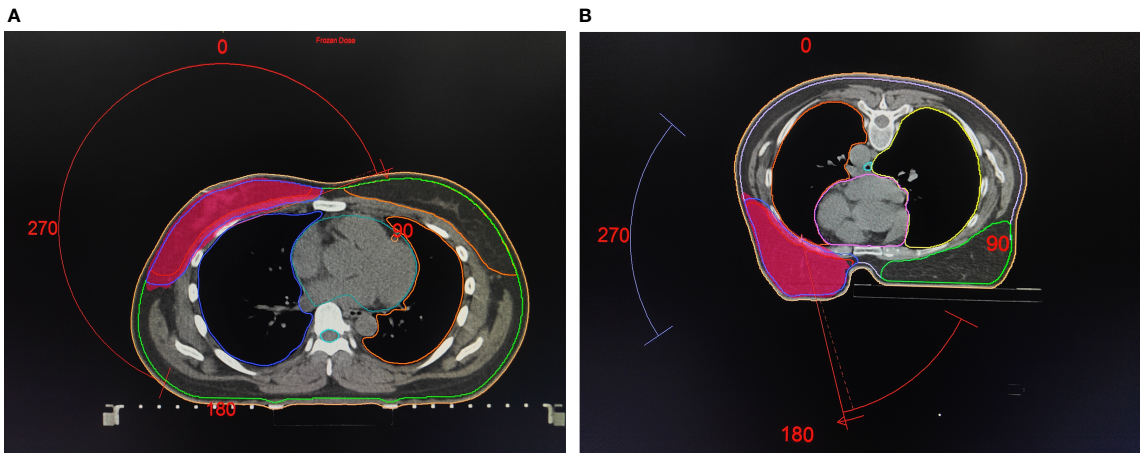

FIGURE 2
Supine/Prone treatment plans with target and organs at risk delineation. **(A)** Treatment plans of the supine position. **(B)** Treatment plans of the prone position.

The RT plans were generated for a Versa HD linear accelerator (Elekta Medical Systems Co., Stockholm, Sweden) with 6 MV photon energy. Previous studies have showed that (15), VMAT could achieve better target conformability and uniformity compared to intensity-modulated radiation therapy (IMRT). Considering the further comparison of dosimetric differences between important normal organs, such as the heart and lung, on the basis of ensuring adequate target coverage, the VMAT irradiation technology being commonly used in our institutions and in this study. Referring to the correlational researches (15, 16), we used a continuous VMAT (cVMAT) treatment plan with one dual arc of (140.0 ± 10.0)∼(320.0 ± 10.0)° for the supine position (Figure 2A). The prone plans consisted of tangential VMAT (tVMAT) plans with two tangential dual arcs of (140.0 ± 10.0)∼(120.0 ± 10.0)° and (340.0 ± 10.0)∼(310.0 ± 10.0)° rotations, accounting for the limitations of the machine boom rotation (Figure 2B). A prescription dose of 50 Gy in 25 fractions was delivered to the whole breast according to the ICRU report 83 (17), with the prescribed dose covering ≥95% of the PTV and ≤7% receiving 105% of the prescribed dose. And according to the relevant research (11) and institutional experience, we constrainted OARs were as follow: V20 < 30% for contralateral lung; mean heart dose < 6 Gy (left and right), and maximum dose of spinal cord <40Gy in the supine position; V20 < 20% for contralateral lung; mean heart dose < 8 Gy (left) or 6Gy (right), and maximum dose of spinal cord < 40 Gy in the prone position. A radiotherapy planning consensus for both sets was achieved by the agreement of more than two physicists. Only the supine treatment plan was used for real-world clinical daily RT.

All plans were compared according to the planning target volume coverage, dose-volume histogram and other dosimetric parameters of normal tissues. For target coverage, we recorded the minimum, maximum and mean doses to the PTV (Dmin, Dmax, Dmean), V95%, V105%, V100%, homogeneity index (HI) (18), and conformity index (CI) (19). The CI and HI were calculated using the following equations: 1) CI=(TV95/TV) × (TV95/V95), where V95 is the total volume receiving 95% of the prescription dose, TV is the target volume, and TV95 is the target volume receiving 95% of the prescription dose, with values closer to 1 indicating optimal conformation; 2) HI= (D2% -D98%)/D50%, where D2%, D50% and D98% are the doses covering 2%, 50% and 98% of the volume of the PTV, with lower values indicating administration of a more homogeneous dose to the target volume. For normal organs, such as the heart and ipsilateral and contralateral lung, we compared Dmax, Dmean, and the percentage of the volume that received more than 5, 10, 20, 30, and 40 Gy (V5, V10, V20, 30, and V40).

Anthropometric body shape parameters

Body shape parameters, including height, weight, body mass index (BMI), body surface area (BSA), bust size and chest size were collected. BMI=weight(kg)/height(m)^2. BSA=0.0073×/height(m)+0.0127×weight(kg)-0.2106. Bust size was measured as the circumference around the chest at the plane of the nipple. Chest size was measured as the circumference around the chest under the fold of the breasts. We also collected general information, including the breast shape (Figure 3) and cup size of all patients.

Supine anatomic feature measurements

Song et al. (20) reported that breast separation (BS) was positively correlated with the mean skin dose and was an important parameter for the selection of electronic tissue compensation radiotherapy. BS was defined as the distance between the entry points of two opposing beams on the central plane. In addition, the central lung distance (CLD) has been said to provide a close estimation of the volumetric lung dose; when the CLD is greater than 3.0 cm, the reduction in the dose delivered to the ipsilateral lung was found to be remarkable when using the medial breast technique (21). The CLD was defined as the perpendicular distance from the chest wall to the posterior border of the tangential fields.

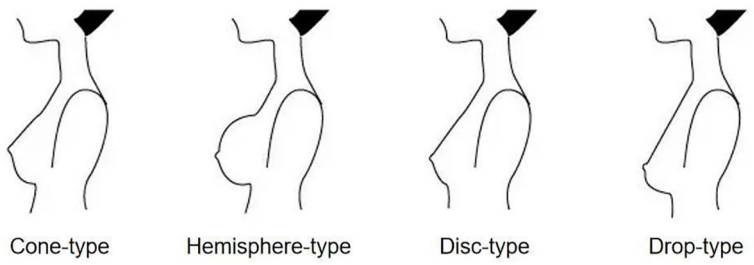

FIGURE 3
Type of breast shape.

Since the BS and the CLD could only be recorded after RT planning, we choose the modified breast separation (mBS) and modified central lung distance (mCLD) as alternative indicators which could be measured on routine chest CT. The mBS was defined as the distance from the border of the sternum and the anterior border of the latissimus dorsi extending to the skin. The mCLD was defined as the maximum perpendicular distance from the mBS to the posterior part of the anterior chest wall. Both parameters were measured on the central plane (similar to the central PTV plane) from the lower edge of the clavicular head to the cardiac apex on supine CT (Figure 4).

Additionally, we creatively assessed a new concept, the central heart distance (CHD), as a predictive parameter for the heart doses. The CHD is the perpendicular distance from the centre point of the heart to the midline on the central heart plane on supine CT (Figure 5). The central heart plane is the middle CT slice from the bifurcation of the pulmonary trunk (superior border) to the last slice containing cardiac tissue (inferior border). The midline was measured from the sternum centre to the posterior margin of the spinous process. The centre point of the heart was automatically computed as a three-dimensional point by Monaco® TPS 5.11.

EUD and fEUD models for plan comparison

The equivalent uniform dose (EUD), defined as the uniform dose giving the same biological effect as a given nonuniform dose distribution, was generalized to normal structures and tumours by Niemierko in 1999 (22). The generalized EUD (gEUD) was calculated based on the power-law dependence of the dose response for the tumour and the OARs with the following simplified formula: $\mathrm{EUD} = (\sum_i v_i D_i^a)^{1/a}$, where vi is the fraction of the reference volume irradiated with dose Di, and a is a free structure-specific parameter that is usually positive for OARs and negative for tumours. Base on the article by a previously published article by Boughalia et al. (23), we set a(PTV)=-6, a(heart)=2, a (ipsilateral lung)=2, a(contralateral lung)=5, a(LADCA)=5, a (contralateral breast)=5, and a(liver)=5. The vi and di values in the prone and supine position plans of each patient were derived from the Monaco TPS and substituted into the EUD formula to calculate the EUD values of the target areas and OARs in the two plans.

Qi et al. (24) created an EUD-based figure-of-merit (fEUD) to quantify the overall plan quality when attempting to use the EUD model to optimize the target and OAR doses. The results showed that the fEUD model can effectively evaluate plans for brain, head and neck, lung, pancreas and prostate tumours. In our previous study, the fEUD model was successfully applied to evaluate the quality of the physical scheme in cervical cancer. The fEUD is computed according to the following equation:

$$\mathrm{fEUD} = 1/\left[1 + k \cdot \frac{\sum_{i=1}^{n} \omega_i \cdot \mathrm{EUD}_{\mathrm{OAR}}^{i}}{\sum_{j=1}^{m} \omega_j' \cdot \mathrm{EUD}_{\mathrm{Target}}^{j}}\right]$$

where n and m are the numbers of OARs and targets, respectively, wi and wj are the corresponding weighting factors, and k is the relative importance factor between the weighted sums of the EUDs for all targets and the OARs. We set wi, wj and k to 1 in this study. The fEUD value ranges from 0 to 1, with greater values indicating superior plan quality. Then, the

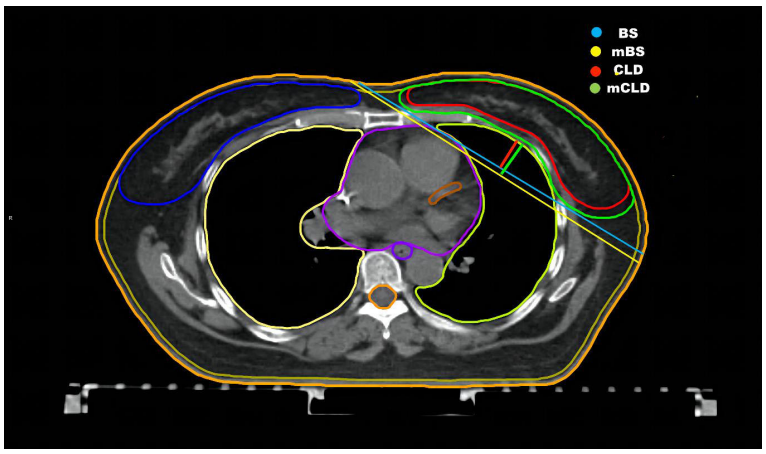

FIGURE 4
Anatomic parameters in the supine CT. The central plane from the low edge of clavicular head to the cardiac apex in the supine CT. The breast separation (BS) is the distance between entry points of two opposing beams on the central plane. The central lung distance (CLD) is the perpendicular distance from chest wall to the posterior boarder of the tangential fields.The modified breast separation (mBS) is the distance from the border of the sternum and the anterior border of latissimus dorsi then extending to skin.The modified central lung distance (mCLD) is the maximum perpendicular distance from BS to the posterior part of the anterior chest wall.

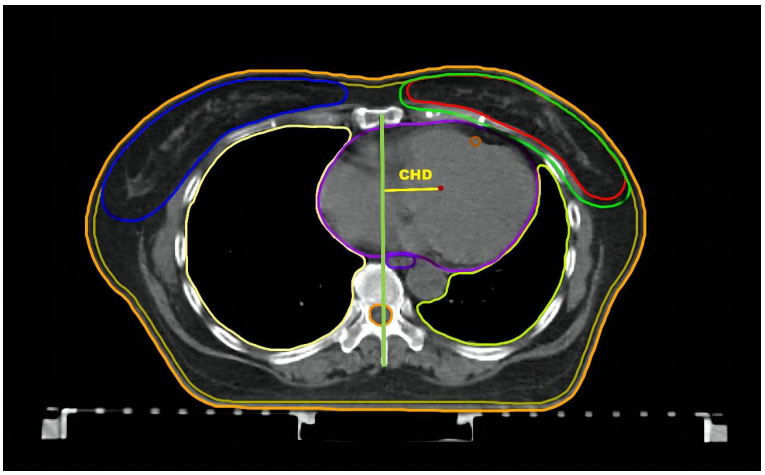

FIGURE 5
CHD in the supine CT. The central heart plane. The central heart distance (CHD) is the perpendicular distance from centre point of heart to the midline.

EUD value is substituted into the fEUD formula to calculate the fEUD value of the prone position and supine position. Finally, we calculated $fEUD_{(prone-supine)}$ to compare the overall quality of the two plans. A positive value of $fEUD_{(prone-supine)}$ indicates that the prone position plan is better, and a negative value indicates that the supine position plan is better.

Statistical analysis

Dosimetric parameters were examined by the paired t test or Wilcoxon signed-rank test. Correlations were measured using Spearman's correlation coefficient (rho). Receiver operating characteristic (ROC) curve analyses were used to examine the predictive validity of the somatotype parameters. Linear regression models were used to explore more conveniently measurable predictors. All statistical analyses were conducted by SPSS Statistics software for Windows ver. 25.0 (IBM Corp., Armonk, NY). Differences were considered significant at p values < 0.05.

Results

Dosimetric analyses

Between June 2020 and June 2021, 160 female patients underwent whole-breast RT after BCS were randomly chosen for this study. Of these patients, 58 did not meet the inclusion criteria, and 39 did not give consent and were excluded. Finally, a total of 63 patients were enrolled (33 with left-sided and 30 right-sided breast cancer). The baseline patient characteristics are shown in Table 1.

We performed comparisons between the prone and supine positions for the entire patient cohort, and the results are summarized in Table 2. For all patients, the prone position reduced the doses to lungs but increased the average volume of the breast and ipsilateral lung and the Dmean of the contralateral breast relative to the supine position ($p< 0.05$). For left-sided BC, compared with those of the supine position, all dose values (Dmean and V5-V40) of the heart and the Dmax and Dmean of LADCA were higher in the prone position ($p \leq 0.001$). For right-sided BC, the Dmax and Dmean of the LADCA was lower in the prone position than in the supine position ($p< 0.001$). The Dmean of the heart was lower in the prone position, although the difference was not significant.

Overall plan figure-of-merit (fEUD)

Table 3 shows the fEUD values for the prone and supine VMAT plans. We found that 19 patients (5 with left-sided and 14 with right-sided BC) benefitted from the prone position according to this quality score. The mean, minimum, maximum volume of the CTV for these 19 patients were found to be 686.45cm^3, 396.98cm^3, 1512.25cm^3, respectively.

Correlation analysis

According to the comparison between the two setups' fEUD values, we used "△fEUD" to assess whether the prone plan was better than the supine plan; if so, the patient was given a value of 1, and otherwise. Correlations between various analysed parameters were calculated using the Pearson test or Spearman rank test, depending on the normality of the distribution. If the

TABLE 1 Patient and tumor characteristics (N=63).

Characteristic	NO. (%)	Mean	Median	Range
Age (year)		48	48	23-70
BMI (kg/m^2)		23.85	23.15	18.73-32.45
BSA (m^2)		1.69	1.73	1.49-2.05
Bust size (cm)		91.33	90.50	73.50-120.00
Chest size (cm)		82.84	79.75	68.00-98.50
CTV (cm^3)				
Supine position		549.24	553.38	129.49-1916.30
Prone position		595.67	661.10	130.04-1823.37
Side				
Left	33(52.38%)			
Right	30(47.62%)			
Quartant				
medial-upper	14(22.22%)			
medial-lower	2(3.17%)			
lateral-upper	37(58.73%)			
lateral-lower	10(15.87%)			
Breast shape				
Disc-type	31(49.21%)			
Cone-type	6(9.52%)			
Drop-type	25(39.68%)			
Hemisphere-type	1(1.58%)			
Cup size				
AA	5(7.94%)			
A	7(11.11%)			
B	29(46.03%)			
C	19(30.16%)			
D	2(3.17%)			
E	0			
F	0			
G	1(1.58%)			

BMI, body mass index; BSA, body surface area. Bust size is measured as the circumference around the chest at the plane of the nipple. Chest size is measured as the circumference around the chest under the fold of the breasts .Breast volume measured by CTV (clinical target volume),in unit of cm^3.

assumption of normality was not fulfilled, we calculated the Spearman correlation coefficients. So Spearman's correlation analysis was conducted between the △fEUD value and the values of the different somatotype parameters (Figure 6). Figure 6 shows the correlation between somatotype parameters and the △fEUD value; for example, the value in the BS grid indicates that the Spearman correlation coefficient (rho) between BS and △fEUD is 0.368, and the corresponding p value is 0.003. We found a weak, positive correlation between BS and △fEUD, and the p value indicates statistical significance. In other words, a longer BS indicates a greater likelihood that the prone position will be better than the supine position. △fEUD was weakly negatively correlated with breast side, bust size, BS and CTV (rho=0.276~0.368, p< 0.05). Subsequently, a multi-index ROC curve was drawn to evaluate the accuracy of these predictors. As shown in Figure 7A, the AUC values for supine CTV, BS, bust size and breast side were 0.702, 0.731, 0.673 and 0.687, respectively; this indicated that supine CTV≥495.996 cm^3 (68.4% sensitivity, 68.2% specificity), BS≥21.735 cm (57.9% sensitivity, 84.1% specificity), bust size≥92.25 cm (84.2% sensitivity, 59.1% specificity) and breast side=right (73.7% sensitivity, 63.6% specificity) could predict a benefit from the prone position.

The above results potentially suggest that right-sided breast cancer patients with a CTV≥495.996 cm^3, BS≥21.735 cm and bust size≥92.25 cm were very likely to benefit from prone RT. However, the CTV and BS values were not available directly from routine chest CT images. Therefore, we attempted to explore the relationship between BS and CTV and other directly measurable somatotype parameters. Positive correlations were identified between BS and breast shape (rho=0.468, p< 0.001) and between CTV and cup size (rho=0.452, p< 0.001), according to the Spearman correlation analysis. Analysis of the linear models (Table 4) demonstrated

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TABLE 2 Comparison between supine and prone positions for left-sided and right-sided groups.

Variable	Left-side			Right-side		
	Supine	Prone	P-value	Supine	Prone	P-value
Volume (cm3)						
CTV	585.44±343.52	631.93±337.47	0.009	509.42±287.42	555.78±280.68	0.014
Ipsilateral lung	1084.34±210.98	1217.28±218.37	0.000	1391.08±229.38	1501.19±248.41	0.000
Heart	540.24±95.30	543.63±103.88	0.794	593.52±93.27	585.02±93.82	0.568
Contralateral lung	1338.88±220.13	1443.73±244.77	0.001	1125.61±221.58	1251.81±218.3	0.000
Contralateral breast	593.66±366.86	661.04±335.47	0.004	470.63±349.55	588.76±334.77	0.000
Target dose						
Dmean (cGy)	5188.08±37.96	5081.25±805.53	0.004	5184.48±31.48	5200.95±39.86	0.098
CI	0.02±0.02	0.02±0.02	0.201	0.02±0.01	0.02±0.01	0.688
HI	0.12±0.04	0.26±0.12	0.000	0.10±0.03	0.10±0.02	0.393
Dose in OARs						
Ipsilateral lung						
Dmean (cGy)	1103.66±835.45	552.84±119.24	0.000	932.30±159.20	643.52±153.67	0.000
V5(%)	51.67±13.44	26.27±6.56	0.000	47.75±10.08	25.09±5.59	0.000
V10(%)	25.64±6.01	10.59±4.43	0.000	25.43±5.38	13.61±4.20	0.000
V20(%)	13.60±4.10	5.82±3.19	0.000	13.71±3.91	8.80±3.30	0.000
Heart						
Dmean (cGy)	309.46±42.67	631.57±126.56	0.000	222.05±60.46	209.34±35.7	0.428
V5(%)	9.07±2.74	26.31±10.88	0.000	3.90±6.66	4.02±3.24	0.472
V10(%)	1.24±0.50	13.10±5.62	0.000	0.28±1.26	0.73±0.61	0.000
V20(%)	0.40±0.33	4.73±2.50	0.000	0.00±0.00	0.06±0.08	0.000
V30(%)	0.15±0.16	2.25±1.69	0.000	0.00±0.00	0.01±0.02	0.018
V40(%)	0.03±0.04	0.43±0.67	0.001	0.00±0.00	0.00±0.01	0.317
LADCA						
Dmin (cGy)	262.51±67.26	298.32±100.30	0.098	182.23±143.69	137.88±19.53	0.092
Dmax (cGy)	2237.98±1303.01	3314.06±1116.54	0.001	414.54±159.77	267.4±120.76	0.000
Dmean (cGy)	656.36±434.15	1459.67±1940.99	0.000	249.72±85.10	169.06±37.23	0.000
Contralateral lung						
Dmean (cGy)	349.38±114.41	184.69±57.98	0.000	272.26±97.93	115.4±14.49	0.000
V5 (%)	21.74±13.95	4.23±6.49	0.000	13.51±11.56	0.09±0.16	0.000
V10 (%)	2.57±2.76	0.86±2.82	0.001	1.18±1.31	0.00±0.00	0.000
V20 (%)	0.05±0.14	0.30±1.66	0.306	0.00±0.01	0.00±0.00	0.157
Contralateral breast						
Dmean (cGy)	401.04±112.35	578.70±202.30	0.000	361.78±107.37	526.69±142.31	0.000
V5 (%)	20.87±13.88	50.80±15.79	0.000	19.56±14.76	28.64±10.35	0.012
Liver						
Dmean (cGy)	158.68±62.20	132.13±78.81	0.085	341.73±136.47	368.23±173.58	0.428

Values are presented as mean±standard deviation. CTV, clinical target volume; LADCA, left anterior descending coronary artery; Dmin, minimum dose; Dmax, maximum dose; Dmean, mean dose; CI, conformity index; HI, homogeneity index; OARs, organs at risk; V_X, percentage of the volume that receives more than X Gy.

that BS≥21.735 cm could represent a breast shape of at least drop type. The model-dependent variable was the BS (linear variable). The independent variable was breast shape (categorical variable), including drop-type, hemisphere-type, cone-type and disc-type, as listed in Table 1. Table 5 shows that CTV≥495.996 cm3 could represent a cup size of at least B. The model-dependent variable was the CTV (linear variable). The independent variable was cup size (categorical variable), including AA, B, C, and G, as listed in Table 1.

Lower doses were delivered to the heart, LADCA and both lungs for right-sided breast cancer patients, and the fEUD model scored 14/30 right-sided breast cancer patients as the "prone beneficial group", as previously described. Based on these data, we found that the CHD was significantly and positively associated with △fEUD among right-sided breast cancer patients (rho=0.506, p =0.004), and ROC curve analyses showed an AUC of 0.792 (Figure 7B). When using 2.215 cm as the cut-off value, the CHD index achieved a sensitivity of

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TABLE 3 fEUD values for prone plans superior to supine plans.

NO.	Side	Prone fEUD	Supine fEUD	fEUD (prone-supine)	Supine-CTV (cm^3)
1	right	0.112893084	0.110414396	0.002478688	415.983
2	right	0.096232005	0.094488975	0.001743031	521.241
3	right	0.120423487	0.091917231	0.028506255	1916.304
4	right	0.091174089	0.089517571	0.001656518	396.978
5	right	0.085721694	0.067475742	0.018245952	524.844
6	left	0.079840625	0.072586274	0.00725435	1512.249
7	left	0.073865475	0.057064462	0.016801013	801.381
8	left	0.105543209	0.089540692	0.016002517	498.615
9	right	0.085468039	0.081230088	0.004237951	496.971
10	right	0.129498717	0.004597929	0.124900788	516.255
11	right	0.091305729	0.001192998	0.090112731	529.179
12	right	0.083498608	0.079928729	0.003569879	623.703
13	right	0.081862464	0.03907797	0.042784494	595.074
14	right	0.089141925	0.085371361	0.003770564	429.055
15	left	0.073702277	0.069422302	0.004279975	1132.620
16	right	0.080514008	0.075874783	0.004639224	515.070
17	left	0.074863464	0.067153439	0.0077100243	578.550
18	right	0.1293878265	0.123577148	0.0058106785	518.390
19	right	0.0924156387	0.083213006	0.0092026320	520.080

19/63 cases were determined as prone-position benefited according to fEUD scores' comparison. The higher the fEUD value, the better the overall quality of plans.Supine-CTV,clinical target volume in supine computed tomography.

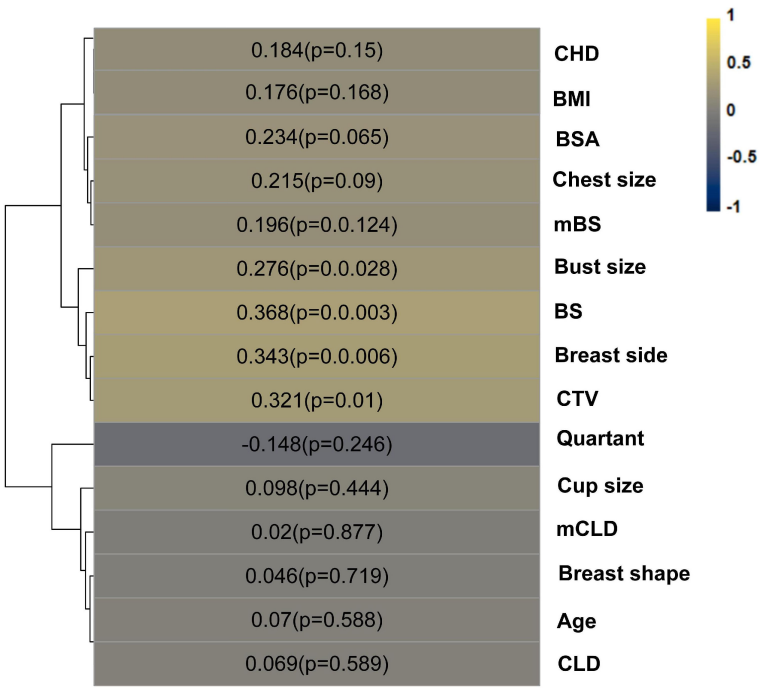

FIGURE 6
Color map of rho between "△fEUD" and somatotype parameters. "△fEUD", whether the prone plan is better than the supine, yes=1, no=0.

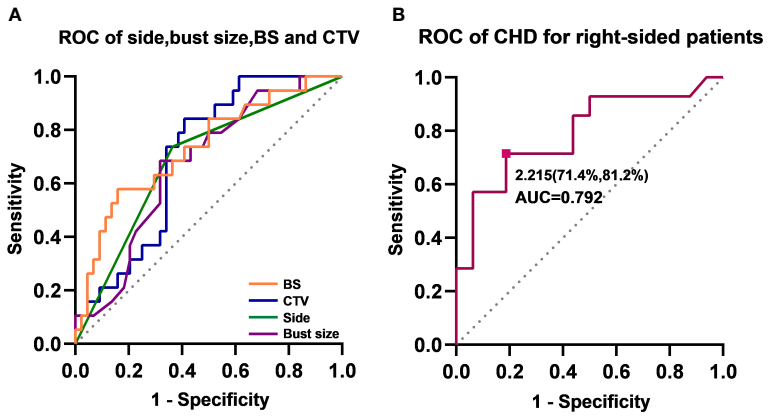

FIGURE 7
Receiver operating characteristic (ROC) curves. **(A)** ROC curves of side, bust size, BS and CTV. Area under the curve (AUC) of BS (orange), supine-CTV (blue), breast side (green) and bust size(purple) were 0.731, 0.702, 0.687, 0.673, respectively. **(B)** ROC curves of CHD for right-sided patients. The cut-off value is 2.215, with a sensitivity of 71.4% and a specificity of 81.2%.

71.4% and a specificity of 81.2% in predicting a successful response to prone RT for right-sided breast cancer patients. The CHD was originally designed as a cardiac dose predictor; Spearman's correlation analysis showed that the CHD was negatively correlated with ΔHeart V10 (prone-supine) among right-sided BC patients (rho=-0.441, $p< 0.05$) but was not correlated with the heart dose values among left-sided BC patients.

Discussion

Prone-position breast RT has previously been confirmed to be more beneficial for women with pendulous or large breasts of volumes ≥750 or 920.3 cm^3 than the supine position (6, 8) because it elongates the treated breast away from the chest wall, which could help to prevent acute skin toxicity, especially along the inframammary fold. This study is one of few about prone breast RT that focus specifically on patients of Eastern ethnicities, such as Chinese, Korean and Japanese, who usually have a smaller breast size and body size than Western women.

Our results suggest that right-sided BC patients with a bust size≥92.25 cm, drop-type breasts and cup size≥ B are highly likely to benefit from prone positioning, while left-sided BC patients conversely are unsuitable for prone RT because of their higher heart and LADCA doses than in the supine position. According to relevant previous studies, the reasons for this phenomenon may include the following. 1) The heart could fall anteriorly towards the chest wall due to gravity in the prone position, moving it closer to the breast target volume and increasing the area that receives higher doses. 2) The average breast size was 549.24 cm^3 (in the supine position) in this research, generally smaller than the recommended prone-beneficial breast volume of 750 cm^3 in some studies (6). Taking the motion of the heart into account, if the breast is not sufficiently large and pendulous enough to be pulled away from the chest wall, the cardiac dose is likely to increase. 3) The RT technique used in this study is VMAT. Compared with IMRT, which was used in the majority of previous prone-position breast RT studies, the VMAT technique has been shown to improve the target dose homogeneity and conformity but inferior in terms of cardiac protection (15, 25). Our institution has been using the VMAT technique for many

TABLE 4 Coefficients of Model BS.

Model BS	Unstandardized coefficients		Standardized coefficients	T	Sig.
	Beta	Std. error	Beta		
Constant	21.926	0.397	–	55.275	0.000
Hemisphere-type	-2.936	2.023	-0.164	-1.452	0.152
Cone-type	-1.901	0.902	-0.249	-2.108	0.039
Disc-type	-2.400	0.533	-0.536	-4.501	0.000

Constant: Drop-type. The dependent variable is the BS. The independent variable were breast shapes (including drop-type, hemisphere-type, cone-type and disc-type).

TABLE 5 Coefficients of Model CTV.

Model CTV	Unstandardized coefficients		Standardized coefficients	T	Sig.
	Beta	Std. error	Beta		
Constant	511.408	46.09	–	11.096	0.000
AA cup	-283.381	120.189	-0.243	-2.358	0.022
A cup	-60.683	104.523	-0.060	-0.581	0.564
C cup	134.293	71.119	0.201	1.888	0.064
G cup	1404.896	252.446	0.557	5.565	0.000

Constant: B cup. The dependent variable is the CTV. The independent variable were cup sizes (including AA, B, 291 C, and G cup).

years for BC patients who receive RT after BCS in the supine position. With the goal of ensuring better target area coverage, there have been ongoing measures and concerted efforts to help reduce the cardiopulmonary dose as much as possible. Nevertheless, the possible benefit from prone RT for left-sided BC patients cannot be completely excluded. Our research found that the minimum CTV of left-sided BC patients in the prone-beneficial group was 498.615 cm^3. A Korean study (11) also showed a dosimetric advantage in prone breast RT for patients with a small breast size (approximately 100 cm^3).

When exploring the relationship between body shape and dosimetry, we chose two methods to collect somatotype parameters, i.e., anthropometric and image CT measurements. Moreover, the fEUD model, proposed by Qi et al. (24) was used to score the prone and supine plans for a quantitative assessment of overall quality. The OARs in the formula do not include the skin, spinal cord, or oesophagus, which are less irradiated within the treatment field. Correlation and ROC curve analyses showed that the possibility of a benefit from the prone position increased for a CHD≥2.215 cm for right-sided BC patients.

Several studies (20, 21, 26) have demonstrated that the maximum heart distance (MHD) is a good predictor of the mean heart dose. The MHD was measured as the maximum width of the heart in the tangent fields. Nonetheless, considering the following limitations of the MHD, we did not use it in this study. 1) The MHD needs to be recorded on beam's eye view of the simulation CT, not on a routine physical examination CT. 2) BC can be either left or right-sided, the MHD in this study was not always a positive value but could also be 0 or negative. Therefore, it cannot be comprehensively and efficiently measured and analysed. 3) The central level of the heart is the distance to the level where the MHD is located, and there is no clear relationship between the two (27). In addition, although it has been demonstrated that other CT lines, such as BS, CLD, mBS and mCLD, are related to cardiopulmonary sparing, they do not yield an obvious prediction.

Therefore, we creatively defined the CHD, which is longer in the prone position than in the supine position because of the left-anterior motion caused by gravity. Logically, if a left-sided BC patient has a longer CHD in the supine position, it means the heart is closer to the target area, and the irradiated volume and dose to the heart will increase when changing from the supine to the prone position. In contrast, the longer the CHD is, the more cardioprotective it is for right-sided BC patients. Consistent with the above hypothesis, our results indicate that the CHD was a good predictive parameter that could be measured on routine chest CT to help select patients with right-sided BC who may benefit from prone-position radiotherapy.

The clinical application and popularization of prone breast RT are mainly restricted for the daily repeatability and stability. Some patients can not tolerate RT in the prone position, especially those with lumbar spine diseases or thoracic malformations. In studies concerning prone BC RT, multiple institutions have modified their prone setups to improve comfort and reduce errors (28). At present, there is no standardized prone-treatment board for breast RT. The prone boards from Orfit, Bionix, and especially Civco have been described in related studies (11, 29). Our prone board was provided by Klarity, and the tendency of the heart to move left anteriorly was less obvious, but the separation of the contralateral breast from the tangential field was not as notably protective as with the board from Civco. No comparison related to comfort and stability could be made.

We first raised the conception of CHD in this study to compare prone vs. supine whole breast radiotherapy for Chinese women, whose somatotype is relatively smaller than that of Western women. We sought to determine whether the smaller body figures and breast size of the Chinese population could benefit from prone radiotherapy. Additionally, we attempted to identify that anatomical characteristics could potentially indicate the benefit of normal tissue, further select the dominant treatment position without two CT simulations,which means more costs for the patients and more workload for physicians and physicists. We also used fEUD models in a innovative and prudent manner to quantitatively evaluate the overall merits of the two plans and the CHD and other geometric lines to explore their correlation with dosimetry.

However, we are aware that the relative small number of cases might increases the contingency of our analysis and some associations might be underestimated. Further studies in a wider cohort are needed to validate our existing results in a greater depth.

Conclusions

For whole-breast irradiation after breast-conserving surgery, compared with the supine position, the prone position resulted in lower heart and ipsilateral lung doses for right-sided BC patients, while higher heart and LADCA doses were observed for patients with left BC. The prone benefit was more prominent for right-sided BC patients with drop-type breasts, greater bust and cup sizes, and, notably, longer CHD.

Data availability statement

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

Ethics statement

All patients were asked to provide their written informed consent before being registered in the study, and the present study was approved by the ethics committee of Tumor Hospital of Yunnan Province (approval number of Institutional Review Board: KYLX2022025). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceived and designed the analysis: YG, HB, LL, YX, LW, YH. Collected the data: LW, WL, YH. Contributed data or analysis tools: LW, LL, LC, Performed the analysis: YG, HB, XP, LC, YX, Wrote the paper: YG, WL, YH. All authors contributed to the article and approved the submitted version.

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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. 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. Lei S, Zheng R, Zhang S, Chen R, Wang S, Sun K, et al. Breast cancer incidence and mortality in women in China: Temporal trends and projections to 2030. *Cancer Biol Med* (2021) 18(3):900–9. doi: 10.20892/j.issn.2095-3941.2020.0523

3. Bao X, Sun K, Tian X, Yin Q, Jin M, Yu N, et al. Present and changing trends in surgical modalities and neoadjuvant chemotherapy administration for fe male breast cancer in Beijing, China: A 10-year (2006-2015) retrospective hospitalization summary re port-based study. *Thorac Cancer* (2018) 9(6):707–17. doi: 10.1111/1759-7714.12636

4. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10 801 women in 17 randomised trials. *Lancet* (2011) 378(9804):1707–16. doi: 10.1016/s0140-6736(11)61629-2

5. Speers C, Pierce LJ. Postoperative radiotherapy after breast-conserving surgery for early-stage breast cancer: A review. *JAMA Oncol* (2016) 2(8):1075–82. doi: 10.1001/jamaoncol.2015.5805

6. Fernandez-Lizarbe E, Montero A, Polo A, Hernanz R, Moris R, Formenti S, et al. Pilot study of feasibility and dosimetric comparison of prone versus supine breast radiotherapy. *Clin Transl Oncol* (2013) 15(6):450–9. doi: 10.1007/s12094-012-0950-8

7. Verhoeven K, Sweldens C, Petillion S, Laenen A, Peeters S, Janssen H, et al. Breathing adapted radiation therapy in comparison with prone position to reduce the doses to the hear t, left anterior descending coronary artery, and contralateral breast in whole breast radiation ther apy. *Pract Radiat Oncol* (2014) 4(2):123–9. doi: 10.1016/j.prro.2013.07.005

8. Gerber NK, Yan SX, Levinson BA, Perez CA, Das IJ, Maisonet OG, et al. A prospective trial to compare deep inspiratory breath hold with prone breast irradiation. *Pract Radiat Oncol* (2020) 10(5):330–8. doi: 10.1016/j.prro.2020.01.001

9. Vakaet V, Van Hulle H, Vergotte M, Schoepen M, Deseyne P, Van Greveling A, et al. 5-year outcomes of a randomized trial comparing prone and supine whole breast irradiation in Large-breasted women. *Int J Radiat Oncol Biol Phys* (2021) 110(3):766–71. doi: 10.1016/j.ijrobp.2021.01.026

10. Veldeman L, Schiettecatte K, De Sutter C, Monten C, van Greveling A, Berkovic P, et al. The 2-year cosmetic outcome of a randomized trial comparing prone and supine whole-breast irradiation in Large-breasted women. *Int J Radiat Oncol Biol Phys* (2016) 95(4):1210–7. doi: 10.1016/j.ijrobp.2016.03.003

11. Chung Y, Yu JI, Park W, Choi DH. Korean First prospective phase II study, feasibility of prone position in postoperative whole breast radiotherapy: A dosimetric comparison. *Cancer Res Treat Oct* (2019) 51(4):1370–9. doi: 10.4143/crt.2018.423

12. Julia W, Douglas A, et al. *Breast cancer atlas for radiation therapy planning: consensus definitions[EB/OL]* (2019). Available at: https://www.rtog.org/LinkClick.aspx?fileticket=SQhssxHu7Jg%3d&tabid=227.

13. Feng M, Moran JM, Koelling T, Chughtai A, Chan JL, Freedman L, et al. Development and validation of a heart atlas to study cardiac exposure to radiation following treatment for breast cancer. *Int J Radiat Oncol* (2011) 79(1):10–8. doi: 10.1016/j.ijrobp.2009.10.058

14. Kong FM, Ritter T, Quint DJ, Senan S, Gaspar LE, Komaki RU, et al. Consideration of dose limits for organs at risk of thoracic radiotherapy: Atlas for lung, proximal bronchial tree, esophagus, spinal cord, ribs, and brachial plexus. *Int J Radiat Oncol* (2011) 81(5):1442–57. doi: 10.1016/j.ijrobp.2010.07.1977

15. Zhao H, He M, Cheng G, Han D, Wu N, Shi D, et al. A comparative dosimetric study of left sided breast cancer after breast-conserving surgery treated wi th VMAT and IMRT. *Radiat Oncol* (2015) 10:231. doi: 10.1186/s13014-015-0531-4

16. Fogliata A, Seppala J, Reggiori G, Lobefalo F, Palumbo V, De Rose F, et al. Dosimetric trade-offs in breast treatment with VMAT technique. *Br J Radiol* (1070) 2017:90. doi: 10.1259/bjr.20160701

17. Hodapp N. The ICRU report 83: prescribing, recording and reporting photon-beam intensity-modulated radiation therapy (IMRT). *Strahlenther onkol.* (2012) 188(1):97–9. doi: 10.1007/s00066-011-0015-x

18. Yoon M, Park SY, Shin D, Lee SB, Pyo HR, Kim DY, et al. A new homogeneity index based on statistical analysis of the dose-volume histogram. *J Appl Clin Med Phys* (2007) 8(2):9–17. doi: 10.1120/jacmp.v8i2.2390

19. Feuvret L, Noël G, Mazeron JJ, Bey P. Conformity index: A review. *Int J Radiat Oncol* (2006) 64(2):333–42. doi: 10.1016/j.ijrobp.2005.09.028

20. Song Y, Zhang M, Gan L, Chen X, Zhang T, Yue NJ, et al. Predictive parameters for selection of electronic tissue compensation radiotherapy in early-stage bre ast cancer patients after breast-conserving surgery. *Oncotarget* (2016) 7 (22):32835–45. doi: 10.18632/oncotarget.9054

21. Kong FM, Klein EE, Bradley JD, Mansur DB, Taylor ME, Perez CA, et al. The impact of central lung distance, maximal heart distance, and radiation technique on the volumetri c dose of the lung and heart for intact breast radiation. *Int J Radiat Oncol Biol Phys* (2002) 54(3):963–71. doi: 10.1016/s0360-3016(02)03741-0

22. Niemierko A. A generalized concept of equivalent uniform dose (EUD). *Med Phys* (1999) 26:1100.

23. Boughalia A, Marcie S, Fellah M, Chami S, Mekki F. Assessment and quantification of patient set-up errors in nasopharyngeal cancer patients and their bi ological and dosimetric impact in terms of generalized equivalent uniform dose (gEUD), tumour contro l probability (TCP) and normal tissue complication probability (NTCP). *Br J Radiol* (2015) 88(1050):20140839. doi: 10.1259/bjr.20140839

24. Qi XS, Semenenko VA, Li XA. Improved critical structure sparing with biologically based IMRT optimization. *Med Phys* (2009) 36(5):1790–9. doi: 10.1118/1.3116775

25. Wu S, Lai Y, He Z, Zhou Y, Chen S, Dai M, et al. Dosimetric comparison of the simultaneous integrated boost in whole-breast irradiation after breast-c onserving surgery: IMRT, IMRT plus an electron boost and VMAT. *PloS One* (2015) 10(3):e0120811. doi: 10.1371/journal.pone.0120811

26. Sakyanun P, Saksornchai K, Nantavithya C, Chakkabat C, Shotelersuk K. The effect of deep inspiration breath-hold technique on left anterior descending coronary artery and heart dose in left breast irradiation. *Radiat Oncol J* (2020) 38 (3):181–8. doi: 10.3857/roj.2020.00094

27. Tan W, Liu D, Xue C, Xu J, Li B, Chen Z, et al. Anterior myocardial territory may replace the heart as organ at risk in intensity-modulated radiother apy for left-sided breast cancer. *Int J Radiat Oncol Biol Phys* (2012) 82(5):1689–97. doi: 10.1016/j.ijrobp.2011.03.009

28. Deseyne P, Speleers B, De Neve W, Boute B, Paelinck L, Van Hoof T, et al. Whole breast and regional nodal irradiation in prone versus supine position in left sided breast cancer. *Radiat Oncol* (2017) 12(1):89. doi: 10.1186/s13014-017-0828-6

29. Yu T, Xu M, Sun T, Shao Q, Zhang Y, Liu X, et al. External-beam partial breast irradiation in a supine versus prone position after breast-conserving surgery for Chinese breast cancer patients. *Sci Rep* (2018) 8(1):15354. doi: 10.1038/s41598-018-33741-z

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Neuro-immune-endocrine mechanisms with poor adherence to aromatase inhibitor therapy in breast cancer

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As the most commonly used endocrine therapy regimen for patients with hormone receptor-positive (HR+) breast cancer (BC) at present, aromatase inhibitors (AIs) reduce the risk of localized and distant recurrence, contralateral BC and secondary cancer, and prolong disease-free survival. Clinical data show that poor adherence during AI treatment is mainly attributed to muscle and joint pain, fatigue, anxiety, depression and sleep disturbances during treatment. The rapid decline of estrogen caused by AIs in a short period of time enhances sympathetic activity, activates T cells in the body, produces inflammatory factors such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukin (IL)-17A, and promotes the occurrence of inflammation and bone loss. This article reviewed the mechanism of poor dependence on AIs in BC patients from the neuro-immuno-endocrine (NIE) perspective and provided clues for clinical intervention against poor adherence.

KEYWORDS

aromatase inhibitors, breast cancer, neuro-immune-endocrine, adherence, stress

Introduction

Global cancer reports show that breast cancer (BC), the highest incidence of cancer, has surpassed lung cancer, whose number of annual new cases is estimated to be 2.3 million (1). About 60% of premenopausal patients and 75% of postmenopausal ones have tumors that are positive for hormone receptors, and estrogen can bind to estrogen receptors (ERs) to accelerate BC development and metastasis (2). Thus, endocrine therapy is the first-line treatment option for such patients, specifically selective ER modulators (SERMs) in premenopausal patients and aromatase inhibitors (AIs) in postmenopausal ones. According to the results of a recent meta-analysis, ovarian function suppression (OFS) + AI was able to lower the absolute risk of recurrence to

five to 10 years for patients with premenopausal hormone receptor-positive (HR+) BC compared with OFS + SERM (3), and bone density could be maintained by using bisphosphonates to reduce fractures resulting from AIs (4). As a result, AIs have become the most commonly used endocrine therapy for patients with HR+ BC. With the publication of clinical findings, the International Breast Group (BIG) 1-98 study had a median follow-up of 12.6 years, and the results showed that taking letrozole alone for five years significantly reduced the incidence of contralateral BC within 10 years (5). The results of a meta-analysis by CHEN J et al. suggest that prolonged AI therapy for two-three years is necessary and sufficient for patients only receiving tamoxifen or tamoxifen + AI treatment for a total of five years, with positive lymph nodes or tumors ≥ 2 cm (6). Despite significantly increasing the risk of cardiotoxicity, osteoporosis, fractures, bone pain, arthralgia, myalgia and ≥ grade 3 hot flashes in patients, extended therapy can reduce the risk of localized and distant recurrence, contralateral BC and secondary cancer (7, 8), and prolong disease-free survival compared with non-prolonged AI therapy (9).

According to their different action mechanisms, AIs are divided into two categories. The first one is nonsteroidal AIs which bind reversibly to aromatase through ionic bonds and prevent the binding of androgens to the enzyme through competition, namely "competitive inhibition". The second one is steroidal AIs which bind irreversibly to aromatase in the form of covalent bonds and cause the permanent inactivation of the enzyme, namely "suicidal inhibition", and such inhibitors are called "lethal inhibitors". Also known as amino hypnosis, Amlumide is the first generation of AIs, which is nonsteroidal and can inhibit the synthesis of all steroid hormones in adrenal glands and display the function of "drug-induced adrenal resection". With large side effects, the drug is inconvenient to use and needs to be taken with hydrocortisone. The second generation of AIs includes nonsteroidal fartrazole and steroidal formessteine that have small side effects due to their selective inhabitation of aromatase and whose efficacy however is not better than tamoxifen. Mainly composed of nonsteroidal drugs like anastrozole and letrozole and steroidal ones like exemestane, the third generation of AIs highly selectively inhibits aromatase, with strong specificity and significantly reduced side effects (10).

Poor compliance status and risk factors such as pain sensation, painful mood and sleep disturbances

A total of 8,769 patients with stage I-III HR+ BC were included in a retrospective study between 1996 and 2007, of whom 43%, 26% and 30% took SERM, AI and at least one of both, respectively. A 4.5-year follow-up was conducted, and only 49% of patients underwent hormone therapy throughout the course (11). Statistical analysis showed that early endocrine discontinuation increased all-cause mortality by 26%, and mortality increased with the decreased level of adherence, with an improvement in the overall survival of women who were married, got a high self-esteem scale score, had no lymph nodes and received radiation therapy (12). Karen et al. conducted a 5-year prospective observational study on 321 patients, among whom 43.6% and 56.4% took SERM and AI, respectively. AI therapy is more likely to be discontinued than SERM therapy, and endocrine symptoms and sleep disturbances present during treatment are the main causes of discontinuation (13).

Naoko et al. conducted a questionnaire survey of 8,875 endocrine-treated patients, and obtained the following results: 56- to 69-year-old patients taking AI exhibited significantly higher knuckle stiffness and vaginal dryness than those taking SERM, but demonstrated significantly lower hot flashes, increased vaginal discharge, weight gain and genital bleeding; ≥70-year-old patients taking AI exhibited significantly more frequent or severe sweating, drowsiness, knuckle stiffness, knee/shoulder pain and limb numbness (14). The survey is consistent with the results of the Malaysian study where the development of musculoskeletal pain in patients using AI was more than twice that in those using SERM, patients with longer menopausal periods were less likely to have musculoskeletal pain and menopausal symptoms, and patients receiving primary or secondary education demonstrated significantly fewer menopause urogenital symptoms (15).

A multicenter phase IV clinical trial showed that musculoskeletal pain during AI treatment occurred primarily in the first six months of treatment, with a higher incidence in patients without a pre-treatment history of musculoskeletal pain and greater post-treatment pain intensity in patients with a prior history of pain (16). Joint pain increased significantly during the first year of AI treatment and the health-related quality of life decreased. Patients switching to AI therapy after two-three years of tamoxifen experienced greater pain and were at greater risk of stopping the drug in the first 12 months (17). The results of a prospective cohort study showed that senescence perceptions related to joint pain and depressive symptoms during AI treatment were significantly associated with AI non-compliance, and AI compliance may be improved by intervention in negative emotions (18). After one year of endocrine therapy, speech memory experienced a significant decrease from baseline (19). The results of an 18-year meta-analysis also indicated that endocrine therapy worsened the speech memory of BC patients (20).

Clinically relevant risk factors

Arimidex, Tamoxifen, alone or in combination (ATAC) was a randomized, double-blind and multicenter clinical trial where patients with early postmenopausal BC were randomly assigned to

the anatrozole alone, tamoxifen alone or anastrozole plus tamoxifen group, and patients with articular symptoms before enrollment were not analyzed. The results suggest that joint symptoms may be correlated with a sharp decrease in estrogen concentration in early endocrine therapy. Specifically, patients had a history of chemotherapy and estrogen replacement therapy, body mass index (BMI) >30 and positive HRs, and received a combination of anastrozole and tamoxifen (21). The integrated employment and skills (IES) trial recruited postmenopausal primary BC patients who had received two-three years of tamoxifen treatment and were assigned at random to continue the use of tamoxifen or switch to exemestane for five years of endocrine therapy. A retrospective analysis of its data found that the risk of carpal tunnel syndrome was increased approximately tenfold after the treatment of exemestane, and the presence or absence of musculoskeletal symptoms in the first six months of treatment appeared not to be related to improved survival. In terms of musculoskeletal symptoms, the results after adjusting for confounding factors showed that some factors were unclear, including weight≥80 kg, geographical area, history of hormone therapy, musculoskeletal diseases, endocrine or metabolic diseases, osteoporosis, ovariectomization (OVX), chemotherapy, radiotherapy and diabetes, pre-treatment hot flashes, arthralgia, myalgia, osteoarthritis (OA) and acquired hypothyroidism as risk factors, no statistically significant length of menopause, type of surgery, age, lymphedema at baseline and diuretic use (22). Combined with these two randomized controlled trials (RCTs), it can be found that BMI >30 or body weight ≥ 80 kg and history of hormone therapy may have a more clear effect on musculoskeletal symptoms.

Paul et al. conducted a multicenter RCT evaluating the advantages and disadvantages of exemestane versus anastrozole in patients with early-stage breast cancer. The results showed no significant differences between the two treatment groups in overall survival, distant metastases, distant disease-free survival, local recurrence, death, contralateral new primary breast cancer, menopausal-like symptoms (hot flashes, arthritis, arthralgia and myalgia), myocardial infarction, stroke, transient ischemic attack, fractures, and depression. Atrial fibrillation, mild bilirubin abnormalities, acne, and virilization were more common in the exemestane group. The anastrozole group had higher rates of anxiety, pain elsewhere (mouth, breast, etc.), postmenopausal vaginal bleeding, hypertriglyceridemia, hypercholesterolemia, and a new diagnosis of self-reported osteoporosis. Minority women in the exemestane group had fewer deaths and lower discontinuation rates than those in the anastrozole group compared with white women (23). Results from a multicenter, randomized, double-blind, phase 3 clinical trial in patients with advanced breast cancer showed that exemestane treatment associated a higher incidence of hot flashes, arthralgias, and musculoskeletal stiffness and most symptoms were grade 1 or 2 compared with the anastrozole group (24). Nazli et al. conducted an RCT of neoadjuvant endocrine therapy in patients with locally advanced postmenopausal breast cancer, randomized to letrozole or exemestaine, and assessed serum levels of 54 cytokines after 16 w. The results showed a significant decrease in serum leptin levels in patients in the exemestane group compared to the non-significant increase caused by letrozole, while the baseline serum leptin level was positively correlated with BMI (25). Therefore, from the comprehensive clinical symptoms and biochemical indicators, the steroid inhibitor exemestane and the non-steroidal inhibitors letrozole and anastrozole lack cross-resistance, which may be related to the metabolism associated with leptin in serum, suggesting that leptin may be a potential predictor of poor patient compliance.

Research has shown that rheumatoid arthritis (RA) patients are less likely to suffer from BC, and patients with a history of BC have a lower risk of recurring RA, but the associated risk has no clear determinants. Endocrine treatments like tamoxifen or AIs seem not to raise the risk of RA (26), which is not in line with the results of cohort studies conducted by Marta et al. From 2004 to 2013, Marta et al. collected data from an administrative healthcare database in Italy to assess the relationship between AI or tamoxifen treatment and an increased risk of RA. A total of 10,493 BC patients were included in the study, of whom 7,533 (71.8%) received AI or tamoxifen treatment. The results showed that exposure to AI was related to a significant increase in the risk of RA compared with exposure to tamoxifen, particularly in patients treated with anastrozole, and RA was not affected by the relationships between cancer severity, age and specific drug indications (27). Other studies have revealed that the simultaneous use of SERMS and AI increases the incidence of rheumatic diseases (28).

Neuro-immune-endocrine mechanisms with poor adherence

First mentioned by Basedovsky in 1977 (29). neuro-immune-endocrine (NIE) networks regulate the normal physiological functions of the body at an overall level and maintain the homeostasis of the body, and the disorder of any of these links inevitably exerts an influence on the functions of other systems.

Research by Ulrich et al. showed that pain neurons can form networks around lymph nodes and regulate two-way communication. It was found that pain neurons increased the distribution density in the enlarged lymph nodes when the immune response in mice was artificially induced. The altered gene expression of specific cells in lymph nodes was observed when pain neurons were activated, suggesting that the pain nerve and lymph nodes surrounded by it can sense and regulate each other (30).

Clinical evidence

In 2010, a case-control genome-wide association study by James et al. determined the association between single nucleotide

polymorphisms (SNPs) and musculoskeletal adverse reactions in females treated with AI for early BC. Enrolling 878 patients, the study noticed that T-cell leukemia/lymphoma 1A (TCL1A) gene was associated with musculoskeletal adverse reactions and the cytokine interleukin (IL)-17) (31). Further research indicates the ability of TCL1A to affect downstream expression across a range of immune mediators, such as Toll-like receptor (TLR)2, TLR7, TLR9, TLR10 and myeloid differentiation factor (MYD)88. MYD88 encodes a functional adapter molecule capable of recruiting IL-1R activating kinase (IRAK)1, IRAK2, IRAK4 and tumor necrosis factor receptor-associated factor 6, ultimately activating nuclear factor kappa-B (NF-κB), secreting pro-inflammatory cytokines and leading to an inflammatory response (32).

In 2015, Joshua et al. conducted a cross-sectional study on an ongoing cohort study of patients undergoing adjuvant AI therapy at the Abramson Cancer Center of the University of Pennsylvania and simultaneously evaluated 34 inflammatory biomarkers in peripheral blood. A total of 203 participants were included, and the results showed a significant association of arthralgia with fatigue and insomnia. Among patients experiencing moderate to severe joint pain, 88.4% and 83.7% went through both fatigue and insomnia, respectively. The coexistence of arthralgia, fatigue and insomnia after adjusting for race, chemotherapy history, nonsteroidal antiinflammatory drugs (NSAIDs), age and BMI was in connection with elevated C-reactive protein (CRP), eotaxin, monocyte chemokine-1 as well as vitamin D-binding protein (VDBP) (33).

The expression of aromatase takes place in the chondrocytes and synovial cells of articular cartilage (34), and decreased estrogen levels increase the production of pro-inflammatory cytokines like IL-6 and -1 in articular chondrocytes, leading to joint pain and swelling (35). While no evidence supports an association of fatigue with pro-inflammatory cytokines IL-1β and -6, the results do show that fatigue is associated with the downstream biomarkers of cytokine activity. In particular, the increased downstream products of IL-6 and -1β, CRP and IL-1 receptor antagonists are related to the increased severity and frequency of fatigue symptoms (36). At the molecular level, IL-6 stimulates the secretion of CRP, whose expression however is blocked by estrogen (37, 38), also explaining the increase of CRP caused by a significant decrease in estrogen levels during AI treatment.

VDBP is not only the primary binding protein for vitamin D but also an acute phase reactant with apparent genetic variability (39). Clinical studies have confirmed the following findings: the incidence of the Fok-I variant of the vitamin D receptor in Caucasian women is about 33%; IL-1β is a cytokine closely related to arthralgia; IL-1β levels are reduced by around 50% in women with this variant; patients are less likely to report abnormal arthralgia and myalgia six months after the initiation of AI therapy (40, 40). Eotaxin and monocyte chemoattractant protein (MCP)-1 are chemokines taking charge of recruiting inflammatory cells to injury sites (41, 42). whose elevated concentrations (43), are seen in fibromyalgia patients and characterized by joint pain, fatigue and low sleep quality (44). Clinical studies have shown that combining hydroxytyrosol, curcumin and omega-3 fatty acids can decrease blood CRP and pain in BC patients undergoing AI after menopause, suggesting the potential role of inflammation in AI-induced musculoskeletal symptoms (45).

Cyclin-dependent kinases (CDKs) are of importance to initiate the cell cycle and regulate transitions in a variety of stages. Binding to cyclin D, CDK4/6 phosphorylates the retinoblastoma (Rb) gene and then releases the transcription factor E2F, promoting the transcription of genes related to the cell cycle and enabling the entrance of cells into the S phase. CDK4/6 inhibitors are effective in blocking tumor cells from G1 to S phase. In ER-positive (ER+) BC, the overactivity of CDK4/6 is very frequent. Preclinical data show that the dual inhibition of CDK4/6 and ER signaling produces a synergistic effect and curbs the growth of ER+ BC cells in the G1 stage. Therefore, adding CDK4/6 inhibitors becomes a better choice for the AI treatment of BC patients with metastasis. As suggested by a systematic review, AI-induced musculoskeletal symptoms experience a relative reduction in incidence after the use of CDK4/6 inhibitors possibly by the mechanism that CDK4/6 inhibitors are capable of attenuating E2F2 activity in the cartilage and synovium and at least partially reversing AI-induced inflammation (46). The same conclusion was also reached in the 18-year study of PALOMA-2 which significantly improved the pain scores of patients compared with letrozole alone (47).

NIE activation

Stress is one of the common factors altering the “steady state” of the environment in the body. In the face of various stressors in both internal and external environments, the stress system of the body is activated and adapts to stressors to maintain the relative stability of the internal environment. Classical stress theory holds that the stress system primarily comprises hypothalamic paraventricular nucleus-corticotropin-releasing hormone (PVN-CRH) and blue-spot-norepinephrine (LC-NE) systems as well as their efferent parts, giving rise to neuroendocrine responses and behavioral changes in stress (48). After the activation of the stress system, the main two major reactions are the sympathetic-adrenal medullary and hypothalamic-pituitary-adrenal cortex systems.

As an enzyme catalyzing the reaction of the last step of epinephrine synthesis, phenylethanolamine-N-methyltransferase (PNMT) is present in certain neurons of the adrenal medulla and central nervous system, where estrogen regulates the expression of c-Fos, indicating that estradiol directly targets many adrenergic neurons. A majority of brainstem PNMT neurons are activated during the initiation of the luteinizing hormone (LH) surge

induced by a hormone, suggesting that estrogen may be a trigger during the GnRH surge (49).

Postmenopausal women have a higher basal level of norepinephrine than premenopausal ones, and also exhibit a greater increase in heart rate, systolic blood pressure and norepinephrine secretion in response to psychological stress (50, 51). Rosano et al. confirmed an increase in the sympathetic impulses of healthy postmenopausal women and a significant decrease in sympathetic activity after chronic estrogen replacement therapy (52). It has been shown that central estrogen administration in de-ovarian rats reduces sympathetic activity (53).

Estrogen plays a complex role in the development of inflammation (37). In a 2001 review of the bimodal effects of estrogen on inflammatory pathways, Calabrese showed that high doses of E2 could inhibit scores of inflammatory mechanisms without or even opposite effects at low concentrations (54). which was also confirmed by Rainer in a 2007 review of E2 suppressing important pro-inflammatory pathways during ovulation/pregnancy, especially in the third trimester. When E2 is reduced to postmenopausal levels, the environment of the body shifts towards inflammation (37).

Women with vasomotor symptoms have lower bone density than those without, and vasomotor symptoms are bound up with sympathetic activity. Drug-induced sympathetic neurological block (via receptor blockers) is conducive to trabecular microstructure, femoral cortex width as well as hip and lumbar vertebrae bone density in postmenopausal women (55). Most effects of E2 on bone cells are mediated by ERα, and subchondral bone mass decreases and is associated with the increased severity of OA despite no change in the cartilage of ERα knockout mice [50]. ERβ does not mediate the bone-sparing activity of estrogen on rat bones or affect ovulation or oophorectomy-induced weight gain, whose function may involve modulating the immune response (56). E2 can induce osteoclasts and inhibit osteoblastic apoptosis (57, 58).

In both mice and humans, thymus structure and function decline with age, and fewer new T cells can be produced and exported to secondary lymphoid organs until old age although most parenchymal tissues are replaced by fat by middle age (59). In the case of the severe depletion of T cells, such as secondary human immunodeficiency virus (HIV) infection, chemotherapy and bone marrow transplantation, an increase occurs in thymic output, which is a phenomenon referred to as thymic rebound essential for the long-term recovery of T-cell homeostasis (60). Estrogen deficiency can also trigger functional thymic rebound and IL-7 elevation after OVX stimulates the thymus-dependent differentiation of bone marrow-derived progenitor cells and mature T cells to regulate the production of T lymphocyte and induce bone loss, while thymectomy can reduce bone loss by 50% and OVX-induced T cell plasia, and the inhibition of IL-7 can completely prevent the production of T lymphocytes and resulting bone loss. Thus, IL-7 mediates T-cell destruction and bone homeostasis after OVX through thymic and extrathymic mechanisms, which is a key upstream target for the estrogen regulation of hematopoietic and immune functions, and is critical for osteosteady (61). Despite not being enough to strengthen thymus production in young mice (62), IL-7 alone plays a vital role in older ones (63). suggesting that IL-7-induced thymic rebound after estrogen deficiency may be the cause of rapid initial bone loss in young females undergoing surgery or females with natural menopause (64, 65). Clinical studies have shown that IL-7R and insulin-like growth factor (IGF-1) associated with T-cell function are significantly expressed in inflammatory arthritis and independent of predictors like CRP used routinely (66).

Most body tissues are innervated by sensory and autonomic nerves to varying degrees, with sympathetic nerves innervating primary (bone marrow and thymus) and secondary (spleen and lymph nodes) lymph organs (67). Changes in these cytokines activate T cells when estrogen is missing and thus result in an increase in IL-7, IGF-1 and reactive oxygen species (ROS) in target organs such as the thymus, spleen and bone, and a decrease in transforming growth factor (TGF)-β. Activated T cells release interferon (IFN)-γ, together with increased ROS, and upregulate major histocompatibility complex (MHC) class II expression through the transcription factor CITITA to increase the antigen presentation of dendritic cells (DC) and macrophages (Mφ) on the one hand, and promote the release of osteoclastic factors TNF-α and IL-17A on the other hand. IL-17A is a potent promoter of bone destruction. TNF-α activates the nuclear factor kappa-B (NF-κB) and c-Fms/macrophage colony-stimulating factor system, produces IL-1β by directly or indirectly upregulating IL-1 to osteoblasts and their precursors, and ultimately leads to an inflammatory response and bone loss (57, 68, 69). Studies have shown that IL-6 produced by bone and bone marrow stromal cells in mice after OVX increases the number of granulocyte and macrophage colony-forming units, facilitates the development of osteoclasts and contributes to the increased number of osteoclasts in the trabecular bone, which may also be one of the mechanisms of increased bone resorption in postmenopausal osteoporosis (70) (Figure 1).

Decreased estrogen after oophorectomy is able to target T cells to produce more TNF-α inducing bone loss, with remission after E2 replacement therapy (71). Ovaryectomy in nude mice deficient in T cells does not induce bone loss, no osteoporosis occurs after the transplantation of T cells in TNF-deficient mice, and bone loss is induced after the transplantation of wild-type mouse T cells, also demonstrating that the presence of TNF-α producing T cells is crucial for the effects of bone or joint metabolism abnormalities after estrogen deficiency (72).

Conclusion

NIE mechanisms play a decisive role in poor adherence to endocrine therapy in BC patients. The sympathetic nervous

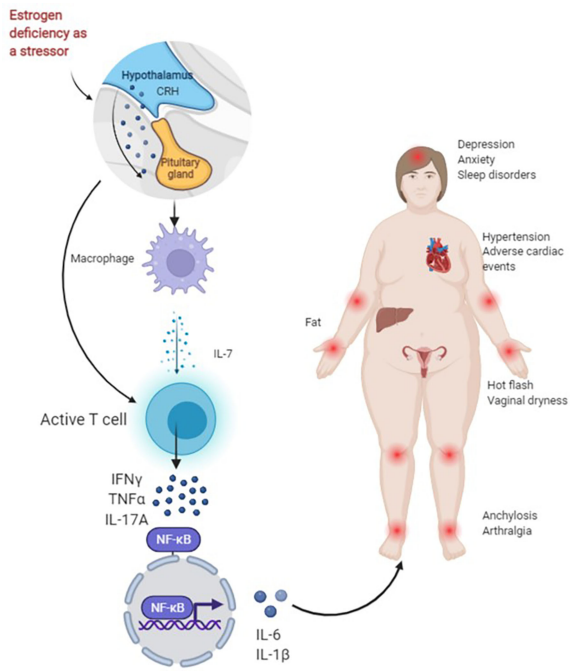

FIGURE 1
Figure 1 presents the possible mechanisms of non-adherence during treatment with AIs in BC patients. As a stressor, AIs lead to a rapid decrease in estrogen in a short period of time. For one thing, the activation of the hypothalamic-pituitary-adrenal (HPA) axis of the body and the enhancement of sympathetic activity can directly activate T cells. For another, the first activation of macrophages helps to release IL-7, activate T cells and produce IFN-γ, TNF-α, IL-17A and other cytokines, and NF-κB nuclear transcription produces IL-6, IL-1β and other downstream factors, and promotes inflammation and bone loss, resulting in joint pain. Joint stiffness, fatigue, sleep disturbances, anxiety, depression, obesity, hypertension and cardiac adverse events increase early discontinuation and lead to poor long-term adherence.

system, which is the total dispatch of the body, participates in the occurrence of adverse reactions by activating or inhibiting the release of inflammatory factors by different immune cells in the rapid decline of estrogen in a short period of time, affecting the compliance of patients and thus determining long-term prognosis. Therefore, the possible mechanisms of poor adherence during patient treatment can be deeply understood to reveal potential pharmacological targets and may be used to guide early clinical intervention, improve adherence and maximize the benefits of BC patients.

Author contributions

FY: Conceptualization, Methodology, Data curation, Supervision and Validation; LH: Visualization, Software and Original draft preparation; GJ: Reviewing and Editing. All authors contributed to the article and approved the submitted version.

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.

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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. Anderson WF, Chatterjee N, Ershler WB, Brawley OW. Estrogen receptor breast cancer phenotypes in the surveillance, epidemiology, and end results database. *Breast Cancer Res Treat* (2002) 76(1):27–36. doi: 10.1023/a:1020299707510

3. Rosie BJB, Richard G, Robert KH, Zu LL, Hong CP, Richard P, et al. Aromatase inhibitors versus tamoxifen in premenopausal women with oestrogen receptor-positive early-stage breast cancer treated with ovarian suppression: A patient-level meta-analysis of 7030 women from four randomised trials. *Lancet Oncol* (2022) 23(3):382–92. doi: 10.1016/s1470-2045(21)00758-0

4. Jeremy B, Mike C, Rory C, David D, Richard G, Richard H, et alAdjuvant bisphosphonate treatment in early breast cancer: Meta-analyses of individual patient data from randomised trials. *Lancet (London England)* (2015) 386 (10001):1353–61. doi: 10.1016/s0140-6736(15)60908-4

5. Ruhstaller T, Giobbie-Hurder A, Colleoni M, Jensen MB, Ejlertsen B, de Azambuja E, et al. Adjuvant letrozole and tamoxifen alone or sequentially for postmenopausal women with hormone receptor-positive breast cancer: Long-term follow-up of the big 1-98 trial. *J Clin Oncol* (2019) 37(2):105–14. doi: 10.1200/jco.18.00440

6. Chen J, Zhang X, Lu Y, Zhang T, Ouyang Z, Sun Q. Optimal duration of endocrine therapy with extended aromatase inhibitors for postmenopausal patients with hormone receptor-positive breast cancer: A meta-analysis. *Breast Cancer (Tokyo Japan)* (2021) 28(3):630–43. doi: 10.1007/s12282-020-01196-8

7. Zhao F, Ren D, Shen G, Ahmad R, Dong L, Du F, et al. Toxicity of extended adjuvant endocrine with aromatase inhibitors in patients with postmenopausal breast cancer: A systemtic review and meta-analysis. *Crit Rev oncology/hematol* (2020) 156:103114. doi: 10.1016/j.critrevonc.2020.103114

8. Burstein HJ, Lacchetti C, Anderson H, Buchholz TA, Davidson NE, Gelmon KA, et al. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: Asco clinical practice guideline focused update. *J Clin Oncol* (2019) 37(5):423–38. doi: 10.1200/jco.18.01160

9. Qian X, Li Z, Ruan G, Tu C, Ding W. Efficacy and toxicity of extended aromatase inhibitors after adjuvant aromatase inhibitors-containing therapy for hormone-Receptor-Positive breast cancer: A literature-based meta-analysis of randomized trials. *Breast Cancer Res Treat* (2020) 179(2):275–85. doi: 10.1007/s10549-019-05464-w

10. Tomao F, Spinelli G, Vici P, Pisanelli GC, Cascialli G, Frati L. Current Role and Safety Profile of Aromatase Inhibitors in Early Breast Cancer. *Expert Rev Anticancer Ther* (2011) 11(8):1253–63. doi: 10.1586/era.11.96

11. Hershman DL, Kushi LH, Shao T, Buono D, Kershenbaum A, Tsai WY, et al. Early discontinuation and nonadherence to adjuvant hormonal therapy in a cohort of 8,769 early-stage breast cancer patients. *J Clin Oncol* (2010) 28(27):4120–8. doi: 10.1200/jco.2009.25.9655

12. Hershman DL, Shao T, Kushi LH, Buono D, Tsai WY, Fehrenbacher L, et al. Early discontinuation and non-adherence to adjuvant hormonal therapy are associated with increased mortality in women with breast cancer. *Breast Cancer Res Treat* (2011) 126(2):529–37. doi: 10.1007/s10549-010-1132-4

13. Smith KL, Verma N, Blackford AL, Lehman J, Westbrook K, Lim D, et al. Association of treatment-emergent symptoms identified by patient-reported outcomes with adjuvant endocrine therapy discontinuation. *NPJ Breast Cancer* (2022) 8(1):53. doi: 10.1038/s41523-022-00414-0

14. Honma N, Makita M, Saji S, Mikami T, Ogata H, Horii R, et al. Characteristics of adverse events of endocrine therapies among older patients with breast cancer. *Supportive Care Cancer* (2019) 27(10):3813–22. doi: 10.1007/s00520-019-04674-8

15. Choo SB, Saifulbahri A, Zullkifli SN, Fadzil ML, Redzuan AM, Abdullah N, et al. Adjuvant endocrine therapy side-effects among postmenopausal breast cancer patients in Malaysia. *Climacteric: J Int Menopause Soc* (2019) 22(2):175–81. doi: 10.1080/13697137.2018.1540563

16. Nabieva N, Häberle L, Brucker SY, Janni W, Volz B, Loehberg CR, et al. Preexisting musculoskeletal burden and its development under letrozole treatment in early breast cancer patients. *Int J Cancer* (2019) 145(8):2114–21. doi: 10.1002/ijc.32294

17. Pineda-Moncusí M, Servitja S, Tusquets I, Diez-Perez A, Rial A, Cos ML, et al. Assessment of early therapy discontinuation and health-related quality of life in breast cancer patients treated with aromatase inhibitors: B-able cohort study. *Breast Cancer Res Treat* (2019) 177(1):53–60. doi: 10.1007/s10549-019-05289-7

18. Brier MJ, Chambless DL, Chen J, Mao JJ. Ageing perceptions and non-adherence to aromatase inhibitors among breast cancer survivors. *Eur J Cancer (Oxford England: 1990)* (2018) 91:145–52. doi: 10.1016/j.ejca.2017.12.006

19. Underwood EA, Jerzak KJ, Lebovic G, Rochon PA, Elser C, Pritchard KI, et al. Cognitive effects of adjuvant endocrine therapy in older women treated for early-stage breast cancer: A 1-year longitudinal study. *Supportive Care Cancer* (2019) 27(8):3035–43. doi: 10.1007/s00520-018-4603-5

20. Underwood EA, Rochon PA, Moineddin R, Lee PE, Wu W, Pritchard KI, et al. Cognitive sequelae of endocrine therapy in women treated for breast cancer: A meta-analysis. *Breast Cancer Res Treat* (2018) 168(2):299–310. doi: 10.1007/s10549-017-4627-4

21. Sestak I, Cuzick J, Sapunar F, Eastell R, Forbes JF, Bianco AR, et al. Risk factors for joint symptoms in patients enrolled in the atac trial: A retrospective, exploratory analysis. *Lancet Oncol* (2008) 9(9):866–72. doi: 10.1016/s1470-2045(08)70182-7

22. Mieog JS, Morden JP, Bliss JM, Coombes RC, van de Velde CJ. Carpal tunnel syndrome and musculoskeletal symptoms in postmenopausal women with early breast cancer treated with exemestane or tamoxifen after 2-3 years of tamoxifen: A retrospective analysis of the intergroup exemestane study. *Lancet Oncol* (2012) 13(4):420–32. doi: 10.1016/s1470-2045(11)70328-x

23. Goss PE, Ingle JN, Pritchard KI, Ellis MJ, Sledge GW, Budd GT, et al. Exemestane versus anastrozole in postmenopausal women with early breast cancer: Ncic ctg Ma.27–a randomized controlled phase iii trial. *J Clin Oncol* (2013) 31(11):1398–404. doi: 10.1200/jco.2012.44.7805

24. Iwata H, Masuda N, Ohno S, Rai Y, Sato Y, Ohsumi S, et al. A randomized, double-blind, controlled study of exemestane versus anastrozole for the first-line treatment of postmenopausal Japanese women with hormone-Receptor-Positive advanced breast cancer. *Breast Cancer Res Treat* (2013) 139(2):441–51. doi: 10.1007/s10549-013-2573-3

25. Bahrami N, Jabeen S, Tahiri A, Sauer T, Ødegård HP, Geisler SB, et al. Lack of cross-resistance between non-steroidal and steroidal aromatase inhibitors in breast cancer patients: The potential role of the adipokine leptin. *Breast Cancer Res Treat* (2021) 190(3):435–49. doi: 10.1007/s10549-021-06399-x

26. Wadström H, Pettersson A, Smedby KE, Askling J. Risk of breast cancer before and after rheumatoid arthritis, and the impact of hormonal factors. *Ann rheum Dis* (2020) 79(5):581–6. doi: 10.1136/annrheumdis-2019-216756

27. Caprioli M, Carrara G, Sakellariou G, Silvagni E, Scire CA. Influence of aromatase inhibitors therapy on the occurrence of rheumatoid arthritis in women with breast cancer: Results from a Large population-based study of the Italian society for rheumatology. *RMD Open* (2017) 3(2):e000523. doi: 10.1136/rmdopen-2017-000523

28. Ray A, Ficek M. Immunomodulatory effects of anti-estrogenic drugs. *Acta Pharm (Zagreb Croatia)* (2012) 62(2):141–55. doi: 10.2478/v10007-012-0012-3

29. Besedovsky H, Sorkin E. Network of immune-neuroendocrine interactions. *Clin Exp Immunol* (1977) 27(1):1–12. doi: 10.3390/ijms232113656

30. Huang S, Ziegler CGK, Austin J, Mannoun N, Vukovic M, Ordovas-Montanes J, et al. Lymph nodes are innervated by a unique population of sensory neurons with immunomodulatory potential. *Cell* (2021) 184(2):441–59.e25. doi: 10.1016/j.cell.2020.11.028

31. Ingle JN, Schaid DJ, Goss PE, Liu M, Mushiroda T, Chapman JA, et al. Genome-wide associations and functional genomic studies of musculoskeletal adverse events in women receiving aromatase inhibitors. *J Clin Oncol* (2010) 28(31):4674–82. doi: 10.1200/jco.2010.28.5064

32. Ho M-F, Ingle JN, Bongartz T, Kalari KR, Goss PE, Shepherd LE, et al. Tcl1a single-nucleotide polymorphisms and estrogen-mediated toll-like Receptor-Myd88–dependent nuclear factor-κb activation: Single-nucleotide polymorphism– and selective estrogen receptor modulator–dependent modification of inflammation and immune response. *Mol Pharmacol* (2017) 92(2):175–84. doi: 10.1124/mol.117.108340

33. Bauml J, Chen L, Chen J, Boyer J, Kalos M, Li SQ, et al. Arthralgia among women taking aromatase inhibitors: Is there a shared inflammatory mechanism with Co-morbid fatigue and insomnia? *Breast Cancer res: BCR* (2015) 17(1):89. doi: 10.1186/s13058-015-0599-7

34. Richmond RS, Carlson CS, Register TC, Shanker G, Loeser RF. Functional estrogen receptors in adult articular cartilage: Estrogen replacement therapy increases chondrocyte synthesis of proteoglycans and insulin-like growth factor binding protein 2. *Arthritis rheum* (2000) 43(9):2081–90. doi: 10.1002/1529-0131(200009)43:9<2081::Aid-anr20>3.0.Co;2-i

35. Le Bail J, Liagre B, Vergne P, Bertin P, Beneytout J, Habrioux G. Aromatase in synovial cells from postmenopausal women. *Steroids* (2001) 66(10):749–57. doi: 10.1016/s0039-128x(01)00104-0

36. Bower JE, Ganz PA, Tao ML, Hu W, Belin TR, Sepah S, et al. Inflammatory biomarkers and fatigue during radiation therapy for breast and prostate cancer. *Clin Cancer Res* (2009) 15(17):5534–40. doi: 10.1158/1078-0432.Ccr-08-2584

37. Straub RH. The complex role of estrogens in inflammation. *Endoc Rev* (2007) 28(5):521–74. doi: 10.1210/er.2007-0001

38. Pfeilschifter J, Köditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endoc Rev* (2002) 23(1):90–119. doi: 10.1210/edrv.23.1.0456

39. Malik S, Fu L, Juras DJ, Karmali M, Wong BY, Gozdzik A, et al. Common variants of the vitamin d binding protein gene and adverse health outcomes. *Crit Rev Clin Lab Sci* (2013) 50(1):1–22. doi: 10.3109/10408363.2012.750262

40. Niravath P, Chen BS, Chapman JAW, Agarwal SK, Welschhans RL, Bongartz T, et al. Vitamin d levels, vitamin d receptor polymorphisms, and inflammatory cytokines in aromatase inhibitor-induced arthralgias: An analysis of cctg Ma.27. *Clin Breast Cancer* (2018) 18(1):78–87. doi: 10.1016/j.clbc.2017.10.009

41. Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* (2006) 117(6):1277–84. doi: 10.1016/j.jaci.2006.02.039

42. Carr MW, Roth SJ, Luther E, Rose SS, Springer TA. Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proc Natl Acad Sci United States America* (1994) 91(9):3652–6. doi: 10.1073/pnas.91.9.3652

43. Zhang Z, Cherryholmes G, Mao A, Marek C, Longmate J, Kalos M, et al. High plasma levels of mcp-1 and eotaxin provide evidence for an immunological basis of fibromyalgia. *Exp Biol Med (Maywood NJ)* (2008) 233(9):1171–80. doi: 10.3181/0712-rm-328

44. Nicassio PM, Moxham EG, Schuman CE, Gevirtz RN. The contribution of pain, reported sleep quality, and depressive symptoms to fatigue in fibromyalgia. *Pain* (2002) 100(3):271–9. doi: 10.1016/s0304-3959(02)00300-7

45. Martínez N, Herrera M, Frías L, Provencio M, Pérez-Carrión R, Díaz V, et al. A combination of hydroxytyrosol, omega-3 fatty acids and curcumin improves pain and inflammation among early stage breast cancer patients receiving adjuvant hormonal therapy: Results of a pilot study. *Clin Trans Oncol* (2018) 21(4):489–98. doi: 10.1007/s12094-018-1950-0

46. Andrikopoulou A, Fiste O, Liontos M, Dimopoulos MA, Zagouri F. Aromatase and Cdk4/6 inhibitor-induced musculoskeletal symptoms: A systematic review. *Cancers* (2021) 13(3):19. doi: 10.3390/cancers13030465

47. Rugo HS, Diéras V, Gelmon KA, Finn RS, Slamon DJ, Martin M, et al. Impact of palbociclib plus letrozole on patient-reported health-related quality of life: Results from the paloma-2 trial. *Ann Oncol* (2018) 29(4):888–94. doi: 10.1093/annonc/mdy012

48. O'Connor TM, O'Halloran DJ, Shanahan F. The stress response and the hypothalamic-Pituitary-Adrenal axis: From molecule to melancholia. *QJM: monthly J Assoc Phys* (2000) 93(6):323–33. doi: 10.1093/qjmed/93.6.323

49. Lee EJ, Moore CT, Hosny S, Centers A, Jennes L. Expression of estrogen receptor-alpha and c-fos in adrenergic neurons of the female rat during the steroid-induced lh surge. *Brain Res* (2000) 875(1-2):56–65. doi: 10.1016/s0006-8993(00)02622-6

50. Owens JF, Stoney CM, Matthews KA. Menopausal status influences ambulatory blood pressure levels and blood pressure changes during mental stress. *Circulation* (1993) 88(6):2794–802. doi: 10.1161/01.cir.88.6.2794

51. Saab PG, Matthews KA, Stoney CM, McDonald RH. Premenopausal and postmenopausal women differ in their cardiovascular and neuroendocrine responses to behavioral stressors. *Psychophysiology* (1989) 26(3):270–80. doi: 10.1111/j.1469-8986.1989.tb01917.x

52. Rosano GM, Patrizi R, Leonardo F, Ponikowski P, Collins P, Sarrel PM, et al. Effect of estrogen replacement therapy on heart rate variability and heart rate in healthy postmenopausal women. *Am J Cardiol* (1997) 80(6):815–7. doi: 10.1016/s0002-9149(97)00528-6

53. Saleh MC, Connell BJ, Saleh TM. Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. *Brain Res* (2000) 879(1-2):105–14. doi: 10.1016/s0006-8993(00)02757-8

54. Calabrese EJ. Estrogen and related compounds: Biphasic dose responses. *Crit Rev Toxicol* (2001) 31(4-5):503–15. doi: 10.1080/20014091111785

55. Bonnet N, Gadois C, McCloskey E, Lemineur G, Lespessailles E, Courteix D, et al. Protective effect of beta blockers in postmenopausal women: Influence on fractures, bone density, micro and macroarchitecture. *Bone* (2007) 40(5):1209–16. doi: 10.1016/j.bone.2007.01.006

56. Harris HA, Albert LM, Leathurby Y, Malamas MS, Mewshaw RE, Miller CP, et al. Evaluation of an estrogen receptor-beta agonist in animal models of human disease. *Endocrinology* (2003) 144(10):4241–9. doi: 10.1210/en.2003-0550

57. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: An inflammatory tale. *J Clin Invest* (2006) 116(5):1186–94. doi: 10.1172/jci28550

58. Eriksen EF, Langdahl B, Vesterby A, Rungby J, Kassem M. Hormone replacement therapy prevents osteoclastic hyperactivity: A histomorphometric study in early postmenopausal women. *J Bone mineral Res* (1999) 14(7):1217–21. doi: 10.1359/jbmr.1999.14.7.1217

59. Douek DC, Koup RA. Evidence for thymic function in the elderly. *Vaccine* (2000) 18(16):1638–41. doi: 10.1016/s0264-410x(99)00499-5

60. Hakim FT, Memon SA, Cepeda R, Jones EC, Chow CK, Kasten-Sportes C, et al. Age-dependent incidence, time course, and consequences of thymic renewal in adults. *J Clin Invest* (2005) 115(4):930–9. doi: 10.1172/jci22492

61. Ryan MR, Shepherd R, Leavey JK, Gao Y, Grassi F, Schnell FJ, et al. An il-7-Dependent rebound in thymic T cell output contributes to the bone loss induced by estrogen deficiency. *Proc Natl Acad Sci United States America* (2005) 102(46):16735–40. doi: 10.1073/pnas.0505168102

62. Chu YW, Memon SA, Sharrow SO, Hakim FT, Eckhaus M, Lucas PJ, et al. Exogenous il-7 increases recent thymic emigrants in peripheral lymphoid tissue without enhanced thymic function. *Blood* (2004) 104(4):1110–9. doi: 10.1182/blood-2003-10-3635

63. Alpdogan O, Schmaltz C, Muriglan SJ, Kappel BJ, Perales MA, Rotolo JA, et al. Administration of interleukin-7 after allogeneic bone marrow transplantation improves immune reconstitution without aggravating graft-Versus-Host disease. *Blood* (2001) 98(7):2256–65. doi: 10.1182/blood.v98.7.2256

64. Riggs BL, Khosla S, Melton LJ3rd. Sex steroids and the construction and conservation of the adult skeleton. *Endoc Rev* (2002) 23(3):279–302. doi: 10.1210/edrv.23.3.0465

65. Hreshchyshyn MM, Hopkins A, Zylstra S, Anbar M. Effects of natural menopause, hysterectomy, and oophorectomy on lumbar spine and femoral neck bone densities. *Obstetrics gynecol* (1988) 72(4):631–8.

66. Niemantsverdriet E, van den Akker EB, Boeters DM, van den Eeden SJF, Geluk A, van der Helm-van Mil AHM. Gene expression identifies patients who develop inflammatory arthritis in a clinically suspect arthralgia cohort. *Arthritis Res Ther* (2020) 22(1):266. doi: 10.1186/s13075-020-02361-2

67. Cleypool CGJ, Mackaaij C, Lotgerink Bruinenberg D, Schurink B, Bleys R. Sympathetic nerve distribution in human lymph nodes. *J Anat* (2021) 239(2):282–9. doi: 10.1111/joa.13422

68. Weitzmann MN. T-Cells and b-cells in osteoporosis. *Curr Opin endocrinol diabetes Obes* (2014) 21(6):461–7. doi: 10.1097/med.0000000000000103

69. Wu D, Cline-Smith A, Shashkova E, Perla A, Katyal A, Aurora R. T-Cell mediated inflammation in postmenopausal osteoporosis. *Front Immunol* (2021) 12:687551. doi: 10.3389/fimmu.2021.687551

70. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, et al. Increased osteoclast development after estrogen loss: Mediation by interleukin-6. *Sci (New York NY)* (1992) 257(5066):88–91. doi: 10.1126/science.1621100

71. Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, et al. Estrogen deficiency induces bone loss by enhancing T-cell production of tnf-alpha. *J Clin Invest* (2000) 106(10):1229–37. doi: 10.1172/jci11066

72. Roggia C, Tamone C, Cenci S, Pacifici R, Isaia GC. Role of tnf-alpha producing T-cells in bone loss induced by estrogen deficiency. *Minerva Med* (2004) 95(2):125–32.

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Quantification of the growth suppression of HER2+ breast cancer colonies under the effect of trastuzumab and PD-1/PD-L1 inhibitor

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Introduction: Immune checkpoint blockade (ICB)-based therapy is revolutionizing cancer treatment by fostering successful immune surveillance and effector cell responses against various types of cancers. However, patients with HER2+ cancers are yet to benefit from this therapeutic strategy. Precisely, several questions regarding the right combination of drugs, drug modality, and effective dose recommendations pertaining to the use of ICB-based therapy for HER2+ patients remain unanswered.

Methods: In this study, we use a mathematical modeling-based approach to quantify the growth inhibition of HER2+ breast cancer (BC) cell colonies (ZR75) when treated with anti-HER2; trastuzumab (TZ) and anti-PD-1/PD-L1 (BMS-202) agents.

Results and discussion: Our data show that a combination therapy of TZ and BMS-202 can significantly reduce the viability of ZR75 cells and trigger several morphological changes. The combination decreased the cell's invasiveness along with altering several key pathways, such as Akt/mTor and ErbB2 compared to monotherapy. In addition, BMS-202 causes dose-dependent growth inhibition of HER2+ BC cell colonies alone, while this effect is significantly improved when used in combination with TZ. Based on the in-vitro monoculture experiments conducted, we argue that BMS-202 can cause tumor growth suppression not only by mediating immune response but also by interfering with the growth signaling pathways of HER2+BC. Nevertheless, further studies are imperative to substantiate this argument and to uncover the potential crosstalk between PD-1/PD-L1 inhibitors and HER2 growth signaling pathways in breast cancer.

KEYWORDS

HER2, PD-1/PD-L1, mathematical model, HER2/PD-1 interaction, breast cancer

Introduction

Recently, the inevitable role of executable, integrated, mathematical, and computational models in cancer research was largely acknowledged and discussed in many recent reviews (1–4). It is apparent that an integrated approach, which involves the analysis of genomic profiles, histopathology, imaging data, immunohistochemistry, proteomics data, drug targets, drug response, and more are imperative to coin translational solutions for cancer management. Specifically, the important role of mathematical and computational models in (1): illustrating highly dynamic biological behaviors (2), quantifying disease characteristics and drug responses (3), allowing easy integration of structured control-theoretic methods for the design of appropriate intervention strategies, and (4) utilizing intelligent algorithms to facilitate reasoning and decision support; are intensively explored recently (2).

$HER2^+$ BC that constitutes 15-20% of all BC types is identified by the overexpression of the HER2 receptor due to *HER2/ERBB2* gene amplification (5, 6). This molecular subtype of BC is associated with poor prognosis, moreover, 30% of patients report metastasis, especially to the brain (2, 7, 8). HER2 targeted therapies have significantly improved post-treatment disease-free survival (DFS) of $HER2^+$ BC patients (9, 10). However, patients undergoing current standard of care treatment (a combination of chemotherapy and anti-HER2 agents) who are under longtime follow-ups report unsatisfactory response rate (20-50%), development of drug resistance, and disease recurrence (9–12). For instance, under TZ therapy, compared to the 3 years (DFS=87.1%) follow-up, a drop of 13.4% in DFS was reported in the case of 10 years (DFS=73.7%) follow-up (13). Similarly, a drop in DFS was reported with a treatment strategy that used a combination of pertuzumab, trastuzumab, docetaxel, and trastuzumab emtansine (14, 15). Hence, there is a quest for the development of computationally and experimentally driven therapeutic strategies for the better management of $HER2^+$ BC patients.

Modern immunotherapeutic strategies which include the use of ICBs are increasingly recommended for the treatment of many types of cancers (16). The fact that scientists behind the identification of programmed death (PD-1) protein were honored with the Nobel prize (2018) signifies the potential benefits of this discovery in cancer therapy. In line with what was expected, several experimental and clinical trials substantiated the credibility of ICBs in terms of (1): safety, potency, and commercial availability (2), memory-lymphocyte mediated long term immunity that leads to durable complete response, and (3) additional advantages in treating advanced and metastatic cancers. For instance, compared to conventional treatment, augmenting ICB-based therapy has shown improved treatment response in many cancers which were otherwise not manageable or relapsing (e.g. melanoma, non-small cell lung cancer). However, the role of ICBs in BC treatment is in its emerging stage. Two important milestones in this regard are the approval of monoclonal antibodies (mAbs) atezolizumab (anti-PD-L1, March 2019) and pembrolizumab (anti-PD-1, November 2020) for the treatment of triple-negative BC (TNBC) (17–21).

Similar to TNBC, the disease progression in $HER2^+$ BC patients have shown a considerable correlation with the immune response and hence it is hypothesized that ICB-based immunomodulation techniques can be used in a favorable way to manage this aggressive cancer as well (19, 20). Many clinical and preclinical experiments associate poor disease prognosis in the case of $HER2^+$ BC with the expression of PD-L1 which might have aided this type of cancers to hide from immune surveillance (19, 20, 22–25). Moreover, studies report increased expression of PD-L1 under treatment with TZ (26). With one of the rationales identified behind the refractory nature of $HER2^+$ BC after anti-HER2 treatment as upregulation of immune checkpoints such as PD-1/PD-L1 and CTLA-4, amending ICB-based treatment is thought to add therapeutic benefits in treating $HER2^+$ BC (22, 27, 28). In line with these indications, reviews suggested that patients with metastatic breast cancer should be tested for response to ICBs for better treatment options (29). Consequently, several ICB-based agents are currently under investigation for the management of $HER2^+$ BC, however, none of them have been approved yet (2, 7). ICB-based drugs being a novel investigational therapeutic option for $HER2^+$ BC, it is imperative to come up with a quantitative comparison against current standard treatment options (4).

Preliminary investigations towards the advantages of combining anti-HER2 treatment with ICB-based therapy also suggest modest and durable outcome in a proportion of $HER2^+$ patients, which is another promising lead that calls for more investigations in this area (25, 30, 31). Apart from mAbs, other drug modalities including small molecules, peptides, and macrocycles are also available for inducing ICB-based therapy (32). Due to the reported resistance to mAb-based therapy and relapse after treatment, there is an increased interest in other drug modalities as well (33–35). Some of the disadvantages of mAbs are difficulty in production, longer half-life, high molecular weight, and less diffusion, on the other hand, small molecules have good affinity, oral bioavailability, and lesser immunotoxicity compared with mAbs (34, 36). Tight binding and retention of mAbs often leads to increased immune-related adverse events (irAEs) compared to small molecule inhibitors (SmIs) (37). Thus, SmIs that block interaction between PD-1 receptor and PD-L1 (ligand) are considered as a promising alternative to many of the currently investigated mAbs. Consequently, there is an apparent need for more research on the development and use of anti-PD-1/PD-L1 SmIs.

Mathematical modeling allows the integration of observed (empirical) results pertaining to a complex biological phenomenon in a simplified way and enables theoretical analysis and simulation studies. Such models can be used for the prediction of future behavior and to study the influence of

each parameter on the overall cancer dynamics. Hence, in this study, we use a mathematical modeling-based approach to develop a new model and quantify the growth inhibition of $HER2^+$ BC cell colonies (ZR75) when treated with anti-HER2 (TZ) and anti-PD-1/PD-L1 (BMS-202) agents.

Materials and methods

Cell culture

The $HER2^+$ cell-line (ZR75) was purchased from the American type culture collection (ATCC) (Rockville, MD, USA) and grown in complete cell culture media, RPMI-1640, (Gibco, Life technologies, Burlington, ON, Canada) augmented with 1% PenStrep antibiotic (Invitrogen, Life Technologies) and 10% fetal bovine serum (FBS; Invitrogen, Life Technologies). Cells were maintained at a temperature of 37°C with a 5% CO2 humidified atmosphere. We confirmed the presence of HER2 in this cell line in our previous study (38).

Cell viability assay

ZR75 cells were seeded in 96-well plates (Thermo Fisher Scientific, Mississauga, ON, Canada) at a density of 8,000 cells/well. After 24 hours, media was replaced with a fresh one with or without the treatment. Cells were treated with TZ (0, 1, 5, 7, 10, 15, and 20 μg/mL), BMS-202 (0, 1, 5, 7, 10, 15, and 20 μM), or a combination of both for 48 hours. Then, media was replaced with Alamar Blue cell viability reagent (Invitrogen, Thermo Fisher Scientific) and cells were incubated with the dye for 4 hours in the dark at 37°C as per the manufacturer protocol. Fluorescence values were recorded at a wavelength of 560 nm (excitation) and 600 nm (emission) using the Infinite m200 PRO fluorescent microplate reader (TECAN, Männedorf, Switzerland), reflecting the number of viable cells in each well.

Morphological examination

ZR75 cells were seeded in 6-well plates at a density of 200,000 cells/well. Changes in morphology of ZR75 cells were recorded after 48 hours of treatment with TZ (5 μg/mL), BMS-202 (5 μM), or a combination of both. Cells were visualized using Leica DMi1 inverted microscope (Leica Microsystems, Wetzlar, Germany). Untreated cells were used as a control.

Cell invasion assay

ZR75 cells were cultured in the upper chamber of 24-wells BioCoat™ Matrigel® Invasion Chambers (Corning, USA) with 8.0μm PET Membrane in a density of 50,000 cells/well. Cells were maintained in serum-free medium with/without treatment. The wells were placed in a base of complete medium with 10% FBS and incubated at 37°C for 48 hours. After that, non-invasive cells in the upper well were removed with a cotton swab. Invasive cells were washed, fixed with 4% formaldehyde, followed by staining with 300 ng/mL of DAPI (Abcam, Cambridge, MA, USA) for 2 minutes in the dark. Then, cells were observed using the fluorescence microscope.

Western blotting

ZR75 cells were seeded in 100 mm petri dishes at a density of 2,000,000 cells/dish. Cells were treated with TZ, BMS-202, or a combination of both for 48 hours. Cell lysates were collected, and 30 μg of proteins were resolved on 10% polyacrylamide SDS PAGE gels and then transferred onto PVDF membranes. Membranes were probed with the following primary antibodies: anti-rabbit Akt (CST: 9272S), anti-rabbit phospho-Akt (Ser473) (CST: 4060S), anti-rabbit mTOR (CST: 2983S), anti-rabbit phospho mTOR (S2448) (Abcam: ab109268), anti-mouse ErbB2 (Abcam: ab16901), anti-rabbit phospho ErbB2 (Abcam: ab53290), and anti-rabbit vimentin (CST: 46173S). Anti-rabbit GAPDH (Cell Signaling: 8480S) was used to ensure equal loading of protein samples. Blots were incubated with ECL Western blotting substrate (Pierce Biotechnology, Rockford, IL, USA) and chemiluminescence was recorded using the iBrightTM CL1000 imaging system (Thermo Fisher Scientific, Wal-tham, MA, USA). Quantification was done using ImageJ software.

Soft agar assay

Colony formation in soft agar was used to determine cells' capacity to colonize in *in-vitro*. A total of 1×10^3 cells of ZR75 were placed in RPMI medium containing 0.2% agar with/without drug(s) (treated and control cells, respectively) and plated in a 6-well plate covered with a layer of 0.4% noble agar in RPMI complete growth media (1 ml solid agar layer/well). A volume of 500 μl of media without (control) or with drug(s) were added to each well on 12^{th} and 14^{th} day of plating for ZR75 to make sure that the agar does not dry. The concentration range for BMS-202 was set to 1-20 μM, as our preliminary experiments on ZR75 colonies revealed no significant drug effect when treated with lower concentrations. Similar ranges were reported in (IC50 15 μM, in PD-L1+ SCC-3 cells and IC50 10 μM, in anti-CD3 activated Jurkat cells) (39), (0.6 nM up to 20 μM) (32), and (2.5-80 μM) (36) for various experiments based on different cell-lines. Colony formation was monitored every two days for a period of three weeks, and pictures of the colonies were taken on the 5^{th}, 7^{th},9^{th}, 12^{th}, 14^{th}, 17^{th}, and 19^{th} day after

seeding from various locations in each well using the inverted light microscope (Leica, Germany).

Model parameter estimation

At least 3 or up to 7 sets (different colonies) of time-series data were collected for each of the 16 samples (15 concentration and 1 control) of ZR75 on every 2nd or 3rd day for up to 19 days. Each time-series data for a particular colony includes up to 7 data points (images captured on 5th, 7th, 9th, 12th, 14th, 17th, and 19th day). All the images required for our study were taken using an inverted microscope (Leica microsystems, Germany) interfaced to LAS EZ software. In order to measure the time-dependent changes in the area of colonies, images were calibrated to 100 µm scale and quantified using ImageJ software. Matlab® *lsqcurvefit()* algorithm was used to estimate model parameters. Mean and standard deviation of parameter estimates were calculated using data sets pertaining to different colonies treated with a particular concentration of drug or drug combination. More than 1200 images were collected for our mathematical modeling experiments alone (excluding preliminary ones) from different wells, out of which around 500 images were omitted as (1) on day one there were no colonies inside or around the marked area to track (2) some colonies inside the marked areas were dormant (3) in some cases at least 4 images (on different days) of the same colony were not captured. Hence, after the experiment, we ended up with 3 to 7 data sets each data set with 4 to 7 data points (days) for various drug concentrations and combinations. Since the growth of breast cancer cell line colonies are nonlinear, we required at least 3 or 4 images of the same colony on different days for model parameter estimation.

Statistical analysis

Data are presented as an average of mean ± SEM (standard error of the mean). Each experiment was repeated at least three times (n=3). One-way ANOVA followed by Tukey's *post-hoc* test was used to compare the difference between treated and untreated cells. The data were analyzed using Microsoft Excel, and differences with $p < 0.05$ were considered statistically significant.

Results

We tested whether our $HER2^+$ BC cell lines (ZR75) express the drug target, PD-L1. FACS analysis of cell surface proteins revealed that 14.2% of ZR75 cells express PD-L1 ligand (data not shown). Thus, we proceeded with the treatment and the following experiments.

We first examined the outcome of TZ and BMS-202 on the viability of ZR75; a $HER2^+$ BC cell line. A significant decrease in the viability of ZR75 cells was observed after mono-treatment with TZ (20µg/mL) and BMS-202 (10µM). Interestingly, combining both treatments resulted in a more significant reduction of cell viability in a dose-dependent fashion, starting from a low dose (5µg/mL of TZ + 5µM of BMS-202) and reaching 13.42 ± 0.37% at high doses (Figure 1).

Afterwards, alterations in ZR75 cell morphology upon treatment with TZ and BMS-202, individually and combined were explored. ZR75 cells show round morphology, forming multilayer colonies as seen in untreated cells (Figure 2A). However, treatment with TZ and BMS-202 shifted cell morphology to a monolayer structure (Figures 2B, C). While, an increase in cell-cell adhesion in a monolayer after treatment with combination therapy was seen, with a lower number of cells (Figure 2D), consistent with our previous experiment.

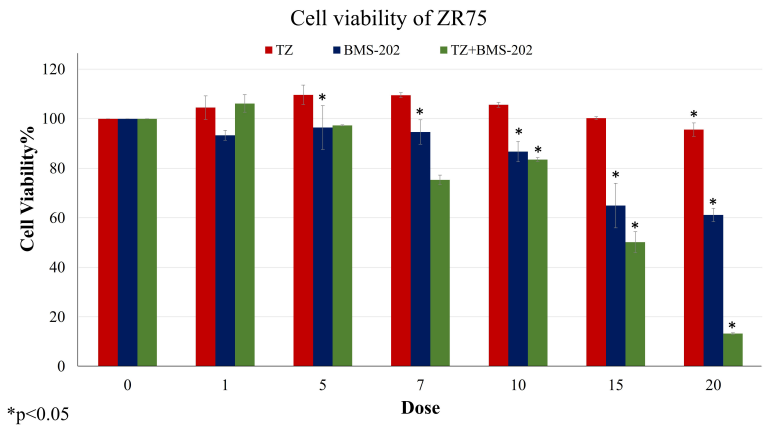

FIGURE 1
The effects of different concentrations of TZ, BMS-202, and a combination of both drugs on cell viability of ZR75 cell line. A significant dose-dependent decrease in cell viability was observed after treatment with the combination therapy. Data are presented as a percentage of viable cells ± SEM.

Next, the impact of TZ, BMS-202 and their combination on cell invasion was investigated using Matrigel® Invasion Chambers. Our data show a significant decrease in the number of invasive cells upon individual treatment with TZ but not with BMS-202. Interestingly, the combination therapy showed a more remarkable decrease in ZR75 cell invasiveness compared to monotherapy and the control (Figures 3A, B). To confirm our finding, we explored alterations in the protein expression of vimentin; a structural protein that plays important roles in cell-cell adhesion and cell invasiveness. We found a significant decrease in the protein expression, mostly in cells treated with the combination therapy of TZ and BMS-202 (Figure 3C).

To gain further understanding of the molecular mechanisms of action of TZ and BMS-202 combination, we explored the expression patterns of key biomarkers critical in pathways related to growth, proliferation, differentiation, and other processes that contribute to cancer progression. Our data revealed that combining TZ with BMS-202 can significantly deregulate several pathways compared to individual treatment in ZR75 cells. For instance, the combination of TZ and BMS-202 decreased the phosphorylation of AKT and mTOR proteins significantly compared to individual treatment, where no such results were observed (Figure 4). In addition, the combination therapy decreased the phosphorylation of HER2, which is a major driver of $HER2^+$ BC growth (Figure 4).

We then explored the effects of TZ and BMS-202 when used alone or in combination and quantified the growth inhibition of $HER2^+$ BC cell colonies in soft agar.

Figure 5 shows the images of the treated and untreated colonies after 14 days of plating.

Figure 6. shows the average number of colonies in matched areas in each well for the control and treated cases. It can be seen that, while there is a considerable number of big colonies in the control case, all treated cases have either a lesser number or no big colonies. Notably, the wells treated with a combination of drugs (H5P5 and H10P5) have no big colonies at all. All these initial experiments with ZR75 cell lines point to the significant growth inhibition of $HER2^+$ BC cells when combination drugs are used.

As the preliminary experiments conducted revealed significant drug effect in the case of combined use of TZ and BMS-202 on $HER2^+$ BC cells, we proceeded to collect time-series data to estimate the parameters for a mathematical model of cancer growth and drug-induced growth inhibition. In order to assess the efficacy of TZ and BMS-202 in the inhibition of colony formation of ZR75 cell lines, we quantified the growth of the same colonies over a period of time. To locate the same colony, markings were made under each well and the area of colonies were measured with images calibrated using LAS EZ software (Figure 7). Colonies with considerable change in size over the period of experiment (big colonies with more than 25 cells and intermediate colonies with 10 to 25 cells) were used for parameter estimation. However, in case of wells treated with drug concentration or combination that caused significant growth inhibition (e.g., P20, H25P10), there were only small, or no colonies left.

In general, exponential, logistic, Gompertz, Michaelis–Menten, Von Bertalanffy, and power-law models are used to represent tumor growth characteristics (2, 40, 41). Based on the comparison of various models for their descriptive power,

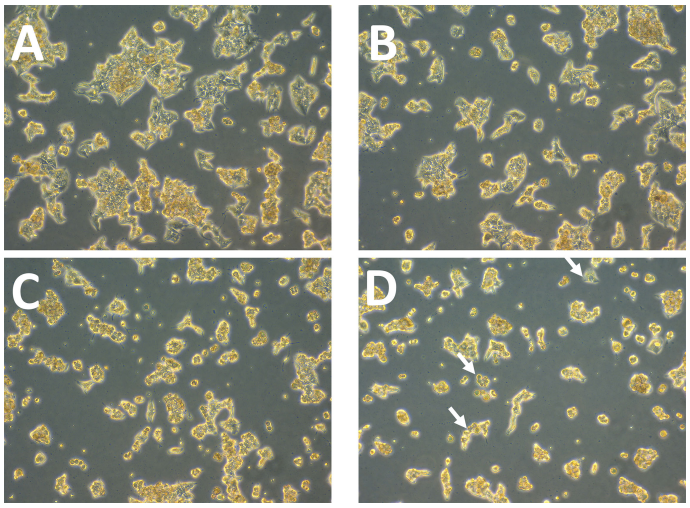

FIGURE 2
(A–D). Effect of TZ and BMS-202 on ZR75 cell morphology. We note that treatment with **(B)** TZ and **(C)** BMS-202 alters cell morphology to a monolayer structure. **(D)** Combining both treatments increases cell-cell adhesion in a monolayer in comparison with the **(A)** control.

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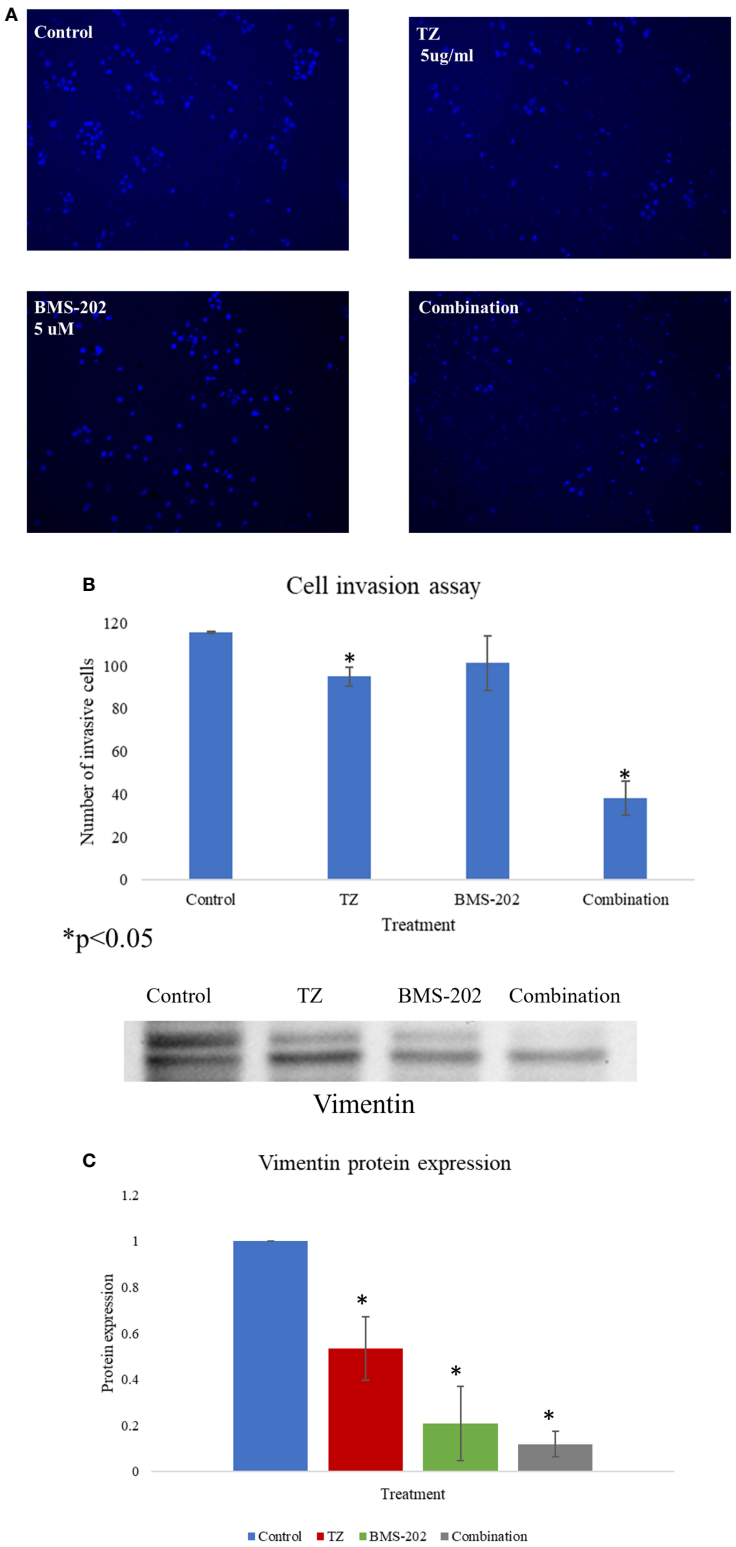

FIGURE 3
(A–C). **(A)** The impact of TZ, BMS-202, and a combination of both on ZR75 cell invasiveness. **(A)** Compared to the control, both TZ and the combination therapy inhibit ZR75 cell invasion, with a more pronounced effect upon treatment with the combination therapy. **(B)** The number of invasive cells was quantified using ImageJ. **(C)** The changes in vimentin expression after treatment with TZ, BMS-202, and their combination. Data are presented as a percentage of the viable cells ± SEM.

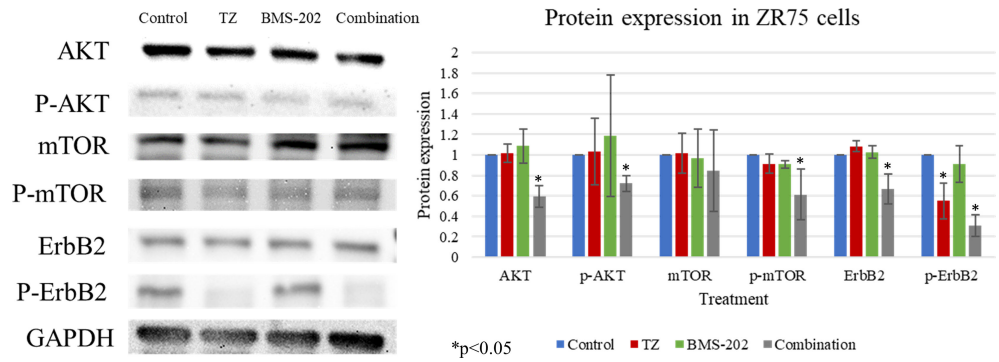

FIGURE 4
Western blot analysis of AKT, mTOR and ErbB2 in ZR75 cells under the effect of TZ and BMS-202. Treatment with both TZ and BMS-202 decreased the phosphorylation of ErbB2, AKT, and mTOR compared to individual treatment and untreated cells. GAPDH was used as a control for the amount of the loaded protein in this assay.

identifiability, and predictability, the literature suggests that no single model is suitable for all types of cancers. Nevertheless, based on the extensive analysis reported in Benzekry et al., 2014, Sarapata et al., and summarized in Padmanabhan et al., 2020, the Gompertz model shows reasonable goodness of fit for cancers in breast, lung, head and neck, liver, bladder, and pancreas. In terms of best fit, power-law is ranked one, for most cancer types. Nonetheless, due to the biologically unjustifiable nature and high sensitivity of the power-law model to parameters, the Gompertz model or logistic model is preferred over the power-law model. In addition, the Gompertz model shows a good predictive ability for breast cancer data.

Out of many possible model options, we choose the Gompertz model as it has already proved to have reasonable fit and predictability with respect to BC data (37–39). The Gompertz model for BC cell colonies growth is given by

$$\frac{dA(t)}{dt} = r\ln\left(\frac{k}{A(t)}\right)A(t), A(0) = A_0 \quad (1)$$

with the solution

$$A(t) = ke^{\ln\left(\frac{A_0}{k}\right)e^{-rt}} = k\left(\frac{A_0}{k}\right)^{e^{-rt}}, \quad (2)$$

where $A(t)$ is the area of the colony in μm^2, r is the growth rate of the colony in $days^{-1}$, and k is the carrying capacity of the environment in μm^2. Gompertz model accounts for both the initial slow growth and saturation in growth towards the end due to space and nutrition (carrying capacity) constraints. Table 1 shows values of k, r, and A_0 obtained by fitting the equivalent form of model (2) given by to the measured data, area of ZR75

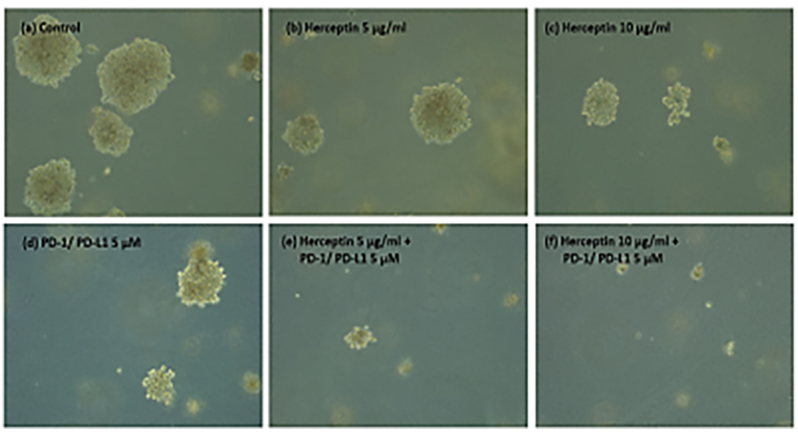

FIGURE 5
(A–F) ZR75 colonies imaged two weeks after treatment. Figure shows **(A)** Control **(B)** H5 **(C)** H10 **(D)** P5 **(E)** H5P5 and **(F)** H10P5 in order. There is a considerable reduction in the number of colonies and size of colonies when treated with combination of TZ and BMS-202.

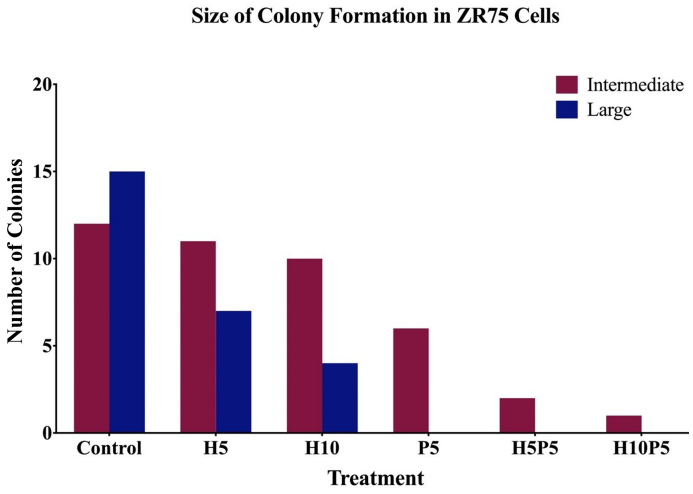

FIGURE 6
The number of big and intermediate colonies after 14 days of seeding in agar gel. It is shown that there is a considerable reduction in the number of colonies when treated with combination of TZ and BMS-202. Note that there are no big colonies in case of H5P5 and H10P5.

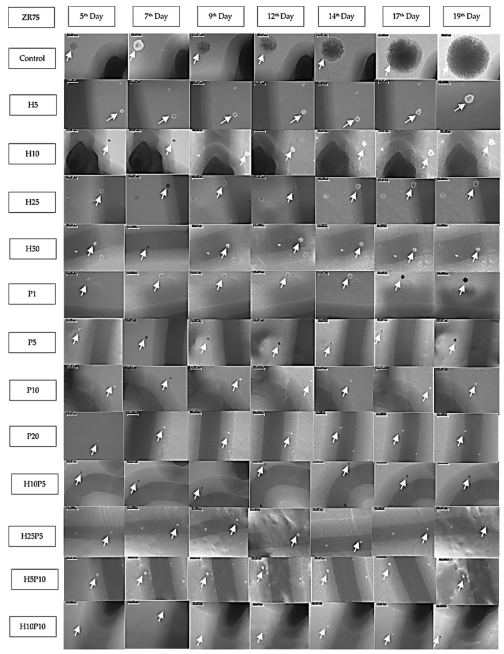

FIGURE 7
Images of ZR75 colonies (1 set) treated with various drug concentrations and combinations. Images are taken using an inverted microscope interfaced to LAS EZ software on 5th, 7th,9th, 12th, 14th, 17th, and 19th day after seeding. White arrow marks show the colonies. Images are calibrated (scale bar=100µm) using LAZ EZ software. Images for higher concentrations (H25P10, H25P20, and H50P20) are not shown as the growth inhibition is close to 100%. Shadows (dark line) of the markings made underneath the 6-well plate to track the colonies are also seen in most of the images.

colonies in agar assay, respectively. Model parameters were estimated using the trust-region-reflective algorithm in Matlab®. Specifically, an in-built function, namely, *lsqcurvefit()* which solves the nonlinear data-fitting problem in a least-squares sense were used to find the coefficients (k, r, and A_0) that best fit the nonlinear function (2). See Appendix (Figs. A1-A18) for model fitting curves obtained using the Matlab® algorithm.

Figure 7 shows one set of time-series data collected over 19 days which were used to quantify the growth of ZR75 colonies under treatment with various drug concentrations and combinations. As given in Table 1, up to 7 sets of such time-series data were obtained 2 or 3 days apart for parameter estimation. There was no colony formation at all in some of the wells (e.g., P20, H25P20).

From Figure 7, it can be seen that the growth rate is reduced for various treated cases compared to the control. However, the value of r in Table 1 does not reflect this growth inhibition, this is due to the fact that the nonlinear least-squares algorithm allows the variables k, r, and A_0 to vary appropriately to find an exact fit to the time-series data. Hence, in order to quantify the growth inhibition due to treatment, the Gompertz model is rewritten as

$$\frac{dA(t)}{dt} = (r-a)\ln\left(\frac{k}{A(t)}\right)A(t) \tag{3}$$

with the solution

$$A(t) = k\left(\frac{A_0}{k}\right)^{e^{-(r-a)t}} \tag{4}$$

where a models the drug effect, that is the per day growth inhibition due to treatment.

Here, note that the input data is the area of the colonies, using which we derived the growth rate, carrying capacity, and

drug effect. As the experiment is conducted in agar gel, on day zero (cell seeding day) the cells were not at all visible in the images, hence curve fitting is conducted using measured area available from 5th day of seeding. The parameter values shown in Table 1 do not directly reveal the difference in growth inhibition caused by different drug concentrations or combination because of the variability in *a*, *k*, and A_0. However, from Figure 7 it is clear that, there is significant growth inhibition in treated colonies compared to the control. For instance, comparing control and H5, when the area of colonies in the control wells was in the range 1000-7500 μm^2 that of H5 was only in the range 250-2250 μm^2(Figures A1, A2 in Supplementary File. Hence, there is a significant reduction in the growth rate in the case of H5. However, due to difference in initial condition (on Day 5) and the wide range of areas of different colonies each day, plotting a single interpolated curve from all replicates did not lead to a conclusive result. Hence, to show the growth pattern in each treatment case and thereby quantify the growth inhibition, we decided to plot the growth curve of each colony separately. As shown in Figures A1-A10 in the Supplementary File, Matlab's *lsqcurvefit()* has successfully derived best-fit parameters, however, as mentioned earlier this significant growth inhibition is not reflected in the value of *r* given in Table 1. This is because, we estimated 3 parameters required for fitting the nonlinear curve such as *r*, *k* and A_0. Hence, to have a clear comparison between the growth inhibition of various drug concentrations and combinations, we fixed two values (*k* and A_0), and re-estimated the growth of control set alone (r_c), then, using $r_c$in equation (4), we estimated the *a* (growth inhibition*)* value for each drug concentration and combination. This is a valid assumption as we used uniform cell seeding density and supplied the same amount of cell culture media to all wells throughout the experiments.

Next, the rationale behind the choice of the value of A_0,is mentioned in Table 2. As shown in Figure 7, we started measuring the area of colonies on the 5th day of seeding i.e. when the colonies were visible. Using the measured data, the fitting algorithm was used to predict the initial area (A_0), the carrying capacity (*k*), and the growth rate (*r*). In order to perform a comparative assessment of the change in growth inhibition between the control and various treated cases, rather than determining the values of A_0 and *k*, we fixed these two parameters for all the cases and re-estimated the value of growth inhibition, *a*, alone. For instance, the initial area A_0 of the colony estimated by the algorithm varied within the range 127.04-414.40 μm^2 for 88 sets in Table 1). Hence, we fixed the value of A_0 as 200 um^2. We chose a value closer to the lower range limit since fixing A_0 greater than the measured value on day 5 would result in negative growth rates for cases with significant growth inhibition (e.g. P20). The value of the carrying capacity (*k*) estimated by the algorithm varied from 2.5e^4 – 2.6e^9 μm^2 for 88 sets in Table 1).

Next, the rationale behind the choice of *k*. Considering space limitation of a single well (34.8 mm diameter, area 3802.66 e^6 μm^2 and seeding density of 1000 cells/well, each colony can have a maximum area of 3.8 e^6 μm^2. Hence, we fixed carrying capacity A_0 as 1 e^6. We tested the algorithm by fixing different reasonable values of A_0 and *k* and in all cases, as expected (due to uniform cell seeding and well size), there is negligible variance in the estimated value of *a* (cases 1 and 2 in Table AT1 in the Supplementary File). Moreover, as shown in Figure 5, small, intermediate, and big colonies were seen in agar assay, hence

TABLE 1 Gompertz model parameters for the growth of the ZR75 colonies in agar assay.

Set	No. of data set	*k* (mean (std. dev)) μm^2	A_0 (mean (std. dev)) μm^2	*r* (mean (std. dev)) days^{-1}
Control	6	5.8e4 (4.8e4)	320.33 (183.85)	0.0911 (0.0880)
H5	7	8.49e8 (2.24e9)	202.247 (247.39)	0.1675 (0.0981)
H10	7	1.4e9 (2.43e9)	375.23 (162.41)	0.0443 (0.0515)
H25	7	1.3e9 (3.46e9)	127.04 (200.9)	0.288 (0.20)
H50	7	4.3e4 (5.2e4)	220.81 (170.80)	0.1562 (0.15)
P1	6	1.2e9 (2.2e9)	227.83 (211.70)	0.0651 (0.10)
P5	6	2.6e9 (3.1e9)	189.05 (152.64)	0.1586 (0.275)
P10	5	8.4e8 (1.0e7)	182.25 (63.98)	-0.259 (0.3)
P20	3	2.3e8 (4e8)	336.28 (241.84)	-0.038 (0.037)
H5P10	4	2.5e4 (4.9e4)	325.56 (45.62)	-0.2191 (0.29)
H10P5	6	3.3e8 (6.7e8)	224.88 (125.49)	-0.367 (0.4)
H10P10	6	1.4e8 (1.1e8)	–	–
H25P5	5	3.5e8 (5.8e8)	213.85 (122.73)	-0.06 (0.12)
H25P10	5	–	–	–
H25P20	4	–	–	–
H50P20	4	3.9e8 (7.8e8)	414.4 (101.21)	-0.03 (0.02)[1]

heterogeneity in the colony size is expected. We excluded very small colonies and used images with intermediate and big colonies. However, even after including both big and intermediate colonies, as shown in Tables 2 and 3 a trend of increased drug effect is seen in the case of combination data.

Table 2 shows the results obtained for ZR75. The overall growth rate of treated colonies is given by $r_{treat} = r\text{-}a$, using r_{treat} the percentage value of growth inhibition (GI) in each case is calculated as % GI=$(1-(r_{treat}/r))\times100$, where r_c is the mean growth rate of the control data set estimated by fixing the values of k and A_0. To summarize, the steps involved in generating Table 2 are: (1) Fix values for k and A_0 and estimate the growth rate (r_c) of control data set, (2) Set $r=r_c$ in equation, (3) and estimate the value of growth inhibition parameter (a) for each data set. From Table 2, it can be seen that BMS-202 can cause dose-dependent growth inhibition of ZR75 colonies. The % GI of ZR75 colonies are 50%, 53.75%, 98.34%, and 100% for P1, P5, P10, and P20, respectively. Moreover, a combination of TZ and BMS-202 resulted in increased growth inhibition of ZR75 colonies compared to respective monotherapies. For instance, %GI for H10P5 was 93.34%, whereas for H10 and P5%GI was 45.42% and 53.75%, respectively. It can also be seen from Table 2 that all combination therapy concentrations resulted in at least 80% GI of ZR75 colonies. Note that these results are for an immune deprived environment. Hence, a synergistic drug combination effect is expected in an immune-competent *in vivo* environment which will have additional effector cell-mediated cytotoxicity as well.

Discussion

It is well known that the mechanism of action behind many of the anti-HER2 agents (trastuzumab, pertuzumab, trastuzumab emtansine, margetuximab, etc.) involve immune effector modulation (10, 31, 42). Moreover, the significant correlation between the presence of TIL (tumor-infiltrating leukocytes) in the tumor microenvironment (TME) and improved survival rate says why disintegration of the immune evasion strategy of cancer cells using ICB is an idea worth exploring for $HER2^+$ BC in particular (2, 27, 43, 44). An interesting study revealed that PD-L1 expression was significantly increased when treated with TZ in HER2-amplified gastric cancer cell lines co-cultured with peripheral blood mononuclear cells (PBMCs). Another study shows that TZ sensitive HER^+ BC reportedly express higher levels of PD-L1 than TZ insensitive BC cells (26). Hence, additional use of ICBs can restore T-cell augmentation and thus enhance antibody-mediated cytotoxicity of TZ. Pre-clinical results report synergy in action when TZ is used with ICB-based (anti-PD-1/anti-CD137 mAb) therapy (45). A combination therapy using margetuximab (anti-HER2) and pembrolizumab (anti-PD-1) showed acceptable safety and tolerability with no dose-limiting toxicities in $HER2^+$ gastro-esophageal adenocarcinoma (32). Similarly, our study reveals that the combination therapy using TZ (anti-HER2, mAb) and BMS-202 (anti-PD-1/PD-L1, SmI) results in improved growth inhibition compared to monotherapies even in an immune cell deprived environment, as shown in contingency Table 3 for % growth inhibition of

TABLE 2 Drug induced growth inhibition of ZR75 colonies in agar assay.

Set	No. of data set	Drug effect (a) $days^{-1}$, (mean (std. dev.))	Growth inhibition (%)
Control	6	0	0
H5	7	0.0081 (0.0026)	33.75 (5.4)
H10	7	0.0109 (0.0054)	45.42 (11.2)
H25	7	0.0055 (0.0032)	22.92 (6.6)
H50	7	0.0053 (0.0071)	22.09 (14.7)
P1	6	0.0120 (0.0058)	50 (12.0)
P5	6	0.0129 (0.0062)	53.75 (12.9)
P10	5	0.0236 (0.0019)	98.34 (3.9)
P20	3	0.0535 (0.0214)	100*
H5P10	4	0.0200 (0.0023)	83.34 (4.7)
H10P5	6	0.0224 (0.0030)	93.34 (6.2)
H10P10	6	0.0224 (0.0055)	93.34 (11.4)
H25P5	5	0.0225 (0.0046)	93.75 (9.5)
H25P10	5	0.0225 (0.0056)	93.75 (11.6)
H25P20	4	0.0315 (0.0073)	100*
H50P20	4	0.0482 (0.0183)	100*

The drug effect parameter a is estimated using model (4) by fixing k=1e6 μm2, A0 = 200 μm2, and the growth rate of the control is set as r=0.0240 (0.0042). The overall growth rate of treated colonies is rtreat =r-a and growth inhibition is calculated as % GI=(1-(rtreat/r)) ×100. * Note that while calculating GI value for P20, H25P20, H50P20, as value of r<a, r-a becomes negative resulting in %GI>100, which is rounded off to 100%.

ZR75 colonies when treated with various drug concentrations and combinations. All these studies serve as a proof of concept for expected synergistic anti-tumor activity in the combination of anti-HER2 and anti-PD-1 agents in an immunocompetent *in vivo* environment (32, 46).

Many mAbs including pembrolizumab and durvalumab, which were FDA approved for many other cancers, are currently under investigation for HER2$^+$ BC particularly to evaluate dose-limiting toxicities, maximum tolerated dose (MTD), recommended phase-II dose (RP2D), and objective response (OR). In a phase 2 trial (PANACEA, pembrolizumab + TZ), it is reported that when 15% (6/40) of PD-L1$^+$ cases achieved OR, none of the PD-L1$^-$ achieved OR. During the 13·6 (for PD-L1$^+$ tumors) and 12·2 (for PD-L1$^-$ tumors) months evaluation period, even though grade 3-5 adverse events (AE) were reported in 50% of patients (with treatment discontinuation due to AE in 8% of the patients), the overall findings suggest that the combination of pembrolizumab and TZ is safe to use and showed continuing clinical benefits in HER2$^+$ BC patients with TZ-resistant and PD-L1$^+$ tumors (47). On a scale of 5, adverse effects in grades 1-2 were reported, RP2D is a full dose of durvalumab and TZ, and no safety issues were reported (25). Other currently ongoing clinical trials include NCT03417544 (atezolizumab, pertuzumab, TZ, HER2$^+$ MBC), NCT03125928 (atezolizumab, paclitaxel, TZ, pertuzumab, HER2$^+$ MBC), NCT03595592, (TZ, pertuzumab, carboplatin, paclitaxel, atezolizumab, HER2$^+$, locally advanced BC), and NCT03199885 (paclitaxel, TZ, pertuzumab, atezolizumab, for HER2$^+$ MBC). Even ICB-based DNA vaccines are under clinical trials for managing HER2$^+$ cancers (48). However, note that in PANACEA only 15% OR is reported which means that we are quite far from figuring out a therapy that ensures 100% complete response or relapse-free survival for HER2$^+$ BC patients (28, 49).

As mentioned earlier resistance to mAb-based therapy and relapse after treatment that were reported in earlier cases calls for more research using other drug modalities such as SmIs, peptides, and macrocycle. BMS-202 is a biphenyl SmI developed by Bristol Myers Squibb (BMS) which can stabilize PD-L1 protein dimers (36, 50). Specifically, BMS-202 can dive deep into the hydrophobic cylindric pocket created by two juxtaposed PD-L1 molecules and stabilize and hide away a PD-L1 homodimer, and thus prevent it from interacting with a PD-1, blocking intracellular signalization which leads to immune evasion of cancer cells (33). Biophysical and crystallographic studies suggest that BMS-202 can inhibit the interaction of the PD-1 receptor with its ligand by facilitating the dimerization of the latter (29, 37, 51–53). Anti-tumor activities and immunomodulatory effects of BMS-202 is studied using *in vitro* (human CD3$^+$ cells) and *in vivo* studies; BMS-202, PD-1/PD-L1 binding is blocked leading to increased IFN-γ secretion *in vitro* (36). Similarly, *in vivo* experiments showed increased IFN-γ levels, cytotoxic T cells, and reduced T regulatory cells in blood (36). Due to the advantages of SmIs over mAbs, there is an increased interest in understanding the usefulness of BMS-202 in treating various cancers (27, 33–35). Study by Zhang et al. (53),, BMS-202 entrapped in nanoparticles (BMS-202 NPs) were used in a BC mice model (4T1 tumor-bearing mice) to study tumor deliverability and anti-cancer activity of BMS-202 NPs. This study showed the impressive anti-tumor and anti-metastatic effects of BMS-202 NPs (53).

In-vitro experiments reveal that BMS-202 can inhibit the proliferation of PD-L1$^+$ SCC-3 cells (IC50 15 μM) and anti-CD3 antibody-activated Jurkat cells (IC50 10 μM) (52). As per this study, BMS-202 does not regulate the expression of PD-1/PD-L1 on cells, rather it inhibits the formation of the PD-1/PD-L1 complex by facilitating the dimerization of PD-L1 (52). Most importantly, BMS-202 showed a clear and direct anti-tumor effect against SCC compared to control in severely immune-deficient (MHC-double knockout) NOG mouse (52). The study using PD-L1$^+$ SCC-3 cells *in vivo* (in NOG mouse) indicate that the antitumor activity of BMS-202 might be partly mediated by immune modulation and partly by the off-target cytotoxic effect (52). In line with these findings, our results also indicate that the anti-tumor activity of BMS-202 on HER2$^+$ BC cells is partly by the off-target cytotoxic effect. More *in vitro* and *in vivo* studies are required to substantiate the synergy in action when BMS-202 is used along with TZ. Note that both drugs increase the level of cytokine interferon in the tumor microenvironment (TME). Another question that remains is whether T cell exhaustion in the TME will limit or saturate the overall efficacy when two drugs are used together *in-vivo*.

The role of vimentin in cancer cell motility, migration and invasion is well established (54). It is a major mediator in the

TABLE 3 Contingency table showing % growth inhibition of ZR75 colonies when treated with various drug concentrations and combinations.

% Growth inhibition P alone	Conc.	% Growth inhibition with combination treatment			
100*	P20	-	-	100	100
98.34*	P10	83.34	93.34	93.75	-
53.75*	P5	-	93.34	93.75	-
50*	P1	-	-	-	-
	Conc.	H5	H10	H25	H50
	% Growth inhibition H alone	33.75*	45.42*	22.92*	22.09*

Values given in bold indicate % Growth inhibitions for combination therapy and those with * are for monotherapy.

epithelial-mesenchymal transition event, which results in cancer dissemination and metastasis (54, 55). Furthermore, knocking out vimentin attenuates tumor cell invasion (56). This highlights the importance of vimentin as a potential target to inhibit tumor progression. In this study, we revealed that vimentin protein levels were significantly decreased upon treatment with the combination of TZ and BMS-202. Accompanied with cell invasion data as well as the deregulation of AKT, mTOR and HER2, which play an important role in carcinogenesis (refs), we suggest that the combination therapy of TZ and BMS-202 may serve as an inhibitor of HER2+ breast cancer cell invasion.

HER2 amplification in HER2+ cancers is considered the major driver of tumor growth and progression. Upon dimerization, HER2 autophosphorylation activates several downstream molecular pathways, such as PKC and AKT/mTOR (57). These pathways control essential biological processes that can work in the favor of cancer cells when deregulated. These processes include cell survival and proliferation, motility, invasion, and differentiation. This shows why targeting HER2 with anti-HER2 drugs or monoclonal antibodies is essential in the management of HER2+ cancers (58). We herein report that treatment with TZ and BMS-202 for 48 hours can suppresses the expression of HER2 receptor, while mostly affecting its phosphorylation. In addition, we noticed a deregulation in the expression patterns of AKT/mTOR upon treatment, which was more pronounced when we used the combination of TZ and BMS-202.

In general, there is a strong indication of the synergistic outcome when anti-HER2 and ICB-based therapies are applied together (17, 21, 22, 27, 28, 59). When it comes to combination therapy, along with empirical experiments, mathematical models can be used to evaluate effective dose combinations and order of treatment (2, 60, 61). Study by Jarrett et al. (61), demonstrated an experimentally-driven mathematical model is used to analyze combination therapy (TZ+paclitaxel) protocols for $HER2^+$ BC. Another mathematical model-based analysis reveals TNF-α induced reduction in drug-resistance to anti-PD-1 (62). Similarly, a mathematical model was developed to represent combination therapy (cancer vaccine and ICB) (51). Thus, it is obvious that mathematical models, if properly devised with appropriate measurable biomarkers can be used to conduct risk-free, cost-effective *in silico* analysis to identify patient cohorts that will benefit from a certain type of treatment (63, 64).

The contribution of this paper comes in many folds. We herein present (1) a feasible methodology to use agar-assay based colony formation experiments to track the growth of the same colony over a period of time and to build a mathematical model based on the time-series data derived (2). Our data revealed improved growth inhibition of colonies in the case of combination treatment compared to single agent cases (3), The Gompertz model is validated as a suitable model to describe the growth pattern of breast cancer cell lines, and (4) the combination treatment with TZ and BMS-202 decreased the cell's invasiveness along with altering several key pathways, such as AKT/mTOR and ErbB2 compared to monotherapy. The application of the mathematical model discussed in this paper is limited to the study of growth patterns of breast cancer cell lines, drug-induced percentage growth inhibition, and combination drug effect. Herein it is important to highlight that a single term Gompertz model is inadequate to reflect the complex dynamics in the tumor microenvironment *in vivo*, which involves the interaction of multiple cells and biochemicals (such as crosstalk between normal, cancer, endothelial and immune cells as well as cytokines, chemokines etc.). Complex models with multiple terms where each term can be linear (such as Gompertz, power law, logistic model) may predict cancer behavior in future timescale as each term in the model equation accommodate (1) growth (2) competition between cells (3) cell differentiation/mutation and (4) the effect of therapy, for each cell type in the tumor microenvironment. However, in this paper, we have used Gompertz model to represent treatment induced growth inhibition alone, not the complete dynamics of a tumor microenvironment. More complex experiments that involve cell-coculture (breast cancer cells with peripheral blood mononuclear cells (PBMCs)) can be used to mimic a tumor microenvironment and thus build more complex mathematical models that can be used to derive critical information regarding immune cell-induced enhancement and saturation of drug effect due to T cell exhaustion. More importantly, we envisage that the results discussed in this paper will lead to more studies that investigate molecular pathways, if any, that improve the potency of TZ when used along with BMS-202 in HER2 treatment.

In this paper, we present a Gompertz model-based method to quantify drug-induced growth inhibition. Development of similar mathematical models which represent the dynamics of $HER2^+$ BC cells, immune cells, and drugs involved are interesting directions for future research. Such models can be used to evaluate the critical threshold of T cell exhaustion that will hinder a patient from getting the potential benefits expected out of ICB-based therapy (16, 65). Apart from the ZR-75 results reported in this paper, we have conducted a similar study using the SKBR3 cell line (please refer to Supplementary Data) wherein the Gompertz model exhibited good fit, however, with slightly different values for variables (r, k, a). Hence, investigating how far we can generalize the model parameters for various cell lines can be an interesting direction for future work. Similarly, deriving a mathematical function that fits the measured growth inhibitions with respect to the two different drug doses used (Table 3) is also desirable for identifying the best dosing combination. In short mathematical model-based approaches can act as a link to facilitate the integration of multiple computational strategies towards tailoring personalized treatment protocols by accommodating patient-specific characteristics (1, 3, 30, 63, 66, 67). Specifically, investigations based on computational approaches which can quantify indications of diagnostic, therapeutic, and prognostic biomarkers pertaining to $HER2^+$ BC can accelerate drug development, drug repositioning, and identification of effective drug combination for managing the disease (2, 68–70).

Conclusions

In order to have a realistic assessment of cancer disease prognosis and predictive outcomes, biomedical research frameworks must adopt more quantitative methods to gain insight on disease mechanisms, therapy options, and prognostic features of biomarkers. The significant correlation between immune response, PD-1/PD-L1 expression, and disease prognosis of $HER2^+$ BC indicates that tailored ICB-based therapies can improve the management of $HER2^+$ BC patients. Our mathematical model-based study points out that the combination therapy using trastuzumab (anti-HER2, mAb) and BMS-202 (anti-PD-1/PD-L1, SmI) results in a significant growth inhibition of $HER2^+$ BC cell lines compared with monotherapies even in an immune cell deprived environment. Nevertheless, further investigations are imperative to uncover the potential crosstalk between PD-1/PD-L1 inhibitors and HER2 growth signaling pathways in 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.

Author contributions

Conceptualization, N.M. and A-EM. Data curation, RP and HK. Formal analysis, RP and HK. Funding acquisition, SV. Methodology, RP and HK. Project administration, A-EM. Resources, A-EM. Supervision, NM and A-EM. Writing – original draft, RP, HK, IG and A-EM. Writing – review and editing, RP, HK, IG, NM, AH, SV and A-EM. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author AH was employed by Hamad Medical Corporation.

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.2022.977664/full#supplementary-material

References

1. Enderling H, Alfonso JCL, Moros E, Caudell JJ, Harrison LB. Integrating mathematical modeling into the roadmap for personalized adaptive radiation therapy. *Trends Cancer* (2019) 5(8):467–74. doi: 10.1016/j.trecan.2019.06.006

2. Padmanabhan R, Kheraldine HS, Meskin N, Vranic S, Al Moustafa A-E. Crosstalk between HER2 and PD-1/PD-L1 in breast cancer: From clinical applications to mathematical models. *Cancers (Basel)* (2020) 12(3):636. doi: 10.3390/cancers12030636

3. Clarke MA, Fisher J. Executable cancer models: successes and challenges. *Nat Rev Cancer* (2020) 20(6):343–54. doi: 10.1038/s41568-020-0258-x

4. Szeto GL, Finley SD. Integrative approaches to cancer immunotherapy. *Trends cancer* (2019) 5(7):400–10. doi: 10.1016/j.trecan.2019.05.010

5. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Sci* (1987) 235(4785):177–82. doi: 10.1126/science.3798106

6. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Sci* (1989) 244(4905):707–12. doi: 10.1126/science.2470152

7. Vranić S, Bešlija S, Gatalica Z. Targeting HER2 expression in cancer: New drugs and new indications. *Bosn J basic Med Sci* (2021) 21(1):1–4. doi: 10.17305/bjbms.2020.4908

8. Kuroiwa Y, Nakayama J, Adachi C, Inoue T, Watanabe S, Semba K. Proliferative classification of intracranially injected HER2-positive breast cancer cell lines. *Cancers (Basel)* (2020) 12(7):1811. doi: 10.3390/cancers12071811

9. Vernieri C, Milano M, Brambilla M, Mennitto A, Maggi C, Cona MS, et al. Resistance mechanisms to anti-HER2 therapies in HER2-positive breast cancer: Current knowledge, new research directions and therapeutic perspectives. *Crit Rev Oncol Hematol* (2019) 139:53–66. doi: 10.1016/j.critrevonc.2019.05.001

10. Puglisi F, Fontanella C, Amoroso V, Bianchi GV, Bisagni G, Falci C, et al. Current challenges in HER2-positive breast cancer. *Crit Rev Oncol Hematol* (2016) 98:211–21. doi: 10.1016/j.critrevonc.2015.10.016

11. Nixon NA, Hannouf MB, Verma S. A review of the value of human epidermal growth factor receptor 2 (HER2)-targeted therapies in breast cancer. *Eur J Cancer* (2018) 89:72–81. doi: 10.1016/j.ejca.2017.10.037

12. Ayoub NM, Al-Shami KM, Yaghan RJ. Immunotherapy for HER2-positive breast cancer: recent advances and combination therapeutic approaches. *Breast Cancer* (2019) 11:53–69. doi: 10.2147/BCTT.S175360

13. Earl H, Hiller L, Vallier A-L, Loi S, McAdam K, Hughes-Davies L, et al. Six versus 12 months' adjuvant trastuzumab in patients with HER2-positive early breast cancer: the PERSEPHONE non-inferiority RCT. *Health Technol Assess* (2020) 24(40):1–190. doi: 10.3310/hta24400

14. Swain SM, Baselga J, Kim S-B, Ro J, Semiglazov V, Campone M, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med* (2015) 372(8):724–34. doi: 10.1056/NEJMoa1413513

15. Krop IE, Kim S-B, Martin AG, LoRusso PM, Ferrero J-M, Badovinac-Crnjevic T, et al. Trastuzumab emtansine versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial. *Lancet Oncol* (2017) 18(6):743–54. doi: 10.1016/S1470-2045(17)30313-3

16. Esteva FJ, Hubbard-Lucey VM, Tang J, Pusztai L. Immunotherapy and targeted therapy combinations in metastatic breast cancer. *Lancet Oncol* (2019) 20 (3):e175–86. doi: 10.1016/S1470-2045(19)30026-9

17. Planes-Laine G, Rochigneux P, Bertucci F, Chrétien A-S, Viens P, Sabatier R, et al. PD-1/PD-L1 targeting in breast cancer: The first clinical evidences are emerging. a literature review. *Cancers (Basel)* (2019) 11(7):1033. doi: 10.3390/cancers11071033

18. Vranic S, Cyprian FS, Gatalica Z, Palazzo J. PD-L1 status in breast cancer: Current view and perspectives. *Semin Cancer Biol* (2021) 72:146–54. doi: 10.1016/j.semcancer.2019.12.003

19. Luen SJ, Savas P, Fox SB, Salgado R, Loi S. Tumour-infiltrating lymphocytes and the emerging role of immunotherapy in breast cancer. *Pathol* (2017) 49 (2):141–55. doi: 10.1016/j.pathol.2016.10.010

20. Muenst S, Schaerli AR, Gao F, Däster S, Trella E, Droeser RA, et al. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. *Breast Cancer Res Treat* (2014) 146(1):15–24. doi: 10.1007/s10549-014-2988-5

21. Kurozumi S, Inoue K, Matsumoto H, Fujii T, Horiguchi J, Oyama T, et al. Clinicopathological values of PD-L1 expression in HER2-positive breast cancer. *Sci Rep* (2019) 9(1):16662. doi: 10.1038/s41598-019-52944-6

22. Krasniqi E, Barchiesi G, Pizzuti L, Mazzotta M, Venuti A, Maugeri-Saccà M, et al. Immunotherapy in HER2-positive breast cancer: state of the art and future perspectives. *J Hematol Oncol* (2019) 12(1):111. doi: 10.1186/s13045-019-0798-2

23. Sobral-Leite M, Van de Vijver K, Michaut M, van der Linden R, Hooijer GKJ, Horlings HM, et al. Assessment of PD-L1 expression across breast cancer molecular subtypes, in relation to mutation rate, BRCA1-like status, tumor-infiltrating immune cells and survival. *Oncoimmunol* (2018) 7(12):e1509820. doi: 10.1080/2162402X.2018.1509820

24. Cimino-Mathews A, Thompson E, Taube JM, Ye X, Lu Y, Meeker A, et al. PD-L1 (B7-H1) expression and the immune tumor microenvironment in primary and metastatic breast carcinomas. *Hum Pathol* (2016) 47(1):52–63. doi: 10.1016/j.humpath.2015.09.003

25. Chia S, Bedard PL, Hilton J, Amir E, Gelmon K, Goodwin R, et al. A phase ib trial of durvalumab in combination with trastuzumab in HER2-positive metastatic breast cancer (CCTG IND.229). *Oncologist* (2019) 24(11):1439–45. doi: 10.1634/theoncologist.2019-0321

26. Triulzi T, Forte L, Regondi V, Di Modica M, Ghirelli C, Carcangiu ML, et al. HER2 signaling regulates the tumor immune microenvironment and trastuzumab efficacy. *Oncoimmunol* (2019) 8(1):e1512942. doi: 10.1080/2162402X.2018.1512942

27. Mittal D, Vijayan D, Neijssen J, Kreijtz J, Habraken MMJM, Van Eenennaam H, et al. Blockade of ErbB2 and PD-L1 using a bispecific antibody to improve targeted anti-ErbB2 therapy. *Oncoimmunol* (2019) 8(11):e1648171. doi: 10.1080/2162402X.2019.1648171

28. Page DB, Bear H, Prabhakaran S, Gatti-Mays ME, Thomas A, Cobain E, et al. Two may be better than one: PD-1/PD-L1 blockade combination approaches in metastatic breast cancer. *NPJ Breast cancer* (2019) 5:34. doi: 10.1038/s41523-019-0130-x

29. Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* (2012) 366(26):2455–65. doi: 10.1056/NEJMoa1200694

30. Goutsouliak K, Veeraraghavan J, Sethunath V, De Angelis C, Osborne CK, Rimawi MF, et al. Towards personalized treatment for early stage HER2-positive breast cancer. *Nat Rev Clin Oncol* (2020) 17(4):233–50. doi: 10.1038/s41571-019-0299-9

31. Catenacci DVT, Kang Y-K, Park H, Uronis HE, Lee K-W, Ng MCH, et al. Margetuximab plus pembrolizumab in patients with previously treated, HER2-positive gastro-oesophageal adenocarcinoma (CP-MGAH22-05): A single-arm, phase 1b-2 trial. *Lancet Oncol* (2020) 21(8):1066–76. doi: 10.1016/S1470-2045(20)30326-0

32. Guzik K, Tomala M, Muszak D, Konieczny M, Hec A, Błaszkiewicz U, et al. Development of the inhibitors that target the PD-1/PD-L1 interaction-a brief look at progress on small molecules, peptides and macrocycles. *Molecules* (2019) 24 (11):2071. doi: 10.3390/molecules24112071

33. Bailly C, Vergoten G. Flurbiprofen as a biphenyl scaffold for the design of small molecules binding to PD-L1 protein dimer. *Biochem Pharmacol* (2020) 178:114042. doi: 10.1016/j.bcp.2020.114042

34. Geng Q, Rohondia SO, Khan HJ, Jiao P, Dou QP. Small molecules as antagonists of co-inhibitory pathways for cancer immunotherapy: a patent review (2018-2019). *Expert Opin Ther Pat* (2020) 30(9):677–94. doi: 10.1080/13543776.2020.1801640

35. Ganesan A, Ahmed M, Okoye I, Arutyunova E, Babu D, Turnbull WL, et al. Comprehensive *in vitro* characterization of PD-L1 small molecule inhibitors. *Sci Rep* (2019) 9(1):12392. doi: 10.1038/s41598-019-48826-6

36. Hu Z, Yu P, Du G, Wang W, Zhu H, Li N, et al. PCC0208025 (BMS202), a small molecule inhibitor of PD-L1, produces an antitumor effect in B16-F10 melanoma-bearing mice. *PloS One* (2020) 15(3):e0228339. doi: 10.1371/journal.pone.0228339

37. Konstantinidou M, Zarganes-Tzitzikas T, Magiera-Mularz K, Holak TA, Dömling A. Immune checkpoint PD-1/PD-L1: Is there life beyond antibodies? *Angew Chem Int Ed Engl* (2018) 57(18):4840–8. doi: 10.1002/anie.201710407

38. Kheraldine H, Gupta I, Alhussain H, Jabeen A, Cyprian FS, Akhtar S, et al. Substantial cell apoptosis provoked by naked PAMAM dendrimers in HER2-positive human breast cancer *via* JNK and ERK1/ERK2 signalling pathways. *Comput Struct Biotechnol J* (2021) 19:2881–90. doi: 10.1016/j.csbj.2021.05.011

39. Jabeen A, Sharma A, Gupta I, Kheraldine H, Vranic S, Al Moustafa A-E, et al. Elaeagnus angustifolia plant extract inhibits epithelial-mesenchymal transition and induces apoptosis *via* HER2 inactivation and JNK pathway in HER2-positive breast cancer cells. *Molecules* (2020) 25(18):4240. doi: 10.3390/molecules25184240

40. Benzekry S, Lamont C, Beheshti A, Tracz A, Ebos JML, Hlatky L, et al. Classical mathematical models for description and prediction of experimental tumor growth. *PloS Comput Biol* (2014) 10(8):e1003800. doi: 10.1371/journal.pcbi.1003800

41. Sarapata EA, de Pillis LG. A comparison and catalog of intrinsic tumor growth models. *Bull Math Biol* (2014) 76(8):2010–24. doi: 10.1007/s11538-014-9986-y

42. Nami B, Maadi H, Wang Z. Mechanisms underlying the action and synergism of trastuzumab and pertuzumab in targeting HER2-positive breast cancer. *Cancers (Basel)* (2018) 10(10):342. doi: 10.3390/cancers10100342

43. Jang B-S, Han W, Kim IA. Tumor mutation burden, immune checkpoint crosstalk and radiosensitivity in single-cell RNA sequencing data of breast cancer. *Radiother Oncol J Eur Soc Ther Radiol Oncol* (2020) 142:202–9. doi: 10.1016/j.radonc.2019.11.003

44. Dirix LY, Takacs I, Jerusalem G, Nikolinakos P, Arkenau H-T, Forero-Torres A, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN solid tumor study. *Breast Cancer Res Treat* (2018) 167(3):671–86. doi: 10.1007/s10549-017-4537-5

45. Stagg J, Loi S, Divisekera U, Ngiow SF, Duret H, Yagita H, et al. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. *Proc Natl Acad Sci U S A* (2011) 108(17):7142–7. doi: 10.1073/pnas.1016569108

46. Janjigian YY, Maron SB, Chatila WK, Millang B, Chavan SS, Alterman C, et al. First-line pembrolizumab and trastuzumab in HER2-positive oesophageal, gastric, or gastro-oesophageal junction cancer: an open-label, single-arm, phase 2 trial. *Lancet Oncol* (2020) 21(6):821–31. doi: 10.1016/S1470-2045(20)30169-8

47. Loi S, Giobbie-Hurder A, Gombos A, Bachelot T, Hui R, Curigliano G, et al. Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): a single-arm, multicentre, phase 1b-2 trial. *Lancet Oncol* (2019) 20(3):371–82. doi: 10.1016/S1470-2045(18)30812-X

48. Arab A, Yazdian-Robati R, Behravan J. HER2-positive breast cancer immunotherapy: A focus on vaccine development. *Arch Immunol Ther Exp (Warsz)* (2020) 68(1):2. doi: 10.1007/s00005-019-00566-1

49. Rom-Jurek E-M, Kirchhammer N, Ugocsai P, Ortmann O, Wege AK, Brockhoff G. Regulation of programmed death ligand 1 (PD-L1) expression in breast cancer cell lines *in vitro* and in immunodeficient and humanized tumor mice. *Int J Mol Sci* (2018) 19(2):563. doi: 10.3390/ijms19020563

50. Zak KM, Grudnik P, Guzik K, Zieba BJ, Musielak B, Dömling A, et al. Structural basis for small molecule targeting of the programmed death ligand 1 (PD-L1). *Oncotarget* (2016) 7(21):30323–35. doi: 10.18632/oncotarget.8730

51. Lai X, Friedman A. Combination therapy of cancer with cancer vaccine and immune checkpoint inhibitors: A mathematical model. *PloS One* (2017) 12(5): e0178479. doi: 10.1371/journal.pone.0178479

52. Ashizawa T, Iizuka A, Tanaka E, Kondou R, Miyata H, Maeda C, et al. Antitumor activity of the PD-1/PD-L1 binding inhibitor BMS-202 in the

humanized MHC-double knockout NOG mouse. *BioMed Res* (2019) 40(6):243–50. doi: 10.2220/biomedres.40.243

53. Zhang R, Zhu Z, Lv H, Li F, Sun S, Li J, et al. Immune checkpoint blockade mediated by a small-molecule nanoinhibitor targeting the PD-1/PD-L1 pathway synergizes with photodynamic therapy to elicit antitumor immunity and antimetastatic effects on breast cancer. *Small* (2019) 15(49):e1903881. doi: 10.1002/smll.201903881

54. Chen Z, Fang Z, Ma J. Regulatory mechanisms and clinical significance of vimentin in breast cancer. *BioMed Pharmacother* (2021) 133:111068. doi: 10.1016/j.biopha.2020.111068

55. Sharma P, Alsharif S, Fallatah A, Chung BM. Intermediate filaments as effectors of cancer development and metastasis: A focus on keratins, vimentin, and nestin. *Cells* (2019) 8(5):497. doi: 10.3390/cells8050497

56. Richardson AM, Havel LS, Koyen AE, Konen JM, Shupe J, Wiles WG4, et al. Vimentin is required for lung adenocarcinoma metastasis *via* heterotypic tumor cell-Cancer-Associated fibroblast interactions during collective invasion. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2018) 24(2):420–32. doi: 10.1158/1078-0432.CCR-17-1776

57. Shah D, Osipo C. Cancer stem cells and HER2 positive breast cancer: The story so far. *Genes Dis* (2016) 3(2):114–23. doi: 10.1016/j.gendis.2016.02.002

58. Ishikawa T, Ichikawa Y, Shimizu D, Sasaki T, Tanabe M, Chishima T, et al. The role of HER-2 in breast cancer. *J Surg Sci* (2014) 2(1):4–9. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4323095/.

59. Özverel CS, Uyanikgil Y, Karaboz İ, Nalbantsoy A. Investigation of the combination of anti-PD-L1 mAb with HER2/neu-loaded dendritic cells and QS-21 saponin adjuvant: effect against HER2 positive breast cancer in mice. *Immunopharmacol Immunotoxicol* (2020) 42(4):346–57. doi: 10.1080/08923973.2020.1775644

60. Unni P, Seshaiyer P. Mathematical modeling, analysis, and simulation of tumor dynamics with drug interventions. *Comput Math Methods Med* (2019) 2019:4079298. doi: 10.1155/2019/4079298

61. Jarrett AM, Shah A, Bloom MJ, McKenna MT, Hormuth DA, Yankeelov TE, et al. Experimentally-driven mathematical modeling to improve combination targeted and cytotoxic therapy for HER2+ breast cancer. *Sci Rep* (2019) 9(1):12830. doi: 10.1038/s41598-019-49073-5

62. Lai X, Hao W, Friedman A. TNF-α inhibitor reduces drug-resistance to anti-PD-1: A mathematical model. *PloS One* (2020) 15(4):e0231499. doi: 10.1371/journal.pone.0231499

63. Turanli B, Altay O, Borén J, Turkez H, Nielsen J, Uhlen M, et al. Systems biology based drug repositioning for development of cancer therapy. *Semin Cancer Biol* (2021) 68:47–58. doi: 10.1016/j.semcancer.2019.09.020

64. Martinez-Morilla S, McGuire J, Gaule P, Moore L, Acs B, Cougot D, et al. Quantitative assessment of PD-L1 as an analyte in immunohistochemistry diagnostic assays using a standardized cell line tissue microarray. *Lab Investig* (2020) 100(1):4–15. doi: 10.1038/s41374-019-0295-9

65. Padmanabhan R, Meskin N, Al Moustafa AE. *Mathematical models of cancer and different therapies: Unified framework*. (2021). (Singapore: Springer).

66. Griguolo G, Pascual T, Dieci MV, Guarneri V, Prat A. Interaction of host immunity with HER2-targeted treatment and tumor heterogeneity in HER2-positive breast cancer. *J Immunother cancer* (2019) 7(1):90. doi: 10.1186/s40425-019-0548-6

67. Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. *Nat Rev Clin Oncol* (2011) 9:16. doi: 10.1038/nrclinonc.2011.177

68. Nagaraj AB, Wang QQ, Joseph P, Zheng C, Chen Y, Kovalenko O, et al. Using a novel computational drug-repositioning approach (DrugPredict) to rapidly identify potent drug candidates for cancer treatment. *Oncogene* (2018) 37(3):403–14. doi: 10.1038/onc.2017.328

69. Kim I-W, Kim JH, Oh JM. Screening of drug repositioning candidates for castration resistant prostate cancer. *Front Oncol* (2019) 9:661. doi: 10.3389/fonc.2019.00661

70. Zhao H, Jin G, Cui K, Ren D, Liu T, Chen P, et al. Novel modeling of cancer cell signaling pathways enables systematic drug repositioning for distinct breast cancer metastases. *Cancer Res* (2013) 73(20):6149–63. doi: 10.1158/0008-5472.CAN-12-4617

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Glossary

ATCC	American type culture collection
BC	breast cancer
BMS	Bristol Myers Squibb
CTLA	cytotoxic T-lymphocyte-associated protein
DFS	disease-free survival
FBS	fetal bovine serum
GI	growth inhibition
HER	human-epidermal growth factor receptor
ICB	immune checkpoint blockade
IFN	interferon
irAEs	immune-related adverse events
mAbs	monoclonal antibodies
MHC	major histocompatibility complex
MTD	maximum tolerated dose
NOG	severely immunodeficient mouse
NP	nanoparticle
OR	objective response
PBS	phosphate buffered saline
PD-1	programmed death receptor
PD-L1	programmed death receptor ligand
PI	propidium iodide
RP2D	recommended phase-II dose
RPMI	Roswell Park Memorial Institute
SCC	squamous cell carcinoma
SmIs	small molecule inhibitors
TIL	tumor-infiltrating leukocytes
TME	tumor microenvironment
TNBC	triple-negative breast cancer
TNF	tumor necrosis factor
ICB	Immune checkpoint blockade
HER	human epidermal growth factor receptor
BC	breast cancer
TZ	trastuzumab

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Clinicopathological characteristics and features of molecular subtypes of breast cancer at high altitudes

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Background: Breast cancer is one of the major malignancies threatening women's health worldwide. The incidence of breast cancer at high altitudes increased over the years. But few studies focused on the characteristics of clinicopathology and molecular subtypes among breast cancer at high altitudes, which are still unknown. Tibet, with an average altitude over 4000 meters, is a representative city at high altitudes, lying in the Qinghai-Tibetan Plateau in southwestern China. This study aimed to identify the clinicopathological characteristics and features of molecular subtypes among Tibetan women with breast cancer, and provide evidence for cancer prevention and personalized therapeutics in high-altitude regions.

Methods: Between May 2013 and March 2022, 104 Tibetan women from high-altitude regions (Tibetan-group) and 34 Han Chinese women from low-altitude regions (Han-group), consecutively diagnosed with breast cancer in the Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region, were included in the study. We retrospectively reviewed the clinical character, altitudes of residence, tumor size, lymph nodes metastasis, distant metastasis, pathological type, immunohistochemical index, and molecular subtype.

Results: In the study, we calculated the patient delay, equal to the period from symptoms onset to hospital visits. The patient delay of Tibetan-group was 7.47 ± 11.53 months, which was significantly longer than that of Han-group, 7.22 ± 22.96 months ($p<0.05$). Body Mass Index (BMI) was significantly different ($p<0.05$). Tumors in Tibetan-group were significantly larger than those in Han-group, 4.13 ± 2.98cm and 2.51 ± 0.82cm in diameter, respectively ($p<0.05$). According to ordinal logistic regression analysis, exposure to high altitudes might result in more advanced T stage (OR=2.45 95%CI 1.10-5.44). 41.3% (43/104) of cases in Tibetan-group had lymph node positive disease, whereas the percentage was found in 38.26% (13/34) in Han-group($p<0.05$). The distribution of molecular subtypes was quite significantly different between two groups ($p<0.05$), according to the comparison of constituent ratios.

Conclusion: Our study verified that breast cancer at high altitudes possessed its own unique clinicopathological characteristics and distinct features of molecular

subtypes. It broadened the understanding of this heterogenous disease and also provided valuable evidence for cancer prevention and personalized therapeutics of breast cancer at high altitudes.

KEYWORDS

molecular subtype, high altitude, pathology, breast cancer, tibet

1 Introduction

Breast cancer is one of the major malignancies, posing a great threat to women's health worldwide. According to statistics, female breast cancer had become the most commonly diagnosed cancer, with an estimated 2.26 million new cases in 2020, surpassing lung cancer as the first of cancer incidence (1). Similarly, the incidence of breast cancer at high altitudes increased over the years (2). But few studies focused on the characteristics of clinicopathology and molecular subtypes among breast cancer patients at high altitudes, which are still unknown. Tibet, with an average altitude over 4000 meters, is a representative city at high altitudes, lying in the Qinghai-Tibetan Plateau in southwestern China. Due to its unique climate, geographical location, ethnicity, lifestyle, religion, and economy, breast cancer patients in Tibet may present special disease features. Nowadays, researchers have realized that breast cancer is not a single disease, but a heterogenous complex disease containing several subtypes (3–5). Since the molecular intrinsic subtypes were first presented, they provided various important information to study the heterogeneity of breast cancer, leading into a new era of classified therapy in breast cancer. This study aimed to identify the clinicopathological characteristics and features of molecular subtypes among Tibetan women with breast cancer, and provide evidence for cancer prevention and personalized therapeutics in high-altitude regions.

2 Materials and methods

2.1 Patients

The study retrospectively reviewed the women who were consecutively diagnosed with breast cancer in the Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region between May 2013 and March 2022. All patients underwent surgical resection or ultrasound-guided core needle biopsy. The diagnosis was confirmed by histopathology or cytopathology. Patients diagnosed pathologically as primary invasive breast cancer were eligible for inclusion. We defined the patients at high altitudes as those who were Tibetan residents, living permanently at altitudes > 2500 m since they were born. And we defined the patients at low altitudes as those who were Han Chinese, living permanently at altitudes ≤ 1000 m since they were born. Patients who migrated from high altitudes to low altitudes or migrated from low altitudes to high altitudes were excluded. Other exclusion criteria included: parents of patients were migrants, and history of other malignancy. According to the altitude of residence, the patients were divided into the Tibetan-group (high-altitude) and the Han-group (low-altitude). The study was approved by the ethic committee of the Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region. Because of the retrospective nature of the study, informed consent was waived.

2.2 Methods

We retrospectively reviewed the following data: age, the altitude of residence, Body Mass Index (BMI), menstrual status, age at menopause, symptoms, time of symptoms onset, time of hospital visit, imaging tests, tumor size, lymph nodes metastasis, distant metastasis, pathological type, immunohistochemical characteristics, and molecular subtype. We calculated the patient delay, equal to the period from symptoms onset to hospital visits. Tumor size was measured by the largest contiguous dimension of a tumor focus according to pathological criteria among patients who underwent radical surgery. It was according to clinical criteria among patients who didn't undergo the surgery due to distant metastasis or refusal to surgery. TNM stage was assessed according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging manual.

Pathological evaluation: Estrogen Receptor (ER)/Progesterone Receptor (PR) was defined positive if it was stained in ≥1% of nuclei in tumor cells, and ER/PR was defined negative if it was stained in <1% of nuclei in tumor cells or non-stained (6). Human Epidermal Growth Factor Receptor 2(HER-2), detected by immunohistochemistry (IHC): +++ was positive and + was negative. When HER-2 was scored ++ by IHC, fluorescent *in situ* hybridization (FISH) should be additionally adopted to evaluate the amplification of HER-2 further. If HER-2 amplification occurred, HER-2 (++) was classified as HER-2 positive. If no HER-2 amplification was found, HER-2 (++) was classified as HER-2 negative (7). Ki-67 was evaluated by the percentage of positive invasive tumor cells with any nuclear staining. When the percentage ≥14%, Ki-67 was recorded high and when the percentage <14%, Ki-67 was recorded low (8).

In the study, cases from both groups were classified into four subtypes, including luminal A (ER -and/or PR-positive, HER2-negative, Ki-67 low), luminal B (ER- and/or PR-positive, HER2-negative, Ki-67 high) or (ER- and/or PR-positive, HER2-positive, any Ki-67), HER2-enriched (ER and PR-negative, HER2-positive), and Triple negative (ER and PR negative, HER2-negative), according to St. Gallen consensus criteria (8).

2.3 Statistical analysis

Values were compared by the student 's t test. Categorical data was compared by Chi-squared test. And when n<5, Fisher's exact test was performed. Ordinal logistic regression analysis was used to calculate the Odds Ratio (OR). Statistical significance was considered by two-tailed test with p<0.05. The statistical analyses were performed using IBM SPSS statistics 26.0 software.

3 Results

3.1 Clinical characters

Between May 2013 and March 2022, 138 female patients were enrolled in the study. Among them, 104 patients were Tibetans from high-altitude regions (Tibetan-group), accounting for 75.4%. Their altitudes of residence ranged from 2720 m to 5200 m, and the median altitude was 3650 m. And 34 patients were Han Chinese from low-altitude regions (Han-group), accounting for 24.6%. Their altitudes of residence ranged from 49 m to 1000 m, and the median altitude was 492 m. There was a significant difference in altitude between two groups (p<0.05). Among Tibetan-group, age at diagnosis ranged from 26 to 80 years, and the average age was 47.96 ± 10.56 years, while age at diagnosis among the Han-group ranged from 19 to 70 years, and the average age was 47.96 ± 10.56years (p>0.05). Post-menopausal cases accounted for 41.3% (43/104) in Tibetan-group, and it was found in 47.1% (16/34) in Han-group. The average age at menopause was 48.40 ± 3.47years in Tibetan-group, and it was 50.56 ± 4.68 years in Han-group. No significant difference was observed in terms of menstrual status and menopause age between two groups(p>0.05). In the study, we calculated the patient delay, equal to the period from symptoms onset to hospital visits. Symptoms such as palpable painless breast lump, breast pain, palpable axillary nodes, nipple retraction, nipple discharge, and changes in skin were included. The patient delay of Tibetan-group was 7.47 ± 11.53 months, which was significantly longer than that of Han-group (7.22 ± 22.96 months) (p<0.05). There was a significant difference in Body Mass Index (BMI) between Tibetan-group (26.17 ± 4.80) and Han-group (23.98 ± 3.17) (p<0.05). Tumors in Tibetan-group were significantly larger than those in Han-group, with 4.13 ± 2.98cm and 2.51 ± 0.82cm in diameter, respectively (p<0.05). According to ordinal logistic regression analysis, exposure to high altitudes might result in more advanced T stage (OR=2.45 95%CI 1.10-5.44).

41.3% (43/104) of cases in Tibetan-group had lymph node positive disease, whereas the percentage was found in 38.26% (13/34) in Han-group(p<0.05). 7.69% (8/104) of patients in Tibetan-group had distant metastasis when they were initially diagnosed, whereas the percentage was found in 5.88% (2/34) in Han-group (p>0.05) (Table 1).

TABLE 1 Clinical characteristics in Tibetan-group and Han-group.

Clinical character	Tibetan-group	Han-group	p value
Age(years) Altitude of residence(m)	47.96 ± 10.56 3650 ± 488.55	47.96 ± 10.56 492 ± 151.35	0.104 [**0.000**]
Menstrual status [n (%)]			
post-menopausal pre-menopausal	43(41.3%) 61(58.7%)	16(47.1%) 18(52.9%)	0.588
Average age at menopause (years)	48.40 ± 3.47	50.56 ± 4.68	0.058
Patient delay(months)	7.47 ± 11.53	7.22 ± 22.96	***0.023***
Body Mass Index (BMI)	26.17 ± 4.80	23.98 ± 3.17	***0.015***
Tumor diameter(cm)	4.13 ± 2.98	2.51 ± 0.82	***0.000***
Lymph nodes metastasis [n (%)] Distant metastasis [n (%)]	58(58.62%) 8(7.69%)	13(38.26%) 2(5.88%)	***0.035*** [1.000]

The bold values were less than 0.05, showing significant difference.

3.2 Clinicopathological characteristics

In Tibetan-group, 94 patients were diagnosed with invasive carcinoma of no special type, accounting for 90.38%. 9 (8.65%) patients were diagnosed with invasive carcinoma of special type, including 1 with cribriform carcinoma, 2 with medullary carcinoma, 2 with papillary carcinoma, 1 with metaplastic carcinoma, 2 with malignant phyllodes tumors and 1 with mucinous carcinoma. And 1 (0.96%) patient was noninvasive carcinoma. In Han-group, 31 patients were diagnosed with invasive carcinoma of no special type, accounting for 91.18%. And 3 (8.82%) patients were diagnosed with invasive carcinoma of special type, including 2 with mucinous carcinoma and 1 with papillary carcinoma. Among Tibetan-group, 66 (65.35%) cases were ER-positive, 35(34.65%) cases were ER-negative, and 3 were unevaluated, while 25 (73.53%) cases were ER-positive, and 9(26.47%) cases were ER-negative in Han-group. As to ER status, no significant difference was found between two groups (p>0.05). In Tibetan-group, 54(53.47%) cases were PR-positive, 47(46.53%) cases were PR-negative, and 3 were unevaluated, while 20 (60.61%) cases were PR-positive, 13(39.39%) cases were PR-negative, and 1 was unevaluated in Han-group. No significant difference was observed between two groups (p>0.05). In Tibetan-group, 23 (24.21%) cases were HER-2-positive, 72(75.79%) cases were HER-2-negative, 3 were uncertain without being retested by FISH, 2 were uncertain after being retested by FISH, and 4 were unevaluated. In Han-group, 12 (36.36%) cases were HER-2-positive, 21(63.64%) were HER-2-negative, and 1 was uncertain after be retested by FISH. There was no significant difference in positive rates of HER-2 status between two groups (p>0.05). Among Tibetan-group, 75 (79.79%) cases were Ki-67 high, 19(20.21%) were Ki-67 low, and 10 were unevaluated. Among Han-group, 31 (91.18%) cases were Ki-67 high, and 3(8.82%) were Ki-67 low. In the term of Ki-67 expression, there was no significant difference between two groups (p>0.05) (Table 2).

3.3 Features of molecular subtypes

Herein, cases from both groups were classified into four molecular subtypes, including Luminal A, Luminal B, HER2-enriched, and Triple negative. Among Tibetan-group, 18 (18.18%) cases were Luminal A, 46 (46.46%) cases were Luminal B, 9 (9.09%) cases were HER2-enriched, and 26 (26.26%) cases were Triple negative. In Han-group, 3 (8.82%) cases were Luminal A, 22 (64.71%) cases were Luminal B, 7 (20.59%) cases were HER2-enriched, and 2 (5.88%)

TABLE 2 Clinicopathological characteristics in Tibetan-group and Han-group[n(%)].

Clinicopathological character	Tibetan-group	Han-group	p value
Pathological type			
Invasive carcinoma of no special type Invasive ductal carcinoma Others	94(90.38%) 69(66.35%) 10(9.62%)	31(91.18%) 24(70.59%) 3(8.82%)	0.747
ER			
Positive Negative	66(65.35%) 35(34.65%)	25(73.53%) 9(26.47%)	0.379
PR			
Positive Negative	54(53.47%) 47(46.53%)	20(60.61%) 13(39.39%)	0.474
Her-2			
Positive Negative	23(24.21%) 72(75.79%)	12(36.36%) 21(63.64%)	0.177
Ki67			
High Low	75(79.79%) 19(20.21%)	31(91.18%) 3(8.82%)	0.186

cases were Triple negative. The constituent ratios of the four molecular subtypes were significantly different between Tibetan-group and Han-group ($p<0.05$) (Table 3).

4 Discussion

Breast cancer is the most common cancer in women worldwide with ever-increasing incidence, and high-altitude regions are no exception (2, 9–11). Previous evidence suggested that high-altitude populations were at higher risk for breast cancer, compared with low-altitude populations. And mounting evidence presented various differences of gene expression during breast cancer process between high- and low-altitude populations (12). Compared to low-altitude regions, the most obvious distinctions of high-altitude regions are intense ultraviolet radiation, hypoxia, and low pressure. Several studies argued whether more exposure to ultraviolet ray would result in higher incidence of breast cancer. However, their conclusions were inconsistent (13, 14). Therefore, the influence of ultraviolet radiation on the development of breast cancer was still unknown. Hypoxia is closely related to tumorigenesis and tumor progression. Zhang et al. (15) induced the breast cancer stem cells by hypoxia. In their study, hypoxic tumor microenviroment greatly promoted the excessive and aberrant angiogenesis. Usually, the tumor vessels formed by angiogenesis were tortuous, dilated and excessively branched (16). Ultimately, the disorganized vasculature was inefficient for blood supply, and contributed to the hypoxic microenviroment in turn, which played a fundamental role for tumor progression (17). Previously, researchers constantly explored the pathogenic factors and gene expression of breast cancer in high-altitude regions, but few studies focused on the clinicopathological characteristics and features of molecular subtypes, which was deserved more attention and further research.

TABLE 3 Molecular subtypes in Tibetan-group and Han-group [n (%)].

Group	Luminal A	Luminal B	HER2-enriched	Triple negative	Total
Tibetan-group	18(18.18%)	46(46.46%)	9(9.09%)	26(26.26%)	99
Han-group	3(8.82%)	22(64.71%)	7(20.59%)	2(5.88%)	34
Total	21	68	16	28	133

In our study, patients in Tibetan-group were all inhabitants of Tibet, which was a representative region at high altitudes. And breast cancer women in the corresponding period at low altitudes were included as controls, forming the Han-group. Our results demonstrated that the two groups had much in common, but the unique characteristics of clinical pathology and distinct features of molecular subtypes were clearly presented in Tibetan-group.

As shown in our study, the majority of patients were between 40 and 50 years. The proportion of post-menopausal patients was over 40%, and the average age at menopause ranged from 45 to 55 years. They all had no significant difference between two groups ($p>0.05$). But the patient delay, Body Mass Index (BMI), tumor size, and lymph node metastasis were all different with statistical significance between the two groups ($p<0.05$). The patient delay, defined as the period between symptoms onset and hospital visits was 7.47 ± 11.53 months in Tibetan-group, which was significantly longer than that in Han-group (7.22 ± 22.96 months) ($p<0.05$). To some extent, this result illustrated the fact that hospital visits of the Tibetan-group might be seriously delayed. The causes of delay were varied. Vast areas of Tibet were economically undeveloped, same as other high-altitude regions. These areas faced a relative shortage of medical resources. And some people had weak health awareness, who paid little attention to the secondary prevention for breast cancer. Besides, the long distance and steepness of the way to hospitals hindered them to seek immediate medical attention, due to the remote location of their settlements. According to the standard deviations of both figures, the patient delay fluctuated widely. However, the reasons of two groups were different. Tibet covered large areas, and districts of Tibet were far apart. There were great distinctions in economic development. In addition, the district distribution of medical resources was greatly uneven. All the reasons mentioned above were attributed to the wide fluctuation in Tibetan-group. But in Han-group, it was probably associated with the small sample size. Women in Tibetan-group were significantly fatter than those in Han-group. As with our results, previous studies found that overweight and obesity were associated not only with a higher risk of developing breast cancer, particularly in postmenopausal women, but also with worse prognosis for women of all ages (18). Compared with Han-group, tumors in Tibetan-group were significantly larger, and patients with positive lymph nodes in Tibetan-group were significantly more, suggesting that patients in Tibetan-group might have more advanced stages, which might result in worse disease outcome. We calculated the OR related to T stage to identify if exposure to altitude could influence breast cancer by ordinal logistic regression analysis. In our results, the OR was 2.45, 95% CI was 1.10-5.44. It demonstrated that

exposure to high altitudes might result in more advanced T stage. Michaelson JS et al. revealed that tumor size was associated with increased lethality, such that each millimeter of tumor diameter was associated with an additional approximately 1% chance of death (19). Zheng S et al. reported that breast cancer in China showed more invasive ductal carcinoma with larger tumor size, later stage than those in the Western ($p<0.001$), and their results indicated that invasive breast cancer of Chinese might be more aggressive than those of the Western population (20). Risk of getting breast cancer was related to living at a higher altitude, as well as an increased risk of death (OR:1.067; $p=0.030$) (21). Several previous studies had reported that the lymph node status, the number of positive lymph nodes and the sites of positive lymph nodes were all important prognostic indicators of breast cancer (22, 23).

According to our results, the major pathological type was invasive carcinoma of no special type in both groups. Among them, invasive ductal carcinoma accounted for the largest proportion. In two groups, the positive rates of ER were 65.35% and 73.53%, respectively. And the positive rates of PR were 53.47% and 60.61%, respectively. They were all found with no significant difference ($p>0.05$). Nuclear ER and PR, also known as members of steroid receptor superfamily, were both essential molecules, coordinately contributing to the development of lobular alveolar epithelial structures of the normal mamma during puberty, menstrual cycle and pregnancy (24). ER positive cancers were reported to be well differentiated, less aggressive, and had a better prognosis (25). The positive rates of HER-2 in two groups were 24.21% and 36.36%, respectively ($p>0.05$). The human epidermal growth factor receptor (HER-2) was a transmembrane tyrosine kinase receptor, and also a proto-oncogene, involved in the proliferation and differentiation of mammary cells. Over-expression of HER-2 allowed mammary cells to proliferate, survive, differentiate through a signal transduction cascade regulated by PI3k/Akt and Ras/Raf/MEK/MAPK pathways (26). Breast cancer with HER-2 over-expression constituted an aggressive type of breast cancer, which tended to grow more rapidly and were at higher risk of lymph node metastasis (27). Over expression of HER-2 was associated with poor prognosis, increased resistance to endocrine therapy and poor responding to non-anthracycline, nontaxane-containing chemotherapy (28, 29). In our two groups, cases with Ki-67 high were found in 79.79% and 91.18%, respectively, with no significant difference ($p>0.05$). Ki-67, also known as an excellent marker of proliferation, remained active during M, G1, S, and G2 phases of the cell cycle, and absent during G0 phase (30–33).

In 2000, Perou et al. proposed molecular classification of breast cancer into intrinsic subtypes, which differed in intrinsic biology, prognosis, and response to therapy (34). It was confirmed by accumulating evidence. In 2013, the St. Gallen international breast cancer conference expert panel refined and re-iterated the value of clinicopathological surrogate definitions resembling intrinsic subtypes to guide selection of systemic adjuvant therapies (7). Ultimately, the criteria of molecular subtypes were established. In addition, molecular subtypes had been confirmed to be the prognostic predictor of breast cancer (35, 36). In previous studies, the distribution of molecular subtypes varied, which was affected by many factors, such as sample size, testing technology, ethnicity, living conditions, economy and so on (37). In our study, Luminal B was the most common subtype both in Tibetan-group and Han-group, found in 46.46% and 64.71%, respectively. Following it, triple negative (26.26%) came to be the second in Tibetan-group, whereas it was HER2-enriched (20.59%) in Han-group. Then Luminal A (18.18%) followed, and HER2-enriched (9.09%) was the least in Tibetan-group. In Han-group, it was followed by Luminal A (8.82%) and triple negative (5.88%). The distribution of subtypes was quite significantly different between two groups ($p<0.05$), according to the comparison of constituent ratios of the four molecular subtypes in two groups.

There are over 140 million people who live at high altitudes worldwide. Tibetans are among them. Tibetans reside in the Qinghai-Tibetan Plateau for centuries. Long-term exposure to this kind of extreme environment brings changes in breast cancer. Our study retrospectively reviewed breast cancer patients from high-altitude regions and low-altitude regions between May 2013 and March 2022 in our hospital. We analyzed the clinicopathological characteristics and features of molecular subtypes of breast cancer at high altitudes. We also compared them with breast cancer at low altitudes. We had found that breast cancer patients at high altitudes showed significantly different in the patient delay, BMI, tumor size, lymph node metastasis and the distribution of subtypes. Our results verified the unique traits of breast cancer at altitudes, which might influence treatment strategies in the future. We will keep following these cases, collecting prognostic data, and observing long-term outcomes. Based on the present study, we recommend taking steps to raise cancer awareness, guide healthy weight maintenance and breast self-examination, improve cancer screening rate, and optimize medical resource allocation. In our study, the sample size of the control group was limited, and it might bring selection bias. We reduced its influence by rigorous statistical methods. Nevertheless, our study had still broadened the understanding of this heterogenous disease and also provided valuable evidence for cancer prevention and personalized therapeutics of breast cancer at high altitudes.

Constituent ratios of the four molecular subtypes were compared between two groups ($p=0.01$).

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 ethic committee of the Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region. Written informed consent from the patients was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

All authors participated in conceptualization, writing, review and editing the manuscript. All authors contributed to the article and approved the submitted version.

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.

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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. Tang RQ, Zou XN, Wang SB, Zhang L, Chen WQ, Ze YG, et al. The primary analysis of cancer registration data of Lhasa, tibet. (article in Chinese). *China Cancer* (2009) 18(6):432–5.

3. Krishnamurti U, Wetherilt CS, Yang J, Peng L, Li X. Tumor-infiltrating lymphocytes are significantly associated with better overall survival and disease-free survival in triple-negative but not estrogen receptor-positive breast cancers. *Hum Pathol* (2017) 64:7–12. doi: 10.1016/j.humpath.2017.01.004

4. Yang ZJ, Yu Y, Chi JR, Guan M, Zhao Y, Cao XC. The combined pN stage and breast cancer subtypes in breast cancer: A better discriminator of outcome can be used to refine the 8th AJCC staging manual. *Breast Cancer* (2018) 25(3):315–24. doi: 10.1007/s12282-018-0833-0

5. Shibuta K, Ueo H, Furusawa H, Komaki K, Rai Y, Sagara Y, et al. The relevance of intrinsic subtype to clinicopathological features and prognosis in 4,266 Japanese women with breast cancer. *Breast Cancer* (2011) 18(4):292–8. doi: 10.1007/s12282-010-0209-6

6. Hammond ME, Hayes DF, Dowsett M, Alfred 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

7. 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(9):2206–23. doi: 10.1093/annonc/mdt303

8. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* (2015) 136(5):E359–86. doi: 10.1002/ijc.29210

9. Chen WQ, Zheng RS, Zhang SW, Zeng HM, Zou XN, He J. Analysis of cancer incidence and mortality in China, 2013. *China Cancer* (2017) 26(1):1–7.

10. Mori N, Mugikura S, Miyashita M, Kudo Y, Suzuki M, Li L, et al. Perfusion contrast-enhanced ultrasound to predict early lymph-node metastasis in breast cancer. *Jpn J Radiol* (2019) 37(2):145–53. doi: 10.1007/s11604-018-0792-6

11. López-Cortés A, Leone PE, Freire-Paspuel B, Arcos-Villacís N, Guevara-Ramírez P, Rosales F, et al. Mutational analysis of oncogenic AKT1 gene associated with breast cancer risk in the high-altitude Ecuadorian mestizo population. *BioMed Res Int* (2018) 2018:7463832. doi: 10.1155/2018/7463832

12. Maryanaji Z. The effect of climatic and geographical factors on breast cancer in Iran. *BMC Res Notes* (2020) 13(1):519. doi: 10.1186/s13104-020-05368-9

13. Hiller TWR, O'Sullivan DE, Brenner DR, Peters CE, King WD. Solar ultraviolet radiation and breast cancer risk: A systematic review and meta-analysis. *Environ Health Perspect* (2020) 128(1):16002. doi: 10.1289/EHP4861

14. Li Y, Ma L. Exposure to solar ultraviolet radiation and breast cancer risk: A dose-response meta-analysis. *Medicine(Baltimore)* (2020) 99(45):e23105. doi: 10.1097/MD.0000000000023105

15. Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, et al. Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m_6A-demethylation of NANOG mRNA. *Proc Natl Acad Sci U S A* (2016) 113(14):E2047–56. doi: 10.1073/pnas.1602883113

16. Shojaei F. Anti-angiogenesis therapy in cancer: current challenges and future perspectives. *Cancer Lett* (2012) 320(2):130–7. doi: 10.1016/j.canlet.2012.03.008

17. Hockel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. *Cancer Res* (1996) 56(19):4509–15.

18. Picon-Ruiz M, Morata-Tarifa C, Valle-Goffin JJ, Friedman ER, Slingerland JM. Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. *CA Cancer J Clin* (2017) 67(5):378–97. doi: 10.3322/caac.21405

19. Michaelson JS, Silverstein M, Sgroi D, Cheongsiatmoy JA, Taghian A, Powell S, et al. The effect of tumor size and lymph node status on breast carcinoma lethality. *Cancer* (2003) 98(10):2133–43. doi: 10.1002/cncr.11765

20. Zheng S, Bai JQ, Li J, Fan JH, Pang Y, Song QK, et al. The pathologic characteristics of breast cancer in China and its shift during 1999-2008: a national-wide multicenter cross-sectional image over 10 years. *Int J Cancer* (2012) 131(11):2622–31. doi: 10.1002/ijc.27513

21. Garrido DI, Garrido SM. Cancer risk associated with living at high altitude in Ecuadorian population from 2005 to 2014. *Clujul Med* (2018) 91(2):188–96. doi: 10.15386/cjmed-932

22. Kuru B, Camlibel M, Gulcelik MA, Alagol H. Prognostic factors affecting survival and disease-free survival in lymph node-negative breast carcinomas. *J Surg Oncol* (2003) 83(3):167–72. doi: 10.1002/jso.10264

23. Taghian A, Jeong JH, Mamounas E, Anderson S, Bryant J, Deutsch M, et al. Patterns of locoregional failure in patients with operable breast cancer treated by mastectomy and adjuvant chemotherapy with or without tamoxifen and without radiotherapy: results from five national surgical adjuvant breast and bowel project randomized clinical trials. *J Clin Oncol* (2004) 22(21):4247–54. doi: 10.1200/JCO.2004.01.042

24. Tanos T, Rojo L, Echeverria P, Brisken C. ER and PR signaling nodes during mammary gland development. *Breast Cancer Res* (2012) 14(4):210. doi: 10.1186/bcr3166

25. Patani N, Martin LA, Dowsett M. Biomarkers for the clinical management of breast cancer: International perspective. *Int J Cancer* (2013) 133(1):1–13. doi: 10.1002/ijc.27997

26. Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. *Nat Rev Clin Oncol* (2011) 9(1):16–32. doi: 10.1038/nrclinonc.2011.177

27. Ross JS, Fletcher JA. The HER-2/neu oncogene in breast cancer: Prognostic factor, predictive factor, and target for therapy. *Oncologist* (1998) 3(4):237–52. doi: 10.1634/theoncologist.3-4-237

28. Rubin I, Yarden Y. The basic biology of HER2. *Ann Oncol* (2001) 12 Suppl 1:S3–8. doi: 10.1093/annonc/12.suppl_1.S3

29. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Alfred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists. American Society of clinical Oncology/College of American pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* (2007) 131(1):18–43. doi: 10.5858/2007-131-18-ASOCCO

30. Gerlach C, Sakkab DY, Scholzen T, Dassler R, Alison MR, Gerdes J. Ki-67 expression during rat liver regeneration after partial hepatectomy. *Hepatology* (1997) 26(3):573–8. doi: 10.1002/hep.510260307

31. Shirendeb U, Hishikawa Y, Moriyama S, Win N, Thu MM, Mar KS, et al. Human papillomavirus infection and its possible correlation with p63 expression in cervical cancer in Japan, Mongolia, and Myanmar. *Acta Histochem Cytochem* (2009) 42(6):181–90. doi: 10.1267/ahc.09030

32. Hooghe B, Hulpiau P, van Roy F, De Bleser P. ConTra: a promoter alignment analysis tool for identification of transcription factor binding sites across species. *Nucleic Acids Res* (2008) 36(Web Server issue):W128–32. doi: 10.1093/nar/gkn195

33. Modlin IM, Moss SF, Chung DC, Jensen RT, Snyderwine E. Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors. *J Natl Cancer Inst* (2008) 100(18):1282–9. doi: 10.1093/jnci/djn275

34. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* (2000) 406(6797):747–52. doi: 10.1038/35021093

35. Ihemelandu CU, Leffall LD Jr, Dewitty RL, Naab TJ, Mezghebe HM, Makambi KH, et al. Molecular breast cancer subtypes in premenopausal and postmenopausal African-American women: age-specific prevalence and survival. *J Surg Res* (2007) 143(1):109–18. doi: 10.1016/j.jss.2007.03.085

36. Montagna E, Maisonneuve P, Rotmensz N, Cancello G, Iorfida M, Balduzzi A, et al. Heterogeneity of triple-negative breast cancer: histologic subtyping to inform the outcome. *Clin Breast Cancer* (2013) 13(1):31–9. doi: 10.1016/j.clbc.2012.09.002

37. Leong SP, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, et al. Is breast cancer the same disease in Asian and Western countries? *World J Surg* (2010) 34(10):2308–24. doi: 10.1007/s00268-010-0683-1

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Relationship between polymorphisms in homologous recombination repair genes RAD51 G172T、XRCC2 & XRCC3 and risk of breast cancer: A meta-analysis

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Background: Genetic variability in DNA double-strand break repair genes such as RAD51 gene and its paralogs XRCC2、XRCC3 may contribute to the occurrence and progression of breast cancer. To obtain a complete evaluation of the above association, we performed a meta-analysis of published studies.

Methods: Electronic databases, including PubMed, EMBASE, Web of Science, and Cochrane Library, were comprehensively searched from inception to September 2022. The Newcastle-Ottawa Scale (NOS) checklist was used to assess all included non-randomized studies. Odds ratios (OR) with 95% confidence intervals (CI) were calculated by STATA 16.0 to assess the strength of the association between single nucleotide polymorphisms (SNPs) in these genes and breast cancer risk. Subsequently, the heterogeneity between studies, sensitivity, and publication bias were performed. We downloaded data from The Cancer Genome Atlas (TCGA) and used univariate and multivariate Cox proportional hazard regression (CPH) models to validate the prognostic value of these related genes in the R software.

Results: The combined results showed that there was a significant correlation between the G172T polymorphism and the susceptibility to breast cancer in the homozygote model (OR= 1.841, 95% CI=1.06–3.21, *P*=0.03). Furthermore, ethnic analysis showed that SNP was associated with the risk of breast cancer in Arab populations in homozygous models (OR=3.52, 95% CI=1.13-11.0, *P*= 0.003). For the XRCC2 R188H polymorphism, no significant association was observed. Regarding polymorphism in XRCC3 T241M, a significantly increased cancer risk was only observed in the allelic genetic model (OR=1.05, 95% CI= 1.00–1.11, *P*=0.04).

Conclusions: In conclusion, this meta-analysis suggests that Rad51 G172T polymorphism is likely associated with an increased risk of breast cancer, significantly in the Arab population. The relationship between the XRCC2 R188H polymorphism and breast cancer was not obvious. And T241M in XRCC3 may be associated with breast cancer risk, especially in the Asian population.

KEYWORDS

breast neoplasms, Rad51 recombinase, single nucleotide polymorphism, DNA repair mechanism mutations, meta-analysis

Introduction

In all countries around the world, cancer is the leading cause of death and an important obstacle to improving life expectancy. Female breast cancer (BC) has overtaken lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases (1). The mechanism of breast carcinogenesis is not yet fully understood. It is considered a polygenic disease and has a component of inheritance due to low-penetrant and common genetic variants. The steady repair of DNA damage is very important for the survival of cells and the maintenance of genetic stability (2).

Over the years, it has been increasingly recognized that variations in the genetic background of individuals combined with environmental exposure can ultimately lead to the occurrence and progression of cancer. DNA repair genes have been considered considerable factors in the prevention of genomic damage and continuously monitor chromosomes to correct injuries caused by exogenous agents such as ultraviolet light or endogenous mutagens (3, 4). Aberrant double-stranded break (DSB) repair leads to genomic instability, a hallmark of malignant cells. Double-stranded breaks are repaired by two pathways: homologous recombination (HR) and non-homologous end joining (NHEJ). Previous analysis has revealed several important features of DSB repair in breast cancer cells: (i) HR is evidently increased in breast cancer cells compared with normal cells; (ii) Non-homologous end joining(NHEJ)repair is the major DSB repair route in both normal and malignant breast epithelial cells; (iii) NHEJ efficiency does not differ significantly between normal and cancerous cells (5). The two pathways of DSB repair are independently controlled, and only HR is increased in breast cancer cells compared with normal breast epithelial cells. RAD51 is a homolog of the E. coli RecA protein, which is essential for maintainability such as meiotic and mitotic recombination, and also plays a critical role in homologous recombination repair (HR) of DNA double-strand breaks (DSB) (6–8).

Researchers recently discovered that the Rad51 promoter in cancer cells is on average 840-fold more active in cancer cells than in normal cells and the fusion of RAD51 promoter and diphtheria toxin gene selectively kills cancer cells. Transcriptional targeting therapy using up-regulated HR gene expression can effectively eliminate cancer cells without toxicity to normal tissues. The human RAD51 gene, located on chromosome 15q15.1, is considered to participate in a common DSB repair pathway and is involved in the development of breast cancer development (9). RAD51 functions by assembling on a single-stranded DNA, inducing homologous pairing, and in turn mediates strand invasion and exchange between homologous DNA and damaged site (10). In recent years, the RAD51 gene polymorphism has attracted a great deal of attention. The RAD51 family of genes, including RAD51 and the five RAD51-like genes, are known to have crucial non-redundant roles in this pathway. Recently, researchers have revealed that RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2, XRCC3) could serve as central proteins during the HRR process. The function of RAD51-like genes is to transduce DNA damage signals to effector kinases that promote break repair. A central player in homologous recombination is the RAD51 recombinase that binds to single-stranded DNA at break sites, the XRCC2 and XRCC3 genes are structurally and functionally related to the RAD51 genes (11). Two commonly studied polymorphisms of the RAD51 gene are G135C (rs1801320), a G to C transversion at position +135, and G172T (rs1801321), a G to T transversion at position +172, both of which are located in the 5 Untranslated region (5'UTR) and appear to be related to functional polymorphisms. Two variants of 135G/C and 172G/T would affect mRNA stability or translational efficiency, resulting in altered levels of polypeptide products, altering the function of encoding the RAD51 protein, and in some way influencing DNA repair capacity and malignancies (12). RAD51 interacts with BRCA1 and BRCA2, acting through HR and NHEJ. For example, down-regulation or mutation of DNA DSB repair proteins involved in the NHEJ pathway was shown to be associated with both BC risk and increased chromosomal radiosensitivity (CRS) (13–15). In addition, RAD51 overexpression is acknowledged to be associated with therapeutic antagonism, aggressiveness, metastatic behavior, and poor prognosis.

X-ray repair cross complementing group 2(XRCC2)gene, located in 7q36.1, is an essential part of the homologous recombination repair pathway and a functional candidate for involvement in cancer progression. Its XRCC2 protein product, together with other proteins encoded by the XRCC2 gene such as RAD51L3, forms a complex that plays a critical role in chromosome segregation and the apoptotic response to DSBs (16, 17). As a member of the RAD51 family of proteins, it is widely acknowledged to mediate HRR (18). However, the exact function of SNPs in the XRCC2 gene in response to different DNA-damaging agents still remains unclear. There is a G-to-A polymorphism located in exon 3 of the XRCC2 gene resulting in a substitution of histidine (His) for arginine (Arg). Known as

Arg188His (R188H, rs3218536), this polymorphism has been widely investigated to explore its potential impact on cancer susceptibility. Furthermore, DNA damage caused by anticancer drugs and radiation have been documented to require XRCC2 for repair in mammalian cells (19–22). Several pieces of evidence stress that high levels of expression of The X-ray repair cross complementing group 3 (XRCC3), another member of the RAD51 family of proteins, are correlated with radioresistance and cytotoxic resistance in human tumor cell lines, suggesting that XRCC2 could also play a relevant role in the effects of oncotherapy (23–25). XRCC3, as we know, is localized on human chromosomes 14q32.325. A coding SNP (T241M, rs861539) has been reported at the 18,067th nucleotide in exon 7 of the XRCC3 gene, resulting in a substitution of methionine (Met) for threonine(Thr) (25). The XRCC3 protein is involved in the joining of single-strand DNA breaks and the joining of double-strand DNA breaks (26). As a member of the Rad51 DNA repair gene family. It functions in the HRR pathway by repairing double-strand breaks. XRCC3 helps the assembly of the nucleofilament protein and its selection and interaction with the appropriate recombination substrates (12). Likewise, XRCC3 controls HR fidelity and is essential to stabilize heteroduplex DNA in HRR. Furthermore, a mutation in XRCC3 generates severe chromosomal instability. The XRCC2 and XRCC3 genes are necessary for HRR and are required for the formation of RAD51 focus (27, 28). In recent studies, common variants of XRCC2, particularly the encoding SNP of exon 3 (Arg188His), have been identified as potential cancer susceptibility sites, although in this case, the association with breast cancer susceptibility remains unclear. Earlier studies have shown that the XRCC3 Thr241Met polymorphism has long been regarded as a risk factor for many cancers.

We examined whether polymorphisms in these three genes involving homologous recombination with DSB were associated with the risk of breast cancer.

Materials and methods

Search strategy and data extraction

All studies investigating the association between polymorphisms in the RAD51 gene and paralog genes, such as the XRCC2 & XRCC3, and the risk of breast cancer, were identified by comprehensive computer-based searches of the PubMed, Embase, Web of Knowledge, and Cochrane Library databases(the last search update on September 2022). The search was carried out using various combinations of keywords such as ('RAD51 gene' OR 'RAD51 recombinase gene' OR 'XRCC3 polymorphism' OR 'XRCC3 Thr241Met polymorphism' OR 'XRCC2' OR 'XRCC2 Arg188His polymorphism') AND ('polymorphism' OR 'variant' OR 'variants').

Eligibility criteria and selection process

Inclusion criteria

Studies included in our meta-analysis needed to have met the following criteria: 1)published in public, full text only; 2) case-control study; 3) sufficient data (genotype distributions for cases and controls) to calculate an odds ratio (OR) with its 95%CI; 4) studies published in English; 5) genotype distribution of the control population consistent with the Hardy-Weinberg Equilibrium (HWE).

Data extraction

Two authors independently extracted information from all eligible publications according to the inclusion criteria listed above. Disagreement was resolved by evaluating a third reviewer and discussing until a consensus was reached. The following characteristics were collected from each study: first author, year of publication, country, ethnicity, methods in experiments, source of control groups and genotype frequencies in case and control groups, and the value of HWE. Duplicated primary studies were deleted and only one version of duplicated documents was kept.

Data collection

The transcriptome data and clinical information of BC patients were obtained from The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov/). In total, 903 patients with BC were selected from the TCGA cohort. For the transcriptome data from TCGA-BRCA, we download their series files. Some important clinical characteristics including age, pathologic stage (I, II, III, IV, V, and NA), and pathology stage (T, N, M) are available. The datasets listed in Table 6 are used to discover and verify prognostic factors of BC patients. We assessed the association of each gene with overall survival by univariate and multivariable Cox proportional hazard regression analysis. All statistical tests were two-sided. The Cox proportional hazard model, including several important factors, was employed to estimate the hazard ratio (HR) and 95% CI for each gene for breast cancer survival. We use normalized P values of <0.05 to define statistical significance. This part of statistical tests was performed using the R software.

Statistical analysis

We first analyze HWE in the controls for each study using a goodness-of-fit test (chi-square or Fisher's exact test) and the departure of HWE genotype frequency among control subjects was determined by P <0.05. Crude odds ratios (OR) with 95% confidence intervals (CI) were used to assess the strength of the association between the RAD51 gene and its paralog polymorphisms and breast cancer susceptibility. The pooled ORs for the RAD51 G172T polymorphism were performed under the dominant model (GG vs. TT+GT), recessive model (TT vs. GG+GT), homozygote model (TT vs. GG), and allelic genetic model (T vs. G). T and G represent the minor and the major alleles, respectively. The same methods were applied to the analysis of other polymorphisms. Stratified analyzes were performed on ethnicity and source of control. A Q-test was performed to assess statistical heterogeneity among studies. The pooled OR was calculated using a fixed effect model if the result of the p-value of the Q test<0.1 indicated significant heterogeneity according to the previous study(Davey and Egger,1997) (29, 30). If the result of the Q test was P>0.1, which indicated that the heterogeneity between studies was not significant. Otherwise, a random-effects model was used. Given the potential heterogeneity

among studies with different ethnicities and sources of control, the random-effects model was adopted (30). Sensitivity analysis was carried out by removing each study at a time to evaluate the stability of the results under either genotypic models or the allelic model. In addition, the Begg test and Egger's linear regression test by visual inspection of the funnel plot were carried out to address the potential publication bias, and $P < 0.05$ was considered an indicator of significant publication bias (30, 31). Cox regression was used to analyze the impact of genes on the prognosis of BC patients and its value in prognostic diagnosis

The Newcastle-Ottawa Scale (NOS) was applied to assess the quality of all studies. The NOS checklist includes three parameters of quality: (i) selected population, (ii) comparability of groups, and (iii) assessment of either the exposure or outcome of interest for case-control studies. The studies scored greater than or equal to 7 were considered to be high quality articles.

Results

Studies included in the meta-analysis

According to our first database search, 272 items were identified (Figure 1). An initial literature search through the PubMed, Embase, Web of Science, and Cochrane database databases yielded 265 published articles after duplicates were removed. When reviewed by titles or abstracts,187 records did not meet the inclusion criteria, leaving 88 potentially relevant studies that were reviewed in full text.

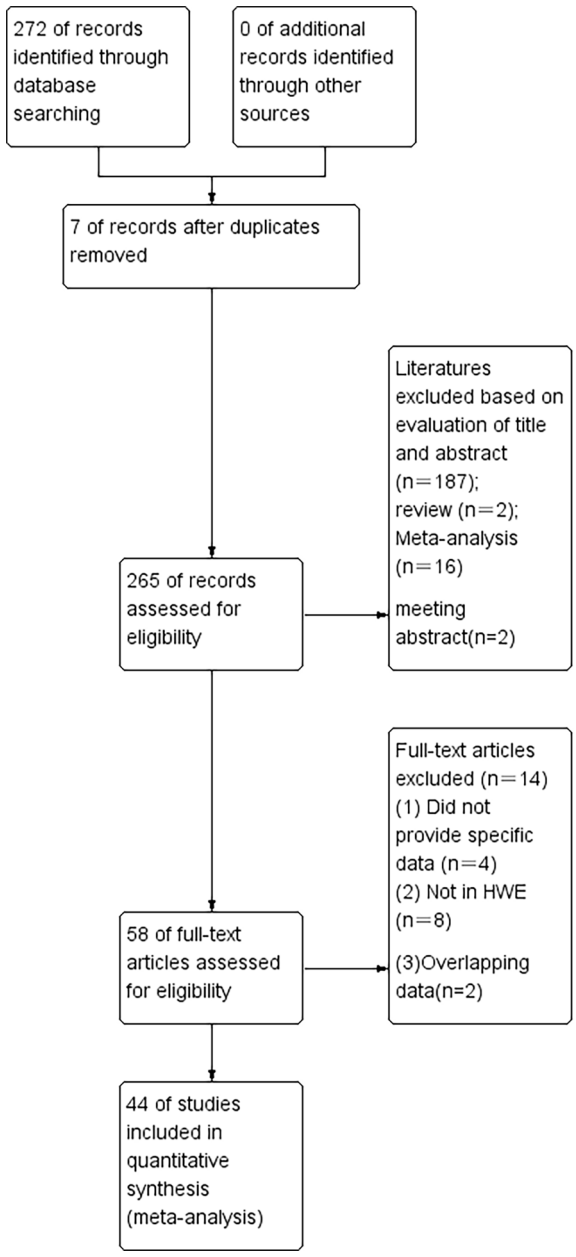

FIGURE 1
The flow diagram of the literature search and the selection of the study.

Among the remaining 88 articles, 2 were reviews, 16 were meta-analyzes and 2 were meeting conferences; these publications were also excluded. Left 58 publications were left, 2 were insufficient data, 4 were overlapping data, and 8 were not in HWE (Tables 1–3). Finally, a total of 44 publications were included in the meta-analysis, among which 9 case-control studies from 9 publications with 4111 cases and 2669 controls for the RAD51 G172T polymorphism, and 20 case-control studies from 11 publications with 20183 cases and 20321 controls for the XRCC2 R188H polymorphism and a total of 47 studies from 38 publications with 26667 cases and 27912 controls for the XRCC3 T241M polymorphism were eventually included in our meta-analysis. We checked the symmetry of the Begg funnel plot and the results of Egger's test to assess publication bias. All statistical analyzes were performed with STATA version 16.0.

Meta-analysis result

Among these 9 case-control studies from 9 publications with 4111 cases and 2669 controls for the RAD51 G172T polymorphism (32–40). The combined results showed that there was no significant correlation between the G172T polymorphism and breast cancer susceptibility in all genetic models except the homozygote model (homozygote model: OR = 1.84, 95% CI = 1.06-3.21, Figure 2; dominant model: OR = 0.97, 95% CI = 0.80–1.18; recessive model: OR = 0.47, 95% CI = 0.22–1.00; allelic genetic model: OR = 1.15, 95% CI = 0.79–1.68). Additionally, ethnic-based analysis showed that SNP was associated with breast cancer risk in Arab populations in homozygous models (OR=3.52, 95% CI=1.13-11.0, *P*= 0.003) (Figure 3). It suggests that the G172T polymorphism may be associated with an increased risk of breast cancer in the Arab population in some cases. When stratified by the source of controls, our results found evidence of an association between cancer risk and the G172T polymorphism in population-based controls in the recessive model (OR=0.25, 95% CI=0.07-0.85, *P*= 0.027), suggesting that it is marginally related to the population-based group.

For the R188H polymorphism XRCC2, 20 case-control studies from 11 articles with 20183 cases and 20321 controls for the XRCC2 R188H polymorphism (41–51). No significant association was observed between this polymorphism and breast cancer susceptibility (homozygote model: OR = 1.13, 95% CI = 0.88–1.46; dominant model: OR = 1.01, 95% CI = 0.92-1.11, recessive model: OR = 0.83, 95% CI = 0.61-1.12; allelic genetic model: OR = 1.05, 95% CI = 0.95-1.17.).

For the polymorphism in XRCC3 Thr241Met, a total of 47 studies of 38 articles with 26667 cases and 27912 controls were eventually included in our meta-analysis (32, 37, 38, 44, 45, 47, 48, 52–80). A significant increase in cancer risk was observed only in the allelic genetic model (homozygote model: OR = 1.08, 95% CI = 0.98–1.20; dominant model: OR = 1.05, 95% CI = 0.99–1.12; recessive model: OR = 0.92, 95% CI = 0.84–1.01; allelic genetic model: OR = 1.05, 95% CI = 1.00–1.11) (Figure 4). In addition, ethnic-based analysis showed that SNP was associated with breast cancer risk in Asian populations in dominant genetic (OR = 1.36,95% CI= 1.11–1.66, *P* = 0.003) and allelic genetic models (OR = 1.32,95% CI 1.07–1.64, *P* = 0.01) (Tables 4, 5).

Prognostic factors

Table 6 depicts the pooled results from the univariable and the multivariable analyses of OS in BC patients (HR). Univariate and multivariate Cox regression analysis was performed to determine whether gene expression is an independent prognostic model of OS in breast cancer patients. As shown in Figure 5, the p values of T, N, M, Stage, and Age were less than 0.05. The results of the univariate Cox regression analysis of OS showed that pathology stage, age, and stage could effectively predict survival in BC patients. Then, we took these factors into the multivariate Cox regression analysis. Furthermore, after the multivariate analyses (Figure 6), the results showed that stage (HR =2.15; 95% CI, 1.42–3.26), age (HR = 1.04; 95% CI, 1.02-1.05) remained independent prognostic factors with an adjusted P value < 0.0001.

TABLE 1 Main characteristics of all studies included in the meta-analysis of the RAD51 G172T polymorphism.

Author [Reference]	Year	Source of control	Ethnicity	Method	Case			Control			HWE	NOS score
					GG	GT	TT	GG	GT	TT		
Kuschel B	2002	PB	Caucasian	Taqman	744	1061	430	226	371	139	0.54	6
Lee	2005	HB	Asian	PCR	721	54	9	533	54	4	0.05	6
Silva	2009	HB	Caucasian	TaqMan	94	139	55	168	275	105	0.69	6
Vral	2011	PB	Caucasian	PCR-RFLP	36	34	30	50	81	23	0.29	2
Sassi	2013	HB	Caucasian	PCR-RLFP/ PCR-CTPP	13	152	139	0	59	260	0.07	5
Michalska	2015	HB	Caucasian	PCR-RFLP	17	11	42	20	40	10	0.16	6
Al Zoubi	2015	PB	Arab	Sequencing	22	14	70	17	29	8	0.44	6
Al Zoubi	2017	PB	Caucasian	PCR-RFLP	5	3	14	6	9	1	0.32	6
Al Zoubi	2021	HB	Arab	sequencing	66	83	53	68	87	26	0.83	6

PB: population-based; HB: hospital-based; HWE: Hardy-Weinberg equilibrium (significant at the 0.05 level) ; NOS: The Newcastle-Ottawa Scale, Quality of studies based on NOS star scoring system: 1–2 stars: poor, 3–5 stars: fair and 6–10 stars: good)
For overlapping studies, only the one with the largest sample numbers was included for meta-analysis.

TABLE 2 Main characteristics of all studies included in the meta-analysis of the XRCC2 R188H polymorphism.

Author [Reference]	Year	Source of control	Ethnicity	Method	Case			Control			HWE	NOS score
					GG	GA	AA	GG	GA	AA		
Millikan-1	2002	PB	African Americans	Taqman	744	21	0	653	25	0	0.63	9
Millikan-2	2002	PB	Caucasian	Taqman	1084	176	8	982	145	7	0.52	9
Han	2004	NA	Caucasian (99%)	TaqMan/ABI PRISM	811	134	7	1066	165	6	0.89	8
BCAC HBCCS	2006	HB	Caucasian (German)	PCR-RFLP	222	31	1	161	32	1	0.66	5
BCAC LSHTM	2006	PB	Caucasian	PCR-RFLP	491	91	3	507	84	7	0.11	6
BCAC Madrid	2006	HB	Caucasian (Spanish)	Taqman	695	152	16	698	136	11	0.14	7
BCAC US3-state	2006	PB	Caucasian	Taqman	1662	198	5	1117	1214	11	0.71	7
BCAC SEARCH	2006	PB	Caucasian (98%)	Taqman	3698	638	32	4385	824	37	0.80	6
BCAC Sheffield	2006	PB	Caucasian	Taqman	818	145	10	807	155	6	0.62	7
BCAC PBSC	2006	PB	Caucasian (Polish)	Taqman	1305	234	10	1983	281	16	0.08	7
BCAC USRTS	2006	HB	Mixed	Taqman	587	122	3	882	161	3	0.12	7
Brooks	2008	NA	Mixed	PCR-RFLP	515	83	4	519	78	5	0.28	8
Webb-1	2008	PB	Mixed	ABI PRISM	1251	187	9	675	101	7	0.15	8
Webb-2	2008	PB	Caucasian	ABI PRISM	1113	177	8	562	90	6	0.26	8
Romanowicz-Makowska	2012	NA	Caucasian	PCR-RFLP	182	344	174	172	376	160	0.09	7
Qureshi	2014	PB	Asian	PCR	131	20	5	137	20	1	0.21	6
Ding	2015	HB	Asian	PCR-LDR	166	280	160	184	305	144	0.41	7
Smolarz	2015	HB	Caucasian	PCR-RFLP	12	8	50	18	40	12	0.21	6
Shadrina	2016	PB	Caucasian	Taqman	594	65	0	587	67	2	0.95	6
Rajagopal	2022	PB	Asian	PCR-RFLP	376	106	9	394	95	4	0.51	7

PB: populaion-based: hospital based; NA: not available; HWE: Hardy-Weinberg equilibrium (significant at the 0.05 level); NOS: The Newcastle-Ottawa Scale, Quality of studies based on NOS star scoring system: 1–2 stars: poor, 3–5 stars: fair and 6–10 stars: good)
For overlapping studies, only the one with the largest sample number was included in the meta-analysis.

Sensitive analysis

Given the significant heterogeneity between studies for the polymorphisms, the random-effect model was used to calculate the pooled results if the heterogeneity was significant. Meanwhile, we also performed a sensitivity analysis to assess the effects of each study on the pooled ORs by omission of individual studies. The sensitivity analysis showed that, for each polymorphism, no single study qualitatively changed the pooled ORs, suggesting that the results of this meta-analysis were statistically stable and reliable.

Publication bias diagnostics

We further identify potential publication biases of the literature using the Egger test and funnel plot. In all studies, no funnel plot asymmetry was found. The results of Egger's test for the RAD51 G172T polymorphism did not show any evidence of publication bias. For the homozygote model, the funnel plot p-value was 0.47, and Egger's test p-value was 0.185. In the dominant model, Begg's test results of the R188H *P* value were 0.67, and Egger's test *P* value was 0.319. Begg's test result of the allelic genetic model in XRCC3 T241M $P = 0.65$ and Egger's test result showed $P = 0.52$, suggesting no publication bias. All *P*-values> 0.05, suggesting that there was no publication bias.

Discussion

Screening for some frequent polymorphisms has improved our understanding of the critical roles that inheritance plays in BC susceptibility. To date, associations between genetic variants in HRR genes and BC development have been investigated, but the results remain unexplained to the best of our knowledge. However, new discoveries in drug research aimed at these gene mutations are always innovative. Some experiments suggest that the inhibition of HR will be selective against breast tumor cells. Inhibitors of HR proteins can be used in combination with radiotherapy or

TABLE 3 Main characteristics of all studies included in the meta-analysis of the XRCC3 T241M polymorphism.

Author [Reference]	Year	Source of control	Ethnicity	Method	Case			Control			HWE	NOS score
					TT	TM	MM	TT	TM	MM		
Millikan	2002	PB	African Americans	Taqman	505	578	101	435	555	142	0.09	9
Rafii S	2002	HB	Caucasian	Taqman	201	248	72	341	416	169	0.87	8
Smith a	2003	HB	Caucasian	PCR-RFLP	96	105	51	104	129	35	0.61	7
Smith b	2003	PB	Caucasian	PCR-RFLP	62	74	26	112	141	49	0.68	7
Jacobsen	2003	PB	Caucasian	Taq-Man / PCR-RFLP	163	203	59	160	198	65	0.77	4
Forsti	2004	PB	Caucasian	PCR-RFLP	72	85	15	89	88	25	0.65	4
Han	2004	NA	Caucasian (99%)	TaqMan/ABI PRISM	388	429	135	468	607	170	0.23	8
Figueiredo	2004	HB	Caucasian (99%)	MALDI-TOF MS	139	186	77	146	200	56	0.34	8
Zhang	2005	HB	Asian	PCR-RFLP	33	80	107	29	115	166	0.17	3
Thyagarajan	2006	PB	Caucasian	PCR-RFLP	160	192	27	126	157	40	0.41	8
BCAC HBCCS	2006	HB	Caucasian (German)	Taq-Man & ARMS	95	119	42	77	88	29	0.64	5
BCAC SEARCH	2006	PB	Caucasian (98%)	Taqman	1177	1462	405	1607	1898	549	0.76	6
BCAC Sheffield	2006	PB	Caucasian	Taqman	458	555	168	437	534	195	0.14	7
BCAC USRTS	2006	HB	Mixed	Taqman	281	336	98	402	480	155	0.55	7
Garcia-Closas-1	2006	PB	Caucasian	Taqman	1102	1419	457	973	1213	368	0.75	7
Garcia-Closas-2	2006	PB	Caucasian	Taqman	785	907	282	980	1039	266	0.71	7
Sangrajrang	2007	HB	Asian	Melting curve analysis	437	69	1	384	38	2	0.32	6
Lee	2007	PB	Asian	Single base extension assay	437	51	1	349	29	0	0.74	6
Brooks	2008	NA	Mixed	PCR-RFLP	254	259	98	249	286	76	0.31	8
Webb-1	2008	PB	Mixed	ABI PRISM	591	656	198	307	375	106	0.61	8
Webb-2	2008	PB	Caucasian	ABI PRISM	500	612	184	248	321	91	0.43	8
Loizidou	2008	PB	Mixed	PCR-RFLP	312	560	220	351	600	226	0.29	8
Sobczuk	2009	HB	Caucasian	PCR-RFLP	29	71	50	24	50	32	0.57	5
Sterpone	2010	HB	Caucasian	PCR-RFLP	18	21	4	15	15	4	0.85	6
Santos	2010	HB	Mixed	PCR-RFLP	28	31	6	49	29	7	0.37	6
Jara	2010	PB	Mixed	CSGE	149	91	27	296	182	22	0.52	7
Silva	2010	HB	Caucasian	PCR-RFLP	109	138	42	178	276	94	0.46	6
Vral	2011	HB	Caucasian	PCR-RFLP or SnapShot technique	60	87	23	54	84	30	0.96	2
Gonzalez-Hormazabal	2012	HB	Mixed	Taqman	187	103	32	335	209	23	0.18	7
Romanowicz-Makowska	2012	PB	Caucasian	PCR-RFLP	190	348	162	158	354	960	0.94	7
Ramadan	2014	HB	Mixed	PCR-RFLP	28	57	15	30	37	38	0.49	7
Qureshi	2014	PB	Asian	PCR	74	67	15	101	44	5	>0.05	6
Ding	2015	HB	Asian	PCR-LDR	510	91	5	557	74	2	0.25	7
Su	2015	HB	Asian	PCR-RFLP	1052	141	39	1131	87	14	0.89	7
Smolarz	2015	HB	Caucasian	PCR-RFLP	19	35	16	15	35	20	0.72	6

(Continued)

TABLE 3 Continued

Author [Reference]	Year	Source of control	Ethnicity	Method	Case			Control			HWE	NOS score
					TT	TM	MM	TT	TM	MM		
Lavanya	2015	HB	Asian	PCR-RFLP	42	7	1	40	8	2	>0.05	6
Al Zoubi	2015	HB	Arab	Sequencing	16	26	4	8	18	5	0.33	5
Shadrina	2016	PB	Caucasian	Taqman	285	284	95	294	278	72	0.59	5
Al Zoubi	2017	HB	Caucasian	Sequencing	8	13	2	4	9	2	0.72	5
Kipen	2017	HB	Caucasian	PCR-RFLP	86	68	15	84	94	7	>0.05	5
Devi	2017	PB	Asian	PCR-RFLP	350	100	14	426	99	9	0.25	9
Ozgoz	2017	HB	Mixed	ultiplex-PCR & MALDI-TOF	42	46	14	37	40	23	0.23	6
Howlader	2020	HB	Asian	PCR-RFLP	70	46	5	96	34	3	0.99	6
Rajagopal	2022	PB	Asian	PCR-RFLP	310	158	23	342	134	17	0.39	7

PB: population-based; HB: hospital-based; NA: not available; HWE: Hardy-Weinberg equilibrium (significant at the 0.05 level); NOS: The Newcastle-Ottawa Scale, Quality of studies based on NOS star scoring system: 1–2 stars: poor, 3–5 stars: fair and 6–10 stars: good)
For overlapping studies, only the one with the largest sample numbers was included in meta-analysis.

chemotherapy to sensitize the cells [5]. A more intriguing possibility would be to use anti-HR agents alone, avoiding the toxicity of DNA-damaging agents. Such a strategy has been applied to selectively kill BRCA2-deficient cells using poly-ADP-ribose-polymerase inhibitors (PARP). The first phase III clinical study of PARP inhibitor for adjuvant treatment of early breast cancer, OlympiA study, aims to evaluate the efficacy and safety of olaparib compared with placebo in the adjuvant treatment of early breast cancer with clinically and pathologically high-risk, HER2-negative, BRCA1/2 mutation. And randomized phase II GeparOLA study showed olaparib plus paclitaxel (PO) in early HER2-negative homologous recombination deficiency (HRD) breast cancer. In conclusion, germline BRCA 1/2 status and HRD predict a higher pathological complete response (pCR) rate in the neoadjuvant treatment (81). The molecular

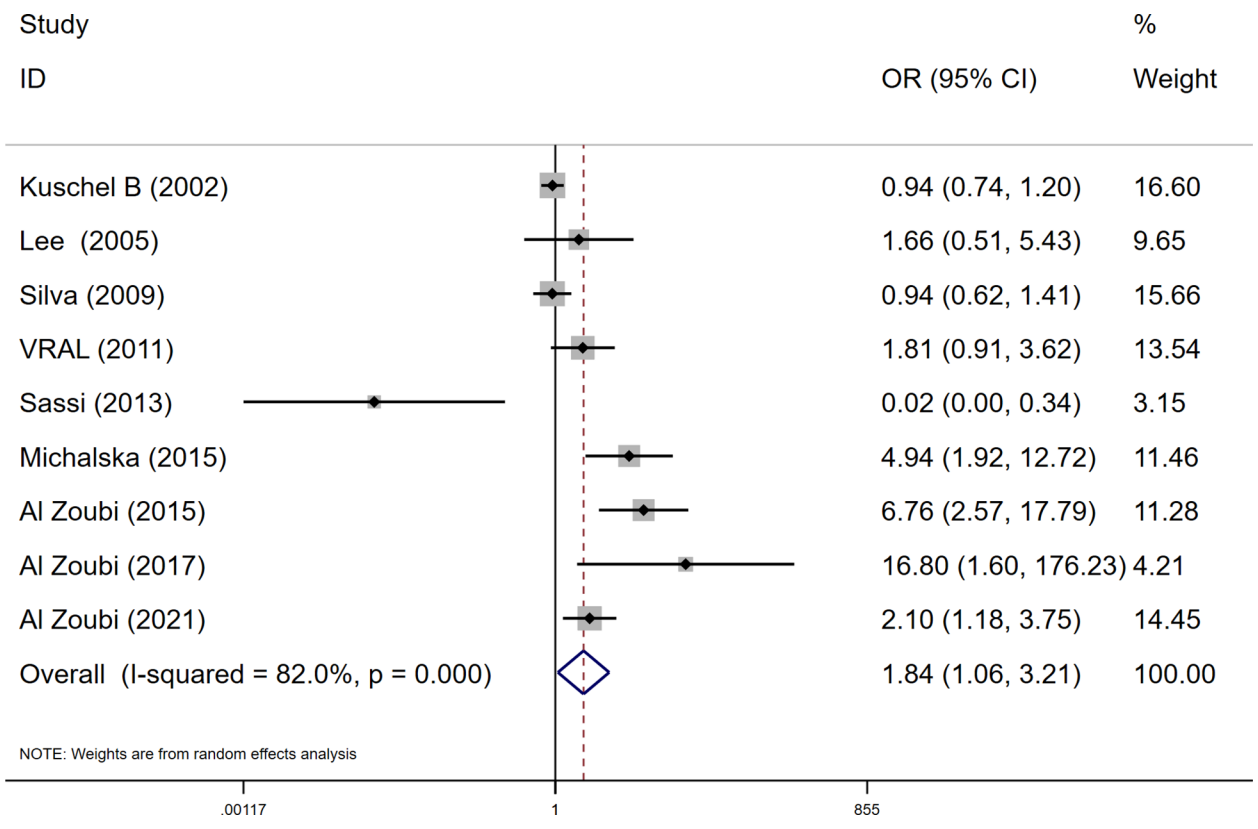

FIGURE 2
Meta-analysis of the RAD51 G172T polymorphism and risk in breast cancer (homozygote model, TT vs. GG).

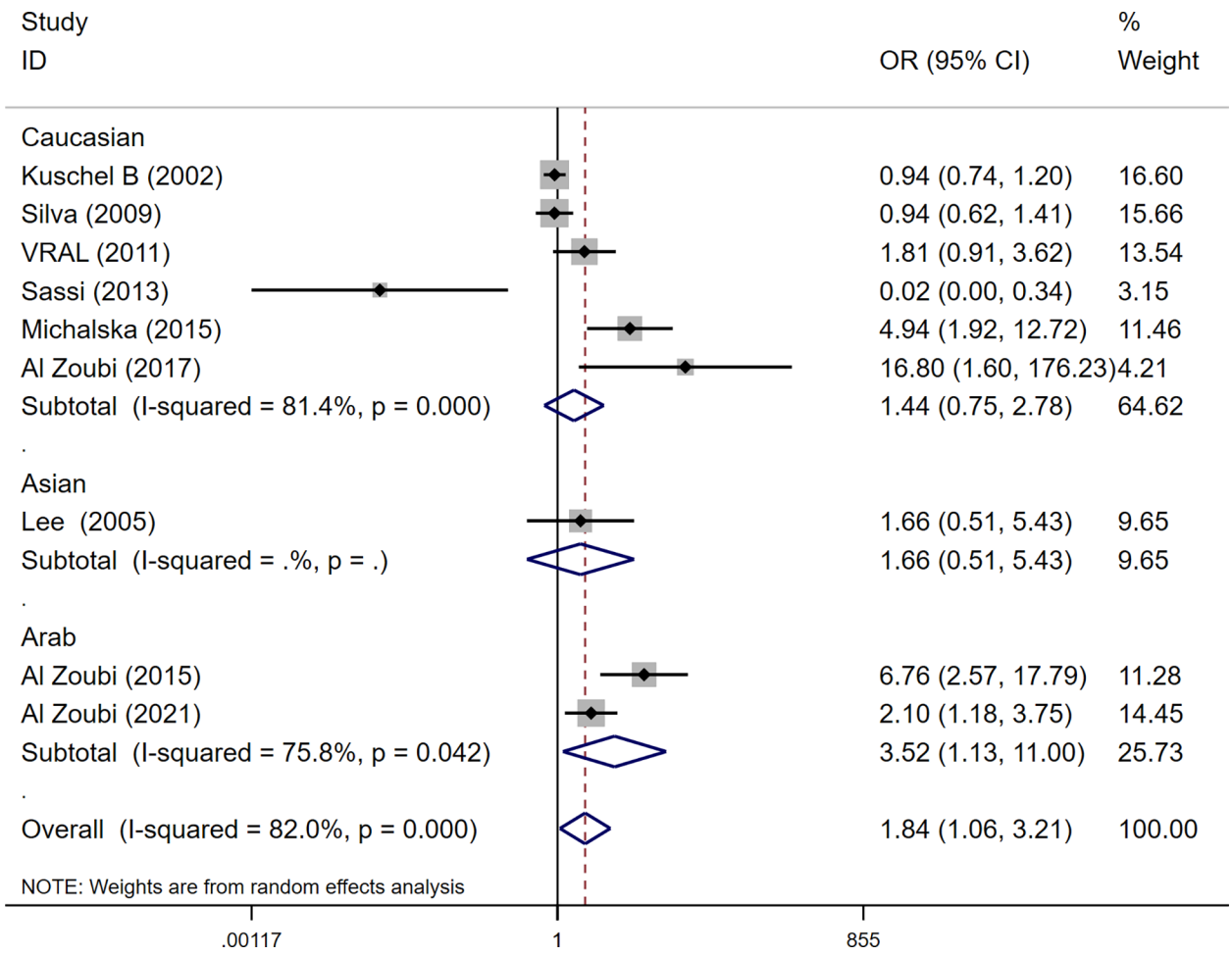

FIGURE 3
Subgroup analysis according to the ethnicity of the RAD51 G172T polymorphism (homozygote model, TT vs. GG).

mechanism of breast cancer is very complex. Therefore, in the post-PARP inhibitor era, there is a great clinical need to find therapeutic targets and analyze prognostic factors to benefit patients, which is conducive to drug development and expansion of new indications and provides the possibility of individualized treatment for breast cancer.

Our analysis demonstrated the importance of recombination repair processes for the fidelity of chromosome segregation and reinforce the functional connection between genes involved in HRR and those that predispose to breast cancer. We also found that patients in our prediction models tended to be older, have an advanced-stage disease, and have a poorer prognosis. Current literature varies widely in experimental methods, stage of disease, family history of cancer, patients with the type of tumor therapy, and the duration since cancer diagnosis, all of which can lead to inconsistent results in case-control studies. Additionally, most of the studies did not specify the immunohistochemical indicators of breast cancer that are relevant to determine which factors can exert a dominating effect. Some research data indicate that double-strand break damage is the most fatal lesion observed in eukaryotic cells because it can cause cell death or create a serious threat to cell viability and genome stability. It has the potential to permanently arrest cell cycle progression and endanger cell survival [10]. Due to the fact that DNA repair mechanisms are crucial to preserving genomic stability and functionality, DNA repair defects can result in the development of chromosomal aberrations that can lead to increased susceptibility to cancer (4, 82, 83). A Japanese study showed that Rad51 gene polymorphisms were found in two patients with bilateral breast cancer (10). It proves that germline mutations in the RAD51 gene may modulate the risk of breast cancer. Previous meta-analyses evaluated the effect of the Rad51 G135C polymorphism on the risk of breast cancer and other cancers. Some experts performed relevant meta-analyses of the analysis and concluded that the Rad51 G172T polymorphism may play a protective role in the development of head and neck cancer, but no significant correlation was found between the Rad51 G172T polymorphism and breast and ovarian cancer (84). It is inconsistent with our conclusion and hypothesized that it was related to inadequate inclusion of the sample size, neglecting gene-gene and gene-environment interactions for some reason. However, there were some approvals on the connection of polymorphism in XRCC2 R188H and the risk of breast cancer before, which has not been confirmed in two population studies in the United States and Poland and several case-control experimental studies (39, 42–44, 50, 51, 68). Moreover, an experiment conducted by RafiiS was hardly replicated in the latest BCAC study (41). Several studies describe a marginally protective effect for rare allele carriers (188His) (64, 85). Interestingly,

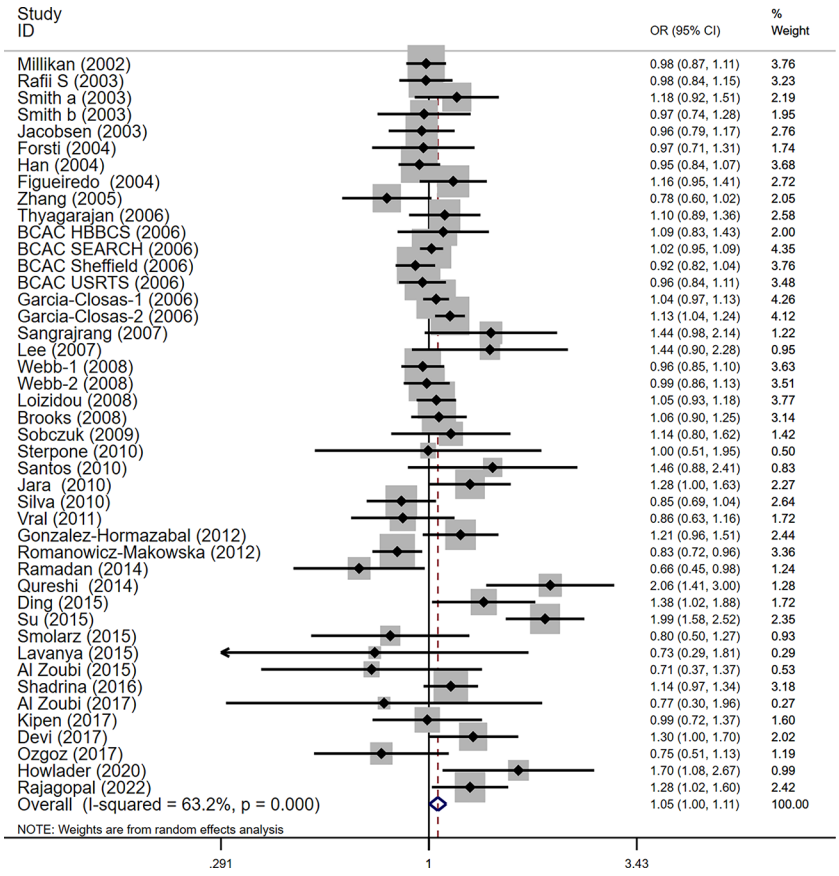

FIGURE 4
Forest plots of the XRCC3 T241M polymorphism and risk of breast cancer (allelic genetic model, M vs. T).

TABLE 4 Meta-analysis of the Rad51 G172T polymorphism on the risk of breast cancer.

Analysis model	Homozygote model	heterogeneity		Dominant model	heterogeneity		Recessive model	heterogeneity		Allelic genetic models	heterogeneity	
	OR(95%CI) *P*	*Ph*	I^2	OR(95% CI) *P*	*Ph*	I^2	OR(95% CI) P	*Ph*	I^2	OR(95% CI)P	*Ph*	I^2
Total	1.84 (1.06,3.21) 0.031*	0.000	82%	0.97 (0.80,1.20) 0.77	0.123	37%	0.47 (0.22,1.00) 0.05	0.000	95.1%	1.15 (0.79,1.68) 0.459	0.000	93.2%
Ethnicity												
Caucasian	1.44 (0.75,2.78) 0.28	0.000	81.4%	0.92 (0.72,1.16) 0.46	0.196	32%	0.60 (0.24,1.49) 0.27	0.000	95.9%	0.97 (0.61,1.53) 0.885	0.000	93.7%
Arab	3.52 (1.13,11.00) 0.03*	0.042	75.%	1.35 (0.94,1.95) 0.11	0.425	0%	0.21 (0.04,1.09) 0.06	0.001	90.7%	2.24 (0.87,5.79) 0.095	0.001	94.1%
Source of control												
PB	2.71 (0.95,7.71) 0.062	0.000	86.5%	1.00 (0.74,1.36) 0.98	0.228	30.8%	0.25 (0.07,0.85) 0.027*	0.000	92.7%	1.80 (0.95,3.41) 0.07	0.000	91.2%
HB	1.39 (0.57,3.35) 0.469	0.000	81.9%	0.95 (0.68,1.32) 0.76	0.076	52.7%	0.72 (0.21,2.46) 0.599	0.000	96.3%	0.85 (0.46,1.56) 0.598	0.000	94.8%

PB: population-based; HB: hospital-based; HWE: Hardy-Weinberg equilibrium (significant at the 0.05 level);
[a]P-values for ORs; Ph values of the Q-test for heterogeneity test; I^2 refers to the proportion of total variation due to between-study heterogeneity;
[b]* mark means the positive results.
[c]Random-effects model was used when the Ph value for the heterogeneity test was <0.05; otherwise, the fixed effects model was used.

TABLE 5 Meta-analysis of the XRCC3 T241M polymorphism on the risk of breast cancer.

Analysis model	Homozygote model	heterogeneity		Dominant model	heterogeneity		Recessive model	heterogeneity		Allelic genetic models	heterogeneity	
	OR (95%CI) *P*	*Ph*	I^2	OR (95% CI) *P*	*Ph*	I^2	OR (95% CI) *P*	*Ph*	I^2	OR (95%CI)*P*	*Ph*	I^2
Total	1.08 (0.98,1.20) 0.125	0.000	54.3%	1.05 (0.99,1.12) 0.09	0.000	50.1%	0.92 (0.84,1.01) 0.09	0.000	55.3%	1.06 (1.00,1.12) 0.04*	0.000	63.2%
Ethnicity												
Caucasian	1.03 (0.94,1.13) 0.578	0.037	36.9%	1.00 (0.96,1/05)0.87	0.577	0.0%	0.96 (0.88,1.05) 0.36	0.04	36.3%	1.01 (0.97,1.05) 0.78	0.145	23.7%
Asian	1.45 (0.83,2.55) 0.193	0.013	58.8%	1.36 (1.11,1.66) 0..003*	0.013	58.6%	0.69 (0.43,1.10) 0.12	0.044	49.7%	1.32 (1.07,1.64) 0.01*	0.000	73.8%
Mixed	1.20 (0.91,1.60) 0.203	0.000	72.7%	1.09 (0.94,1.27) 0.30	0.044	48.0%	0.88 (0.65,1.17) 0.37	0.000	78.2%	1.07 (0.95,1.21) 0.26	0.001	68.8%
Source of control												
PB	1.07 (0.96,1.20) 0.23	0.002	55.9%	1.04 (0.97,1.11) 0.24	0.021	44.7%	0.94 (0.85,1.04) 0.22	0.008	50.3%	1.04 (0.99,1.10) 0.15	0.001	59.8%
HB	1.08 (0.86,1.36) 0.495	0.000	57.5%	1.09 (0.96,1.23) 0.19	0.001	53.4%	0.93 (0.75,1.15) 0.50	0.000	61.6%	1.07 (0.96,1.19) 0.25	0.000	67.7%
NA	1.07 (0.82,1.40) 0.60	0.212	35.9%	0.91 (0.79,1.04) 0.17	0.497	0.0%	0.8 (0.68,1.10) 0.24	0.222	33.0%	0.370	N	N

PB: population-based; HB: hospital-based; HWE: Hardy-Weinberg equilibrium (significant at the 0.05 level); NA: not available
[a]*P*-values for ORs; *Ph* values of the Q-test for heterogeneity test; I^2 refers to the proportion of total variation due to between-study heterogeneity
* refers to P<0.05 and had a statistical significance.

Silva suggested that the potential protective role of the variant allele of XRCC2, in women who have never breastfed, could be related to a more efficient DNA repair activity (37). On the other hand, Han described a protective effect for women with high plasma α-carotene levels. However, current evidence shows that in most studies the XRCC2 R188H polymorphism is considered to have little relationship with the risk of breast cancer. According to our meta-analysis of breast cancer, we did not find a significant association between this polymorphism and breast cancer susceptibility, which is consistent with the previous meta-analysis. In previous studies, a relevant study reported their results with significant unexplained heterogeneity (*Ph* = 0.014) (86). Furthermore, studies that depart from the

TABLE 6 RAD51 univariate Cox regression analyses of OS in BC patients.

Clinicopathologic parameters	OS					
	Univariate Cox analysis			Multivariate Cox analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age	1.03	1.02-1.05	<0.0001****	1.04	1.02-1.05	<0.0001****
M	1.32	1.02-1.7	0.036*	0.88	0.62-1.26	0.495
N	1.68	1.39-2.04	<0.0001****	1.09	0.81-1.46	0.577
RAD51	2.65	0.37-19.01	0.334			
Stage	1.69	1.43-2.01	<0.0001****	2.15	1.42-3.26	<0.0001***
T	1.23	1.07-1.41	0.004**	0.97	0.82-1.15	0.747
XRCC2	0	0-Inf	0.994			

OS: overall survival, HR: Hazard ratio.
Only one mutated sample in XRCC3, but there was no survival information, it was rounded off in the analysis.
P value < 0.05 was considered significant. The asterisk (*) indicates p < 0.05 ; two asterisks (* *) represent p < 0.01, and four asterisks (* * * *) represent p < 0.0001.

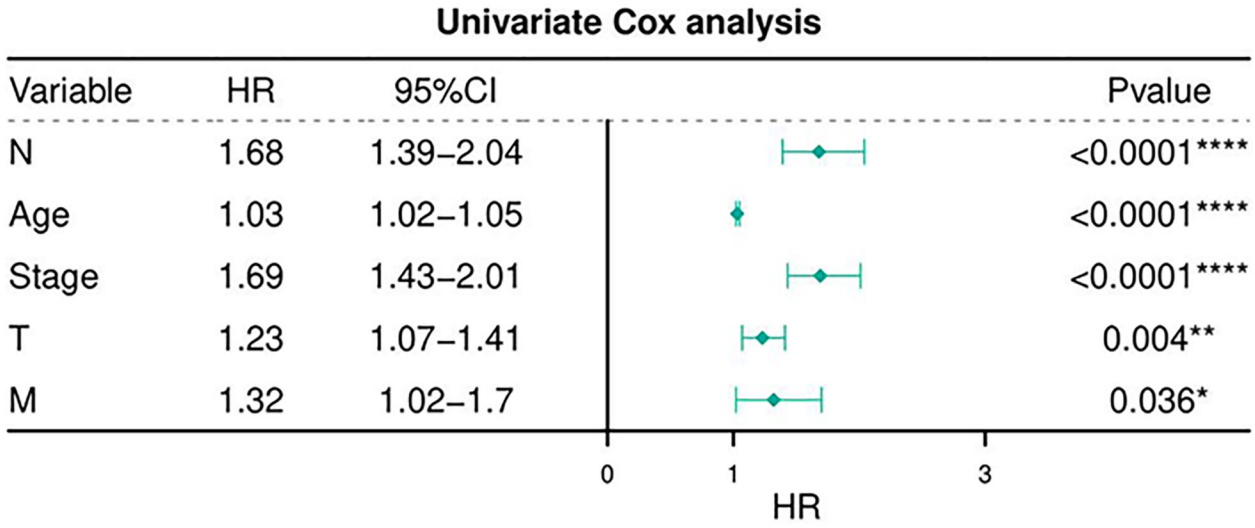

FIGURE 5
Univariate Cox regression analyses of OS in BC patients.

Hardy-Weinberg equilibrium (HWE) were included in the meta-analysis, which may lead to potential bias. Current evidence suggests that XRCC2 R188H polymorphism is considered to have a weak protective effect against breast cancer development in most studies, but the association did not reach statistical significance. As we mentioned above, since this effect is very weak and R188H may serve as a positional marker for other potentially functional SNPs or haplotypes, it is not surprising that this SNP is not associated with breast cancer, or even in an inverse relationship. Therefore, limited by the above factors, the interpretation of the results of previous research should be cautious. A common polymorphism in the XRCC3 gene is at nucleotide 1,8607C/T which results in the substitution of the amino acid threonine for methionine at codon 241 (Thr241Met) of exon 7 of the XRCC3 gene, which may affect the function of the encoding enzyme or/and its interaction with other proteins involved in DNA repair. Inheritance of functional polymorphisms in DNA repair genes may influence the capacity of the DNA repair process, thus leading to increased cancer risk. Due to a C18607T transition at exon 7 of the XRCC3 gene, the substitution of amino acids Thr241Met is functionally active, as it is associated with an increase in the number of micronuclei in human lymphocytes exposed to ionizing radiation (59, 67, 72, 87, 88). The variant allele (241Met) is associated with high levels of DNA adducts in lymphocyte DNA, which could be associated with reduced DNA repair capacity (88). A case-control study in Pakistan found that homozygous (TT) and heterozygous (TM) genotypes of the T241M polymorphism were associated with an increased risk of breast cancer compared to controls (47). Similar results have previously been observed in different studies, suggesting an association between Met allele variants and breast cancer in Caucasian and Asian populations (63, 65). Interestingly, Rajagopal

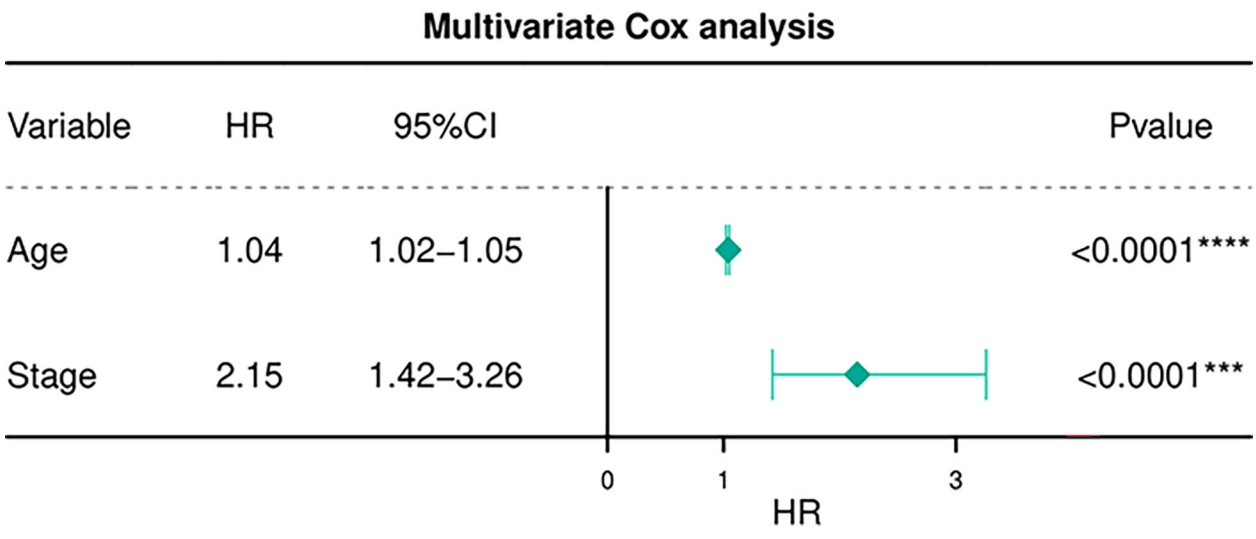

FIGURE 6
Multivariate Cox regression analyses of OS in BC patients.

found that heterozygous genotype (TM) and homozygous mutant genotype (MM) were not significantly associated with breast cancer risk when it comes to the role of the T241M variation in XRCC3 (48). Chai performed a meta-analysis of 23 case-control studies on the association of XRCC3 SNPs with the risk of breast cancer in the above SNPs and the general population and the Asian population in both recessive and homozygous models (89). Our results based on racial stratification analysis are consistent with their observed correlations in Asian populations, but not the same with their associated models. Although they found an association between this SNP and the risk of sporadic breast cancer, based on the conclusive results obtained, we believe that this association is not accurate enough. Although other studies have not shown an association between T241M polymorphism and the risk of breast cancer (52, 54). Therefore, more studies are needed to confirm these associations.

Compared with studies before, our study has some improvements. First, Our study had the advantage of including higher numbers of cases and controls. Second, these polymorphisms in RAD51 and paralog genes were analyzed and associated with the risk of specific cancer, breast cancer. Third, we provided a more comprehensive analysis of the data by calculating four different genetic models and performing a subgroup analysis by ethnicity, and source of controls (population or hospital-based). Finally, we excluded studies in which the distribution of genotypes in the control group was inconsistent with HWE because they might influence the results. The results of this study further revealed the correlation between the polymorphism in these genes and the occurrence and development of breast cancer, providing a direction for the study of molecular mechanisms of cancer in the future.

The main limitations of our meta-analysis are: 1) This meta-analysis only searched published studies in English, ignoring some unpublished studies or studies in other languages that may also meet the inclusion criteria. 2) Some studies did not provide enough clinical data such as patient family history, ER/PR, HER-2 hormone receptor status, tissue type, and tumor grade, leading to failure to conduct a comprehensive subgroup analysis to explore the source of heterogeneity. 3) Gene-gene and gene-environment interactions were not considered in current meta-analyses. Possible gene-gene and gene-environment interactions between Rad51 gene polymorphism and cancer susceptibility need to be further studied. 4) some patients were chosen from hospital-based groups, and these women may have benign breast disease, corresponding to an increased potential risk of breast cancer. 5) Most of the patients in our study were Caucasian, which may limit the general application of our results.

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

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by JY and C-GW. Manuscript drafting and reviewing: All authors. All authors contributed to the article and approved the submitted version.

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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.2023.1047336/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. Mohindra A, Hays LE, Phillips EN, Preston BD, Helleday T, Meuth M., et al. Defects in homologous recombination repair in mismatch-repair-deficient tumour cell lines. *Hum Mol Genet* (2002) 11(18):2189–200. doi: 10.1093/hmg/11.18.2189

3. Dixon K, Kopras E. Genetic alterations and DNA repair in human carcinogenesis. *Semin Cancer Biol* (2004) 14(6):441–8. doi: 10.1016/j.semcancer.2004.06.007

4. Yu Z, Chen J, Ford BN, Glickman BW. Human DNA repair systems: an overview. *Environ Mol Mutagen* (1999) 33(1):3–20. doi: 10.1002/(SICI)1098-2280(1999)33:1<3::AID-EM2>3.0.CO;2-L

5. Mao Z, Jiang Y, Liu X, Seluanov A, Gorbunova V. DNA Repair by homologous recombination, but not by nonhomologous end joining, is elevated in breast cancer cells. *Neoplasia (New York NY).* (2009) 11(7):683–91. doi: 10.1593/neo.09312

6. Kuznetsov SG, Haines DC, Martin BK, Sharan SK. Loss of Rad51c leads to embryonic lethality and modulation of Trp53-dependent tumorigenesis in mice. *Cancer Res* (2009) 69(3):863–72. doi: 10.1158/0008-5472.CAN-08-3057

7. Richardson C. RAD51, genomic stability, and tumorigenesis. *Cancer Lett* (2005) 218 (2):127–39. doi: 10.1016/j.canlet.2004.08.009

8. Khanna KK, Jackson SP. DNA Double-strand breaks: signaling, repair and the cancer connection. *Nat Genet* (2001) 27(3):247–54. doi: 10.1038/85798

9. Kato M, Yano K, Matsuo F, Saito H, Katagiri T, Kurumizaka H, et al. Identification of Rad51 alteration in patients with bilateral breast cancer. *J Hum Genet* (2000) 45 (3):133–7. doi: 10.1007/s100380050199

10. Vispé S, Defais M. Mammalian Rad51 protein: a RecA homologue with pleiotropic functions. *Biochimie* (1997) 79(9-10):587–92. doi: 10.1016/S0300-9084(97)82007-X

11. Suwaki N, Klare K, Tarsounas M. RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis. *Semin Cell Dev Biol* (2011) 22 (8):898–905. doi: 10.1016/j.semcdb.2011.07.019

12. Thacker J. The RAD51 gene family, genetic instability and cancer. *Cancer Lett* (2005) 219(2):125–35. doi: 10.1016/j.canlet.2004.08.018

13. Willems P, Claes K, Baeyens A, Vandersickel V, Werbrouck J, De Ruyck K, et al. Polymorphisms in nonhomologous end-joining genes associated with breast cancer risk and chromosomal radiosensitivity. *Genes Chromosomes Cancer* (2008) 47(2):137–48. doi: 10.1002/gcc.20515

14. Vandersickel V, Depuydt J, Van Bockstaele B, Perletti G, Philippe J, Thierens H, et al. Early increase of radiation-induced γH2AX foci in a human Ku70/80 knockdown cell line characterized by an enhanced radiosensitivity. *J Radiat Res* (2010) 51(6):633–41. doi: 10.1269/jrr.10033

15. Willems P, De Ruyck K, Van Den Broecke R, Makar A, Perletti G, Thierens H, et al. A polymorphism in the promoter region of Ku70/XRCC6, associated with breast cancer risk and oestrogen exposure. *J Cancer Res Clin Oncol* (2009) 135(9):1159–68. doi: 10.1007/s00432-009-0556-x

16. Braybrooke JP, Spink KG, Thacker J, Hickson ID. The RAD51 family member, RAD51L3, is a DNA-stimulated ATPase that forms a complex with XRCC2. *J Biol Chem* (2000) 275(37):29100–6. doi: 10.1074/jbc.M002075200

17. Griffin CS, Simpson PJ, Wilson CR, Thacker J. Mammalian recombination-repair genes XRCC2 and XRCC3 promote correct chromosome segregation. *Nat Cell Biol* (2000) 2(10):757–61. doi: 10.1038/35036399

18. Rajesh C, Gruver AM, Basrur V, Pittman DL. The interaction profile of homologous recombination repair proteins RAD51C, RAD51D and XRCC2 as determined by proteomic analysis. *Proteomics* (2009) 9(16):4071–86. doi: 10.1002/pmic.200800977

19. Tsaryk R, Fabian K, Thacker J, Kaina B. Xrcc2 deficiency sensitizes cells to apoptosis by MNNG and the alkylating anticancer drugs temozolomide, fotemustine and mafosfamide. *Cancer Lett* (2006) 239(2):305–13. doi: 10.1016/j.canlet.2005.08.036

20. Sprong D, Janssen HL, Vens C, Begg AC. Resistance of hypoxic cells to ionizing radiation is influenced by homologous recombination status. *Int J Radiat Oncol Biol Phys* (2006) 64(2):562–72. doi: 10.1016/j.ijrobp.2005.09.031

21. Evans JW, Chernikova SB, Kachnic LA, Banath JP, Sordet O, Delahoussaye YM , et al. Homologous recombination is the principal pathway for the repair of DNA damage induced by tirapazamine in mammalian cells. *Cancer Res* (2008) 68(1):257–65. doi: 10.1158/0008-5472.CAN-06-4497

22. De Silva IU, Mchugh PJ, Clingen PH, Hartley JA. Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. *Mol Cell Biol* (2000) 20(21):7980–90. doi: 10.1128/MCB.20.21.7980-7990.2000

23. Wang ZM, Chen ZP, Xu ZY, Christodoulopoulos G, Bello V, Mohr G, et al. *In vitro* evidence for homologous recombinational repair in resistance to melphalan. *J Natl Cancer Inst* (2001) 93(19):1473–8. doi: 10.1093/jnci/93.19.1473

24. Bello VE, Aloyz RS, Christodoulopoulos G, Panasci LC. Homologous recombinational repair vis-à-vis chlorambucil resistance in chronic lymphocytic leukemia. *Biochem Pharmacol* (2002) 63(9):1585–8. doi: 10.1016/S0006-2952(02)00954-1

25. Yanagisawa T, Urade M, Yamamoto Y, Furuyama J. Increased expression of human DNA repair genes, XRCC1, XRCC3 and RAD51, in radioresistant human KB carcinoma cell line N10. *Oral Oncol* (1998) 34(6):524–8. doi: 10.1016/S1368-8375(98)00045-1

26. Izumi T, Wiederhold LR, Roy G, Roy R, Jaiswal A, Bhakat KK, et al. Mammalian DNA base excision repair proteins: their interactions and role in repair of oxidative DNA damage. *Toxicology* (2003) 193(1-2):43–65. doi: 10.1016/S0300-483X(03)00289-0

27. Bishop DK, Ear U, Bhattacharyya A, Calderone C, Beckett M, Weichselbaum RR, et al. Xrcc3 is required for assembly of Rad51 complexes *in vivo*. *J Biol Chem* (1998) 273 (34):21482–8. doi: 10.1074/jbc.273.34.21482

28. O'regan P, Wilson C, Townsend S, Thacker J. XRCC2 is a nuclear RAD51-like protein required for damage-dependent RAD51 focus formation without the need for ATP binding. *J Biol Chem* (2001) 276(25):22148–53. doi: 10.1074/jbc.M102396200

29. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* (1959) 22(4):719–48.

30. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *Bmj* (1997) 315(7109):629–34. doi: 10.1136/bmj.315.7109.629

31. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* (1994) 50(4):1088–101. doi: 10.2307/2533446

32. Al Zoubi MS, Zavaglia K, Mazanti C, Al Hamad M, Al Batayneh K, Aljabali AAA , et al. Polymorphisms and mutations in GSTP1, RAD51, XRCC1 and XRCC3 genes in breast cancer patients. *Int J Biol Markers* (2017) 32(3):e337–43. doi: 10.5301/ijbm.5000258

33. Al-Zoubi MS, Mazzanti CM, Zavaglia K, Al Hamad M, Armogida I, Lisanti MP, et al. Homozygous T172T and heterozygous G135C variants of homologous recombination repairing protein RAD51 are related to sporadic breast cancer susceptibility. *Biochem Genet* (2016) 54(1):83–94. doi: 10.1007/s10528-015-9703-z

34. Michalska MM, Samulak D, Romanowicz H, Smolarz B. Single nucleotide polymorphisms (SNPs) of RAD51-G172T and XRCC2-41657C/T homologous recombination repair genes and the risk of triple- negative breast cancer in polish women. *Pathol Oncol Res* (2015) 21(4):935–40. doi: 10.1007/s12253-015-9922-y

35. Salim Al Zoubi MS, Al-Eitan LN, Rababa'h DM, Al-Batayneh K, Farzand R, Quinn GA, et al. RAD51-UTR haplotype genetic polymorphisms and susceptibility to breast cancer in women from Jordanian population. *Exp Oncol* (2021) 43(2):149–54. doi: 10.32471/exp-oncology.2312-8852.vol-43-no-2.16338

36. Sassi A, Popielarski M, Synowiec E, Wozniak K. BLM and RAD51 genes polymorphism and susceptibility to breast cancer. *Pathol Oncol Res* (2013) 19(3):451–9. doi: 10.1007/s12253-013-9602-8

37. Silva SN, Tomar M, Paulo C, Gomes BC, Azevedo AP, Teixeira V, et al. Breast cancer risk and common single nucleotide polymorphisms in homologous recombination DNA repair pathway genes XRCC2, XRCC3, NBS1 and RAD51. *Cancer Epidemiol* (2010) 34(1):85–92. doi: 10.1016/j.canep.2009.11.002

38. Vral A, Willems P, Claes K, Poppe B, Perletti G, Thierens H. Combined effect of polymorphisms in Rad51 and Xrcc3 on breast cancer risk and chromosomal radiosensitivity. *Mol Med Rep* (2011) 4(5):901–12. doi: 10.3892/mmr2011523

39. Kuschel B, Auranen A, Mcbride S, Novik KL, Antoniou A, Lipscombe JM, et al. Variants in DNA double-strand break repair genes and breast cancer susceptibility. *Hum Mol Genet* (2002) 11(12):1399–407. doi: 10.1093/hmg/11.12.1399

40. Lee KM, Choi JY, Kang C, Kang CP, Park SK, Cho H, et al. Genetic polymorphisms of selected DNA repair genes, estrogen and progesterone receptor status, and breast cancer risk. *Clin Cancer Res* (2005) 11(12):4620–6. doi: 10.1158/1078-0432.CCR-04-2534

41. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the breast cancer association consortium. *J Natl Cancer Inst* (2006) 98(19):1382–96.

42. Brooks J, Shore RE, Zeleniuch-Jacquotte A, Currie D, Afanasyeva Y, Koenig KL, et al. Polymorphisms in RAD51, XRCC2, and XRCC3 are not related to breast cancer risk. *Cancer Epidemiol Biomarkers Prev* (2008) 17(4):1016–9. doi: 10.1158/1055-9965.EPI-08-0065

43. Garcia-Closas M, Egan KM, Newcomb PA, Brinton LA, Titus-Ernstoff L, Chanock S, et al. Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses. *Hum Genet* (2006) 119(4):376–88. doi: 10.1007/s00439-006-0135-z

44. Han J, Hankinson SE, Ranu H, De Vivo I, Hunter DJ. Polymorphisms in DNA double-strand break repair genes and breast cancer risk in the nurses' health study. *Carcinogenesis* (2004) 25(2):189–95. doi: 10.1093/carcin/bgh00244

45. Millikan RC, Player JS, Decotret AR, Tse CK, Keku T. Polymorphisms in DNA repair genes, medical exposure to ionizing radiation, and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* (2005) 14(10):2326–34. doi: 10.1158/1055-9965.EPI-05-0186

46. Pelttari LM, Kiiski JI, Ranta S, Vilske S, Blomqvist C, Aittomäki K, et al. RAD51, XRCC3, and XRCC2 mutation screening in Finnish breast cancer families. *Springerplus* (2015) 4:92. doi: 10.1186/s40064-015-0880-3

47. Qureshi Z, Mahjabeen I, Baig R, Kayani M. Correlation between selected XRCC2, XRCC3 and RAD51 gene polymorphisms and primary breast cancer in women in Pakistan. *Asian Pac J Cancer Prev* (2014) 15(23):10225–9. doi: 10.7314/apjcp.2014.15.23.10225

48. Rajagopal T, Seshachalam A, Rathnam KK, Talluri S, Venkatabalasubramanian S, Dunna NR, et al. Homologous recombination DNA repair gene RAD51, XRCC2 & XRCC3 polymorphisms and breast cancer risk in south Indian women. *PloS One* (2022) 17(1):e0259761. doi: 10.1371/journal.pone.0259761

49. Ramadan RA, Desouky LM, Elnaggar MA, Moaaz M, Elsherif AM. Association of DNA repair genes XRCC1 (Arg399Gln), (Arg194Trp) and XRCC3 (Thr241Met) polymorphisms with the risk of breast cancer: a case-control study in Egypt. *Genet Test Mol Biomarkers* (2014) 18(11):754–60. doi: 10.1089/gtmb.2014.0191

50. Romanowicz-Makowska H, Smolarz B, Zadrozny M, Westfal B, Baszczynski J, Polac I, et al. Single nucleotide polymorphisms in the homologous recombination repair genes and breast cancer risk in polish women. *Tohoku J Exp Med* (2011) 224(3):201–8. doi: 10.1620/tjem.224.201

51. Webb PM, Hopper JL, Newman B, Chen X, Kelemen L, Giles GG, et al. Double-strand break repair gene polymorphisms and risk of breast or ovarian cancer. *Cancer Epidemiol Biomarkers Prev* (2005) 14(2):319–23. doi: 10.1158/1055-9965.EPI-04-0335

52. Jacobsen NR, Nexø BA, Olsen A, Overvad K, Wallin H, Tjønneland A, et al. No association between the DNA repair gene XRCC3 T241M polymorphism and risk of skin cancer and breast cancer. *Cancer Epidemiol Biomarkers Prev* (2003) 12(6):584–5.

53. Rafii S. A naturally occurring mutation in an ATP-binding domain of the recombination repair gene XRCC3 ablates its function without causing cancer susceptibility. *Hum Mol Genet* (2003) 12(8):915–23. doi: 10.1093/hmg/ddg102

54. Smith TR, Miller MS, Lohman K, Lange EM, Case LD, Mohrenweiser HW, et al. Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer. *Cancer Lett* (2003) 190(2):183–90. doi: 10.1016/S0304-3835(02)00595-5

55. Su CH, Chang WS, Hu PS, Hsiao CL, Ji HX, Liao CH, et al. Contribution of DNA double-strand break repair gene XRCC3 genotypes to triple-negative breast cancer risk. *Cancer Genomics Proteomics* (2015) 12(6):359–67.

56. Al Zoubi MS. X-Ray repair cross-complementing protein 1 and 3 polymorphisms and susceptibility of breast cancer in a Jordanian population. *Saudi Med J* (2015) 36 (10):1163–7. doi: 10.15537/smj.2015.10.12659

57. Costa S, Pinto D, Pereira D, Rodrigues H, Cameselle-Teijeiro J, Medeiros R, et al. DNA Repair polymorphisms might contribute differentially on familial and sporadic breast cancer susceptibility: a study on a Portuguese population. *Breast Cancer Res Treat* (2007) 103(2):209–17. doi: 10.1007/s10549-006-9364-z

58. Devi KR, Ahmed J, Narain K, Mukherjee K, Majumdar G, Chenkual S, et al. DNA Repair mechanism gene, XRCC1A (Arg194Trp) but not XRCC3 (Thr241Met) polymorphism increased the risk of breast cancer in premenopausal females: A case-control study in northeastern region of India. *Technol Cancer Res Treat* (2017) 16 (6):1150–9. doi: 10.1177/1533034617736162

59. Ding P, Yang Y, Cheng L, Zhang X, Cheng L, Li C, et al. The relationship between seven common polymorphisms from five DNA repair genes and the risk for breast cancer in northern Chinese women. *PloS One* (2014) 9(3):e92083. doi: 10.1371/journal.pone.0092083

60. Gonzalez-Hormazabal P, Reyes JM, Blanco R, Bravo T, Carrera I, Peralta O, et al. The BARD1 Cys557Ser variant and risk of familial breast cancer in a south-American population. *Mol Biol Rep* (2012) 39(8):8091–8. doi: 10.1007/s11033-012-1656-2

61. Jara L, Dubois K, Gaete D, de Mayo T, Ratkevicius N, Bravo T, et al. Variants in DNA double-strand break repair genes and risk of familial breast cancer in a south American population. *Breast Cancer Res Treat* (2010) 122(3):813–22. doi: 10.1007/s10549-009-0709-2

62. Krupa R, Synowiec E, Pawlowska E, Morawiec Z, Sobczuk A, Zadrozny M, et al. Polymorphism of the homologous recombination repair genes RAD51 and XRCC3 in breast cancer. *Exp Mol Pathol* (2009) 87(1):32–5. doi: 10.1016/j.yexmp.2009.04.005

63. Lee SA, Lee KM, Park SK, Choi JY, Kim B, Nam J, et al. Genetic polymorphism of XRCC3 Thr241Met and breast cancer risk: case-control study in Korean women and meta-analysis of 12 studies. *Breast Cancer Res Treat* (2007) 103(1):71–6. doi: 10.1007/s10549-006-9348-z

64. Loizidou MA, Michael T, Neuhausen SL, Newbold RF, Marcou Y, Kakouri E, et al. Genetic polymorphisms in the DNA repair genes XRCC1, XRCC2 and XRCC3 and risk of breast cancer in Cyprus. *Breast Cancer Res Treat* (2008) 112(3):575–9. doi: 10.1007/s10549-007-9881-4

65. Romanowicz-Makowska H, Smolarz B, Polac I, Sporny S. Single nucleotide polymorphisms of RAD51 G135C, XRCC2 Arg188His and XRCC3 Thr241Met homologous recombination repair genes and the risk of sporadic endometrial cancer in polish women. *J Obstet Gynaecol Res* (2012) 38(6):918–24. doi: 10.1111/j.1447-0756.2011.01811.x

66. Sangrajrang S, Schmezer P, Burkholder I, Boffetta P, Brennan P, Woelfelschneider A, et al. The XRCC3 Thr241Met polymorphism and breast cancer risk: a case-control study in a Thai population. *Biomarkers* (2007) 12(5):523–32. doi: 10.1080/13547500701395602

67. Shadrina AS, Ermolenko NA, Boyarskikh UA, Sinkina TV, Lazarev AF, Petrova VD , et al. Polymorphisms in DNA repair genes and breast cancer risk in Russian population: a case-control study. *Clin Exp Med* (2016) 16(1):21–8. doi: 10.1007/s10238-014-0329-y

68. Smolarz B, Makowska M, Samulak D, Michalska MM, Mojs E, Wilczak M, et al. Association between single nucleotide polymorphisms (SNPs) of XRCC2 and XRCC3 homologous recombination repair genes and triple-negative breast cancer in polish women. *Clin Exp Med* (2015) 15(2):151–7. doi: 10.1007/s10238-014-0284-7

69. Sterpone S, Cornetta T, Padua L, Mastellone V, Giammarino D, Testa A, et al. DNA Repair capacity and acute radiotherapy adverse effects in Italian breast cancer patients. *Mutat Res* (2010) 684(1-2):43–8. doi: 10.1016/ j.mrfmmm.2009.11.009

70. Thyagarajan B, Anderson KE, Folsom AR, Jacobs DR Jr, Lynch CF, Bargaje A, et al. No association between XRCC1 and XRCC3 gene polymorphisms and breast cancer risk: Iowa women's health study. *Cancer Detect Prev* (2006) 30(4):313–21. doi: 10.1016/j.cdp.2006.07.002

71. Zhang L, Ruan Z, Hong Q, Gong X, Hu Z, Huang Y, et al. Single nucleotide polymorphisms in DNA repair genes and risk of cervical cancer: A case-control study. *Oncol Lett* (2012) 3(2):351–62. doi: 10.3892/ol.2011.463

72. Aka P, Mateuca R, Buchet JP, Thierens H, Kirsch-Volders M. Are genetic polymorphisms in OGG1, XRCC1 and XRCC3 genes predictive for the DNA strand break repair phenotype and genotoxicity in workers exposed to low dose ionising radiations? *Mutat Res* (2004) 556(1-2):169–81. doi: 10.1016/ j.mrfmmm.2004.08.002.

73. Figueiredo JC, Knight JA, Briollais L, Andrulis IL, Ozcelik H. Polymorphisms XRCC1-R399Q and XRCC3-T241M and the risk of breast cancer at the Ontario site of the breast cancer family registry. *Cancer Epidemiol Biomarkers Prev* (2004) 13(4):583–91.

74. Försti A, Angelini S, Festa F, Sanyal S, Zhang Z, Grzybowska E, et al. Single nucleotide polymorphisms in breast cancer. *Oncol Rep* (2004) 11(4):917–22.

75. Santos RA, Teixeira AC, Mayorano MB, Carrara HH, Andrade JM, Takahashi CS, et al. DNA Repair genes XRCC1 and XRCC3 polymorphisms and their relationship with the level of micronuclei in breast cancer patients. *Genet Mol Biol* (2010) 33(4):637–40. doi: 10.1590/S1415-47572010005000082

76. Dashti S, Taherian-Esfahani Z, Keshtkar A, Ghafouri-Fard S. Associations between XRCC3 Thr241Met polymorphisms and breast cancer risk: systematic-review and meta-analysis of 55 case-control studies. *BMC Med Genet* (2019) 20(1):79. doi: 10.1186/s12881-019-0809-8

77. Dufloth RM, Costa S, Schmitt F, Zeferino LC. DNA Repair gene polymorphisms and susceptibility to familial breast cancer in a group of patients from campinas, Brazil. *Genet Mol Res* (2005) 4(4):771–82.

78. Zhang L, Zhang Z, Yan W. Single nucleotide polymorphisms for DNA repair genes in breast cancer patients. *Clin Chim Acta* (2005) 359(1-2):150–5. doi: 10.1016/j.cccn.2005.03.047

79. Howlader NR, Rahman MM, Hossain MA, Sultana R, Hossain SM, Mazid MA, et al. Genetic polymorphisms in DNA repair genes XRCC1 and 3 are associated with increased risk of breast cancer in Bangladeshi population. *Breast Cancer Res Treat* (2020) 182(3):739–50. doi: 10.1007/s10549-020-05738-8

80. Sobczuk A, Romanowicz-Makowska H, Fiks T, Baszczyński J, Smolarz B. XRCC1 and XRCC3 DNA repair gene polymorphisms in breast cancer women from the lodz region of Poland. *Pol J Pathol* (2009) 60(2):76–80.

81. Fasching PA, Link T, Hauke J, Seither F, Jackisch C, Klare P, Seither F, Jackisch C, Klare P, et al. Neoadjuvant paclitaxel/olaparib in comparison to paclitaxel/carboplatinum in patients with HER2-negative breast cancer and homologous recombination deficiency (GeparOLA study). *Ann Oncol Off J Eur Soc Med Oncol* (2021) 32:49–57. doi: 10.1016/j.annonc.2020.10.471

82. Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J Natl Cancer Inst* (2000) 92(11):874–97. doi: 10.1093/jnci/92.11.874

83. Wood RD, Mitchell M, Lindahl T. Human DNA repair genes, 2005. *Mutat Res* (2005) 577(1-2):275–83. doi: 10.1016/j.mrfmmm.2005.03.007

84. Zhao M, Chen P, Dong Y, Zhu X, Zhang X. Relationship between Rad51 G135C and G172T variants and the susceptibility to cancer: a meta-analysis involving 54 case-control studies. *PloS One* (2014) 9(1):e87259. doi: 10.1371/journal.pone.0087259

85. Pooley KA, Baynes C, Driver KE, Tyrer J, Azzato EM, Pharoah PD, et al. Common single-nucleotide polymorphisms in DNA double-strand break repair genes and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* (2008) 17(12):3482–9. doi: 10.1158/1055-9965.EPI-08-0594

86. Yu KD, Chen AX, Qiu LX, Fan L, Yang C, Shao ZM.. XRCC2 Arg188His polymorphism is not directly associated with breast cancer risk: evidence from 37,369 subjects. *Breast Cancer Res Treat* (2010) 123(1):219–25. doi: 10.1007/s10549-010-0753-y

87. Kipen VN, Melnov SB, Smolyakova RM. The role of the XRCC1, XRCC3, and PALB2 genes in the genesis of sporadic breast cancer. *Russian J Genetics: Appl Res* (2017) 7(6):705–11. doi: 10.1134/S2079059717060090

88. Angelini S, Kumar R, Carbone F, Maffei F, Forti GC, Violante FS, et al. Micronuclei in humans induced by exposure to low level of ionizing radiation: influence of polymorphisms in DNA repair genes. *Mutat Res* (2005) 570(1):105–17. doi: 10.1016/j.mrfmmm.2004.10.007

89. Chai F, Liang Y, Chen L, Zhang F, Jiang J. Association between XRCC3 Thr241Met polymorphism and risk of breast cancer: Meta-analysis of 23 case-control studies. *Med Sci Monit* (2015) 21:3231–40. doi: 10.12659/MSM.894637

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Locoregional treatment of *de novo* stage IV breast cancer in the era of modern oncology

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Approximately 6% of metastatic breast cancers arise *de novo*. While systemic therapy (ST) remains the treatment backbone as for patients with metachronous metastases, locoregional treatment (LRT) of the primary tumor remains a controversial method. The removal of the primary has an established role for palliative purposes, but it is unclear if it could also determine a survival benefit. Retrospective evidence and pre-clinical studies seem to support the removal of the primary as an effective approach to improve survival. On the other hand, most randomized evidence suggests avoiding LRT. Both retrospective and prospective studies suffer several limitations, ranging from selection bias and outdated ST to a small sample of patients. In this review we discuss available data and try to identify subgroups of patients which could benefit the most from LRT of the primary, to facilitate clinical practice decisions, and to hypothesize future studies design on this topic.

KEYWORDS

breast cancer, stage IV, primary tumor, locoregional treatment, surgery, radiotherapy

Introduction

Approximately 6% of metastatic breast cancers (BC) arise *de novo* (1). In these patients, systemic therapy (ST), based on hormone receptor (HR) and HER2 expression, is the pillar of treatment as for patients with metachronous metastases. However, the presence of the primary tumor raises questions among clinicians about the potential benefit deriving from a local approach. Palliative removal of the primary is an established procedure as it can relieve BC patients from pain, skin ulceration, bleeding, and infections.

Surgery can also be useful to remove an ST-resistant primary tumor in presence of responsive metastatic disease.

On the other hand, it is unclear if surgery of the primary, with eventual lymph node dissection and consolidative radiotherapy, translates into a survival benefit that could justify such an invasive approach.

Pre-clinical data suggest that locoregional therapy (LRT) could be beneficial by several mechanisms. First of all, tumor burden reduction may increase CD4 and CD8 cells, improving immunologic response to cancer (2, 3). It can also minimize the dissemination of metastatic BC stem cells from the primary tumor which may act as a source of seeding (4, 5). Furthermore, some data suggest that mesenchymal stem cells released from the bone marrow may populate primary tumor more efficiently compared to metastatic sites, enhancing the metastatic potential of primary tumor cells (6).

These biological assumptions were also supported by retrospective studies that showed an association between primary tumor resection and improved survival in patients with synchronous metastases (7–10).

However, in addition to the intrinsic limitation of retrospective evidence, it is important to note that the timing of surgery is rarely specified. Patients which underwent LRT of the primary and are defined metastatic afterward because of post-operative systemic staging could have a better prognosis compared to patients who were diagnosed as metastatic before surgery. The potential influence of this stage migration bias is also outpointed by a retrospective study by Bafford et al. which highlighted a survival benefit only in those patients who underwent surgery of the primary before a diagnosis of metastatic disease (11). Consequently, randomized studies were designed to verify this hypothesis (Table 1).

Evidence from randomized trials

The most recent published study which investigated the impact of primary surgery on survival is the ECOG-ACRIN 2108. A total of 256 patients with metastatic BC who did not progress during 4-8 months of ST were assigned (from February 2011 to July 2015) to LRT of the primary plus ST or ST-only continuation. Overall Survival (OS) was chosen as the primary endpoint. The statistical analysis showed no difference in 3-year OS (68.4% vs 67.9%) (HR, 1.11; 90% CI, 0.82–1.52; p=0.57). No progression-free survival (PFS) difference was observed either; only locoregional progression was reduced in the LRT group (3-year rate: 16.3% v 39.8%; P < 0.001).

Subset analysis based on HR and HER2 status did not show any subgroup which benefited from the locoregional approach (16).

An open-label randomized controlled trial with a similar study design, conducted in Mumbai, compared LRT of the primary plus ST vs ST alone in a population of stage IV BC patients with *de novo* disease.

Patients with unresectable primary underwent chemotherapy before randomization while, in presence of a resectable primary tumor eligible for endocrine therapy, the assignment was conducted upfront. A total of 350 patients were randomized. The primary endpoint was OS as for the previous study. Even in this case, no statistically different median overall survival (mOS) was reported between the two groups: 19.2 months for the LRT group vs 20.5 months in the ST alone group (HR 1.04, 95% CI 0·81-1·34; p=0·79) (13).

However, it is worth noting that the reported mOS values were considerably lower in comparison to the previous trial, in which the mOS was about 55% in both groups (16). This discrepancy can be justified by the lack of tailored therapy in this Indian trial, such as HER2-directed therapy for HER-2 positive patients and endocrine therapy for HR-positive subtypes.

The ABCSG-28 POSYTIVE trial is another phase 3 randomized study with negative results but a different design. The random assignment of *de novo* stage IV BC patients was performed before ST and patients assigned to the intervention arm underwent upfront surgery followed by ST. Only 95 patients were included between 2011 and 2015. The mOS (primary endpoint) in the surgery plus ST arm was consistently lower compared to the ST-only arm (34.6 months vs 54.8 months, HR=0.0691, p=0.267). Whilst cT3 and cN2 tumors were more represented in the surgery arm (22.2% vs 6.7% and 15.6% vs 4.4% respectively), the two groups were balanced in relation to the ST schedule.

Even if the results of this trial seem unequivocal, it must be addressed that this study was stopped early due to poor recruitment, with consequently very low statistical power, and the control arm (ST alone) performed better than expected (54.8 months vs 24 months) (14).

The Turkish Federation's MF07-01 trial is the unique randomized study that showed a survival benefit in favor of LRT.

Similarly to the ABCSG-28 POSYTIVE study, patients were randomized to upfront surgery followed by ST or ST alone. The statistical analysis demonstrated a benefit in OS at the median 40-months follow-up which was confirmed at 10-year follow-up: mOS for the LRT arm and ST-only arm was respectively 46 months and 35 months (HR 0.7, p=0.0003). However, the two groups were unbalanced for the BC subtype, as HR-positive disease was more represented in the LRT (86% vs 73%), and the control arm included more triple negative BC (18% vs 7%) (15, 17).

These data seem to rule out a potential role for LRT of the primary in *de novo* stage IV BC patients given that the majority of randomized studies did not show a survival benefit. However, these trials are not free from inherent limits, are heterogeneous and, last but not least, there are subgroups of patients which deserve in-depth analysis.

Oligometastatic vs polymetastatic disease

The oligometastatic disease is defined by the presence of no more than five metastatic lesions, assessed with high-resolution imaging and safely treatable with metastases-directed therapy (18).

The hypothesis that metastases-directed treatment (MDT) in oligometastatic disease could be beneficial is supported by retrospective and prospective data which showed long-term survival (19). The available randomized data rely only on two studies with conflicting results (20, 21). Waiting for data from numerous ongoing randomized trials, the current practice is to discuss oligometastatic BC patients in a multidisciplinary setting.

The chance to achieve long-term survival in oligometastatic BC patients legitimates an aggressive approach aimed at eradicating the detectable disease, making this subgroup of patients a suitable

TABLE 1 Randomized controlled trials investigating the role of locoregional treatment of the primary tumor in *de novo* stage IV breast cancer patients.

STUDY NAME	NCT	COUNTRY	ACCRUAL PERIOD	NO. OF PATIENTS	HER2-POSITIVE PATIENTS	HR-POSITIVE PATIENTS	RANDOMIZATION	SURGERY TIMING	BCS RATE	RT	MDT	BONE-ONLY MTS	PRIMARY ENDPOINT	RESULTS
ECOG-ACRIN 2108 (12)	NCT01242800	United States	2011-2025	256	LRT arm - 33% ST arm - 32%	LRT arm - 58% ST arm - 61%	After ST	After ST	25%	As per standard of early BC	Permitted	LRT arm - 44% ST arm - 39%	OS	No significant benefit
Tata Memorial (13)	NCT0019377	India	2005-2012	350	LRT arm - 26% ST arm - 35%	LRT arm - 59% ST arm - 60%	After ST*	After ST	23%	As per standard of early BC	Not specified	LRT arm - 29% ST arm - 28%	OS	No significant benefit
ABCSG – 28 POSYTIVE (14)	NCT01015625	Austria	2010-2019	95	LRT arm - 27% ST arm - 18%	LRT arm - 62% ST arm - 67%	Before ST	Before ST	29%	As per standard of early BC	Permitted	LRT arm - 49% ST arm - 36%	OS	No significant benefit
MF07-01 (15)	NCT00557986	Turkey	2008-2012	278	LRT arm - 30% ST arm - 31%	LRT arm – 85% ST arm – 72%	Before ST	Before ST	26%	As per standard of early BC	Permitted	LRT arm - 51% ST arm - 40%	OS	mOS 46 months vs 35 months (HR 0.7, p=0.0003)

HR, hormone receptor; BC, breast cancer; BCS, breast conserving surgery; RT, radiotherapy; MDT, metastases-directed treatment; MTS, metastases; ST, systemic therapy; LRT, locoregional treatment; OS, overall survival; mOS, median overall survival.
*In presence of resectable primary tumor eligible for endocrine therapy the assignment was conducted upfront.

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candidate for the surgery of the primary in case of *de novo* presentation.

Unfortunately, literature data regarding the survival impact of surgical resection of the primary in oligometastatic BC patients is lacking. This is probably due to the main use of LRT of the primary in clinical practice which is palliation.

In the ECOG-ACRIN 2108 trial, no survival difference was reported for oligometastatic patients (HR, 1.18; 95% CI, 0.38 to 3.67) which represented 16.3% of the study population (16).

A Similar result was shown in the Indian randomized trial in which 25% of patients had less than four metastases and were balanced between the intervention and control arm (13).

In the MF07-01 trial, the only randomized trial showing a survival benefit deriving from LRT, there was no clear distinction between oligometastatic and polymetastatic disease but no survival benefit for patients with solitary lung/liver metastases was reported for those treated with LRT, probably due to the poor representation of this subgroup (15).

However, when assessing the impact of local treatment of the primary in oligometastatic BC we cannot ignore if the limited metastases were treated with MDT. The aforementioned randomized trials generally did not specify this information, but it can be noted that MDT was generally permitted in accordance with clinical practice. In the Turkish trial it is only mentioned that irradiation rates and surgical interventions to metastatic sites were similar among the two arms (17).

On the other hand, even if the majority of randomized trials investigating MDT impact in oligometastatic BC does not include patients with uncontrolled primary (22–24), there are some exceptions (25, 26) in which it does not constitute an exclusion criterion if accessible to curative-intent treatment.

If the population of oligometastatic BC patients with synchronous metastases will be properly represented in these trials, we may have some insight into the potential survival benefit deriving from the combination of LRT of the primary and MDT with eradication intent.

Bone-only disease

Metastatic BC patients with bone-only disease have an excellent prognosis compared to those with visceral involvement, showing an mOS that can exceed 5 years after the detection of the metastases (27, 28), thus prompting clinicians to consider the possibility of primary tumor surgery during the therapeutic process.

The BOMET MF 14-01 is a prospective multicenter registry study that evaluated the role of LRT of the primary tumor in addition to ST in *de novo* stage IV BC patients with bone-only metastases. This study included 505 patients and highlighted a prolonged survival in the median 3-year follow-up in favor of LRT of the primary (HR 0.40, p<0.0001). At 34-months median follow-up, 85 (35.4%) patients in the ST-only group and 28 (10.5%) in the LRT group died (29).

The potential survival benefit of LRT is also suggested by retrospective evidence (30–32).

In a large cohort retrospective study including 3956 BC patients with bone metastases, surgery of the primary tumor in addition to ST significantly improved OS with a median survival of 50 months versus 31 months in ST-only patients (p<0.001) (33).

Regarding randomized trials, in the Turkish study, 51% and 40% of patients presented bone-only metastases in the LRT group and ST group respectively. Notably, 23% and 15% of patients had solitary bone metastasis in the LRT and ST groups respectively. At unplanned subgroup analysis patients with solitary bone, metastasis showed a lower risk of death if treated with LRT in addition to ST (15).

Conversely, in the ECOG-ACRIN 2108 trial, which did not demonstrate any benefit of LRT in addition to ST, patients with bone-only disease (37.7%) were less represented (12).

Even if available data are not enough to conclude that LRT of the primary tumor is beneficial among patients with bone-only disease, we can affirm that this population deserves more focus.

The STEREO-OS trial, which is aimed to demonstrate that Stereotactic Body Radiotherapy of the metastases can improve survival in patients with 1 to 3 bone metastases, will also include patients with a primary tumor accessible to curative-intent treatment and might provide some information in this regard.

What locoregional treatment modality should we prefer?

As previously mentioned, the rationale behind LRT of the primary tumor includes the reduction of tumor burden and the removal of cancer stem cells which may propagate the disease (7).

This implies that a complete removal of locoregional disease could be of utmost importance to achieve the best survival benefit, justifying surgery with clear margins and excision of involved axillary nodes.

In a retrospective study conducted by Rapiti et al. showing a survival benefit in metastatic BC patients treated with surgery of the primary, women with positive surgical margins exhibited the same survival as non-surgery ones (32).

The presence of free margins was generally associated with better survival in retrospective studies, while no clear difference was found between mastectomy and breast-conserving surgery (34–36).

Similarly, BC patients with synchronous metastases seem to benefit from axillary dissection in presence of nodal involvement even though evidence on this topic is lacking (32, 34).

As for surgery with clean margins and axillary dissection for patients with nodal metastases, local radiotherapy represents an important method in the pursuit of complete removal of locoregional disease in stage IV BC patients, considering its role in local relapse prevention and mortality reduction in early BC setting (37).

Some retrospective evidence pointed out that the omission of radiotherapy was associated with worse survival (36).

Looking at randomized studies, in the negative study published in 2022 by Khan et al., LRT consisted of surgery and radiotherapy as per standard of early-stage BC. Radiotherapy use followed NCCN guidelines and axillary dissection was reserved for patients with involved lymph nodes.

Among 107 patients which underwent surgery, 75 (70.1%) received mastectomy and 32 (29.9%) breast-conserving surgery. Radiotherapy has been employed in 44 patients (58.7%) after mastectomy and in 27 patients (84.4%) after breast-conserving surgery.

Notably, of 125 patients randomly assigned to the LRT arm, 18 (14.4%) did not receive it for various reasons, ranging from physician advice to progressive disease.

Furthermore, of 131 patients assigned to the ST-only arm, 22 (16.8%) received surgery, which was permitted for palliation, with postoperative radiotherapy in 10 cases (12). This displacement raises concerns about the negative result of the trial. LRT for primary tumor consisted of mastectomy or breast-conserving surgery with eventual postoperative radiotherapy also in the other three randomized trials.

In the Turkish trial 102 patients (74%) underwent a mastectomy, 36 (26%) breast-conserving surgery and the majority of patients received axillary dissection (92.8%) (15).

Given that also the timing of LRT could influence the outcomes, it is worth noting that in the ECOG-ACRIN 2108 trial surgery was carried out after a period of ST, while in the Turkish and ABCSG-28 POSYTIVE trials it was performed upfront.

Thus, considering the OS benefit reported in the Turkish trial (17), it might be thought that upfront surgery could provide some advantage over delayed one. In addition, this hypothesis is in accordance with previously reported biological assumptions. An upfront LRT could be convenient as it can stop the dissemination of metastatic BC stem cells from the primary earlier in the disease course (4, 5).

Surgery was performed upfront also in the ABCSG-28 POSYTIVE trial and no survival benefit for LRT was reported. However, it must be considered that this study was underpowered as it was stopped early due to poor recruitment (14).

Biological subtypes

It is well known that, between BC subtypes, HR-positive tumors feature the best prognosis (38). As HR-positive disease tends to progress with more indolence, it is not uncommon to consider primary surgery in *de novo* stage IV patients in clinical practice.

In confirmation of this trend, HR-positive *de novo* stage IV BC patients demonstrated to benefit the most from LRT in retrospective studies (11, 39–41).

Some retrospective evidence seems also to support the use of LRT in HER2-positive subtype (7, 40, 42, 43).

In the randomized trial by Soran et al., 86% of patients were HR-positive, 30% HER2-positive, and 7% triple negative in the LRT arm, while in the ST-only arm, 73% were HR-positive, 28% HER-2 positive, and 18% triple negative.

The imbalance in biological subtypes distribution, with aggressive ones being more represented in the ST-only arm, questions the positive result of this trial.

However, in accordance with retrospective evidence, an unplanned subgroup analysis showed a benefit in OS for HR-positive patients (15).

The exploratory *post hoc* subgroup analyses of the ECOG-ACRIN 2108 trial, which was well balanced for disease subtype distribution, reported similar results across all the subgroups except for disease subtype: LRT was clearly unfavorable for triple negative patients (HR 3.33) (12).

Based on this data, HR-positive BC seems to be the best candidate for LRT in presence of synchronous metastases, while for triple-negative tumors primary surgery could be even detrimental. Any opinion on HER2-positive patients must be weighed with caution as HER2-directed therapy was not used with the same frequency in these studies.

In addition, we should consider that the usual classification of BC subtypes is being revolutionized due to the introduction of HER2-low subtype, which is forcing us to reconsider the treatment approach in every setting of BC (44).

Modern therapy implications

ST for metastatic BC patients has dramatically evolved over the last twenty years for every disease subtype.

The recent introduction of cyclin-dependent kinase 4/6 inhibitors for metastatic HR-positive BC treatment has carried to PFS and OS improvement, further ameliorating the prognosis of this indolent subgroup (45).

Even if HER2 expression results in a more aggressive disease with a poor prognosis, the use of HER2-targeted therapy led to outstanding survival benefit in these patients. In particular, the combination of trastuzumab, pertuzumab, and docetaxel increased the number of HER2-positive long survivors with an 8-year survival rate of 37% for patients treated with dual HER-2 blockade therapy (46).

The breakthrough of antibody-drug conjugates is the best example of modern ST progress. Trastuzumab deruxtecan is changing the treatment paradigm of both HER2-positive (47, 48) and HER2-low (48) disease and sacituzumab govitecan are improving triple negative and HR-positive BC survival (49).

However, most retrospective and prospective studies investigating the role of LRT of the primary tumor in stage IV BC included patients treated with outdated ST.

The example of the open-label randomized trial conducted at Tata Memorial Hospital in India is explicative. In this study, anti-HER2 therapy was omitted in approximately 92% of HER2-positive patients (13).

On one hand, LRT of the primary tumor and modern ST seem the perfect partners for an aggressive approach aimed at eradicating the disease and reaching long-term survival. On the other hand, the development of highly effective systemic drugs may mitigate the benefits of primary tumor surgery, thus making it useless for survival benefit improvement. It is also possible that both hypotheses are true, but for different groups of patients.

The association of LRT and ST could also have a synergistic effect. Immune checkpoint inhibitors, which boost the immune response against cancer cells by targeting either programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1), are establishing themselves in triple-negative disease, becoming the first line of therapy in association with chemotherapy in case PD-L1 positive disease (50, 51).

Pre-clinical data suggest that tumor promotes metastasis by systemic inflammation and cytotoxic CD8+ T cell effector function suppression (52). At the same time surgery of the primary tumor led to the rebound of antibody and cell-mediated response, restoring immunocompetence and increasing CD4 and CD8 cells in mice with metastatic BC (2, 3). Consequently, the combination of immune checkpoint inhibitors and LRT of the primary tumor could determine a strong immune response with enhanced tumor response.

Final considerations

Retrospective studies seem consistent in supporting LRT for *de novo* stage IV BC patients (Table 2). However, retrospective data suffer from several limitations. The selection bias is one of the most relevant as patients who were candidates for LRT was younger, had better access to care, and a lower burden of disease (53). In addition, it is also plausible that these patients underwent more aggressive ST, thus unbalancing survival outcomes (54).

Even though some preclinical data provide a rationale for LRT of the primary there are also concerns about the possibility that surgery may lead to cancer cells shedding into the circulation (55), a hypothesis that seems consistent with the increased incidence of distant metastases in patients which underwent LRT, highlighted in the randomized trial by Badwe et al. (13).

Randomized trials did not support LRT of the primary altogether, as confirmed by a metanalysis by Reinhorm et al. (56), but, as previously discussed, they suffer major limitations as well, ranging from outdated ST to a small sample of patients.

We must take these results with caution, and we must not label LRT of the primary as a pointless technique, also considering that advances in ST and radiotherapy/surgery methodic require continuous testing of the possible benefit deriving from LRT in stage IV BC.

We should identify the best candidate for LRT and design randomized trials accordingly. Based on the retrospective evidence and the randomized Turkish trial, oligometastatic patients, with bone-only disease and HR-positive disease could be the best candidates for studies investigating the role of LRT in stage IV BC. Regarding oligometastatic patients, the combination of LRT of the primary and metastases-directed therapy, aimed at complete eradication of detectable disease, should be investigated. This aggressive approach in combination with highly effective modern ST could provide long-term survival and, in some cases, even the cure for metastatic BC patients (Figure 1).

The best timing for LRT of the primary remains an issue. Upfront surgery might be the correct approach according to the potential role of metastatic BC stem cells dissemination from the primary and the significant OS benefit observed in the randomized trial by Soran et al., in which metastatic BC patients underwent upfront surgery (4, 5, 15).

On the other hand, upfront surgery could represent an overtreatment for those patients destined to progress early in the disease course.

In this context, biomarkers, such as circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), could help us characterize the metastatic disease.

In a retrospective analysis including 2436 patients with stage IV BC, a CTCs threshold of 5 cells per 7.5 ml was able to differentiate aggressive from indolent metastatic disease (57). ctDNA percentages were correlated with prognosis as well, with high levels being associated with shorter OS (58, 59).

Metastatic BC patients with high CTCs or ctDNA levels could be at higher risk of fast disease progression and, consequently, the rationale behind LRT of the primary tumor in those patients might be invalidated. Thus, the implementation of these biomarkers for patients' stratification in future studies is suitable.

Results of two randomized trials investigating the role of LRT in *de novo* stage IV BC are awaited (60, 61). In addition, a single-arm

TABLE 2 Main Retrospective studies and outcome in locoregional treatment of the primary tumor in the novo stage IV breast cancer patients.

STUDY NAME	ACCRUAL PERIOD	NO. OF PATIENTS	LRT Vs No LRT	BONE-ONLY MTS	PRIMARY ENDPOINT	RESULTS
SEER (2010-2016) (33)	2010-2016	3956	Surgery Group arm – 82% Not Surgery Group– 18%	All	OS	mOS 50 months in Surgery group VS 31 months in Non-Surgery Group (p <0.001)
Geneva Cancer Registry (32)	1977-1996	300	NA	145	OS	HR=0.6, P= 0.046 (Surgery of Primary Tumor and negative margins)
French Epidemiological Strategy and Medical Economics MBC database	2008-2014	4276	LRT arm – 77.2%	2556 (40%)	OS	HR 0.65 (0.55-0.76) p 0.0001
BOMET MF 14-01 (29)	NA	505	LRT 52.5% No LRT 47.5%	All	OS	HR 0.40 (0.30-0.54) p <0.0001
SEER database (10)	1988-2011	29916	Surgery Group- 51% Not Surgery Group - 49%	NA	OS	mOS 34 months vs 18 months (HR 0.7, p=0.0003)
SEER database (8)	1988-2003	9734	Surgery Group 47% Not Surgery Group 53%	NA	OS	mOS 36 months vs 21 months (p <0.001)
Blanchard KD et al (42)	NA	395	LRT 61.3% No LRT 38.7%	NA	OS	OS 27.1 vs 16.8 (p <0.0001)

LRT, locoregional treatment; MTS, metastases; OS, overall survival; mOS, median overall survival; NA, not applicable; HR, hazard ratio.

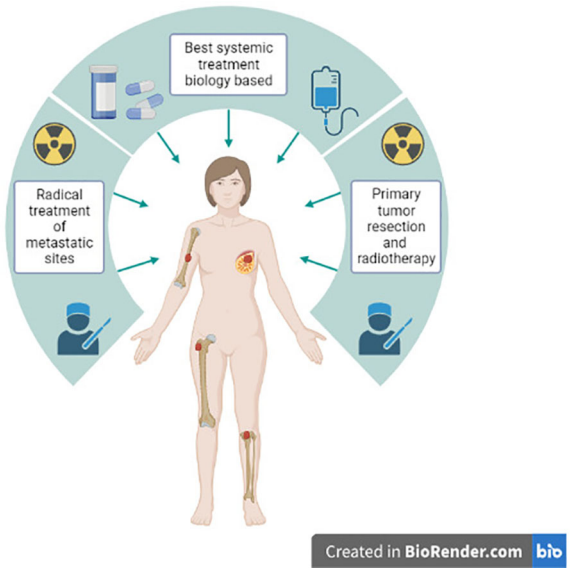

FIGURE 1
Aggressive approach combining modern systemic therapy, locoregional treatment of the primary tumor and metastases-directed therapy in stage IV *de novo* oligometastatic breast cancer patients.

trial investigating the role of palbociclib and LRT combination in HR-positive/HER2-negative metastatic BC is still recruiting (62).

Conclusions

The purpose of our review is to underline the limitations and strengths of LRT of the primary tumor, to design future randomized trials, more precisely and accurately. The design of new randomized clinical trials should include modern ST, a properly selected population, and new biomarkers are strongly encouraged.

Meanwhile, in the absence of robust evidence, LRT of the primary tumor should be discussed in a multidisciplinary context for every patient with *de novo* stage IV BC

Author contributions

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

Conflict of interest

UD received honoraria for advisory boards or speaker fees for Pfizer, BMS, MSD, PharmaMar, Astellas, Bayer, Ipsen, Roche, Novartis, Clovis, GSK, AstraZeneca, Institutional research grants from AstraZeneca, Sanofi and Roche. MP has received advisory board fees from Novartis.

All other authors confirm that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. O'Shaughnessy J. Extending survival with chemotherapy in metastatic breast cancer. *Oncologist* (2005) 10(S3):20–9. doi: 10.1634/theoncologist.10-90003-20

2. Rashid OM, Nagahashi M, Ramachandran S, Graham L, Yamada A, Spiegel S, et al. Resection of the primary tumor improves survival in metastatic breast cancer by reducing overall tumor burden. *Surg (United States)* (2013) 153(6):771–8. doi: 10.1016/j.surg.2013.02.002

3. 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

4. Liu H, Patel MR, Prescher JA, Patsialou A, Qian D, Lin J, et al. Cancer stem cells from human breast tumors are involved in spontaneous metastases in orthotopic mouse models. *Proc Natl Acad Sci USA* (2010) 107(42):18115–20. doi: 10.1073/pnas.1006732107

5. Norton L, Massagué J. Is cancer a disease of self-seeding? *Nat Med* (2006) 12 (8):875–8. doi: 10.1038/nm0806-875

6. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* (2007) 449(7162):557–63:7162. doi: 10.1038/nature06188

7. Gera R, Chehade HELH, Wazir U, Tayeh S, Kasem A, Mokbel K. Locoregional therapy of the primary tumour in *de novo* stage IV breast cancer in 216 066 patients: A meta-analysis. *Sci Rep* (2020) 10(1):1–11. doi: 10.1038/s41598-020-59908-1

8. Gnerlich J, Jeffe DB, Deshpande AD, Beers C, Zander C, Margenthaler JA. Surgical removal of the primary tumor increases overall survival in patients with metastatic breast cancer: analysis of the 1988-2003 SEER data. *Ann Surg Oncol* (2007) 14(8):2187–94. doi: 10.1245/s10434-007-9438-0

9. Lang JE, Tereffe W, Mitchell MP, Rao R, Feng L, Meric-Bernstam F, et al. Primary tumor extirpation in breast cancer patients who present with stage IV disease is associated with improved survival. *Ann Surg Oncol* (2013) 20(6):1893. doi: 10.1245/s10434-012-2844-y

10. Vohra NA, Brinkley J, Kachare S, Muzaffar M. Primary tumor resection in metastatic breast cancer: A propensity-matched analysis, 1988-2011 SEER data base. *Breast J* (2018) 24(4):549–54. doi: 10.1111/tbj.13005

11. Bafford AC, Burstein HJ, Barkley CR, Smith BL, Lipsitz S, Iglehart JD, et al. Breast surgery in stage IV breast cancer: impact of staging and patient selection on overall survival. *Breast Cancer Res Treat* (2008) 115(1):7–12. doi: 10.1007/s10549-008-0101-7

12. Khan SA, Zhao F, Solin LJ, Goldstein LJ, Cella D, Basik M, et al. A randomized phase III trial of systemic therapy plus early local therapy versus systemic therapy alone in women with de novo stage IV breast cancer: A trial of the ECOG-ACRIN research group (E2108). *J Clin Oncol* (2020) 38(18_suppl). doi: 10.1200/JCO.2020.38.18_suppl.LBA2

13. 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

14. 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

15. 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):742–751.e5. doi: 10.1016/j.jamcollsurg.2021.08.686

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. 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

18. Lievens Y, Guckenberger M, Gomez D, Hoyer M, Iyengar P, Kindts I, et al. Defining oligometastatic disease from a radiation oncology perspective: An ESTRO-ASTRO consensus document. *Radiother Oncol* (2020) 148:157–66. doi: 10.1016/j.radonc.2020.04.003

19. Makhlin I, Fox K. Oligometastatic breast cancer: Is this a curable entity? a contemporary review of the literature. vol. 22. *Curr Oncol Rep* (2020) 22(2):15. doi: 10.1007/s11912-020-0867-2

20. Palma DA, Olson R, Harrow S, Gaede S, v. LA, Haasbeek C, et al. Stereotactic ablative radiotherapy for the comprehensive treatment of oligometastatic cancers: Long-term results of the SABR-COMET phase II randomized trial. *J Clin Oncol* (2020) 38 (25):2830–8. doi: 10.1200/JCO.20.00818

21. Chmura SJ, Winter KA, Al-Hallaq HA, Borges VF, Jaskowiak NT, Matuszak M, et al. NRG-BR002: A phase IIR/III trial of standard of care therapy with or without stereotactic body radiotherapy (SBRT) and/or surgical ablation for newly oligometastatic breast cancer (NCT02364557). *J Clin Oncol* (2019) 37(15_suppl):TPS1117–TPS1117. doi: 10.1200/JCO20193715_supplTPS1117

22. Stereotactic ablative radiotherapy for comprehensive treatment of oligometastatic (1-3 metastases).

23. Stereotactic ablative radiotherapy for comprehensive treatment of 4-10 oligometastatic tumors.

24. Trial of superiority of stereotactic body radiation therapy in patients with breast cancer.

25. Thureau S, Marchesi V, Vieillard MH, Perrier L, Lisbona A, Leheurteur M, et al. Efficacy of extracranial stereotactic body radiation therapy (SBRT) added to standard treatment in patients with solid tumors (breast, prostate and non-small cell lung cancer) with up to 3 bone-only metastases: study protocol for a randomised phase III trial (STEREO-OS). *BMC Cancer* (2021) 21(1):117. doi: 10.1186/s12885-021-07828-2

26. Krug D, Vonthein R, Illen A, Olbrich D, Barkhausen J, Richter J, et al. Metastases-directed radiotherapy in addition to standard systemic therapy in patients with oligometastatic breast cancer: Study protocol for a randomized controlled multi-national and multi-center clinical trial (OLIGOMA). *Clin Transl Radiat Oncol* (2021) 28:90–6. doi: 10.1016/j.ctro.2021.03.012

27. Coleman RE, Rubens RD. The clinical course of bone metastases from breast cancer. *Br J Cancer* (1987) 55(1):61–6. doi: 10.1038/bjc.1987.13

28. Zengel B, Kilic M, Tasli F, Simsek C, Karatas M, Ozdemir O, et al. Breast cancer patients with isolated bone metastases and oligometastatic bone disease show different survival outcomes. *Sci Rep* (2021) 11(1):20175. doi: 10.1038/s41598-021-99726-7

29. 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

30. Pons-Tostivint E, Kirova Y, Lusque A, Campone M, Geffrelot J, Mazouni C, et al. Survival impact of locoregional treatment of the primary tumor in *De novo* metastatic breast cancers in a Large multicentric cohort study: A propensity score-matched analysis. *Ann Surg Oncol* (2018) 26(2):356–65:2. doi: 10.1245/s10434-018-6831-9

31. Wang K, Shi Y, Li ZY, Xiao YL, Li J, Zhang X, et al. Metastatic pattern discriminates survival benefit of primary surgery for *de novo* stage IV breast cancer: A real-world observational study. *Eur J Surg Oncol* (2019) 45(8):1364–72. doi: 10.1016/j.ejso.2019.02.013

32. Rapiti E, Verkooijen HM, Vlastos G, Fioretta G, Neyroud-Caspar I, Sappino AP, et al. Complete excision of primary breast tumor improves survival of patients with metastatic breast cancer at diagnosis. *J Clin Oncol* (2006) 24(18):2743–9. doi: 10.1200/JCO.2005.04.2226

33. Huang Z, Zhou X, Tong Y, Zhu L, Zhao R, Huang X. Surgery for primary tumor benefits survival for breast cancer patients with bone metastases: a large cohort retrospective study. *BMC Cancer* (2021) 21(1):222. doi: 10.1186/s12885-021-07964-9

34. Khan SA, Stewart AK, Morrow M. Does aggressive local therapy improve survival in metastatic breast cancer? *Surgery* (2002) 132(4):620–7. doi: 10.1067/msy.2002.127544

35. Kommalapati A, Tella SH, Goyal G, Ganti AK, Krishnamurthy J, Tandra PK. A prognostic scoring model for survival after locoregional therapy in *de novo* stage IV breast cancer. *Breast Cancer Res Treat* (2018) 170(3):677–85. doi: 10.1007/s10549-018-4802-2

36. 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

37. Darby S, McGale P, Correa C, Taylor C, Arriagada R, Clarke M, 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 10 801 women in 17 randomised trials. *Lancet* (2011) 378(9804):1707–16. doi: 10.1016/S0140-6736(11)61629-2

38. McAndrew NP, Finn RS. Clinical review on the management of hormone receptor–positive metastatic breast cancer. *JCO Oncol Pract* (2022) 18(5):319–27. doi: 10.1200/OP.21.00384

39. Tan Y, Li X, Chen H, Hu Y, Jiang M, Fu J, et al. Hormone receptor status may impact the survival benefit of surgery in stage iv breast cancer: a population-based study. *Oncotarget* (2016) 7(43):70991–1000. doi: 10.18632/oncotarget.11235

40. Neuman HB, Morrogh M, Gonen M, van Zee KJ, Morrow M, King TA. Stage IV breast cancer in the era of targeted therapy: does surgery of the primary tumor matter? *Cancer* (2010) 116(5):1226–33. doi: 10.1002/cncr.24873

41. 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. doi: 10.1001/jamasurg.2015.4539

42. Blanchard DK, Shetty PB, Hilsenbeck SG, Elledge RM. Association of surgery with improved survival in stage IV breast cancer patients. *Ann Surg* (2008) 247(5):732–8. doi: 10.1097/SLA.0b013e3181656d32

43. Chen PY, Cheng SHC, Hung CF, Yu BL, Chen CM. Locoregional therapy in luminal-like and HER2-enriched patients with *de novo* stage IV breast cancer. *Springerplus* (2013) 2(1):589. doi: 10.1186/2193-1801-2-589

44. Eiger D, Agostinetto E, Saúde-Conde R, de Azambuja E. The exciting new field of HER2-low breast cancer treatment. *Cancers (Basel)* (2021) 13(5):1–18. doi: 10.3390/cancers13051015

45. Piezzo M, Chiodini P, Riemma M, Cocco S, Caputo R, Cianniello D, et al. Progression-free survival and overall survival of CDK 4/6 inhibitors plus endocrine therapy in metastatic breast cancer: A systematic review and meta-analysis. *Int J Mol Sci* (2020) 21(17):1–17. doi: 10.3390/ijms21176400

46. Swain SM, Miles D, Kim SB, Im YH, Im SA, Semiglazov V, et al. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA): end-of-study results from a double-blind, randomised, placebo-controlled, phase 3 study. *Lancet Oncol* (2020) 21(4):519–30. doi: 10.1016/S1470-2045(19)30863-0

47. Cortés J, Kim SB, Chung WP, Im SA, Park YH, Hegg R, et al. Trastuzumab deruxtecan versus trastuzumab emtansine for breast cancer. *N Engl J Med* (2022) 386 (12):1143–54. doi: 10.1056/NEJMoa2115022

48. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *New Engl J Med* (2022) 387(1):9–20. doi: 10.1056/NEJMoa2203690

49. Bardia A, Hurvitz SA, Tolaney SM, Loirat D, Punie K, Oliveira M, et al. Sacituzumab govitecan in metastatic triple-negative breast cancer. *New Engl J Med* (2021) 384(16):1529–41. doi: 10.1056/NEJMoa2028485

50. Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im SA, Yusof MM, et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet* (2020) 396(10265):1817–28. doi: 10.1016/S0140-6736(20)32531-9

51. Schmid P, Rugo HS, Adams S, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* (2020) 21(1):44–59. doi: 10.1016/S1470-2045(19)30689-8

52. Janssen LME, Ramsay EE, Logsdon CD, Overwijk WW. The immune system in cancer metastasis: friend or foe? *J Immunother Cancer* (2017) 5(1):79. doi: 10.1186/s40425-017-0283-9

53. 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):3384–95:12. doi: 10.1245/s10434-008-0085-x

54. Khan SA. Primary tumor resection in stage IV breast cancer: consistent benefit, or consistent bias? *Ann Surg Oncol* (2007) 14(12):3285–7. doi: 10.1245/s10434-007-9547-9

55. Tohme S, Simmons RL, Tsung A. Surgery for cancer: A trigger for metastases. *Cancer Res* (2017) 77(7):1548–52. doi: 10.1158/0008-5472.CAN-16-1536

56. Reinhorn D, Mutai R, Yerushalmi R, Moore A, Amir E, Goldvaser H. Locoregional therapy in *de novo* metastatic breast cancer: Systemic review and meta-analysis. *Breast* (2021) 58:173–81. doi: 10.1016/j.breast.2021.05.003

57. Cristofanilli M, Pierga JY, Reuben J, Rademaker A, Davis AA, Peeters DJ, et al. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): International expert consensus paper. *Crit Rev Oncol / Hematol* (2019) 134:39–45. doi: 10.1016/j.critrevonc.2018.12.004

58. Sant M, Bernat-Peguera A, Felip E, Margelí M. Role of ctDNA in breast cancer. *Cancers (Basel)* (2022) 14(2):310. doi: 10.3390/cancers14020310

59. Fiste O, Liontos M, Koutsoukos K, Terpos E, Dimopoulos MA, Zagouri F. Circulating tumor DNA-based predictive biomarkers in breast cancer clinical trials: a narrative review. *Ann Transl Med* (2020) 23):1603–3. doi: 10.21037/atm-20-1175

60. Compare the efficacy of surgery combined with systemic therapy and pure systemic therapy in patients with stage IV breast cancer.

61. Shien T, Mizutani T, Tanaka K, Kinoshita T, Hara F, Fujisawa T, et al. A randomized controlled trial comparing primary tumor resection plus systemic therapy with systemic therapy alone in metastatic breast cancer (JCOG1017 PRIM-BC). *J Clin Oncol* (2017) 35(15_suppl):TPS588–8. doi: 10.1200/JCO20173515_supplTPS588

62. PALbociclib in advanced breast cancer: Therapy INtegrating locorEgional treatment and palbociclib in de Novo, treatment naive, stage IV ER+, HER2- breast cancer patients | clinical research trial listing (Radiotherapy | surgery | breast cancer stage IV.

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Analysis of prognostic factors and construction of prognostic models for triple-positive breast cancer

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Objective: By identifying the clinicopathological characteristics and prognostic influences of patients with triple-positive breast cancer (TPBC) at Xijing Hospital in China compared with those in the United States, this study aims to construct a nomogram model to forecast the overall survival rate (OS) of TPBC patients.

Method: The Surveillance, Epidemiology, and End Results (SEER) database was used to screen 5769 patients as the training cohort, and 191 patients from Xijing Hospital were used as the validation cohort. Cox risk-proportional model was applied to select variables and the nomogram model was constructed based on the training cohort. The performance of the model was evaluated by calculating the C-index and generating calibration plots in the training and validation cohorts.

Results: Cox multifactorial analysis showed that age, chemotherapy, radiotherapy, M-stage, T-stage, N-stage, and the mode of surgery were all independent risk factors for the prognosis of TPBC patients (all $P<0.05$). With this premise, the nomogram model was constructed and evaluated. The C-index value of the nomogram model was 0.830 in the training group and 0.914 in the validation group. Moreover, both the calibration and ROC curves for the proposed model exhibited reliable performance, and the clinical decision curve analysis showed that the proposed model can bring clinical benefits.

Conclusions: The constructed nomogram can accurately predict individual survival probabilities and may serve as a clinical decision support tool for clinicians to optimize treatment in individuals.

KEYWORDS

triple positive breast cancer, prognostic model, nomogram, overall survival, SEER

Introduction

Breast cancer comprises a highly diverse set of systemic illnesses on a molecular level. According to the 2011 St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer, breast cancer can be classified into four subtypes based on immunohistochemical evaluation of hormone receptors (HRs), including the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) (1). Among these subtypes is the triple-positive breast cancer (TPBC) subtype, which is immunohistochemically expressed as ER+/PR+/HER2+ and any Ki-67 status and accounts for approximately 10% of all breast cancer cases (2).

Individuals with TPBC were found to have higher tumor grades, larger tumors, and poorer prognoses than those with other subtypes, and their tumors exhibited aggressive behavior (3). To date, only a few studies have explicitly focused on the clinical features and prognosis of TPBC. According to Anderson et al., the age of onset of TPBC was concentrated between 45 and 75 years, and the prognosis was poorer than that of HR(+) and HER-2(-) subtypes but better than that of HER2-enriched ones (4). Treatment typically consists of adjuvant chemotherapy combined with trastuzumab, followed by endocrine therapy in TPBCs that express both hormonal receptors and HER2 (5). Although patients benefit from multiple treatment options, interactions among various treatment regimens may reduce the therapeutic impact, most likely due to crosstalk between the HER-2 and ER gene signaling pathways at multiple points (6). Additionally, You et al. observed that the overall survival rate of patients with TPBC was higher than the survival rate of those with HER2-enriched ones and similar to those with the luminal A subtype (7).

For many years, the prognosis of patients with breast cancer has been assessed using the TMN prognostic staging method. The eighth edition of the American Joint Committee on Cancer's prognostic staging method integrates the state of estrogen and progesterone receptors, HER2 expression, and histological grading based on TNM staging (8). Its prognostic value and availability in patients with breast cancer have been validated since the development of this revolutionary breast cancer staging system. However, the prognostic staging system appears quite complex for clinical application due to the recurrent grouping (9–12). Furthermore, because genetic testing technology is still not extensively employed, the new system's clinical usefulness is limited. In addition, breast cancer is highly heterogeneous and the individual prognosis is affected by a wide range of factors (13). According to He et al. (14), the novel prognostic staging approach did not outperform the anatomical staging system in terms of prediction power for triple-negative breast cancer. Adjustment and optimization of the prognostic staging system are still needed. Hence, building adequate predictive models for the various molecular subtypes of breast cancer can benefit clinical practice.

The Surveillance, Epidemiology, and End Results (SEER) database is one of the most representative large tumor registry databases in North America, collecting a large amount of relevant data on evidence-based medicine and covering approximately 1/3 of the US population. The nomogram is a commonly used method for survival prediction that combines intuition, accuracy, dependability, and practicality (15). It has been successfully used to predict the prognosis of various malignancies, including breast cancer (16). In this study, data from the Fourth Military Medical University Affiliated Xijing Hospital and the SEER database were synthesized to construct a nomogram model for predicting the overall survival (OS) of TPBC patients, aiming to provide a basis for clinical treatment.

Patients and methods

Patient selection

SEER is a large-scale cancer registration database that covers approximately 34.6% of the U.S. population (17). The data for this study were selected from 17 registries of the SEER program (with an additional treatment field), which is supported by the National Cancer Institute. The data of TPBC patients from January 2013 to December 2017 in the SEER database were extracted and screened by SEER*Stat version 8.4.0.1 software. The inclusion criteria were as follows: (1) patients with pathologically confirmed breast cancer, based on malignant behavior of the International Classification of Diseases (ICD)-O-3; (2) female; (3) molecular subtype is ER+/PR+/HER2+; (4) older than 18 years; (5) survival data with complete and available dates and more than 0 days of survival; and (6) clear clinicopathological information for all the variables of interest including age at diagnosis, breast subtype, tumor size, laterality, lymph node metastasis status, distant metastatic status, type of surgery, pathological type, histological grading, chemotherapy, and radiotherapy information. According to the inclusion and exclusion criteria, cases meeting the criteria were gradually screened out, and 5769 patients with TPBC were ultimately included (Figure 1). Moreover, 191 patients with triple-positive breast cancer at Xijing Hospital in China hospitalized for surgery from January 2013 to December 2017 were collected, and the clinicopathological characteristics and prognosis of the patients were determined.

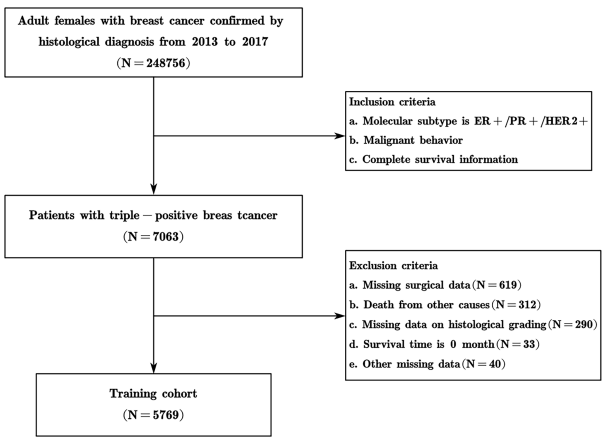

FIGURE 1
Flowchart of the cohort selection process.

Follow-up was performed by in-hospital review, telephone consultations, and instructional activities.

Study variables

The following variables at diagnosis were selected as the potential prognostic factors: age(less than 60 years old or older than 60 years old), laterality (right or left side), pathological type (infiltrative ductal carcinoma or other types), histological grading (well-differentiated, moderately differentiated, poorly differentiated, undifferentiated or anaplastic), tumor size, lymph node metastasis status, distant metastatic status, type of surgery, chemotherapy (yes or none) and radiotherapy (yes or none). The values of tumor size, lymph node metastasis status, distant metastasis status, and surgery type were transformed into grouped categorical variables according to routine practice.

Overall survival (OS) was used as the primary endpoint for this study. OS was defined as the time between the date of diagnosis and the date of death caused by BC. For the validation cohort, the deadline for follow-up was September 14, 2021.

Statistical analysis

The data were analyzed using R software (4.1.1). All variables were transformed into categorical variables. The baseline characteristics of the modeled and validated sets were compared using the Pearson χ^2 test, where the Mann–Whitney U test was performed for the rank data. The Kaplan–Meier curve was used to describe the OS, and the differences between the curves were analyzed by the log-rank test. Univariate and multivariate Cox regression models were performed to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) to analyze the independent prognostic factors associated with OS in TPBC patients. Based on the independent prognostic factors of TPBC, the Rms and Survival packages in R software (4.1.1) were used to construct the nomogram. To confirm the predictive accuracy of the nomogram, both internal (200 bootstraps resamples based on the training cohort) and external (based on the validation cohort) validations were performed. The differentiation of the model was evaluated by the concordance index (C-index) and the receiver operating characteristic (ROC) curve, and the calibration of the model was checked by drawing calibration curves to ensure that the model was accurate and reliable. Finally, decision curve analysis (DCA) was performed for the model to check the clinical benefit and application value of the model. Two-sided P<0.05 was deemed statistically significant.

Results

Baseline characteristics of TPBC patients

The Pearson χ^2 test and Mann–Whitney U test were used to compare the baseline characteristics of the training and validation sets. The results showed that the patients in the validation set were younger at onset and had a lower proportion of poorly differentiated histology compared to the training set. In addition, there were significant differences in pathological staging, tumor size, lymph node metastasis, distant metastasis, and choice of treatment modality (all p<0.05) (Table 1).

Effect of variables on the prognosis of TPBC

For each variable in the training set, a COX univariate survival analysis was performed. The results showed that nine variables, including age, tumor grade, radiotherapy, chemotherapy, pathological staging, T-stage, N-stage, M-stage, and mode of surgery, were factors influencing the prognosis of TPBC (all p<0.05). Multivariate analysis using Cox proportional risk regression (variable screening method: forward: LR, variable inclusion criterion = 0.05, exclusion criterion = 0.1) was performed with the above risk factors as independent variables, and the results indicated that age, radiotherapy, chemotherapy, T-stage, N-stage, M-stage, and mode of surgery were independent risk factors for the prognosis of TPBC (all p<0.05) (Table 2).

Based on Kaplan-Meier and Log-rank tests, the survival curves for the key variables were plotted using the Cox risk model (Figure 2).

Construction of a nomogram for the prognosis of TPBC patients

Based on the results of the Cox univariate and multivariate regression analysis of the training group (Figures 3A, B), the seven variables screened were used to construct a nomogram of the OS prognosis of 5769 TPBC patients (Figure 4). By summing the scores obtained for each variable to obtain an overall score, the nomogram prediction model predicts the 3-year and 5-year OS for TPBC patients.

Validation of the nomograms

The C-index values of the nomograms in the training cohort were 0.830 (95% CI, 0.795-0.864) for OS. In the validation cohort, the C-index value for OS was 0.914 (95% CI, 0.816-0.999). In addition, the ROC curves and calibration curves of the 3-year and 5-year OS were plotted in the training and validation sets. The results showed that the area under the curve (AUG) was greater than 0.8 in both the training and validation sets, while the calibration curves presented excellent consistency between the actual and nomogram-predicted survival probabilities, indicating that the model predicted with decent accuracy (Figures 5, 6).

DCA analysis

Unlike traditional statistical methods, which only evaluate the accuracy of a model, decision curve analysis (DCA) can tell us whether using a model to aid clinical decision-making would improve outcomes for our patients (18). In this study, DCA was plotted against 3-year and 5-year survival for the training and validation

TABLE 1 Baseline characteristics of TPBC patients.

Characteristics	Training cohort (n=5769)	Validation cohort (n=191)	χ^2 value	P value
	Number of patients (%)	Number of patients (%)		
Age				
<60	3376 (58.5)	168 (86.6)	61.37	<0.001
≥60	2393 (41.5)	26 (13.4)		
Laterality				
Left	2998 (52)	103 (53.1)	0.095	0.758
Right	2771 (48)	91 (46.9)		
Pathological type				
Infiltrative ductal carcinoma	4909 (85.1)	191 (98.5)	27.068	<0.001
Other types	860 (14.9)	3 (1.5)		
Histological grading				
Grade I	386 (6.7)	7 (3.6)		<0.001[a]
Grade II	2575 (44.6)	146 (75.3)		
Grade III+IV	2808 (48.7)	41 (21.1)		
T				
T≤2	3170 (54.9)	140 (72.2)		<0.001[a]
2<T≤5	2050 (35.5)	46 (23.7)		
5<T	549 (9.6)	8 (4.1)		
N				
0	3822 (66.3)	115 (59.4)		<0.003[a]
1	1495 (25.9)	42 (21.6)		
2	300 (5.2)	22 (13.3)		
3	152 (2.6)	15 (7.7)		
M				
0	5655 (98)	175 (90.2)	52.603	<0.001
1	114 (2)	19 (9.8)		
Type of surgery				
Total mastectomy	1692 (29.3)	35 (18)	311.515	<0.001
Breast-conserving surgery	3287 (57)	43 (22.2)		
Modified radical surgery	790 (13.7)	116 (59.8)		
Chemotherapy				
None	1232 (21.4)	26 (13.4)	7.132	0.008
Yes	4537 (78.6)	168 (86.6)		
Radiotherapy				
None	2403 (41.7)	119 (61.3)	29.804	<0.001
Yes	3366 (58.3)	75 (38.7)		

[a]Mann-Whitney U test.

sets, respectively. The results show that the net clinical benefit of the model at 3 and 5 years is elevated within a suitable threshold in both the training and validation sets, especially in the validation set, indicating that the model has excellent clinical efficacy (Figure 7).

Discussion

TPBC is a subtype of breast cancer that falls within the luminaI B molecular type (19), accounting for approximately 10% of hormone

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TABLE 2 Univariate and multivariate analysis of TPBC patients.

Characteristics	Univariate analysis		Multivariate analysis	
	HR(95%CI)	P value	HR(95%CI)	P value
Age				
<60	1.00	<0.001	1.00	<0.001
≥60	1.93(1.50-2.57)		1.86(1.37-2.53)	
Histological grading				
Grade I	1.00	0.002		
Grade II	1.15(0.57-2.31)			
Grade III+IV	1.89(0.96-3.72)			
T				
T≤2	1.00	<0.001	1.00	<0.001
2<T≤5	3.35(2.33-4.81)		2.63(1.77-3.89)	
5<T	8.37(5.65-12.40)		4.27(2.68-6.80)	
N				
0	1.00	<0.001	1.00	<0.001
1	3.11(2.19-4.41)		2.96(1.99-4.42)	
2	8.62(5.70-13.03)		5.59(3.44-9.09)	
3	12.31(7.83-19.35)		6.68(3.83-11.64)	
M				
0	1.00	<0.001	1.00	<0.001
1	6.66(4.23-10.48)		2.47(1.52-4.00)	
Type of surgery				
Total mastectomy	1.00	<0.001		<0.001
Breast-conserving surgery	0.63(0.43-0.91)		0.87(0.58-1.31)	
Modified radical surgery	3.50(2.47-4.98)		1.60(1.08-2.38)	
Chemotherapy				
None	1.00	<0.001	1.00	<0.001
Yes	0.43(0.32-0.57)		0.28(0.20-0.39)	
Radiotherapy				
None	1.00	0.001		0.005
Yes	0.63(0.47-0.84)		0.58(0.42-0.80)	

receptor-positive breast cancers (20). Currently, there are few studies on the prognosis of triple-positive breast cancer. Kast et al. (21) stated that TPBC is aggressive cancer, with ductal carcinoma being the most common. Pathologically, most cases were classified as grade III, with an elevated prevalence of lymph node metastases and giant tumors. Additionally, Guan et al. showed that patients with TPBC tended to be younger and exhibit pathological characteristics such as vascular or nerve infiltration and an elevated rate of lymph node metastases, proliferation index, and tumor load (22). In this study, TPBC patients had a younger age of onset, a higher percentage of histological grade III, and significant lymph node metastases, consistent with previous studies. In summary, triple-positive breast cancer is a relatively aggressive molecular subtype.

Since TPBC is a relatively rare and clinically neglected condition, assessment of the prognosis of patients with TPBC is essential for the integrated management of TPBC. Numerous studies have investigated breast cancer prognosis, and molecular type, tumor size, lymph node status, and histological grading are often used as prognostic indications in clinical practice. Additionally, while both the 21-gene and 70-gene recurrence scores are approved for clinical

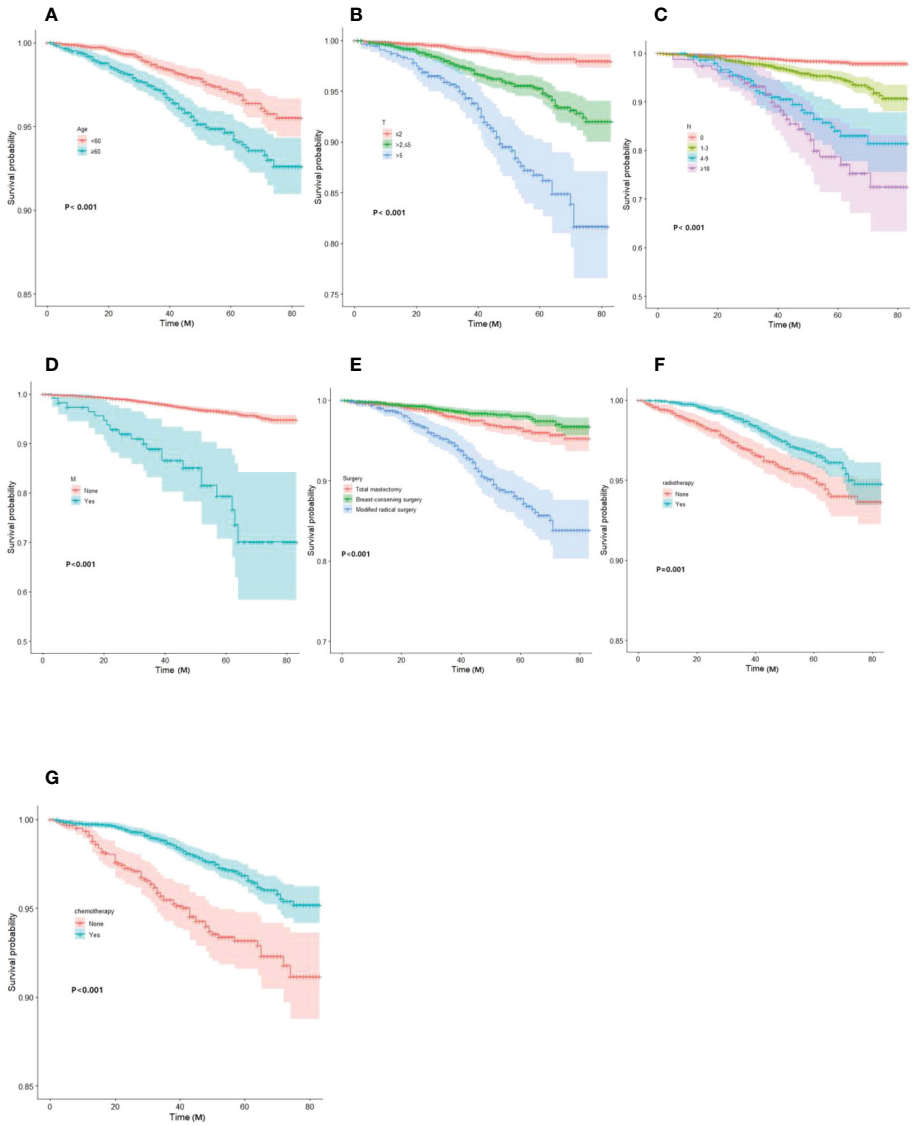

FIGURE 2
Kaplan-Meier curves of OS for each predictor. **(A)** Age; **(B)**T-stage; **(C)** N-stage; **(D)** M-stage; **(E)** mode of surgery; **(F)** radiotherapy; **(G)** chemotherapy. OS, overall survival.

use, the use of multiple testing to predict recurrence remains contentious because of the limited clinical benefit and extreme cost (16). There is currently a shortage of simple and effective prognostic and predictive assessment methods that may be used in clinical practice. No acceptable model for prognostic assessment has been created in prior investigations, especially for TPBC. The nomogram is a graphical representation of the multivariate prognostic model, which can be used to individually predict the survival situation at a specific time point (23). As a contemporary forecasting model, nomograms have higher accuracy and wider applicability and are easy to popularize compared with traditional forecasting methods (24). As reported in the literature, Zhou et al. constructed and validated well-calibrated nomograms for predicting disease-free survival and OS in patients with TNBC (25). In addition, as one of the largest cancer registries in the United States, the SEER database contains a wealth of evidence-based medical data, including basic information, clinical characteristics, treatments, and patient follow-up. Therefore, this study developed a prognostic prediction nomogram model for TPBC patients based on data from the SEER database, which is reduplicative.

In this study, we developed a nomogram-based Cox regression model to predict the 3-year and 5-year OS of TPBC patients. The ROC and calibration curves showed that the nomogram could accurately predict the OS of TPBC patients. At the same time, decision curve analysis showed that the clinical efficacy of the model was excellent. Multivariate analysis showed that age, tumor grade, radiotherapy, chemotherapy, stage T, stage N, stage M, and surgical modality were independent risk factors for TPBC prognosis. These independent risk factors were essentially consistent with clinical observations.

It has been shown that HR-positive, HER2-positive breast cancer patients older than 75 years have significantly increased mortality compared to other populations (26). Our findings also suggested a poor prognosis for TPBC patients over the age of 60. Currently, TNM

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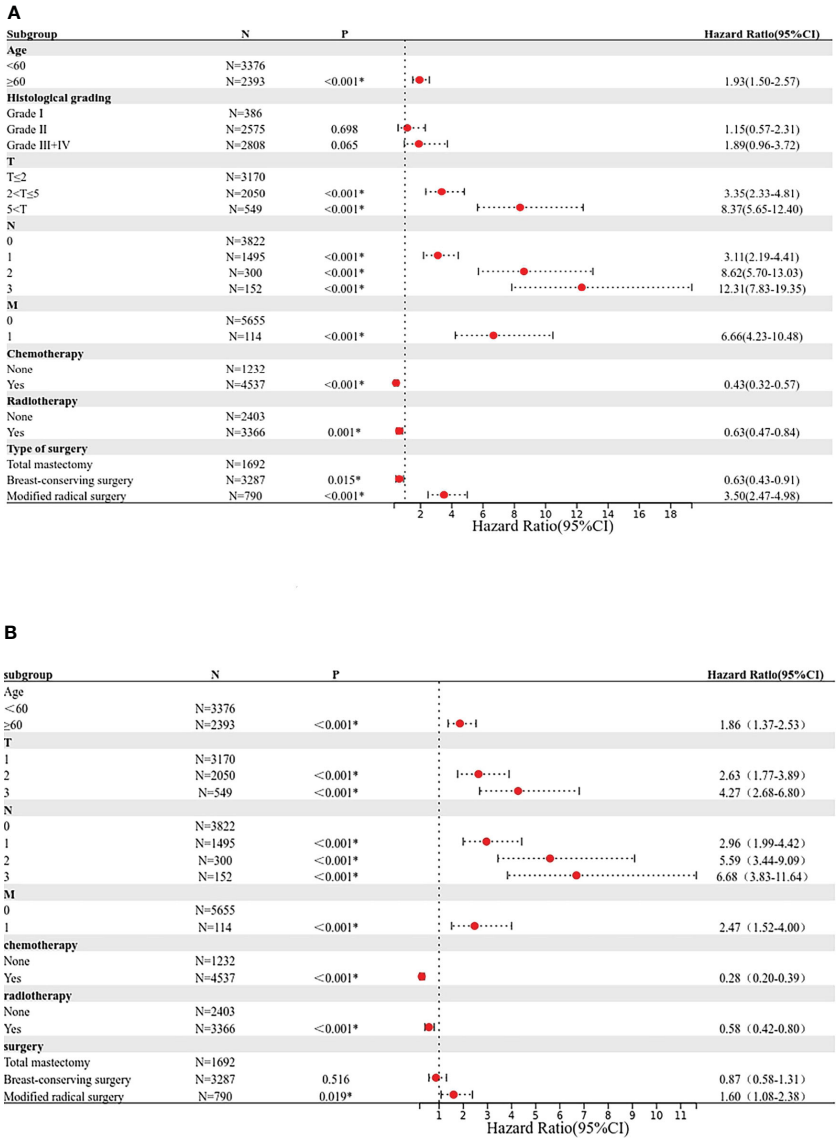

FIGURE 3
Proportional hazard model forest map of overall survival in TPBC patients in SEER. **(A)** Forest map (univariate analysis). **(B)** Forest map (multivariate analysis). * means $p < 0.05$.

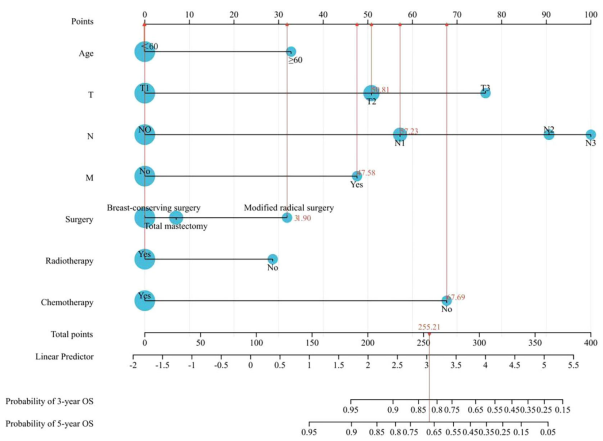

FIGURE 4
Nomogram prediction model for prognosis of TPBC patients.

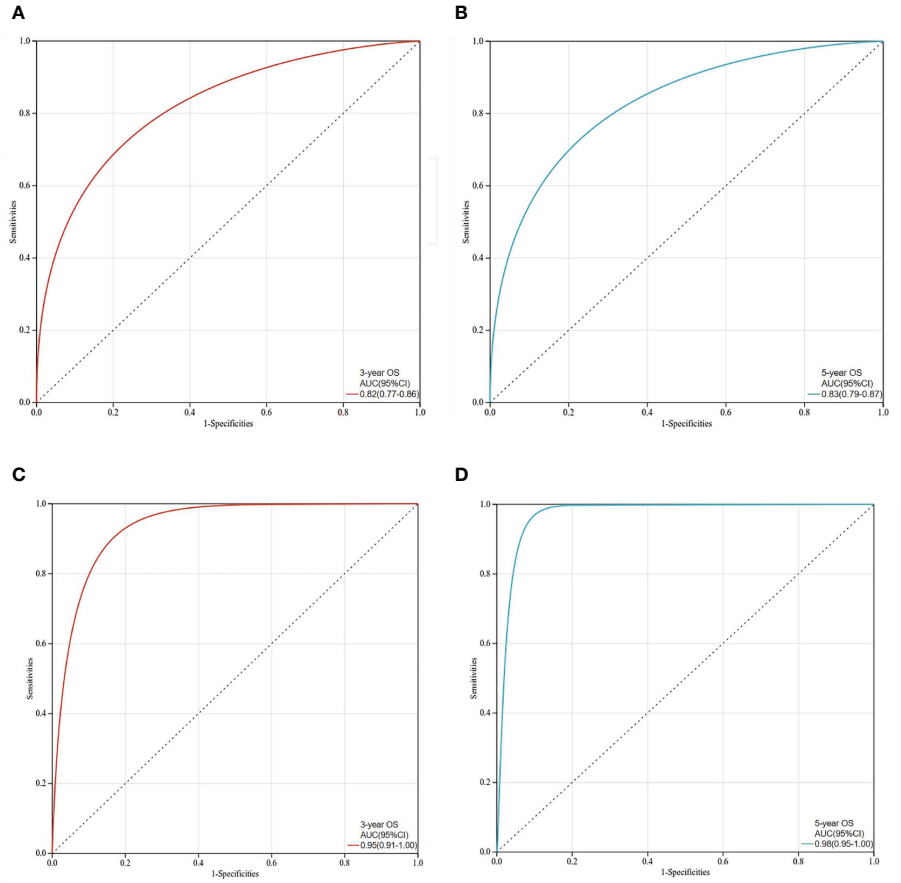

FIGURE 5
ROC curves for prediction of 3-year and 5-year overall survival in the training set and validation set. **(A)**The 3-year overall survival of the training set; **(B)**The 5-year overall survival of the training set; **(C)** The 3-year overall survival of the validation set; **(D)** The 5-year overall survival in the validation set.

prognostic staging is commonly used to assess the prognosis of breast cancer patients. In this study, patients had a worse prognosis as the TNM stage increased, which is consistent with previous studies (27). Breast-conserving therapy (BCT) had similar long-term survival outcomes to mastectomy in patients with early breast cancer, and recent studies had reported similar rates of recurrence compared with mastectomy (28). However, the latest research showed that BCT was associated with superior overall survival compared with mastectomy for early-stage breast cancer (29), consistent with this study. In addition, by comparing data, we observed a relatively elevated BCT rate in the United States. The reason lies in the uniformity of diagnosis and treatment levels and treatment standards among American doctors and in the fact that doctors can follow treatment standards very well.

Theoretically, endocrine therapy, chemotherapy, and targeted therapy have a significant impact on the prognosis of patients with TPBC. In 2017, NCCN guidelines recommended chemotherapy in combination with anti-HER-2 therapy and endocrine therapy as a treatment regimen for TPBC (30). Targeted therapy is vital in the adjuvant treatment of HER2-positive early-stage breast cancer. The clinical trial HERA study revealed that 79.4% of patients survived for >10 years and were at a lower risk of death after 1 year of trastuzumab adjuvant therapy (31). However, in clinical practice, we found that patients with TPBC had less benefit from trastuzumab, which may have been due to drug resistance. Studies have demonstrated that the presence or absence of HRs is a crucial component in determining breast cancer diagnosis, therapy, and prognosis (32). A secondary analysis (33) of the HERA study published in 2016 confirmed the lower benefit of trastuzumab in patients with TPBC with high ER expression. This was demonstrated by the interaction between ERs and the intracellular signaling pathway regulated by HER-2 (34).

Although other researchers have done similar work (27), Compared with previous studies, the innovations of this study are as follows. Firstly, by including a Chinese cohort, the variability of clinical characteristics of TPBC patients by race was explored. Secondly, to externally validate the model, this study used a Chinese cohort, and the results were more compelling. Finally, to improve the model's construction and validation, this study included survival analysis and clinical decision curves.

Through internal and external validation, our constructed nomogram showed excellent accuracy and clinical benefit. However, there were still some limitations to this study. First, due to the limitations of the data in the SEER database, the predictive model cannot include some crucial clinical factors, such as chemotherapy protocol, targeted therapy regimen, endocrine therapy regimen, and Ki-67 expression, and additional studies may be needed to optimize the model. At the same time, 1294 of the 7063 identified TPBC patients were excluded due to insufficient data, which may have contributed to selection bias. Furthermore, the constructed nomogram had only been externally validated with a single sample in China, so caution should be

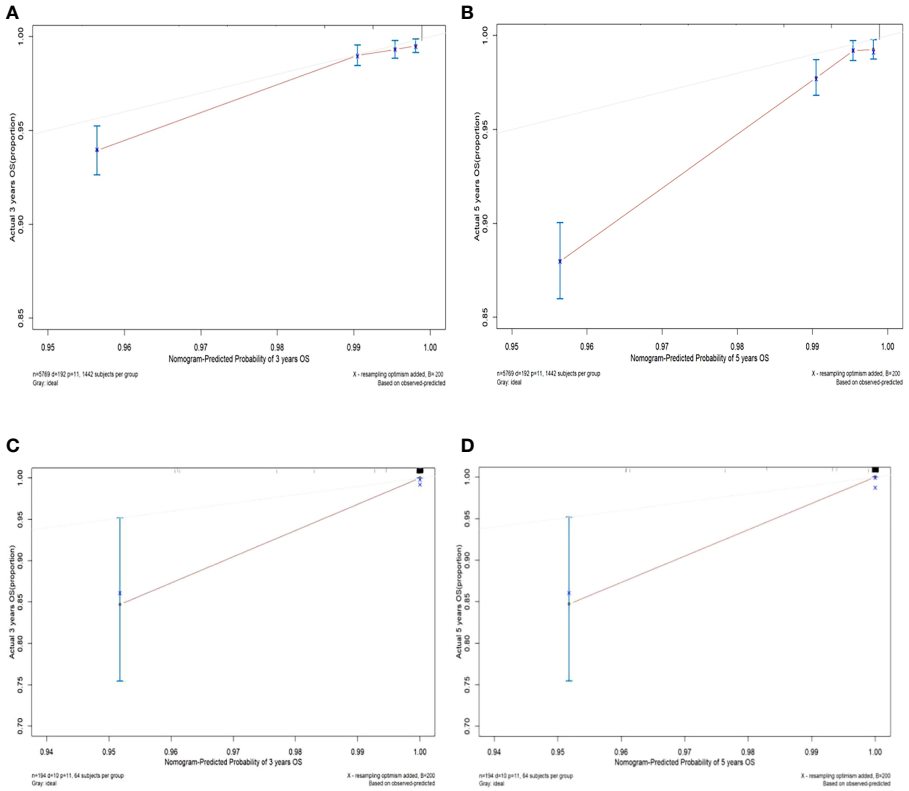

FIGURE 6
Calibration curves of 3-year and 5-year overall survival in the training set and validation set. **(A)** The 3-year overall survival of the training set; **(B)** The 5-year overall survival of the training set; **(C)** The 3-year overall survival of the validation set; **(D)** The 5-year overall survival in the validation set.

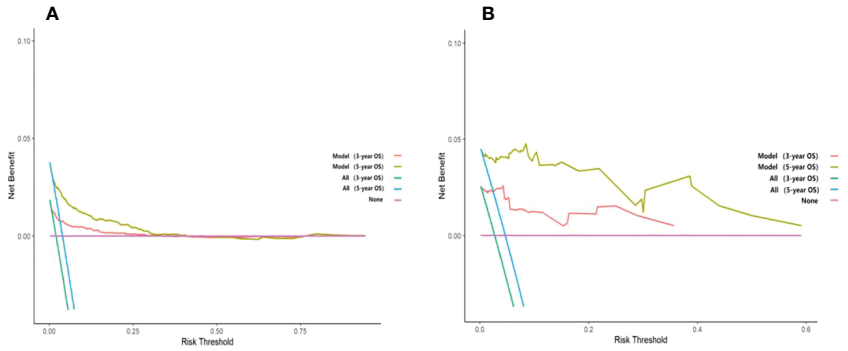

FIGURE 7
Decision curve analysis curves of 3-year and 5-year overall survival in the training set and validation set. **(A)** The OS of the training set; **(B)** The OS of the validation set.

exercised in extending our results to patients from different geographic regions or with other ethnic backgrounds.

Conclusion

In summary, this study developed a nomogram model to predict the overall survival of TPBC patients based on data from the SEER database in the United States and Xijing Hospital in China. Both the calibration curves and ROC curves for the model exhibited reliable performance, and the clinical decision curve analyses showed that the model can bring clinical benefit. Therefore, the constructed nomogram can accurately predict individual survival probabilities and may serve as a clinical decision support tool for clinicians to optimize treatment in individuals.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conception and design, SY. Collection and assembly of data, JX. Acquisition of study materials or patients, BD. Data analysis and interpretation, AG. Manuscript writing, AG. Final approval of manuscript, all authors. All authors contributed to the article and approved the submitted version.

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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.2023.1071076/full#supplementary-material

References

1. 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. *Ann Oncol* (2011) 22:1736–47. doi: 10.1093/annonc/mdr304

2. Vici P, Pizzuti L, Natoli C, Gamucci T, Di Lauro L, Barba M, et al. Triple positive breast cancer: A distinct subtype? *Cancer Treat Rev* (2015) 41:69–76. doi: 10.1016/j.ctrv.2014.12.005

3. Negi P, Kingsley PA, Jain K, Sachdeva J, Srivastava H, Marcus S, et al. Survival of triple negative versus triple positive breast cancers: Comparison and contrast. *Asian Pac J Cancer Prev* (2016) 17:3911–6. doi: 10.1002/14651858.CD011220.pub2

4. Anderson WF, Rosenberg PS, Prat A, Perou CM, Sherman ME. How many etiological subtypes of breast cancer: two, three, four, or more? *J Natl Cancer Inst* (2014) 106:dju165. doi: 10.1093/jnci/dju165

5. Tripathy D, Kaufman PA, Brufsky AM, Mayer M, Yood MU, Yoo B, et al. First-line treatment patterns and clinical outcomes in patients with HER2-positive and hormone receptor-positive metastatic breast cancer from registHER. *Oncologist* (2013) 18:501. doi: 10.1634/theoncologist.2012-0414

6. Geyer FC, Rodrigues DN, Weigelt B, Reis-Filho JS. Molecular classification of estrogen receptor-positive/luminal breast cancers. *Adv Anat Pathol* (2012) 19:39–53. doi: 10.1097/PAP.0b013e31823fafa0

7. You SH, Chae BJ, Eom YH, Yoo TK, Kim YS, Kim JS, et al. Clinical differences in triple-positive operable breast cancer subtypes in Korean patients: An analysis of Korean breast cancer registry data. *J Breast Cancer* (2018) 21:415–24. doi: 10.4048/jbc.2018.21.e53

8. Giuliano AE, Connolly JL, Edge SB, Mittendorf EA, Rugo HS, Solin LJ, et al. Breast cancer–major changes in the American joint committee on cancer eighth edition cancer staging manual. *CA Cancer J Clin CA Cancer J Clin* (2017) 67:290–303. doi: 10.3322/caac.21393

9. Zombori T, Lehóczky L, Cserni B, Nyári T, Cserni G. Evaluation of anatomic and prognostic stages of breast cancer according to the 8th edition of the TNM staging system-retrospective analysis based on data from deceased patients once diagnosed with breast cancer. *Orv Hetil* (2017) 158:1373–81. doi: 10.1556/650.2017.30849

10. Weiss A, Chavez-MacGregor M, Lichtensztajn DY, Yi M, Tadros A, Hortobagyi GN, et al. Validation study of the American joint committee on cancer eighth edition prognostic stage compared with the anatomic stage in breast cancer. *JAMA Oncol* (2018) 4:203–9. doi: 10.1001/jamaoncol.2017.4298

11. Wang M, Chen H, Wu K, Ding A, Zhang M, Zhang P. Evaluation of the prognostic stage in the 8th edition of the American joint committee on cancer in locally advanced breast cancer: An analysis based on SEER 18 database. *Breast* (2018) 37:56–63. doi: 10.1016/j.breast.2017.10.011

12. Abdel-Rahman O. Validation of the 8th AJCC prognostic staging system for breast cancer in a population-based setting. *Breast Cancer Res Treat* (2018) 168:269–75. doi: 10.1007/s10549-017-4577-x

13. Siegel RL, Miller KD, Fuchs H, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71:7–33. doi: 10.3322/caac.21654

14. He J, Tsang JY, Xu X, Li J, Li M, Chao X, et al. AJCC 8th edition prognostic staging provides no better discriminatory ability in prognosis than anatomical staging in triple negative breast cancer. *BMC Cancer* (2020) 20:1–9. doi: 10.1186/s12885-019-6494-3

15. Rossi PG, Lebeau A, Canelo-Aybar C, Saz-Parkinson Z, Quinn C, Langendam M, et al. Recommendations from the European commission initiative on breast cancer for multigene testing to guide the use of adjuvant chemotherapy in patients with early breast cancer, hormone receptor positive, HER-2 negative. *Br J Cancer* (2021) 124:1503–12. doi: 10.1038/s41416-020-01247-z

16. Balachandran VP, Gonen M, Smith JJ, Dematteo RP. Nomograms in oncology: More than meets the eye. *Lancet Oncol* (2015) 16(4):e173–e80. doi: 10.1016/S1470-2045(14)71116-7

17. Chu J, Yang D, Wang L, Xia J. Nomograms predicting survival for all four subtypes of breast cancer: A SEER-based population study. *Ann Transl Med* (2020) 8(8):544. doi: 10.1001/jamaoncol.2017.4298

18. Vickers AJ, Holland F. Decision curve analysis to evaluate the clinical benefit of prediction models. *Spine J* (2021) 21(10):1643–8. doi: 10.1016/j.spinee.2021.02.024

19. 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

20. Dowsett M, Allred C, Knox J, Quinn E, Salter J, Wale C, et al. Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the arimidex, tamoxifen, alone or in combination trial. *J Clin Oncol* (2008) 26:1059–65. doi: 10.1200/JCO.2007.12.9437

21. Kast K, Link T, Friedrich K, Petzold A, Niedostatek A, Schoffer O, et al. Impact of breast cancer subtypes and patterns of metastasis on outcome. *Breast Cancer Res Treat* (2015) 150:621–9. doi: 10.1007/s10549-015-3341-3

22. Guan XQ, SC GU, Ji WU, Jiang XL, Shi XH, Yuan M, et al. Pathological features and survival of triple positive breast cancer in 271 cases. *Chin J Curr Adv Gen Surg* (2018) 21:262–6. doi: 10.3969/j.issn.1009-9905.2018.04.003

23. Park SY. Nomogram: An analogue tool to deliver digital knowledge. *J Thorac Cardiovasc Surg* (2018) 155(4):1793. doi: 10.1016/j.jtcvs.2017.12.107

24. Li G, Tian M-L, Bing Y-T, Wang H-Y, Yuan C-H, Xiu D-R. Nomograms predict survival outcomes for distant metastatic pancreatic neuroendocrine tumor: A population based STROBE compliant study. *Med (Baltimore)* (2020) 99(13):e19593. doi: 10.1097/MD.0000000000019593

25. Luo WQ, Huang QX, Huang XW, Hu HT, Zeng FQ, Wang W. Predicting breast cancer in breast imaging reporting and data system (BI-RADS) ultrasound category 4 or 5 lesions: A nomogram combining radiomics and BI-RADS. *Sci Rep* (2019) 9:1–1. doi: 10.1038/s41598-019-48488-4

26. Kim HJ, Kim S, Freedman RA, Partridge AH. The impact of young age at diagnosis (age <40 years) on prognosis varies by breast cancer subtype: A U.S. SEER database analysis. *Breast (Edinburgh Scotland)* (2022) 61:77–83. doi: 10.1016/j.breast.2021.12.006

27. Wang X, Wang H, Liu F, Jie L, Song Z. Establishment and verification of a nomogram for predicting survival in patients with triple-positive breast cancer. *Ann Transl Med* (2022) 10(16):884. doi: 10.21037/atm-22-3560

28. Johns N, Dixon JM. Should patients with early breast cancer still be offered the choice of breast conserving surgery or mastectomy? *Eur J Surg Oncol* (2016) 42(11):1636–41. doi: 10.1016/j.ejso.2016.08.016

29. Wrubel E, Natwick R, Wright GP. Breast-conserving therapy is associated with improved survival compared with mastectomy for early-stage breast cancer: A propensity score matched comparison using the national cancer database. *Ann Surg Oncol* (2021) 28(2):914–9. doi: 10.1245/s10434-020-08829-4

30. 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(3):310–20. doi: 10.6004/jnccn.2018.0012

31. Goldhirsch A, Gelber RD, Piccart-Gebhart MJ, De Azambuja E, Procter M, Suter TM, et al. 2 years versus 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): An open-label, randomised controlled trial. *Lancet* (2013) 382:1021–8. doi: 10.1016/S0140-6736(13)61094-6

32. Hwang KT, Kim J, Jung J, Kim BH, Park JH, Jeon SY, et al. Long-term prognostic effect of hormone receptor subtype on breast cancer. *Breast Cancer Res Treat* (2020) 179:139–51. doi: 10.1007/s10549-019-05456-w

33. Loi S, Dafni U, Karlis D, Polydoropoulou V, Young BM, Willis S, et al. Effects of estrogen receptor and human epidermal growth factor receptor-2 levels on the efficacy of trastuzumab: A secondary analysis of the HERA trial. *JAMA Oncol* (2016) 2:1040–7. doi: 10.1001/jamaoncol.2016.0339

34. Montemurro F, Di Cosimo S, Arpino G. Human epidermal growth factor receptor 2 (HER2)-positive and hormone receptor-positive breast cancer: New insights into molecular interactions and clinical implications. *Ann Oncol* (2013) 24(11):2715–24. doi: 10.1093/annonc/mdt287

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Clinicopathologic characteristics and prognostic significance of HER2-low expression in patients with early breast cancer: A systematic review and meta-analysis

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Background: HER2-low expression breast cancer (BC) accounts for approximately 45%-55% of all BC cases. The purpose of this study was to investigate the prognostic difference between patients with HER2-low expression and HER2-zero BC.

Methods: An electronic search of Pubmed, Embase, Cochrane Library, and Web of Science databases was performed to screen studies that included prognostic comparisons between HER2-zero and HER2-low expression groups. A total of 14 studies involving 52106 patients were included.

Results: Our results indicated that HER2-low expression was associated with a significant benefit in OS among all patients with early BC (HR, 0.83; 95% CI, 0.78–0.88), patients with hormone-receptor positive BC (HR, 0.83; 95% CI, 0.77–0.89), and patients with TNBC (HR, 0.78; 95% CI, 0.70–0.87). HER2-low expression was associated with a significant benefit in DFS among all patients (HR, 0.81; 95% CI, 0.71–0.93), patients with hormone receptor-positive BC (HR, 0.81; 95% CI, 0.72–0.90), but no significant difference in DFS was found in patients with TNBC (HR, 0.87; 95% CI, 0.65–1.17). HER2-low expression was associated with a significant benefit in RFS among all patients (HR, 0.90; 95% CI, 0.85–0.95), patients with hormone receptor-positive BC (HR, 0.90; 95% CI, 0.84–0.96), but no significant difference in RFS was found in patients with TNBC (HR, 0.80; 95% CI, 0.55–1.16).

Conclusions: Among patients with early-stage BC, patients with HER2-low expression BC had better OS in the overall population, hormone receptor-

positive and TNBC subgroups. Besides, favorable DFS and RFS were observed in both the overall population and hormone receptor-positive subgroup.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier (CRD 42022349458).

KEYWORDS

HER2-low, HER2-zero, breast cancer, prognosis, meta-analysis

1 Introduction

Breast cancer (BC) is the most commonly diagnosed cancer among women worldwide. According to the Global Cancer Statistics 2020, there were an estimated 2.3 million new cases of female BC worldwide in 2020 (1). Human epidermal growth factor receptor 2 (HER2) detection is essential for BC treatment planning. HER2-positive BC accounts for approximately 15% of all BC cases, in which multiple agents targeting HER2 have provided significant clinical benefits in patients with both early and advanced BC (2, 3). However, 85% of patients with BC were classified as HER2-negative and were therefore ineligible for anti-HER2 treatment (4). Recently, antibody-drug conjugates (ADCs) have been proved to have antitumor activity in patients with classical HER2-positive BC (5, 6), as well as BC with low HER2 expression (7). These results have led to the concept of "HER2-low expression" which includes tumors with HER2 expression indicated by a immunohistochemistry (IHC) score of 1+ or 2+/fluorescence *in-situ* hybridization (FISH)-negative.

In the past, HER2-low expression and HER2-zero BC have been combined and considered HER2 negative BC. Moreover, HER2-low expression BC accounts for approximately 45%–55% of all BC cases, indicating that the number of new HER2 low-expression BC cases could be approximately 1 million worldwide annually, which is almost equivalent to that of all new annual gastric cancer cases worldwide (1, 4). Because the population of patients with BC with HER2-low expression is very large, understanding the associations of different clinicopathologic characteristics and prognosis between patients with HER2-low expression and HER2-zero BC is significant, and will help clinicians develop more precise treatment strategies and avoid overtreatment or undertreatment in patients with HER2-low expression BC in the future. In addition, it may guide the design of future clinical trials for HER2-low expression BC.

Several studies have shown that compared with HER2-zero BC, HER2-low expression BC has a specific biology with varying responses to therapy and prognosis (8–10). However, other studies have found that HER2-low expression is indistinct from HER2-zero BC in terms of clinicopathologic characteristics and prognosis (11). Thus, whether HER2-low expression BC varies in biological and prognostic significance from that of HER2-zero BC remains unclear. This study aimed to evaluate the biological and prognostic significance of HER2-low expression in patients with BC.

2 Method

The study protocol adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guidelines (12, 13). This systematic review was prospectively registered with The International Prospective Register of Systematic Reviews (CRD 42022349458). Because this study was based exclusively on published literature, ethics approval and informed consent were not required.

2.1 Study objectives

The primary objective was to identify associations between prognosis, including overall survival (OS), disease-free survival (DFS), and relapse-free survival (RFS), and early HER2-low expression (HER2 IHC 1/2+ with FISH negative) and HER2-zero BC, including hormone receptor-positive BC and triple-negative BC (TNBC). The secondary objective was to identify associations between prognosis, including OS and RFS, and early HER2 IHC 0, HER2 IHC 1+, and HER2 IHC 2+ (IHC 2+ in the following text refers to IHC 2+/FISH-negative) BC, including hormone receptor-positive BC and TNBC. In addition, subgroup analyses were performed. The association of DFS and distant DFS (DDFS) with HER2-low in high-genetic-risk and low-genetic-risk groups was analyzed.

2.2 Literature search

We conducted an electronic search of PubMed, Embase, Cochrane Library, and Web of Science databases. The search strategy combined Medical Subject Heading terms and keywords encompassing two key concepts: BC and HER2-low expression (Supplementary Table 1). All titles were initially screened independently and the appropriate abstracts were reviewed independently by two authors (T.W. and DY.W.). Abstracts that met the criteria were retained for full-text review. Disagreements were resolved through discussion during the screening and extraction period.

2.3 Study selection

The selected studies had to meet the following inclusion criteria simultaneously: (1) published from January 1, 2015, to July 21, 2022

in English; (2) study population included patients with early BC; (3) analysis included prognostic comparisons between HER2-zero and HER2-low expression groups or between any two groups among HER2-zero, HER2 IHC 1+, and HER2 IHC 2+ groups (e.g., HER2-zero and HER2 IHC 1+ vs. HER2 IHC 2+); (4) OS, DFS or RFS were reported as hazard ratios (HRs) (If no HRs were presented for OS, DFS, or RFS, the Kaplan-Meier [K-M] curve of any OS, DFS, or RFS outcome must be provided to facilitate data extraction of K-M curves to calculate HRs); (5) retrospective study, randomized controlled trial (RCT), or pool analysis study. Regarding studies with populations comprised of patients with both early and advanced or metastatic BC, the prognostic analysis must have been performed separately for patients with early BC; otherwise, the proportion of patients with advanced or metastatic BC must be less than 10%.

The exclusion criteria were studies (1) published in a language other than English or before January 1, 2015 (2) in which populations included mainly advanced or metastatic BC without separate prognostic analysis of patients with early BC or (3) without survival comparisons of OS, DFS, or RFS between patients with HER2-zero and HER2-low expression BC,or among HER2-zero, HER2 IHC 1+, and HER2 IHC 2+ groups.

2.4 Data extraction

Study and participant characteristics and outcome measures were extracted by two authors (T.W. and DY.W.) independently. Disagreements were resolved by discussion until consensus. The following variables were extracted: title and study details (year, journal, and location), study population characteristics (sample size, median age, median follow-up, tumor size, lymph node status, tumor grade, stage), and outcome data. The HRs for OS, DFS, and RFS were extracted from each eligible study. If K-M curves were provided without HRs in the reported literature, we used Engauge Digitizer to extract data from K-M curves and calculate the respective HRs using the practical methods described by Tierney et al. (14).

2.5 Statistical analysis

Statistical analysis was performed from August 15, 2022, to August 25, 2022. Outcome data were reported as HRs; If K-M curves were provided without HRs, HRs were calculated using data extracted from K-M curves. The 95% confidence intervals (CIs) were estimated using the Mantel-Haenszel method. The 95% CIs that did not cross unity were considered statistically significant. I^2 statistics were used to estimate statistical heterogeneity, with greater than 50% indicating significant heterogeneity. When no significant heterogeneity ($I^2 \leq 50\%$) was observed, a fixed-effects model was used. In contrast, when significant heterogeneity ($I^2 > 50\%$) was observed, a random-effects model was used to calculate the pooled effect estimate (HR) to explain any possible inter-study heterogeneity. Sensitivity analysis was performed to assess the robustness of the meta-analysis conclusions. Two-sided and P values <0.05 were considered statistically significant in all analyses. All statistical analyses were performed using R, version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

2.6 Risk of bias

Risk of bias (RoB) was assessed by two authors (T.W. and DY.W.). Retrospective studies were assessed using the Newcastle-Ottawa Scale based on several parameters, including patient selection, ascertainment of exposure, outcome assessment, cohort comparability, and follow-up duration and adequacy (15). Points were calculated for each study and classified as low, high, or unclear RoB accordingly. Disagreements regarding these categories were resolved through discussion until consensus between the authors was reached. The Egger test was used for funnel plot asymmetry and to visualize publication bias (16).

3 Results

3.1 Characteristics of the included studies

The flow diagram (Figure 1) outlines the study selection process and reasons for exclusion. In total, 2398 publications were identified using the predefined search terms, of which 14 studies met the inclusion criteria (17–30).

Among the 14 selected articles, 52106 participants were ultimately included in the analysis. One pool analysis (17) and one RCT (30) were included, whereas the remaining 12 studies were retrospective cohort studies. For 3 studies that did not include K-M curves with HRs, the HRs were estimated using data extracted from the K-M curves. The two reviewers were in 100% agreement regarding the extracted data. Supplementary Table 2 provides an overview of the main characteristics and relevant outcomes of the included studies. Included studies were assessed according to Newcastle-Ottawa scores, which are summarized in Supplementary Table 3. None of the included studies was classified as having a high RoB for objective outcomes. The included studies differed in their methodology. The periods ranged from 0.8 to 10.3 years. The sample sizes ranged from 296 to 5235 patients. Moreover, 4 studies were conducted in Europe, 6 studies in Asia, 1 study in North America, 1 study in South America, and 2 studies in Multi-continents. The mean age of the patients varied from 45.3 to 66.1 years old.

3.2 OS

In this meta-analysis, 7 studies with 37466 patients were included to assess the association of HER2-low expression and HER2-zero BC with OS among all patients (including patients with hormone receptor-positive BC and TNBC) with early BC. Our results indicated that among all patients with early BC, HER2-low expression was associated with a significant benefit in OS (HR, 0.83; 95% CI, 0.78–0.88), with low heterogeneity observed across studies ($I^2 = 40\%$; $P = 0.13$) (Figure 2A).

Furthermore, 7 studies with 34229 patients and 7 studies with 7482 patients were included to assess the association of HER2-low expression and HER2-zero BC with OS in patients with hormone receptor-positive BC and TNBC, respectively. HER2-low expression was significantly associated with longer OS in patients with hormone-receptor positive BC (HR, 0.83; 95% CI, 0.77–0.89), with low

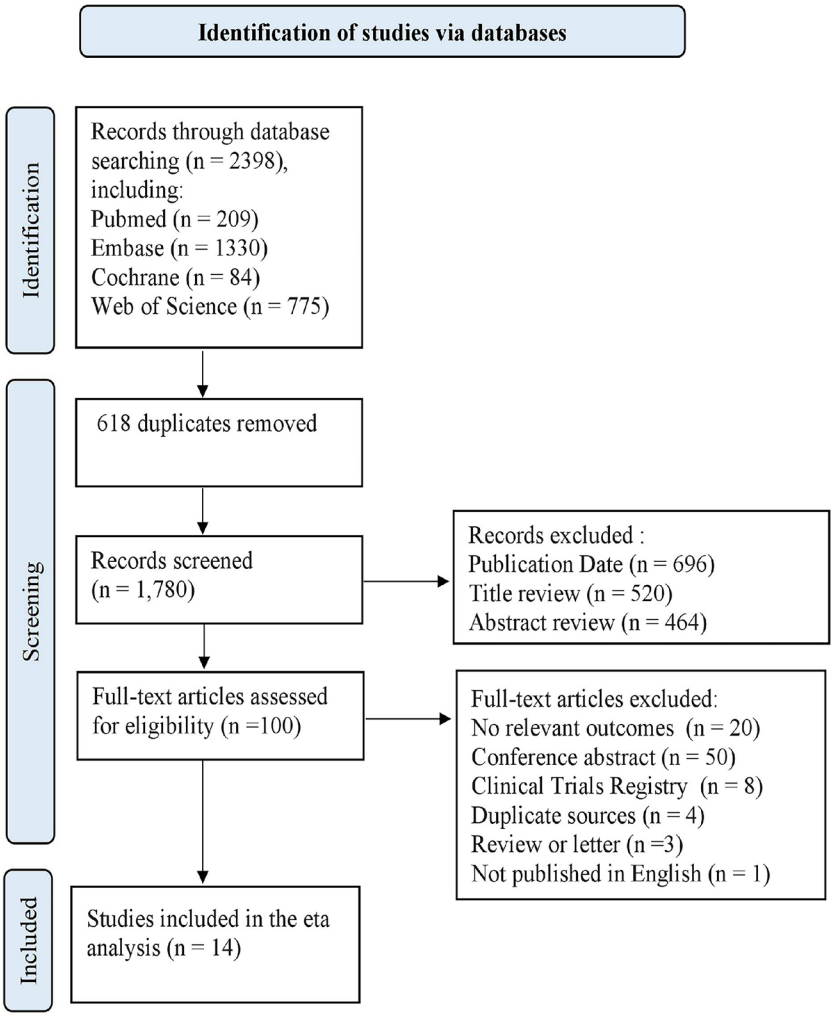

FIGURE 1
Flow diagram of search strategy and study selection.

heterogeneity observed across studies (I^2 = 0%; P = 0.42) (Figure 2B). Similarly, in patients with TNBC, HER2-low expression was significantly associated with longer OS (HR, 0.78; 95% CI, 0.70–0.87), with moderate heterogeneity observed across studies (I^2 = 43%; P = 0.10) (Figure 2C).

To determine whether HER2-zero, HER2 IHC 1+, and HER2 IHC 2+ BC were associated with OS among all patients (including patients with hormone receptor-positive BC and TNBC), further analyses were performed. Two studies with 3490 patients revealed no significant difference in OS between BC patients with HER2 IHC 2+ and HER2 IHC 1+ (HR, 1.01; 95% CI, 0.69–1.46), with no considerable heterogeneity (I^2 = 0%; P = 0.33) (Figure 2D). However, data obtained from three studies with 20407 patients revealed no significant difference in OS between patients with HER2 IHC 2+ and HER2-zero BC (HR, 0.89; 95% CI, 0.78–1.00),with no considerable heterogeneity (I^2 = 27%; P = 0.26) (Figure 2E). Significantly longer OS was observed in patients with HER2 IHC 1+ than that in HER2-zero based on three studies with 25910 patients (HR, 0.85; 95% CI, 0.79–0.92), with no considerable heterogeneity (I^2 = 0%; P = 0.51) (Figure 2F).

3.3 DFS

Among all patients (including patients with hormone receptor-positive BC and TNBC), significantly longer DFS was observed in patients with HER2-low expression compared with that in patients with HER2-zero BC (HR, 0.81; 95% CI, 0.71–0.93) based on three studies with 7667 patients, with no considerable heterogeneity observed (I^2 = 0%; P = 0.46) (Figure 3A).

Regarding patients with hormone receptor-positive BC, the analysis based on six studies with 12283 patients revealed significantly longer DFS among patients with HER2-low expression compared with that in patients with HER2-zero BC (HR, 0.81; 95% CI, 0.72–0.90), with no considerable heterogeneity observed (I^2 =0%; P = 0.73) (Figure 3B). The association of HER2-low expression and DDFS in hormone receptor-positive BC was analyzed (Supplementary Figure 1A) and no significant difference was observed based on two studies with 5146 patients (HR, 0.73; 95% CI, 0.59–0.91), with no considerable heterogeneity observed (I^2 = 0%; P = 0.79).

However, among patients with TNBC, no significant difference in DFS was found in patients with HER2-low expression and HER2-zero

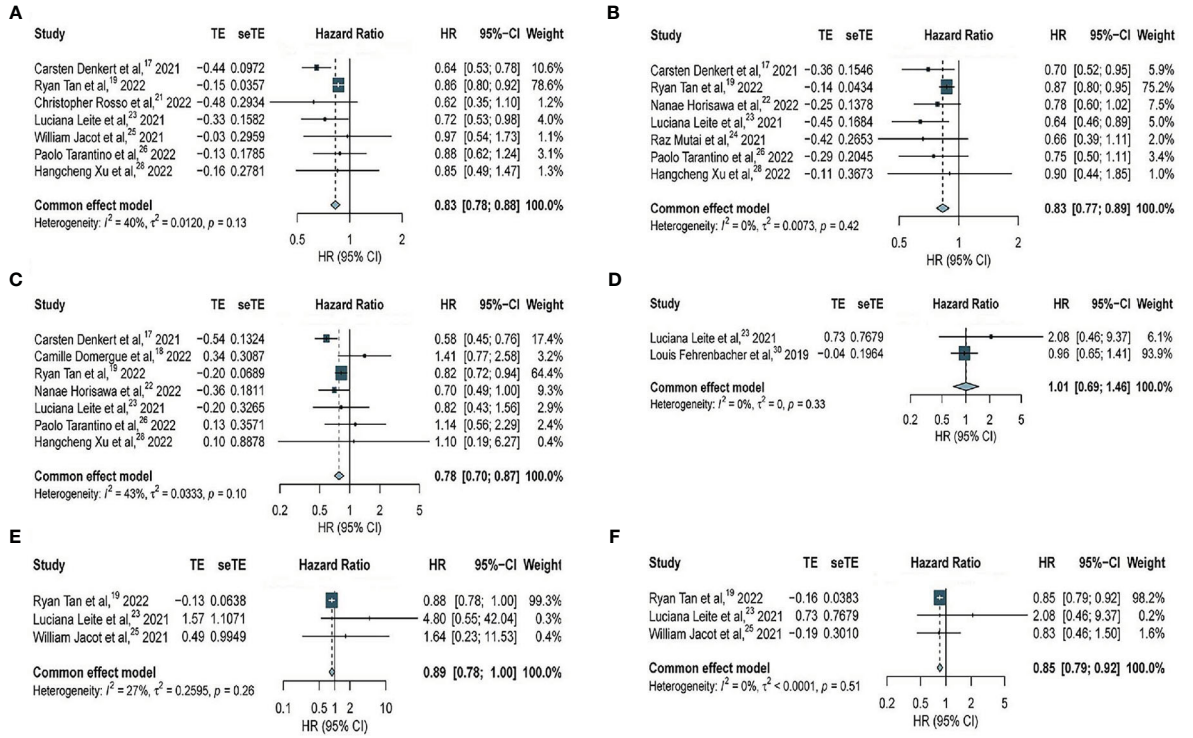

FIGURE 2
Forest plot of **(A)** OS in overall EBC population (HER2 low vs. HER2 0); **(B)** OS in hormone receptor positive subgroup (HER2 low vs. HER2 0); **(C)** OS in TNBC subgroup (HER2 low vs. HER2 0); **(D)** OS in overall EBC population (HER2 IHC 2 vs. IHC 1); **(E)** OS in overall EBC population (HER2 IHC 2 vs. IHC 0); **(F)** OS in overall EBC population (HER2 IHC 1 vs. IHC 0).

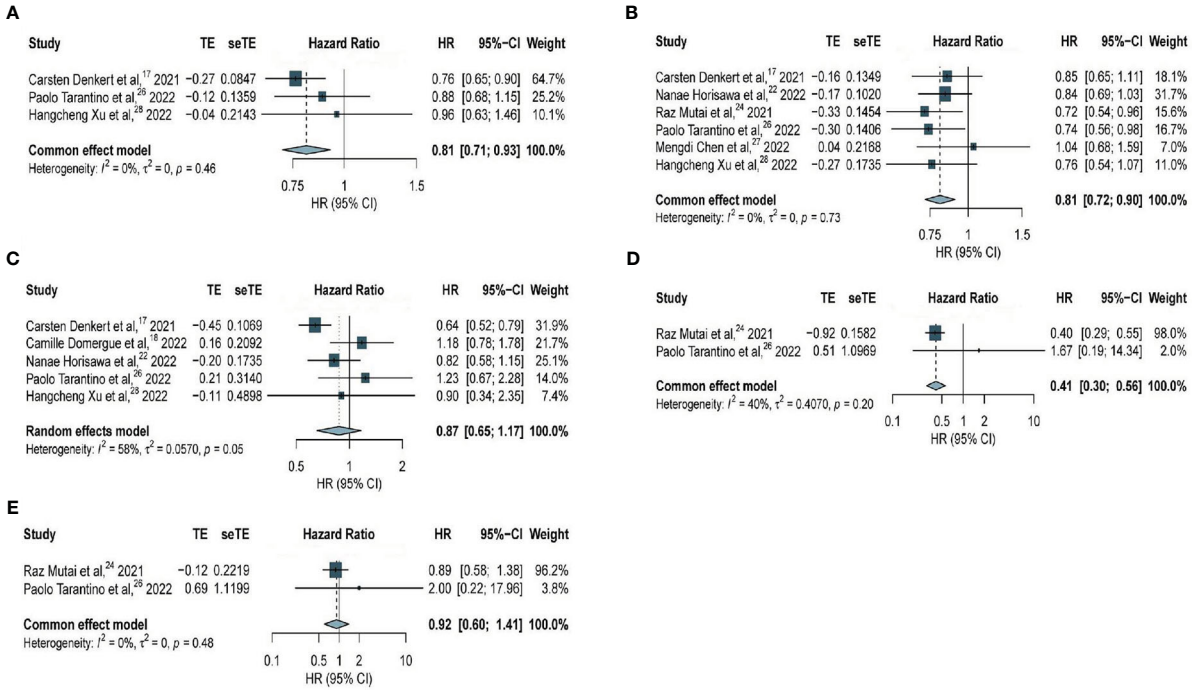

FIGURE 3
Forest plot of **(A)** DFS in overall EBC population (HER2 low vs. HER2 0); **(B)** DFS in hormone receptor positive subgroup (HER2 low vs. HER2 0); **(C)** DFS in TNBC subgroup (HER2 low vs. HER2 0); **(D)** DFS in high genetic risk EBC population (HER2 low vs. HER2 0); **(E)** DFS in low genetic risk EBC population (HER2 low vs. HER2 0).

BC (HR, 0.87; 95% CI, 0.65–1.17) based on five studies with 2535 patients, and this outcome was statistically insignificant within a very heterogeneous study group (I^2 = 58%, P = 0.05) (Figure 3C).

Further analyses were performed to determine whether genetic risk was associated with DFS among all patients. Among all patients with high genetic risk, significantly longer DFS was observed among patients with HER2-low expression compared with that in patients with HER2-zero BC (HR, 0.41; 95% CI, 0.30–0.56) based on data obtained from two studies with 392 patients, with no considerable heterogeneity observed (I^2 = 40%; P = 0.20) (Figure 3D). Among all patients with low genetic risk, data obtained from two studies with 1956 patients revealed no significant difference in DFS between patients with HER2-low expression and HER2-zero BC (HR, 0.92; 95% CI, 0.60–1.41), with no considerable heterogeneity observed (I^2 =0%; P = 0.48) (Figure 3E).

The same association was observed for DDFS. No significant difference was observed in patients with low genetic risk based on two studies with 1956 patients (Supplementary Figure 1B), whereas patients with HER2-low expression had significantly better DDFS compared with that in patients with high genetic risk based on two studies with 392 patients (Supplementary Figure 1C).

3.4 RFS

Among all patients, patients with HER2-low expression had significantly longer RFS compared with that in patients with HER2-zero BC (HR, 0.90; 95% CI, 0.85–0.95) based on four studies with 30380 patients, with no considerable heterogeneity observed (I^2 = 0%; P = 0.62) (Figure 4A). Regarding patients with hormone receptor-positive BC, our analysis of two studies with 24045 patients revealed significantly longer RFS among patients with HER2-low expression compared with that in patients with HER2-zero BC (HR, 0.90; 95% CI, 0.84–0.96), with no considerable heterogeneity observed (I^2 = 0%; P = 0.65) (Figure 4B). However, among patients with TNBC, no significant difference was seen in patients with HER2-low expression and HER2-zero BC (HR, 0.80; 95% CI, 0.55–1.16) based on two studies with 4947 patients and within a very heterogeneous study group (I^2 = 68%, P = 0.053) (Figure 4C).

An analysis of the association of HER2-zero, HER2 IHC 1+, and HER2 IHC 2+ BC with RFS was performed. Among all patients, data obtained from four studies with 20884 patients revealed no significant difference in RFS between patients with HER2 IHC 2+ and HER2-zero BC (HR, 0.95; 95% CI, 0.86–1.05), with no considerable heterogeneity observed (I^2 = 0%; P = 0.53) (Figure 4D). However, significantly longer RFS was observed in patients with HER2 IHC 1+ than that in patients with HER2-zero BC (HR, 0.90; 95% CI, 0.84–0.96) based on four studies with 26699 patients, with no considerable heterogeneity observed (I^2 = 0%; P = 0.61) (Figure 4E).

It's worth mentioning that sensitivity analyses were performed for each of these analyses (Supplementary Figures 2-4). The sensitivity analysis suggested that the study by Denkert et al. (17) was the source of heterogeneity in the analysis of TNBC. Denkert et al. (17) is a pool analysis of four RCTs, and different study design types may be the source of heterogeneity. Therefore, the analysis was performed again after removing the study by Denkert et al. (17), and the results showed that HER2-low expression was still significantly associated with longer OS (HR, 0.83; 95% CI, 0.74–0.94), with low heterogeneity observed across studies (I^2 = 0%; P = 0.45), consistent with our previous results (Supplementary Figure 5A). And HER2-low expression was still significantly associated with longer DFS (HR, 0.99; 95% CI, 0.77–1.28), with low heterogeneity observed across studies (I^2 =0%; P = 0.49) which is consistent with the results of the analysis of keeping the study by Denkert et al. (17) (Supplementary Figure 5B). Besides, Egger test

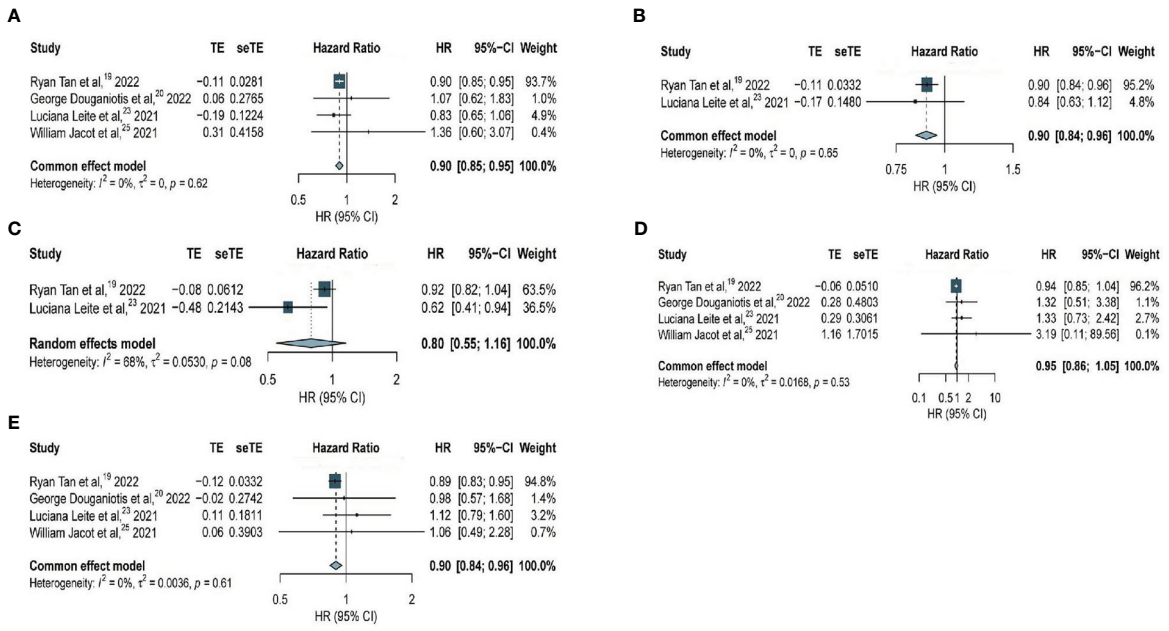

FIGURE 4
Forest plot of **(A)** RFS in overall EBC population (HER2 low vs. HER2 0); **(B)** RFS in hormone receptor positive subgroup (HER2 low vs. HER2 0); **(C)** RFS in TNBC subgroup (HER2 low vs. HER2 0); **(D)** RFS in overall EBC population (HER2 IHC 2 vs. IHC 0); **(E)** RFS in overall EBC population (HER2 IHC 1 vs. IHC 0).

was used for funnel plot asymmetry and no significant publication bias was found except the analysis for OS in patients with hormone-receptor positive BC (Supplementary Figure 6).

4 Discussion

Recently, the remarkable therapeutic effect of novel ADCs on HER2-low expression BC has generated great interest for this new subtype. Nevertheless, the prognosis of HER2-low expression BC remains controversial. In our systematic review and meta-analysis of the published data, the prognostic difference between patients with HER2-low expression and HER2-zero BC was analyzed among patients with early-stage BC, both in the overall population and hormone receptor-positive and TNBC subgroups.

We found that compared with patients with HER2-zero BC, patients with HER2-low expression BC had better OS, DFS, and RFS both in the overall population and hormone receptor-positive subgroup, suggesting distinct biological subtype of HER2-low expression. In the TNBC subgroup, OS was superior in patients with HER2-low expression compared with that for patients with HER2-zero BC, whereas no significant differences in DFS and RFS were observed between patients with HER2-low expression and HER2-zero BC.

Among all patients with BC, significantly longer OS and RFS was observed in patients with HER2 IHC 1+ compared with that in patients with HER2-zero BC. However, no significant difference in OS and RFS was observed between patients with HER2 IHC 2+ and HER2-zero BC. No significant difference in OS was observed between patients with HER2 IHC 1+ and HER2-zero BC. The prognostic difference in RFS between patients with HER2 IHC 1+ and HER2-zero BC was not analyzed because of data leakage.

In addition, the Oncotype Dx risk score was compared between HER2-low expression and HER2-zero BC among patients with hormone receptor-positive BC (24, 26). Interestingly, the prognosis of HER2-low expression BC differs significantly in patients with high genetic risk (Oncotype Dx risk score > 26), although not for patients with low genetic risk (Oncotype Dx risk score ≤ 25). In early hormone receptor-positive BC with high genomic risk, HER2-low expression was associated with more favorable DFS and DDFS compared with that for HER2-zero BC. However, for early hormone receptor-positive BC with low genomic risk, no significant differences were observed in DFS or DDFS between patients with HER2-low expression and HER2-zero BC.

The findings of a recent study involving 30491 patients support that HER2-low expression has a better prognosis than that of HER2-zero BC, although this study used BC-specific survival as an outcome indicator, which was not included in our meta-analysis. This conclusion is consistent with our findings and further validates our conclusion (8).

Several reasons may explain why HER2-low expression has a more favorable prognosis in patients with hormone receptor-positive BC, whereas only OS was superior in patients with TNBC. First, the PAM50 intrinsic subtype profiles of HER2-low expression BC were demonstrated in a recent study (9), which concluded that in hormone receptor-positive BC, the gene expression of patients with HER2-zero and HER2-low expression tumors was obviously different. However, no significant difference in gene expression was observed between HER2-zero and HER2-low expression in patients with TNBC. This indicates that HER2-low expression is more likely to be a distinct biological entity in hormone receptor-positive than in TNBC tumors.

What's more, several studies have reported an association between hormone receptor status and HER2-low expression. The percentage of HER2-low expression differed between the hormone receptor-positive and TNBC groups. Interestingly, the prevalence of HER2-low expression was higher in patients with hormone receptor-positive BC than that in TNBC (9, 31). HER2-low expression BC tends to be luminal-like with high estrogen receptor (ER) expression, whereas HER2-zero BC is generally more basal-like, with low ER expression (26). ER expression may play a confounding role when analyzing the prognostic difference between patients with HER2-low expression and HER2-zero BC in some studies. However, this hypothesis requires further statistical analysis and verification in future studies.

Further, HER2-low expression BC is reportedly associated with indicators of decreased aggressiveness, such as lower histological grade, lower Ki-67 status, and fewer TP53 mutations compared with that of HER2-zero BC (17). Whether the prognostic differences are driven by HER2-low expression or the varying distribution of other clinicopathological characteristics, such as ER expression, requires further investigation.

HER2-low expression and HER2-zero BC vary in the somatic mutation landscape. In patients with HER2-low expression BC, the frequency of phosphatidylinositol 3-kinase/protein kinase B signaling mutations was higher, and the frequency of p53 signaling and cell cycle pathway mutations was lower. This conclusion supports that HER2-low expression and HER2-zero BC are two different entities (10). Another study with similar findings reported that PIK3CA and TP53 mutation frequencies differed between patients with HER2-low expression and HER2-zero BC. Moreover, BRCA1/2 and other BC predisposition gene mutations have different frequencies (17). Studies on the PAM50 intrinsic subtype also found significant differences in gene expression between HER2-low expression and HER2-zero among patients with hormone receptor-positive BC, although no significant differences were observed in patients with TNBC (9). Further studies are needed to verify whether different gene expression profiles lead to different prognoses, and whether these differences are sufficient for classification into independent molecular subtypes.

Owing to the promising future of ADCs in treating HER2-low expression BC, researchers are conducting clinical trials to investigate the therapeutic effect of advanced treatment with novel ADCs in patients with early stage BC. However, we observed significant survival differences between patients with HER2-low expression and HER2-zero BC. This study suggests the possibility that patients with HER2-low expression BC may receive de-escalated treatment to achieve the desired therapeutic effect, which could guide the design of future clinical trials. Our results provide new directions for future research.

Many studies have found poor concordance between different pathologists when using IHC to assess HER2-low expression and HER2-zero BC. One study found that there was only 26% agreement when IHC was used to assess low levels of HER2 (i.e., IHC 0 and IHC 1+) (32). The phase 1b trastuzumab deruxtecan study reported consistency of 40% for HER2 IHC 2+ and 70% for HER2 IHC 1+ between local and central pathology reports (33). This suggests that

pathologists need to use more accurate methods to distinguish HER2-low expression from HER2-zero in the future, such as the detection of mRNA expression or quantitative automated chemistry.

5 Limitations

Several limitations of this study should be considered when interpreting the results. First of all, considering the accessibility and quality of the available literature, only studies published in English were included in our study. Considering the integrity of the data, conference reports were not included in the report, which potentially affected the interpretation of our results. Secondly, to fully utilize the data, if K-M curves were provided without HRs, we used Engauge Digitizer to extract data from K-M curves and calculated the HRs using practical methods and a small data set. Owing to inevitable human errors when using measurement tools, a certain degree of deviation might exist between the extracted and real HRs. Thirdly, this meta-analysis included retrospective studies, RCT, and pool analysis, which may have increased the heterogeneity among studies. And the only RCT (30) was treated as a cohort study and the RCT was assessed using the Newcastle-Ottawa Scale. In the analysis of OS and DFS among patients with TNBC, the heterogeneity was large (with I^2 values of 43% and 58%, respectively). To reduce the impact of this possible heterogeneity on the results, the sensitivity analysis was performed. After the sensitivity analysis, we concluded that the main source of heterogeneity was from the included pool analysis study (17). Therefore, we excluded this article and conducted another meta-analysis of patients with TNBC with OS and DFS as outcome indicators. Nevertheless, we reached similar conclusions. In addition, there were relatively small number of studies for each analysis which limited further analysis for whether the length of follow-up duration or the different therapy method have influence on the conclusion. Further studies are needed. Lastly, in the analysis of the prognostic differences among genetic risk types and HER2 IHC groups, the number of included studies was small. The sensitivity analysis was conducted to evaluate the robustness of the meta-analysis results.

Despite these limitations, we believe that this analysis provides significant implications for future treatment strategies and research directions.

6 Conclusion

Whether HER2 low is a prognostic factor in early BC is widely discussed and has attracted the attention of many scholars. Nevertheless, the prognosis of HER2-low expression BC is still controversial at present. Therefore, the study aimed to evaluate the prognostic significance of HER2-low expression in patients with BC. Overall, this meta-analysis revealed that among patients with early-stage BC, patients with HER2-low expression BC had better OS in the overall population and hormone receptor-positive and TNBC subgroups. In particular, favorable DFS and RFS were observed in both the overall population and hormone receptor-positive subgroup. The results of this meta-analysis support that there are distinct subtypes of HER2-low expression BC, although further studies are necessary to verify whether differences in genetic profiles are sufficient for classification into independent molecular subtypes. The results of this meta-analysis will deepen the general understanding of HER2-low expression BC and have important implications for future therapeutic strategies.

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

TW, DW, SG, and PY contributed to the conception of the study, performed the data analyses and wrote the manuscript. XW, JY, YK, JJ, ZY, and YS helped perform the analysis with constructive discussions. All authors contributed to the article and approved the submitted version.

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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.2023.1100332/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Forest plot of **(A)** DDFS in hormone receptor positive subgroup (HER2 low vs. HER2 0) **(B)** DDFS in low genetic risk EBC population (HER2 low vs. HER2 0) **(C)** DDFS in high genetic risk EBC population (HER2 low vs. HER2 0)

SUPPLEMENTARY FIGURE 2
Sensitivity analysis of **(A)** OS in overall EBC population (HER2 low vs. HER2 0); **(B)** OS in hormone receptor positive subgroup (HER2 low vs. HER2 0); **(C)** OS in TNBC subgroup (HER2 low vs. HER2 0); **(D)** OS in overall EBC

population (HER2 IHC 2 vs. IHC 0); **(E)** OS in overall EBC population (HER2 IHC 1 vs. IHC 0).

SUPPLEMENTARY FIGURE 3

Sensitivity analysis of **(A)** DFS in overall EBC population (HER2 low vs. HER2 0); **(B)** DFS in hormone receptor positive subgroup (HER2 low vs. HER2 0); **(C)** DFS in TNBC subgroup (HER2 low vs. HER2 0).

SUPPLEMENTARY FIGURE 4

Sensitivity analysis of **(A)** RFS in overall EBC population (HER2 low vs. HER2 0); **(B)** RFS in overall EBC population (HER2 IHC 2 vs. IHC 0); **(C)** RFS in overall EBC population (HER2 IHC 1 vs. IHC 0).

SUPPLEMENTARY FIGURE 5

Forest plot of **(A)** OS in TNBC subgroup (HER2 low vs. HER2 0) removed an article with potential heterogeneity; **(B)** DFS in TNBC subgroup (HER2 low vs. HER2 0) removed an article with potential heterogeneity;

SUPPLEMENTARY FIGURE 6

Egger test of **(A)** OS in overall EBC population (HER2 low vs. HER2 0), p=0.3675; **(B)** OS in hormone receptor positive subgroup (HER2 low vs. HER2 0), p=0.0435; **(C)** OS in TNBC subgroup (HER2 low vs. HER2 0), p=0.5758; **(D)** DFS in hormone receptor positive subgroup (HER2 low vs. HER2 0), p=0.7471; **(E)** DFS in TNBC subgroup (HER2 low vs. HER2 0), p=0.1468.

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. von Minckwitz G, Huang CS, 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(7):617–28. doi: 10.1056/NEJMoa1814017

3. Piccart M, Procter M, Fumagalli D, de Azambuja E, Clark E, Ewer MS, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer in the APHINITY trial: 6 years' follow-up. *J Clin Oncol* (2021) 39(13):1448–57. doi: 10.1200/JCO.20.01204

4. Tarantino P, Hamilton E, Tolaney SM, Cortes J, Morganti S, Ferraro E, et al. HER2-low breast cancer: Pathological and clinical landscape. *J Clin Oncol* (2020) 38(17):1951–62. doi: 10.1200/JCO.19.02488

5. Tamura K, Tsurutani J, Takahashi S, Iwata H, Krop IE, Redfern C, et al. Trastuzumab deruxtecan (DS-8201a) in patients with advanced HER2-positive breast cancer previously treated with trastuzumab emtansine: a dose-expansion, phase 1 study. *Lancet Oncol* (2019) 20(6):816–26. doi: 10.1016/S1470-2045(19)30097-X

6. Modi S, Saura C, Yamashita T, Park YH, Kim SB, Tamura K, et al. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. *N Engl J Med* (2020) 382 (7):610–21. doi: 10.1056/NEJMoa1914510

7. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *New Engl J Med* (2022). doi: 10.1056/NEJMoa2203690

8. Won HS, Ahn J, Kim Y, Kim JS, Song JY, Kim HK, et al. Clinical significance of HER2-low expression in early breast cancer: a nationwide study from the Korean breast cancer society. *Breast Cancer Res* (2022) 24(1):22. doi: 10.1186/s13058-022-01519-x

9. Schettini F, Chic N, Brasó-Maristany F, Paré L, Pascual T, Conte B, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. *NPJ Breast Cancer* (2021) 7(1):1. doi: 10.1038/s41523-020-00208-2

10. Zhang G, Ren C, Li C, Wang Y, Chen B, Wen L, et al. Distinct clinical and somatic mutational features of breast tumors with high-, low-, or non-expressing human epidermal growth factor receptor 2 status. *BMC Med* (2022) 20(1):142. doi: 10.1186/s12916-022-02346-9

11. Tarantino P, Gandini S, Nicolò E, Trillo P, Giugliano F, Zagami P, et al. Evolution of low HER2 expression between early and advanced-stage breast cancer. *Eur J Cancer* (2022) 163:35–43. doi: 10.1016/j.ejca.2021.12.022

12. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PloS Med* (2009) 6(7):e1000097.

13. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj* (2021) 372:n71.

14. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* (2007) 8:16. doi: 10.1186/1745-6215-8-16

15. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* (2010) 25(9):603–5. doi: 10.1007/s10654-010-9491-z

16. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *Bmj* (1997) 315(7109):629–34. doi: 10.1136/bmj.315.7109.629

17. Denkert C, Seither F, Schneeweiss A, Link T, Blohmer JU, Just M, et al. Clinical and molecular characteristics of HER2-low-positive breast cancer: pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. *Lancet Oncol* (2021) 22(8):1151–61. doi: 10.1016/S1470-2045(21)00301-6

18. Domergue C, Martin E, Lemarié C, Jézéquel P, Frenel JS, Augereau P, et al. Impact of HER2 status on pathological response after neoadjuvant chemotherapy in early triple-negative breast cancer. *Cancers (Basel)* (2022) 14(10). doi: 10.3390/cancers14102509

19. Tan R, Ong WS, Lee KH, Lim AH, Park S, Park YH, et al. HER2 expression, copy number variation and survival outcomes in HER2-low non-metastatic breast cancer: an international multicentre cohort study and TCGA-METABRIC analysis. *BMC Med* (2022) 20(1):105. doi: 10.1186/s12916-022-02284-6

20. Douganiotis G, Kontovinis L, Markopoulou E, Ainali A, Zarampoukas T, Natsiopoulos I, et al. Prognostic significance of low HER2 expression in patients with early hormone receptor positive breast cancer. *Cancer Diagn Progn* (2022) 2(3):316–23. doi: 10.21873/cdp.10111

21. Rosso C, Voutsadakis IA. Characteristics, clinical differences and outcomes of breast cancer patients with negative or low HER2 expression. *Clin Breast Cancer* (2022) 22 (4):391–7. doi: 10.1016/j.clbc.2022.02.008

22. Horisawa N, Adachi Y, Takatsuka D, Nozawa K, Endo Y, Ozaki Y, et al. The frequency of low HER2 expression in breast cancer and a comparison of prognosis between patients with HER2-low and HER2-negative breast cancer by HR status. *Breast Cancer (Tokyo Japan)* (2022) 29(2):234–41. doi: 10.1007/s12282-021-01303-3

23. de Moura Leite L, Cesca MG, Tavares MC, Santana DM, Saldanha EF, Guimarães PT, et al. HER2-low status and response to neoadjuvant chemotherapy in HER2 negative early breast cancer. *Breast Cancer Res Treat* (2021) 190(1):155–63. doi: 10.1007/s10549-021-06365-7

24. Mutai R, Barkan T, Moore A, Sarfaty M, Shochat T, Yerushalmi R, et al. Prognostic impact of HER2-low expression in hormone receptor positive early breast cancer. *Breast* (2021) 60:62–9. doi: 10.1016/j.breast.2021.08.016

25. Jacot W, Maran-Gonzalez A, Massol O, Sorbs C, Mollevi C, Guiu S, et al. Prognostic value of HER2-low expression in non-metastatic triple-negative breast cancer and correlation with other biomarkers. *Cancers (Basel)* (2021) 13(23). doi: 10.3390/cancers13236059

26. Tarantino P, Jin Q, Tayob N, Jeselsohn RM, Schnitt SJ, Vincuilla J, et al. Prognostic and biologic significance of ERBB2-low expression in early-stage breast cancer. *JAMA Oncol* (2022) 8(8):1177–83. doi: 10.1001/jamaoncol.2022.2286

27. Chen M, Chen W, Liu D, Chen W, Shen K, Wu J, et al. Prognostic values of clinical and molecular features in HER2 low-breast cancer with hormonal receptor overexpression: features of HER2-low breast cancer. *Breast Cancer (Tokyo Japan)* (2022) 29(5):844–53. doi: 10.1007/s12282-022-01364-y

28. Xu H, Han Y, Wu Y, Wang Y, Li Q, Zhang P, et al. Clinicopathological characteristics and prognosis of HER2-low early-stage breast cancer: A single-institution experience. *Front Oncol* (2022) 12:906011. doi: 10.3389/fonc.2022.906011

29. Kim MH, Kim GM, Kim JH, Kim JY, Park HS, Park S, et al. Intermediate HER2 expression is associated with poor prognosis in estrogen receptor-positive breast cancer patients aged 55 years and older. *Breast Cancer Res Treat* (2020) 179(3):687–97. doi: 10.1007/s10549-019-05505-4

30. Fehrenbacher L, Cecchini RS, Geyer CEJr., Rastogi P, Costantino JP, Atkins JN, et al. NSABP b-47/NRG oncology phase III randomized trial comparing adjuvant chemotherapy with or without trastuzumab in high-risk invasive breast cancer negative for HER2 by FISH and with IHC 1+ or 2. *J Clin Oncol* (2020) 38(5):444–53. doi: 10.1200/JCO.19.01455

31. Miglietta F, Griguolo G, Bottosso M, Giarratano T, Lo Mele M, Fassan M, et al. Evolution of HER2-low expression from primary to recurrent breast cancer. *NPJ Breast Cancer* (2021) 7(1):137. doi: 10.1038/s41523-021-00343-4

32. Fernandez AI, Liu M, Bellizzi A, Brock J, Fadare O, Hanley K, et al. Examination of low ERBB2 protein expression in breast cancer tissue. *JAMA Oncol* (2022) 8(4):1–4. doi: 10.1001/jamaoncol.2021.7239

33. Modi S, Park H, Murthy RK, Iwata H, Tamura K, Tsurutani J, et al. Antitumor activity and safety of trastuzumab deruxtecan in patients with HER2-Low-Expressing advanced breast cancer: Results from a phase ib study. *J Clin Oncol* (2020) 38(17):1887–96. doi: 10.1200/JCO.19.02318

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Efficacy and safety of treatment regimens for patients with metastatic, locally advanced, or recurrent breast cancer carrying *BRCA1/BRCA2* pathogenic variants: A network meta-analysis

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Objective: Patients with breast cancer carrying *BRCA1* and *BRCA2* genetic alterations show poor prognoses. However, the efficacy of pharmacotherapies for patients with advanced breast cancer carrying *BRCA1/2* pathogenic variants remains unclear. This study aimed to conduct a network meta-analysis to assess the efficacy and safety of various pharmacotherapies for patients with metastatic, locally advanced, or recurrent breast cancer carrying *BRCA1/BRCA2* pathogenic variants.

Methods: A literature search was conducted using Embase, PubMed, and Cochrane Library (CENTRAL), from inception to 11th May 2022. The references of included articles were screened to identify relevant literature. This network meta-analysis included patients with metastatic locally advanced or recurrent breast cancer who received pharmacotherapy and carried deleterious variants of *BRCA1/2*. The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines were followed for conducting and reporting this systematic meta-analysis. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) method was employed to evaluate evidential certainty. Frequentist random-effect model was applied. Results of objective response rate (ORR), progression-free survival (PFS), overall survival (OS), and rates of any-grade adverse events were presented.

Results: Nine randomized controlled trials were obtained comprising six treatment regimens, including 1912 patients with pathogenic variants of *BRCA1* and *BRCA2*. The orchestration of PARP inhibitors with platinum-based chemotherapy was found to be the most effective with a pooled odds ratio (OR) of 3.52 (95% CI 2.14, 5.78) for ORR; 1.53 (1.34,1.76), 3.05 (1.79, 5.19), and 5.80 (1.42, 23.77) for 3-, 12-, and 24-month PFS, respectively, and 1.04 (1.00, 1.07), 1.76 (1.25, 2.49) and 2.31 (1.41, 3.77) for 3-, 12-, and 36-month OS, respectively compared to those receiving non-platinum-based chemotherapy. However, it posed an elevated risk of some adverse events. Platinum-based chemotherapy alone or PARP inhibitors markedly

improved ORR, PFS, and OS compared to non-platinum-based chemotherapy. Interestingly, platinum-based chemotherapy surpassed PARP inhibitors in terms of efficacy. Evidence on programmed death-ligand 1(PD-L1) inhibitors and sacituzumab govitecan (SG) suggested low quality and insignificant results.

Conclusions: Among all treatment regimens, PARP inhibitors with platinum exhibited the best efficacy, although with a trade-off of elevated risk of some types of adverse events. Future research on direct comparisons between different treatment regimens specifically targeting patients with breast cancer carrying *BRCA1/2* pathogenic variants with a pre-specified adequate sample size is warranted.

KEYWORDS

breast neoplasms, genes, BRCA1, BRCA2, network meta-analysis

Introduction

Breast cancer is the most diagnosed cancer in women and the fifth leading cause of cancer mortality worldwide, with an estimated 685,000 deaths in 2020 (1). Breast cancer is also the leading cause of cancer-related disability-adjusted life years (DALYs) for females globally, as reported in 2019 (2). It has a rapidly rising incidence rate in transitioning countries in South America, Africa, and Asia, as well as high-income Asian countries (1).

Pathogenic variants of breast cancer susceptibility genes 1 or 2 (*BRCA1*/*BRCA2*) reportedly occur in nearly 5% of patients with breast cancer (3, 4). These patients are more likely to have a family history, receive an early diagnosis, or show a worse prognosis, especially at an advanced cancer stage (5, 6). Genetic alterations in *BRCA1*/*BRCA2* cause the weakening of DNA double-strand break (DSB) repair ability, making the tumor cells highly dependent on the pathways involved in single-strand break repair (7, 8). The enzyme, poly(adenosine diphosphate-ribose) polymerase (PARP) crucially controls this pathway, making PARP inhibitors a promising treatment strategy for patients with breast cancer carrying *BRCA1/2* pathogenic variants (9). The U.S. Food and Drug Administration approved two PARP inhibitors, olaparib and talazoparib, as treatment options for patients with metastatic or advanced breast cancer carrying germline *BRCA1/2* pathogenic variants. Other PARP inhibitors have also been tested for breast cancer therapy, including veliparib and niraparib. As a class, PARP inhibitors share some similarities (10). Platinum agents are reportedly more effective for patients with breast cancer carrying germline *BRCA1/2* pathogenic variants (11). These treatments are recommended as preferred treatment options for recurrent or stage IV TNBC in the updated guidelines (12).

Platinum-based chemotherapy and PARP inhibitors are common regimens for patients with breast cancer carrying *BRCA* pathogenic variants. Randomized controlled trials (RCTs) using PARP inhibitors or platinum for treating patients with metastatic, locally advanced, or recurrent breast cancer carrying *BRCA1/2* pathogenic variants have shown efficacy, as evidenced by improved survival duration (13–15). However, the comparative performances of these regimens remain unknown.

A previous network meta-analysis compared the efficacy and safety of various drug regimens for patients with *BRCA*-pathogenic variant-associated breast cancer. However, the primary analysis mixed studies on patients at different disease stages, and no comparative results were provided for patients with advanced breast cancer and *BRCA1/2* pathogenic variants (16).

We undertook this network meta-analysis to assess the efficacy and safety of pharmacotherapies for patients with metastatic, locally advanced, or recurrent breast cancer carrying *BRCA1/2* pathogenic variants.

Methods

The network meta-analysis was performed following the guidelines of the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (17).

Data sources and search strategies

From inception until May 11th, 2022, a systematic literature search was conducted in Embase, PubMed, and Cochrane Library (CENTRAL). To identify relevant studies, we screened the references cited in the included publications. Terms related to breast cancer and its synonyms, *BRCA1/2* pathogenic variants, and RCTs were used (please refer to the detailed search string in Appendix 1).

Study selection and data extraction

Eligibility criteria included the following: (1) studies of patients with advanced or metastatic breast cancer. (2) Studies targeting patients carrying *BRCA1/2* pathogenic variants or those reporting

relevant subgroup results. (3) Studies with chemotherapy or targeted therapies as the treatment strategy. (4) Studies reporting at least one of the following outcomes: objective response rate (ORR), progression-free survival (PFS), or overall survival (OS). (5) Studies with an RCT design.

Exclusion criteria were as follows: (1) studies including patients with *BRCA* methylation. (2) Studies including patients treated with non-platinum-based chemotherapy both in the intervention and control arms. (3) Trials published in languages other than English. Only reports with the most updated results were used to retrieve information for studies derived from the same trial.

The screening was conducted by meticulously reading the titles and abstracts of each potential article, and full texts were scrupulously scrutinized when necessary. The following data were collected: author's names, publication year, study's abbreviation, registration number, sample size, *BRCA* pathogenic variant type, the proportion of TNBC patients, patients' indication, treatment regimens, patients' median age, and efficacy and safety outcomes. Two investigators (ZY and LY) independently conducted study selection and data extraction. Any disparity was adjudicated by a senior reviewer (LW).

Outcomes and measures

Efficacy outcomes included ORR and PFS rates at 3-, 12-, and 24 months, and OS rates at 3-, 12-, 24, and 36 months. Raw data on the number of patients experiencing/not experiencing the outcome were obtained from Kaplan-Meier survival curves. The toxicological effects were measured as rates of any-grade adverse events (thrombocytopenia, neutropenia, anemia, leukopenia, nausea, vomiting, diarrhea, constipation, decreased appetite, fatigue, headache, alopecia, and back pain).

Data analysis and evidential quality assessment

All eligible studies were included in the network meta-analysis utilizing the frequentist method and the random-effects model (18). The network estimates were visualized using net-league tables and forest plots. Odds ratios (ORs) with 95% confidence intervals (CIs) were created to quantify outcomes. The P-score, measuring the degree to which one therapy was guaranteed to be superior compared to its counterparts, was used to rank various treatment regimens (19).

Two independent reviewers (ZY and LY) evaluated the risk of bias in each study using the Cochrane risk of bias tool 2.0 for RCTs. All efficacy outcomes were assessed, and the effect of assignment to intervention was regarded as the effect of interest. The study's overall risk of bias was divided into three categories as follows: low risk of bias if all domains showed low risk; some concerns if there was at least one domain showing some concerns but not at high risk, and high if there was at least one domain at high risk or multiple domains showing some concerns (20). We applied the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach to assess the certainty of the evidence and rated it as high, moderate, low, or very low (21).

Cochran's Q statistic was decomposed into within-design and between-design values to test the heterogeneity (22). Local inconsistency was tested by splitting and comparing indirect and direct effects, and the former estimates were calculated by back-calculation method (23). We also assessed the transitivity by comparing the distributions of potential effect modifiers across treatment regimens. Comparison-adjusted funnel plots were applied to detect publication biases for direct comparisons with treatment ranked by their P-scores (24). Egger's regression and Begg's rank tests were also performed to test for asymmetry in any potential publication biases. To assess the robustness of these results, sensitivity analyses were conducted using the surface under the cumulative ranking curve (SUCRA) values to rank the treatments and excluding studies reporting somatic *BRCA* deleterious variants, as the corresponding patients may not share the same advantage as those carrying germline mutations (12). The R package, netmeta, in R version 4.2.0, was used for data analyses.

Results

We identified 786 records, and after screening the titles and abstracts, 216 reports were retrieved for screening their full-text (Figure 1). Nine RCTs involving 1912 participants with six treatment regimens, including non-platinum-based chemotherapy, platinum-based chemotherapy, PARP inhibitor-containing regimen, PARP inhibitor plus platinum-based chemotherapy, programmed death-ligand 1 (PD-L1) inhibitor, and sacituzumab govitecan (SG), with one multi-arm study, were included. For EMBRACA and OlympiAD studies, additional final analysis reports from updated survival data were included. Finally, 11 reports were included (25–35).

The features of the included studies are summarized in Table 1. The range of publications dated from 2018 to 2021, suggesting recent research attention has been drawn toward *BRCA1/2* deleterious variants. Eight studies explicitly reported the results of patients carrying germline *BRCA* deleterious variants, and one reported a mix of patients carrying germline or somatic mutations. Three of the nine studies targeted only TNBC patients. Eight studies provided the outcome as ORR, while another set of eight studies provided the outcome for survival rate as PFS; seven stated the outcome as the OS rate, and five offered comprehensive information on adverse events included in the analysis.

Figure 2 illustrates the network of available direct comparisons for efficacy outcomes. Network plots for safety outcomes are provided in Appendix 2. Table 2 shows the network meta-analysis results for the efficacy outcomes of eligible trials. Rankings of efficacy outcomes are shown in Table 3.

ORR comparison

In terms of ORR, the treatment regimens containing both PARP inhibitors and platinum-based chemotherapies yielded the best benefit versus SG (OR 1.04, 95% CI 0.11 to 9.93), platinum-based chemotherapy (OR 1.18, 95% CI 0.87 to 1.60), PARP inhibitors (OR 2.09, 1.31 to 3.34), and non-platinum-based chemotherapy (OR 3.52, 95% CI 2.14, 5.78). Additionally, platinum-based chemotherapy markedly improved ORR compared to PARP inhibitors (OR 1.77, 95% CI 1.15 to 2.75) and non-platinum-based chemotherapy (OR

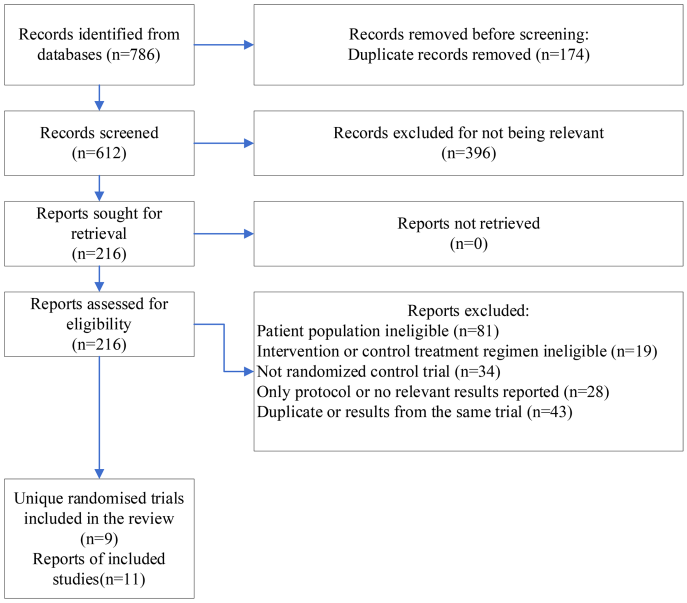

FIGURE 1
PRISMA flowchart describing the study selection process.

1.68, 95% CI 1.24 to 2.28). PARP inhibitors showed a significantly higher ORR compared to non-platinum-based chemotherapy (OR 1.68, 95% CI 1.24 to 2.28).

PFS comparison

For the outcome of PFS, the treatment regimens containing both PARP inhibitor and platinum-based chemotherapy were most likely to be ranked the best among all treatments. The PFS improved significantly at months 3 (OR 1.20, 95% CI 1.08, 1.33), 12 (OR 2.02, 95% CI 1.31, 3.10), and 24 (OR 3.44, 95% CI 1.10, 10.72) with PARP inhibitor plus platinum compared to other regimens comprising PARP inhibitors alone. The orchestration of PARP inhibitors with platinum-based chemotherapy also showed a significantly better PFS than those of the non-platinum-based chemotherapy at months 3 (OR 1.53, 95% CI 1.34,1.76), 12 (OR 3.05, 95% CI 1.79, 5.19), and 24 (OR 5.80, 95% CI 1.42, 23.77). A significant advantage of PARP inhibitor plus platinum over PD-L1 inhibitor was found for 3-month PFS (OR 1.33 95% CI 1.03, 1.71) but not 12-month (OR 2.22, 95% CI 0.83, 5.94) or 24-month (OR 1.52 95% CI 0.19, 12.41) PFS rates. Furthermore, platinum-based chemotherapy showed significantly higher 3-month and 12-month PFS rates than PARP inhibitor alone (OR 1.18, 95% CI 1.07, 1.32; OR 1.79, 95% CI 1.16, 2.76); however, the relative effect was statistically insignificant for 24-month PFS with a wider confidence interval (OR 1.97, 95% CI 0.62, 6.27). Platinum-based chemotherapy also had a higher 3-month PFS than PD-L1 inhibitor (OR 1.32, 95% CI1.02, 1.69), as well as significantly higher 3-month and 12-month PFS rates than non-platinum-based chemotherapy (OR 1.52, 95% CI 1.33, 1.74; OR 2.70, 95% CI 1.58, 4.62). The treatment regimen containing PARP inhibitors alone showed better 3-month PFS (OR 1.28, 95% CI 1.16, 1.41) and 12-month PFS (OR 1.51, 95% CI 1.09, 2.08) rates than non-platinum-based chemotherapy.

OS comparison

In terms of the 3-month OS, treatment regimens containing both PARP inhibitors and platinum-based chemotherapy (OR 1.04, 95% CI 1.00, 1.07), platinum-based chemotherapy (OR 1.03, 95% CI 1.00, 1.06), and the treatment using PARP inhibitors (OR 1.04, 95% CI 1.01, 1.07) were significantly superior to non-platinum-based chemotherapy. For the 12-month OS, the treatment regimens containing both PARP inhibitors and platinum demonstrated a significant advantage over treatment with PARP inhibitors alone (OR 1.22, 95% CI 1.04, 1.42). At month 24, the treatment regimen containing both PARP inhibitors and platinum showed a higher OS rate than treatment with PARP inhibitors alone (OR 1.66, 95% CI 1.24, 2.23) and treatment using non-platinum-based chemotherapy (OR 1.76, 1.25, 2.49). Platinum-based chemotherapy also showed a better 24-month OS compared to PARP inhibitors alone (OR 1.57, 95% CI 1.17, 2.11) and non-platinum-based chemotherapy (OR 1.66, 95% CI 1.18, 2.36). For 36-month OS, only four treatments were included in the analysis. The treatment regimen containing both PARP inhibitors and platinum showed a significantly higher 36-month OS rate versus platinum-based chemotherapy (OR 1.21, 95% CI 1.01, 1.46), PARP inhibitors (OR 1.77, 95% CI 1.19, 2.63), and non-platinum-based chemotherapy (OR 2.31, 95% CI 1.41, 3.77). Platinum-based chemotherapy also showed a better 36-month OS compared to non-platinum-based chemotherapy (OR 1.91, 95% CI 1.16, 3.13).

Results from the IMpassion130 trial, whereby some of the patients carried somatic *BRCA* variants, were excluded from the sensitivity

TABLE 1 Characteristics of the included studies.

Study	Study abbreviation	Registration number	Sample size	*BRCA* pathogenic variant type	Proportion of TNBC*	Patients' indication	Intervention	Control	Outcomes
A. Bardia 2021 (25)	ASCENT	NCT02574455	34	Germline	0.68	metastatic breast cancer	Sacituzumab govitecan	Non-platinum-based chemotherapy	ORR
Nicholas C. Turner 2021 (26)	BRAVO	NCT01905592	206	Germline	0.54	advanced breast cancer	PARP inhibitor	Non-platinum-based chemotherapy	ORR; PFS; OS
Leisha A. Emens 2021 (27)	IMpassion130	NCT02425891	89	Germline or somatic	1	advanced breast cancer	PD-L1 inhibitor	Non-platinum-based chemotherapy	PFS; OS
Véronique Diéras 2020 (28)	BROCADE3	NCT02163694	509	Germline	0.57	metastatic or locally advanced breast cancer	PARP inhibitor + Platinum	Platinum-based chemotherapy	ORR; PFS; OS
J. K. Litton 2020 (29); J. K. Litton 2018 (30)	EMBRACA	NCT01945775	431	Germline	0.44	locally advanced breast cancer or metastatic breast cancer	PARP inhibitor	Non-platinum-based chemotherapy	ORR; PFS; OS
M.E.Robson 2019 (31); Mark Robson 2017 (32)	OlympiAD	NCT02000622	302	Germline	0.5	metastatic breast cancer	PARP inhibitor	Non-platinum-based chemotherapy	ORR; PFS; OS
Andrew Tutt 2018 (33)	TNT	NCT00532727	43	Germline	1	advanced breast cancer	Platinum-based chemotherapy	Non-platinum-based chemotherapy	ORR; PFS; OS
H. S. Han 2018 (34)	BROCADE	NCT01506609	284	Germline	0.41	locally recurrent or metastatic breast cancer	Arm1: PARP inhibitor + Platinum; arm2: PARP inhibitor	Platinum-based chemotherapy	ORR; PFS; OS
J.Zhang 2018 (35)	CBCSG006	NCT01287624	14	Germline	1	metastatic breast cancer	Platinum-based chemotherapy	Non-platinum-based chemotherapy	ORR; PFS

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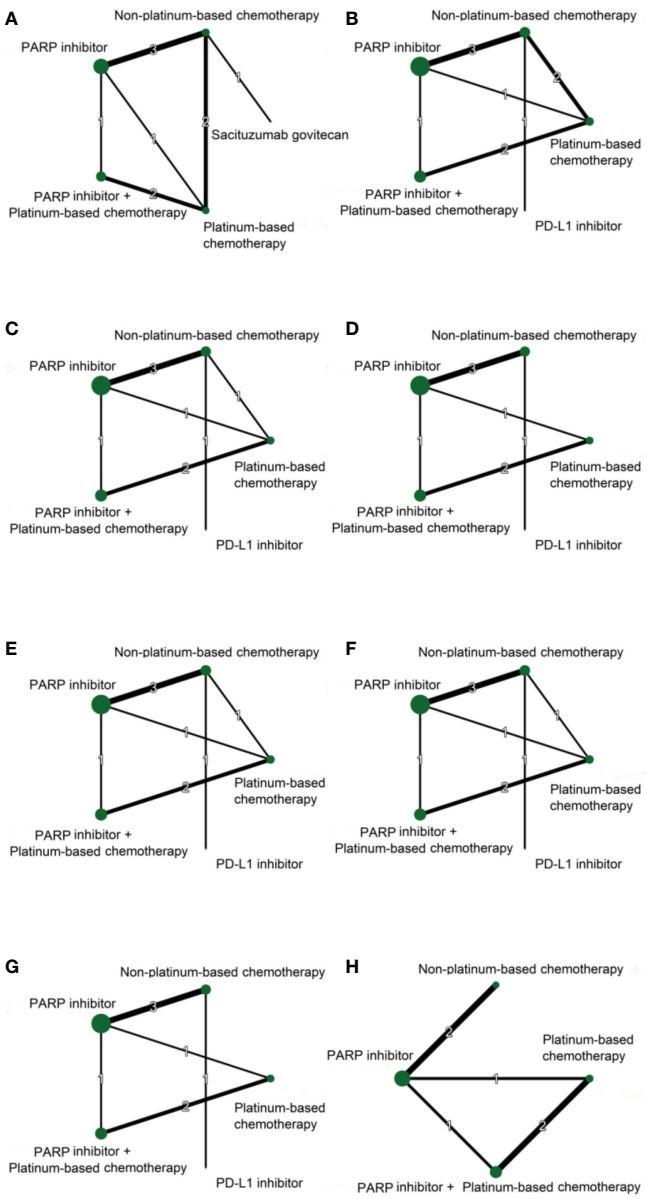

FIGURE 2
Network plots of direct comparisons for ORR **(A)**, 3-month PFS **(B)**, 12-month PFS **(C)**, 24-month PFS **(D)**, 3-month OS **(E)**, 12-month OS **(F)**, 24-month OS **(G)**, and 36-month OS **(H)** Each node represents a treatment regimen. The thickness of the lines is related to the number of randomized trials that included relevant direct comparisons, and the size of the nodes is proportional to the number of individuals allocated to the corresponding intervention group.

TABLE 2 League tables of network estimates of odds ratios for efficacy outcome analyses.

(A) objective response rate				
PARP inhibitor + Platinum-based chemotherapy				
1.04 (0.11, 9.93)ǂ	Sacituzumab govitecan			
1.18 (0.87, 1.60)§	1.13 (0.12, 10.70)ǂ	Platinum-based chemotherapy		
2.09 (1.31, 3.34)§	2.01 (0.22, 18.51)ǂ	**1.77 (1.15, 2.75)§**	PARP inhibitor	
3.52 (2.14, 5.78)‡	3.38 (0.37, 30.43)ǂ	**2.98 (1.89, 4.69)‡**	**1.68 (1.24, 2.28)‡**	Non-platinum-based chemotherapy

(Continued)

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TABLE 2 Continued

(B) 3-month PFS(lower triangle); 12-month PFS(upper triangle)				
PARP inhibitor + Platinum-based chemotherapy	1.13 (0.88, 1.44)§	**2.02 (1.31, 3.10)§**	2.22 (0.83, 5.94)ǂ	**3.05 (1.79, 5.19)ǂ**
1.01 (0.98, 1.05)§	Platinum-based chemotherapy	**1.79 (1.16, 2.76)§**	1.97 (0.73, 5.28)ǂ	**2.70 (1.58, 4.62)ǂ**
1.20 (1.08, 1.33)§	**1.18 (1.07, 1.32)§**	PARP inhibitor	1.10 (0.45, 2.68)ǂ	**1.51 (1.09, 2.08)ǂ**
1.33 (1.03, 1.71)ǂ	**1.32 (1.02, 1.69)ǂ**	1.11 (0.88, 1.40)ǂ	PD-L1 inhibitor	1.37 (0.60, 3.14)ǂ
1.53 (1.34, 1.76)ǂ	**1.52 (1.33, 1.74)ǂ**	**1.28 (1.16, 1.41)ǂ**	1.15 (0.93, 1.43)ǂ	Non-platinum-based chemotherapy
(C) 24-month PFS(lower triangle); 3-month OS(upper triangle)				
PARP inhibitor + Platinum-based chemotherapy	1.05 (0.95, 1.16)ǂ	1.01 (0.99, 1.02)§	1.00 (0.98, 1.02)§	**1.04 (1.00, 1.07)ǂ**
1.52 (0.19, 12.41)*	PD-L1 inhibitor	0.96 (0.87, 1.06)ǂ	0.95 (0.86, 1.05)ǂ	0.99 (0.90, 1.08)ǂ
1.75 (0.84, 3.63)§	1.15 (0.14, 9.46)ǂ	Platinum-based chemotherapy	0.99 (0.97, 1.01)§	**1.03 (1.00, 1.06)ǂ**
3.44 (1.10, 10.72)ǂ	2.26 (0.39, 13.17)*	1.97 (0.62, 6.27)§	PARP nhibitor	**1.04 (1.01, 1.07)ǂ**
5.80 (1.42, 23.77)ǂ	3.81 (0.81, 18.04)*	3.32 (0.80, 13.84)*	1.69 (0.73, 3.88)ǂ	Non-platinum-based chemotherapy
(D) 12-month OS(lower triangle); 24 month OS(upper triangle)				
PARP inhibitor + Platinum-based chemotherapy	1.06 (0.94, 1.20)§	1.16 (0.66, 2.05)ǂ	**1.76 (1.25, 2.49)ǂ**	**1.66 (1.24, 2.23)§**
1.06 (0.96, 1.16)§	Platinum-based chemotherapy	1.09 (0.62, 1.94)ǂ	**1.66 (1.18, 2.36)ǂ**	**1.57 (1.17, 2.11)§**
1.09 (0.81, 1.48)ǂ	1.03 (0.76, 1.40)ǂ	PD-L1 inhibitor	1.52 (0.97, 2.39)ǂ	1.43 (0.88, 2.33)ǂ
1.16 (0.98, 1.39)ǂ	1.10 (0.93, 1.31)ǂ	1.07 (0.83, 1.37)ǂ	Non-platinum-based chemotherapy	0.94 (0.79, 1.13)ǂ
1.22 (1.04, 1.42)§	1.15 (0.99, 1.35)§	1.12 (0.85, 1.46)ǂ	1.05 (0.94, 1.16)ǂ	PARP inhibitor
(E) 36-month OS				
PARP inhibitor + Platinum-based chemotherapy				
1.21 (1.01, 1.46)§	Platinum-based chemotherapy			
1.77 (1.19, 2.63)§	1.46 (0.97, 2.19)§	PARP inhibitor		
2.31 (1.41, 3.77)§	**1.91 (1.16, 3.13)§**	1.31 (0.98, 1.74)§	Non-platinum-based chemotherapy	

The relative effects are measured as OR and 95% CI. All tables list the treatments in the order of p-scores of the treatments for the outcome in the lower triangle. According to GRADE, the certainty of evidence was classified as *very low, ǂlow, ǂmoderate, and §high.
The bold values are the values with statistical significance.

analysis. The principal findings were supported by the results of our sensitivity analysis (Appendix 9).

Safety analysis

Figure 3 shows the network estimates of ORs for adverse events of any grade. Compared to non-platinum-based chemotherapy, the treatment regimen of PARP inhibitor plus platinum had a significantly higher OR for thrombocytopenia (OR 2.96, 95% CI 2.00, 4.39), anemia (OR 3.40, 95% CI 1.61, 7.20), leukopenia (OR 2.12, 95% CI 1.10, 4.10), headache (OR 1.82, 95% CI 1.13, 2.92), and alopecia (OR 4.07, 95% CI 1.71, 9.68), while treatment with PARP inhibitors showed a significantly increased risk of thrombocytopenia (OR 3.15, 95% CI 2.19, 4.54), anemia (OR 1.65, 95% CI 1.13, 2.41), nausea (OR 1.44 95% CI 1.08, 1.92), vomiting (OR 1.62, 95% CI 1.07, 2.44), headache (OR 1.52 95% CI 1.16, 2.01), and back pain (OR 1.48 95% CI 1.05, 2.08), compared to non-platinum-based chemotherapy. Platinum-based chemotherapy had higher ORs for thrombocytopenia (OR 2.69 95% CI 1.82, 3.99), anemia (OR 2.99, 95% CI 1.41, 6.35),

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TABLE 3 Network rankings of efficacy outcomes by p-score.

Treatments	ORR	3-month PFS	12-month PFS	24-month PFS	3-month OS	12-month OS	24-month OS	36-month OS
PARP inhibitor + Platinum-based chemotherapy	0.84	0.93	0.94	0.89	0.80	0.89	0.88	0.99
Platinum-based chemotherapy	0.65	0.81	0.77	0.59	0.50	0.63	0.70	0.66
Sacituzumab govitecan	0.66	NA	NA	NA	NA	NA	NA	NA
PARP inhibitor	0.32	0.45	0.40	0.30	0.80	0.11	0.20	0.33
Non-platinum-based chemotherapy	0.03	0.02	0.06	0.05	0.17	0.32	0.07	0.01
PD-L1 inhibitor	NA	0.28	0.33	0.67	0.23	0.54	0.64	NA

P-score values are represented by the numbers. NA indicates no available treatment included for the analysis of the specific outcomes.

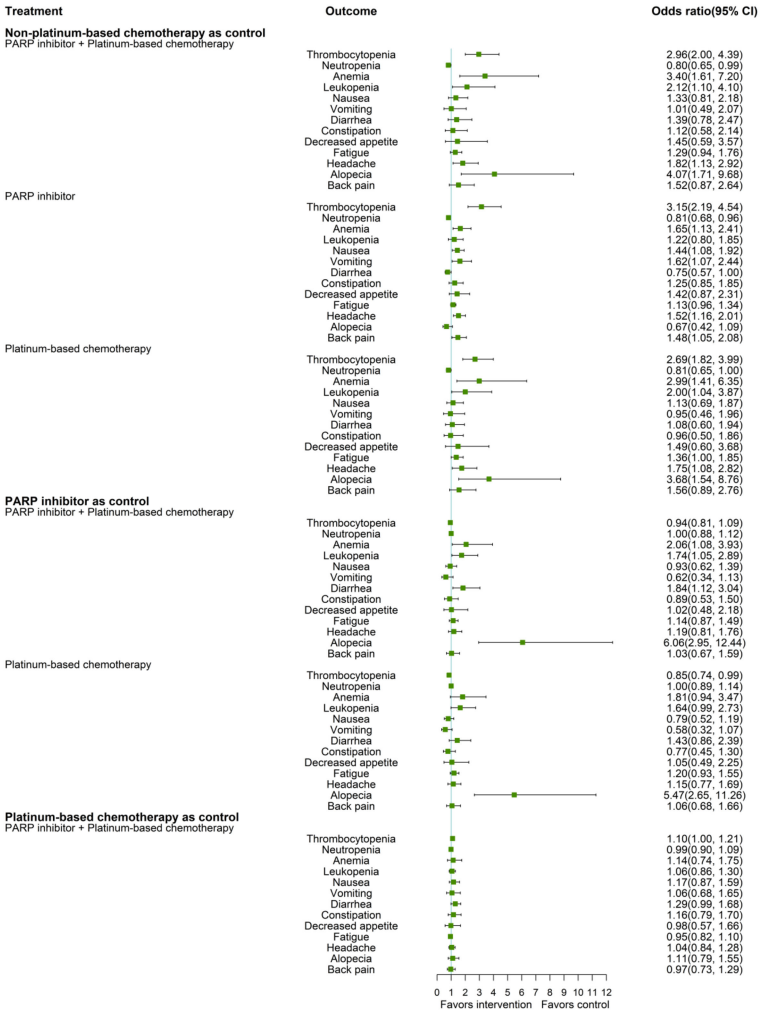

FIGURE 3
Forest plot for any-grade adverse events.

leukopenia (OR 2.00, 95% CI 1.04, 3.87), fatigue (OR 1.36, 95% CI 1.00, 1.85), headache (OR 1.75, 95% CI 1.08, 2.82), and alopecia (OR 3.68, 95% CI 1.54, 8.76), compared to non-platinum-based chemotherapy. All three treatment regimens, PARP inhibitor plus platinum (OR 0.8, 95% CI 0.65, 0.99), PARP inhibitor (OR 0.81 95% CI 0.68, 0.96), and platinum-based chemotherapy (OR 0.81 95% CI 0.65, 1.00), had a significantly lower risk of neutropenia than non-platinum-based chemotherapy.

Compared to a treatment regimen containing PARP inhibitor, significantly higher ORs of anemia (OR, 2.06, 95% CI 1.08, 3.93), leukopenia (OR 1.74, 95% CI 1.05, 2.89), diarrhea (OR 1.84, 95% CI 1.12, 3.04), and alopecia (OR 6.06, 95% CI 2.95, 12.44) were observed

for the treatment regimens including both PARP inhibitors and platinum.

Platinum-based chemotherapy had a significantly higher OR for alopecia (OR 5.47, 95% CI 2.65, 11.26) than PARP inhibitor but a significantly lower OR for thrombocytopenia (OR 0.85, 95% CI 0.74, 0.99). Compared to platinum-based chemotherapy, the treatment with PARP inhibitors plus platinum showed a substantially higher OR for thrombocytopenia (OR 1.10, 95% CI 1.00, 1.21) but no significant differences were noted for most adverse events.

Supplementary Appendix 3 summarizes the results of the risk of bias assessment. The network meta-analysis' heterogeneity, intransitivity, inconsistency, and publication bias were also assessed (Supplementary Appendices 4–7). No evidence of significant inconsistency was detected.

Discussion

This network meta-analysis revealed that incorporating a PARP inhibitor in platinum-based chemotherapy was the most efficient treatment plan for all specified efficacy outcomes, that is ORR, PFS, and OS. Additionally, platinum-based chemotherapy was superior to PARP inhibitor alone in terms of ORR, 3-month PFS, 12-month PFS, and 24-month OS. Among safety outcomes, the treatment regimens comprising both PARP inhibitors and platinum, PARP inhibitor alone, or platinum-based chemotherapy were all associated with a significantly elevated risk for hematological and non-hematological side effects compared to non-platinum-based chemotherapy. The treatment regimen comprising both a PARP inhibitor and platinum showed a higher risk of anemia, leukopenia, diarrhea, and alopecia, compared to PARP inhibitors without platinum; the safety profile to platinum-based chemotherapy was comparable to PARP inhibitor plus platinum. Thus, adding PARP inhibitors to platinum-based chemotherapy would hardly cause more safety burdens.

A previous network meta-analysis of hazard ratios for PFS and ORR found that for patients with advanced breast cancer carrying germline *BRCA* variants, treatment with PARP inhibitor plus platinum were ideal regimens (16). Our study included updated articles and additional treatment regimens. We further evaluated more outcomes of OS and PFS rates at different times and various types of adverse events in detail with a specialized focus on patients with metastatic, locally advanced, or recurrent breast cancer. We found similar results, whereby treatment with PARP inhibitor plus platinum was the most effective. Furthermore, we also identified that platinum-based chemotherapy had a better prognosis in terms of most efficacy outcomes than the treatment with PARP inhibitors alone. Nevertheless, there was only one study that included a direct comparison between platinum-based chemotherapy and PARP inhibitors, which had a major contribution to the pooled results. Further verification in the future is needed.

BRCA1/2 are crucial for homologous recombination (HR) during DSB repair, and pathogenic variants are linked to genome instability and the progression of cancer (36). It was reported that HR deficiency assays, such as detecting nuclear RAD51 foci in tumor cells, could identify patients with *BRCA* pathogenic variants that are more likely to respond to platinum-containing therapy and PARP inhibitors (37–40). Platinum drugs, like cisplatin and carboplatin, act as DNA cross-linking agents forming intra-strand crosslinks, and in turn inhibiting DNA synthesis, function, and transcription (41). *BRCA* pathogenic variant carriers without sufficient DNA repair ability are, therefore, more sensitive to platinum (42). PARP1 and PARP2 enzymes are critical to the DNA damage response (DDR), and HR deficiency and PARP inhibitors result in synthetic lethality through mechanisms related to catalytic inhibition of the PARP enzyme and trapping of PARP-DNA complexes (9). Our findings showed that the treatment combining both PARP inhibitors and platinum had better efficacy than either regimen alone; however, there may be an increased risk of some adverse events in the former. Since both PARP inhibitors and platinum target and impede DNA synthesis, identifying which of the two treatments is more effective for patients carrying *BRCA* pathogenic variants would be an interesting topic. Although treating patients with advanced breast cancer carrying pathogenic variants of *BRCA* with platinum-based chemotherapy is more advantageous than PARP inhibitors according to our analyses, the results need further verification in a sizable RCT that includes direct comparisons.

Sacituzumab govitecan is a Trop-2-directed antibody-drug conjugate that can increase double-stranded DNA breaks (43). It benefits metastatic TNBC patients regardless of germline *BRCA1/2* variants, as evidenced in an original trial (25). SG was included in the network analysis for ORR comparison, yet the related results were all statistically insignificant, and the evidence was of low certainty. As for PD-L1 inhibitors, the original study also found that *BRCA1/2* status was not a prognostic factor for PFS or OS outcomes (27). Network meta-analysis results for PD-L1 revealed that the quality of evidence was relatively poor. Thus, further investigation is warranted and should be more pertinent to the specific corresponding biomarker (Trop-2 and PD-L1) expression than the *BRCA1/2* variants.

There are also some limitations of our study. First, the sample sizes of several trials were small, which may have led to a broader estimate of the CIs of effects and impaired the evidence quality. This happens mainly because the targeted group with *BRCA1/2* pathogenic variants is only a subgroup from the original trial. Second, not all studies were included for comparing each outcome, as some data were unavailable from the initial studies.

Conclusion

In conclusion, PARP inhibitor combined with platinum-based chemotherapy was proved as the optimal treatment for patients with metastatic, locally advanced, or recurrent breast cancer carrying *BRCA1/2* pathogenic variants in terms of efficacy outcomes, namely ORR, PFS, and OS. Although the combination of both PARP inhibitor and platinum resulted in more adverse events compared to PARP inhibitor alone and non-platinum-based chemotherapy regimens, which should raise caution in clinical settings, adding PARP inhibitor to platinum barely caused an extra risk of unfavorable events compared to platinum-based chemotherapy alone. Thus, this combined regimen should be considered for patients with advanced breast cancer carrying *BRCA* pathogenic variants for better prognostic outcomes. Confirmatory RCTs of sufficient, pre-specified sample sizes that directly compare currently available treatment regimens and are explicitly aimed at patients carrying *BRCA1/2* pathogenic variants should be conducted in the future.

Author contributions

WL had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. YZ: Conceptualization, Methodology, Software, Investigation, Formal analysis, Writing - Original Draft, Visualization. YL: Conceptualization, Investigation, Data Curation Writing - Review & Editing. WDL: Software, Validation, Visualization, Writing - Review & Editing. RZ: Writing - Review & Editing, Conceptualization. LT: Writing - Review & Editing Supervision. YW: Methodology Supervision. WL: Supervision, Writing - Review & Editing, Acquisition of the financial support for the project leading to this publication, Project administration All authors contributed to the article and approved the submitted version.

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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.2023.1080297/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. Kocarnik JM, Compton K, Dean FE, Fu W, Gaw BL, Harvey JD, et al. Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups from 2010 to 2019. *JAMA Oncol* (2022) 8(3):420. doi: 10.1001/jamaoncol.2021.6987

3. Malone KE, Daling JR, Doody DR, Hsu L, Bernstein L, Coates RJ, et al. Prevalence and predictors of BRCA1 and *BRCA2* mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. *Cancer Res* (2006) 66 (16):8297–308. doi: 10.1158/0008-5472.CAN-06-0503

4. Kurian AW, Gong GD, John EM, Miron A, Felberg A, Phipps AI, et al. Performance of prediction models for BRCA mutation carriage in three Racial/Ethnic groups: Findings from the northern California breast cancer family registry. *Cancer Epidemiol Biomark Ampmathsemicolon Prev* (2009) 18(4):1084–91. doi: 10.1158/1055-9965.epi-08-1090

5. Kwong A, Shin VY, Ho JCW, Kang E, Nakamura S, Teo S-H, et al. Comprehensive spectrum of BRCA1 and *BRCA2* deleterious mutations in breast cancer in Asian countries. *J Med Genet* (2016) 53(1):15–23. doi: 10.1136/jmedgenet-2015-103132

6. Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a Large triple-negative breast cancer cohort unselected for family history of breast cancer. *J Clin Oncol* (2015) 33(4):304 –11. doi: 10.1200/jco.2014.57.1414

7. Zhao W, Wiese C, Kwon Y, Hromas R, Sung P. The BRCA tumor suppressor network in chromosome damage repair by homologous recombination. *Annu Rev Biochem* (2019) 88(1):221–45. doi: 10.1146/annurev-biochem-013118-111058

8. Tarsounas M, Sung P. The antitumorigenic roles of BRCA1–BARD1 in DNA repair and replication. *Nat Rev Mol Cell Biol* (2020) 21(5):284–99. doi: 10.1038/s41580-020-0218-z

9. Lord CJ, Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. *Science* (2017) 355(6330):1152–8. doi: 10.1126/science.aam7344

10. Palleschi M, Tedaldi G, Sirico M, Virga A, Ulivi P, De Giorgi U. Moving beyond PARP inhibition: Current state and future perspectives in breast cancer. *Int J Mol Sci* (2021) 22(15):7884. doi: 10.3390/ijms22157884

11. Isakoff SJ, Mayer EL, He L, Traina TA, Carey LA, Krag KJ, et al. TBCRC009: A multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. *J Clin Oncol* (2015) 33:1902–9. doi: 10.1200/jco.2014.57.6660

12. Network NCC. Breast cancer (Version 2.2022), in: *NCCN website* (2022). Available at: https://www.nccn.org/guidelines/category_1 (Accessed July 11, 2022).

13. Wang C-J, Xu Y, Lin Y, Zhu H-J, Zhou Y-D, Mao F, et al. Platinum-based neoadjuvant chemotherapy for breast cancer with BRCA mutations: A meta-analysis. *Front Oncol* (2020) 10:592998. doi: 10.3389/fonc.2020.592998

14. Caramelo O, Silva C, Caramelo F, Frutuoso C, Almeida-Santos T. The effect of neoadjuvant platinum-based chemotherapy in BRCA mutated triple negative breast cancers -systematic review and meta-analysis. *Hered Cancer Clin Pract* (2019) 17 (1):11–21. doi: 10.1186/s13053-019-0111-y

15. Wang J, Zhang Y, Yuan L, Ren L, Zhang Y, Qi X. Comparative efficacy, safety, and acceptability of single-agent poly (ADP-ribose) polymerase (PARP) inhibitors in BRCA-mutated HER2-negative metastatic or advanced breast cancer: A network meta-analysis. *Aging* (2020) 13(1):450–9. doi: 10.18632/aging.202152

16. Jiang Y, Meng X-Y, Deng N-N, Meng C, Li L-H, He Z-K, et al. Effect and safety of therapeutic regimens for patients with germline BRCA mutation-associated breast cancer: A network meta-analysis. *Front Oncol* (2021) 11:718761. doi: 10.3389/fonc.2021.718761

17. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev* (2021) 10(1):89–100. doi: 10.1186/s13643-021-01626-4

18. Rücker G. Network meta-analysis, electrical networks and graph theory. *Res Synth Methods* (2012) 3(4):312–24. doi: 10.1002/jrsm.1058

19. Rücker G, Schwarzer G. Ranking treatments in frequentist network meta-analysis works without resampling methods. *BMC Med Res Methodol* (2015) 15(1):58–67. doi: 10.1186/s12874-015-0060-8

20. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* (2019) 366:l4898. doi: 10.1136/bmj.l4898

21. Puhan MA, Schunemann HJ, Murad MH, Li T, Brignardello-Petersen R, Singh JA, et al. A GRADE working group approach for rating the quality of treatment effect estimates from network meta-analysis. *BMJ* (2014) 349(sep24 5):g5630–g5630. doi: 10.1136/bmj.g5630

22. Jackson D, White IR, Riley RD. Quantifying the impact of between-study heterogeneity in multivariate meta-analyses. *Stat Med* (2012) 31(29):3805–20. doi: 10.1002/sim.5453

23. Dias S, Welton NJ, Caldwell DM, Ades AE. Checking consistency in mixed treatment comparison meta-analysis. *Stat Med* (2010) 29(7–8):932–44. doi: 10.1002/sim.3767

24. Chaimani A, Salanti G. Using network meta-analysis to evaluate the existence of small-study effects in a network of interventions. *Res Synth Methods* (2012) 3(2):161–76. doi: 10.1002/jrsm.57

25. Bardia A, Tolaney SM, Punie K, Loirat D, Oliveira M, Kalinsky K, et al. Biomarker analyses in the phase III ASCENT study of sacituzumab govitecan versus chemotherapy in patients with metastatic triple-negative breast cancer. *Ann Oncol* (2021) 32(9):1148–56. doi: 10.1016/j.annonc.2021.06.002

26. Turner NC, Balmaña J, Poncet C, Goulioti T, Tryfonidis K, Honkoop AH, et al. Niraparib for advanced breast cancer with germline BRCA1 and *BRCA2* mutations: the EORTC 1307-BCG/BIG5–13/TESARO PR-30–50–10-C BRAVO study. *Clin Cancer Res* (2021) 27(20):5482–91. doi: 10.1158/1078-0432.CCR-21-0310

27. Emens LA, Molinero L, Loi S, Rugo HS, Schneeweiss A, Diéras V, et al. Atezolizumab and *nab* -paclitaxel in advanced triple-negative breast cancer: Biomarker evaluation of the IMpassion130 study. *JNCI J Natl Cancer Inst* (2021) 113(8):1005–16. doi: 10.1093/jnci/djab004

28. Diéras V, Han HS, Kaufman B, Wildiers H, Friedlander M, Ayoub J-P, et al. Veliparib with carboplatin and paclitaxel in BRCA-mutated advanced breast cancer (BROCADE3): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* (2020) 21(10):1269–82. doi: 10.1016/s1470-2045(20)30447-2

29. Litton JK, Hurvitz SA, Mina LA, Rugo HS, Lee K-H, Gonçalves A, et al. Talazoparib versus chemotherapy in patients with germline BRCA1/2-mutated HER2-negative advanced breast cancer: Final overall survival results from the EMBRACA trial. *Ann Oncol* (2020) 31(11):1526–35. doi: 10.1016/j.annonc.2020.08.2098

30. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee K-H, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *N Engl J Med* (2018) 379(8):753–63. doi: 10.1056/NEJMoa1802905

31. Robson ME, Tung N, Conte P, Im S-A, Senkus E, Xu B, et al. OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. *Ann Oncol* (2019) 30(4):558–66. doi: 10.1093/annonc/mdz012

32. Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *N Engl J Med* (2017) 377(6):523–33. doi: 10.1056/NEJMoa1706450

33. Tutt A, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P, et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT trial. *Nat Med* (2018) 24(5):628–37. doi: 10.1038/s41591-018-0009-7

34. Han HS, Diéras V, Robson M, Palácová M, Marcom PK, Jager A, et al. Veliparib with temozolomide or carboplatin/paclitaxel versus placebo with carboplatin/paclitaxel in patients with BRCA1/2 locally recurrent/metastatic breast cancer: randomized phase II study. *Ann Oncol* (2018) 29(1):154–61. doi: 10.1093/annonc/mdx505

35. Zhang J, Lin Y, Sun XJ, Wang BY, Wang ZH, Luo JF, et al. Biomarker assessment of the CBCSG006 trial: A randomized phase III trial of cisplatin plus gemcitabine compared with paclitaxel plus gemcitabine as first-line therapy for patients with metastatic triple-negative breast cancer. *Ann Oncol* (2018) 29(8):1741–7. doi: 10.1093/annonc/mdy209

36. Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat Rev Cancer* (2011) 12(1):68–78. doi: 10.1038/nrc3181

37. Telli ML, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, et al. Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer HRD predicts response to platinum therapy in TNBC. *Clin Cancer Res* (2016) 22(15):3764–73. doi: 10.1158/1078-0432.CCR-15-2477

38. Chopra N, Tovey H, Pearson A, Cutts R, Toms C, Proszek P, et al. Homologous recombination DNA repair deficiency and PARP inhibition activity in primary triple negative breast cancer. *Nat Commun* (2020) 11(1):1–12. doi: 10.1038/s41467-020-16142-7

39. Pellegrino B, Herencia-Ropero A, Llop-Guevara A, Pedretti F, Moles-Fernández A, Viaplana C, et al. Preclinical *in vivo* validation of the RAD51 test for identification of homologous recombination-deficient tumors and patient stratification. *Cancer Res* (2022) 82(8):1646–57. doi: 10.1158/0008-5472.CAN-21-2409

40. Llop-Guevara A, Loibl S, Villacampa G, Vladimirova V, Schneeweiss A, Karn T, et al. Association of RAD51 with homologous recombination deficiency (HRD) and clinical outcomes in untreated triple-negative breast cancer (TNBC): Analysis of the GeparSixto randomized clinical trial. *Ann Oncol Off J Eur Soc Med Oncol* (2021) 32:1590–6. doi: 10.1016/j.annonc.2021.09.003

41. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: Molecular mechanisms of action. *Eur J Pharmacol* (2014) 740:364–78. doi: 10.1016/j.ejphar.2014.07.025

42. Torrisi R, Zuradelli M, Agostinetto E, Masci G, Losurdo A, Sanctis RD, et al. Platinum salts in the treatment of BRCA-associated breast cancer: A true targeted chemotherapy? *Crit Rev Oncol Hematol* (2019) 135:66–75. doi: 10.1016/j.critrevonc.2019.01.016

43. Cardillo TM, Sharkey RM, Rossi DL, Arrojo R, Mostafa AA, Goldenberg DM. Synthetic lethality exploitation by an anti–Trop-2-SN-38 antibody–drug conjugate, IMMU-132, plus PARP inhibitors in *BRCA1/2* –wild-type triple-negative breast cancer. *Clin Cancer Res* (2017) 23(13):3405–15. doi: 10.1158/1078-0432.CCR-16-2401

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Evolution and clinical significance of HER2-low status after neoadjuvant therapy for breast cancer

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Background: The emergence of HER2 antibody-drug conjugates provides new treatment decisions for breast cancer patients, especially those with HER2-low expression. In order to explore the biological characteristics of breast cancer with HER2-low expression, the HER2-low category in primary breast cancer and residual tumor after neoadjuvant therapy was investigated to reflect the evolution of HER2 expression.

Methods: HER2 was assessed according to the latest ASCO/CAP guidelines. The cut-off value for staining of HER2-positive cells was >10%. HER2-negative cases were divided into HER2-low (IHC=1+/2+ and no ISH amplification) and HER2-zero (IHC-0), and the clinicopathological characteristics of the cases were collected.

Results: This study included 1140 patients with invasive breast cancer who received preoperative neoadjuvant therapy from 2018 to 2021, of which 365 patients achieved pCR and 775 were non-pCR. In the non-pCR cohort, HER2-low cases accounted for 59.61% of primary tumors and 55.36% of residual tumors. Among HER2-negative cases, HR-positive tumors had a higher incidence of low HER2 expression compared with triple-negative tumors (80.27% vs 60.00% in primary tumors and 72.68% vs 50.77% in residual tumors). The inconsistency rate of HER2 expression was 21.42%, mainly manifested as the conversion of HER2-low cases to HER2-zero (10.19%) and the conversion of HER2-zero to HER2-low (6.45%). Among the HER2-negative cases in the primary tumor, the HER2 discordance rate of HR-positive cases was lower than that of triple-negative cases (23.34% VS 36.92%). This difference was mainly caused by the case switching from HER2-low to HER2-zero. Compared with HER2-zero cases, there were statistically significant differences in RCB grade, MP grade and the number of metastatic lymph nodes in HER2-low cases. Patients with low HER2 expression had a lower pathological response rate and a higher number of metastatic lymph nodes.

Conclusion: HER2-low breast cancer is highly unstable during disease evolution and has certain biological characteristics. HER2-low breast cancer is not only correlated with positive HR, but also has a certain correlation with positive AR.

Re-detection of HER2 in breast cancer after neoadjuvant therapy may lead to new treatment opportunities for a certain proportion of patients.

KEYWORDS

breast cancer, human epidermal growth factor receptor 2, hormone receptor, neoadjuvant therapy, pathological complete response

Introduction

Human epidermal growth factor receptor-2 (HER2) is a proto-oncogene and has a high response rate in breast cancer and other types of cancers. Beyond that, HER2 status defines a distinct breast cancer subtype with aggressive biological behavior and historically worse prognosis, a reality that was changed after the incorporation of HER2 therapy (1). 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines recommend a binary distinction between HER2-positive and HER2-negative breast cancers to guide clinicians' treatment decision. However, the emergence of the antibody-drug conjugates (ADCs) has provided new treatment decisions for patients with low HER2 expression. Breast cancer classified as negative in a certain proportion (approximately 45-55%) (2–4) actually belong to the newly proposed HER2-low. Breast cancer with an immunohistochemistry (IHC) score of 1+ or 2+ and unamplified by *in situ* hybridization (ISH) is referred to as HER2-low breast cancer. Recently, the results of non-randomized trials with novel antibody–drug conjugates targeting HER2 (trastuzumab–deruxtecan and trastuzumab–duocarmazine) have suggested a level of efficacy in HER2-low patients with advanced breast cancer, with objective response rates ranging between 32% and 37% in a heavily pretreated population (5, 6). Trastuzumab deruxtecan (DS8201a), for instance, has achieved an objective response rate (ORR) of 37% in highly pretreated patients with HER2-low metastatic breast cancer (5), whereas in a similar population ORR with trastuzumab duocarmazine (SYD985) was 28-40% depending on HR expression (6). This led to the hypothesis that HER2-low tumors might represent a separate disease subset, distinct from other luminal and triple-negative breast cancers (TNBC). Indeed, several trials are currently exploring the potential of anti-HER2 agents in HER2-low patients.

In this study, the evolution and clinicopathological characteristics of HER2-low expression tumors were analyzed based on neoadjuvant breast cancer patients in China.

Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor; AR, androgen receptor; IHC, immunohistochemistry; ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; ADCs, the antibody-drug conjugates; ISH, *in situ* hybridization; pCR, pathological complete response; RCB, residual cancer burden; MP, Miller Payen classification; ORR, objective response rate; TNBC, triple-negative breast cancers; HE, hematoxylin-eosin; SP, streptavidin-perosidase.

Materials and methods

General information

A total of 1140 patients who received preoperative neoadjuvant therapy in the Fourth Hospital of Hebei Medical University from January 2018 to December 2021 were screened, and all patients underwent surgery in this hospital. Neoadjuvant therapy includes preoperative chemotherapy and endocrine therapy. Of these, 775 patients did not achieve pathological complete response (pCR), and 365 patients achieved pCR. The clinicopathological characteristics of the cases were collected and analyzed. In the non-pCR cohort, there were 773 females and 2 males, ranging in age from 24 to 86 years old. In the pCR cohort, there were 365 females and 0 males. Two or more attending pathologists performed double-blind follow-up on hematoxylin-eosin (HE) sections and HER2 IHC sections of all patients to improve the clinicopathological data.

Methods

Retrospective analysis of patients with breast cancer that met the criteria was performed by IHC and ISH. The IHC method used Roche's rabbit monoclonal primary antibody and the BenchMarK XT automatic IHC instrument was used for detection. The clinicopathological data of the non-pCR cohort of patients were collected, and the biological characteristics of HER2 low expression cases and HER2 0 cases were analyzed.

Interpretation criteria

According to the ASCO/CAP guidelines (7), HER2 IHC staining results were determined, HER2 0: no staining is observed HER2-null or membrane staining that is incomplete and is faint/barely perceptible and in <10% tumor cells; HER2 1+: incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells; HER2 2+: weak to moderate complete membrane staining in >10% of tumor cells; or circumferential membrane staining that is complete, intense, and in ≤10% of tumor cells; HER2 3+: circumferential membrane staining that is complete, intense, and in >10% of tumor cells. For HER2 2+ cases, the ISH method was used for further testing, where HER2-zero was determined as HER2 negative; 1+ and 2+ with no ISH

amplification as HER2-low, 2+ with ISH amplification and 3+ as HER2 positive. Hormone receptor (HR)-positive, at least 1% of infiltrating tumor cells showed immunostaining. Androgen receptor (AR)-positive, at least 1% of infiltrating tumor cells showed immunostaining.

Statistical methods

Statistical software SPSS 23.00 was used for statistical analysis and processing, Kappa was used for consistency analysis, and $\chi 2$ test was used to test the significance of differences. $P<0.05$ was considered statistically significant.

Results

Clinical data

1140 breast cancer patients after neoadjuvant therapy were collected, including 1138 females and 2 males, aged 24-86 years. There were 775 patients with invasive breast cancer in the non-pCR group, including 405 left breast masses, 368 right breast masses, and 2 double breast masses. Among the HER2-negative cases, 505 were invasive ductal carcinoma after neoadjuvant therapy, 7 were mucinous adenocarcinoma, and 62 were of undetermined type; 583 were <70 years old, and 10 were ≥70 years old. Residual cancer burden (RCB) Grading: 63 cases of grade I, 172 cases of grade II, and 358 cases of grade III; Miller Payen classification (MP classification): grades 1-5 were 9, 71, 425, 53, and 12 cases, respectively.

Consistent analysis of HER2 status after neoadjuvant therapy

775 patients with invasive breast cancer were all tested for HER2. The interpretation was based on the ASCO guidelines. HER2-negative cases were divided into HER2-low (IHC=1+/2+ and no ISH amplification) and HER2-zero (IHC- 0). HER2 status of primary tumors: 130 cases of HER2-zero, 462 cases of HER2-low, and 183 cases of HER2-positive; HER2 status of residual tumors after neoadjuvant therapy: 164 cases of HER2-zero, 429 cases of HER2-low, and 182 cases of HER2-positive (Table 1). There was indeed a difference in the HER2 status of breast cancer before and after neoadjuvant therapy, and the difference was statistically significant (P=0.014), and the HER2 status was inconsistent (Kappa=0.630, P<0.001). The inconsistency rate was 21.42%, and the main difference: cases of HER2-low were switched to HER2-zero (Figures 1–3).

HER2 low expression status and HR status

In the non-pCR cohort (N=775), HER2-low cases accounted for 59.61% (n=462) of primary breast cancer, 55.36% (n=429) of residual breast cancer after neoadjuvant therapy, respectively 78.04% and 71.92% of HER2-negative primary and residual breast cancers. In the analysis of HER2-negative cases, 527 were HR-positive cases and 65 were HR-negative cases among the primary breast cancers. Among the residual breast cancers after neoadjuvant therapy, there were 512 HR-positive cases and 62 HR-negative cases. The low expression of HER2 was 71.45% and 6.59% in the HR-positive/HER2-negative cohort and triple-negative cohort of primary breast cancer, respectively ($p<0.01$), and the residual breast cancer HR-positive/HER2-negative cohort and triple-negative cohort after neoadjuvant therapy were 66.72% and 5.75% respectively ($p <0.01$). After statistical chi-square test, low HER2 expression was positively correlated with HR-positive/HER2-negative breast cancer subtypes, and the difference was statistically significant ($p<0.05$) (Table 2). Compared with TNBC, the incidence of HER2-low tumors was higher in HR-positive tumors (80.27% vs. 60.00%; $p<0.01$). HR-positive tumors were characterized by a higher incidence of IHC 1+ and 2+ than TNBC (32.76% vs. 23.94% and 41.38% vs. 35.21%; $p<0.05$) (Figure 4).

Analysis of HER2 1+ and HER2 2+ and HR status after neoadjuvant therapy in HER2-low cases, after statistical analysis, there was no statistical difference in HR status between HER2 1+ and HER2 2+ ($P>0.05$) (Table 3).

In the cohort, there were 592 HER2-negative cases in primary breast cancer, including 527 HR-positive cases and 65 triple-negative cases. Compared with residual breast cancer, the inconsistency rate of HR-positive cases was 123/527, 23.34%; the inconsistency rate of triple-negative cases was 24/65,

TABLE 1 HER2 expression evolution from primary breast cancer to residual breast cancer.

	HER2 expression on residual breast cancer n (%)			
	HER2-zero	HER2-low	HER2-pos	Total
HER2 expression on primary breast cancer n(%)				
HER2-zero	79 (10.19)	50 (6.46)	1 (0.13)	130 (16.78)
HER2-low	79 (10.19)	366 (47.23)	17 (2.19)	462 (59.61)
HER2-pos	6 (0.77)	13 (1.68)	164 (21.16)	183 (23.61)
Total	164 (21.16)	429 (55.36)	182 (23.48)	775

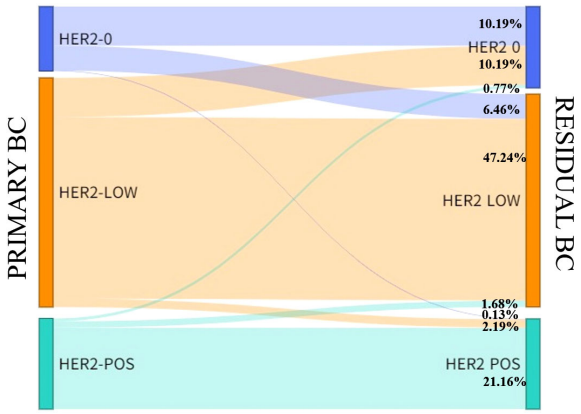

FIGURE 1
HER2 expression evolution from primary breast cancer to residual breast cancer.

36.92% (Table 4). The HER2 discordance rate of HR-positive cases was lower than that of triple-negative cases (23.34% vs. 36.92%) (Figure 5).

HER2 low expression status and AR status

Of the 775 patients in the non-PCR group after neoadjuvant therapy, 677 cases had definite AR status, 432 cases were AR positive, and the positive rate was 63.81%. After neoadjuvant therapy, 677 of the 775 patients in the non-PCR group had definite AR status, and 432 were AR positive. In AR positive cases, 80 cases were HER2-zero, 261 cases were HER2-low and 91 cases were HER2 positive. Among AR negative cases, 67 cases were HER2-zero, 116 cases were HER2-low and 62 cases were HER2 positive. Among them, 524 were HER2-negative. HER2 low expression in both AR positive and AR negative cases were 76.54% and 63.38%, respectively. Chi-square test showed that AR-positive breast cancer had a higher incidence of HER2-low than AR-negative breast cancer ($p < 0.01$) (Table 5).

To further analyze the correlation between HER2 low expression and AR status, In 124 HR negative cases after neoadjuvant therapy, 27 cases were HER2-zero, 46 cases were HER2-low, and 51 cases were HER2-positive. There were 45 AR

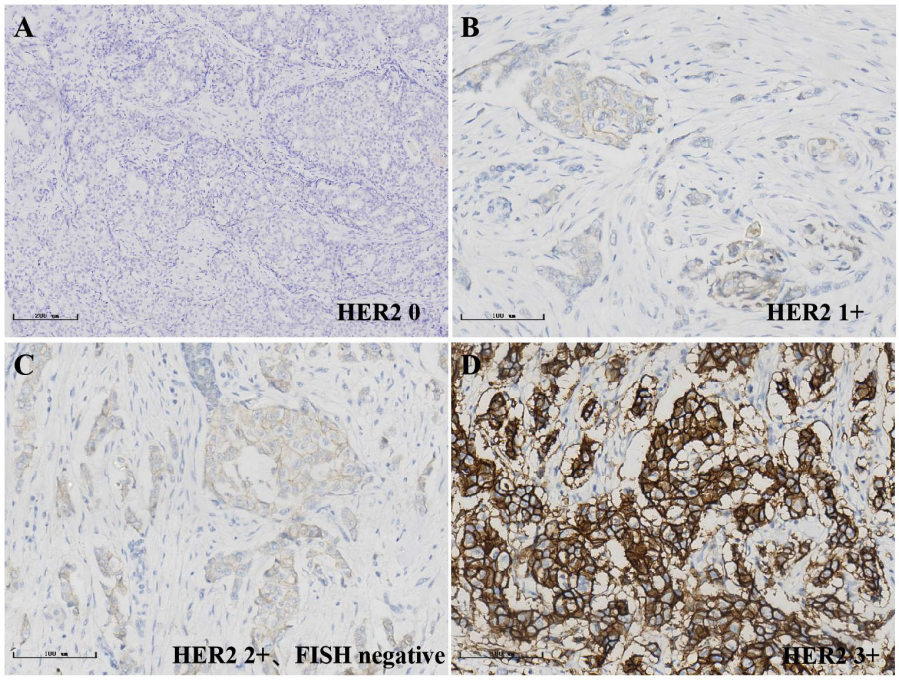

FIGURE 2
Immunohistochemical detection of HER2 status in breast cancer, streptavidin-perosidase (SP); **(A)**0; **(B)**1+; **(C)**2+; **(D)**3+.

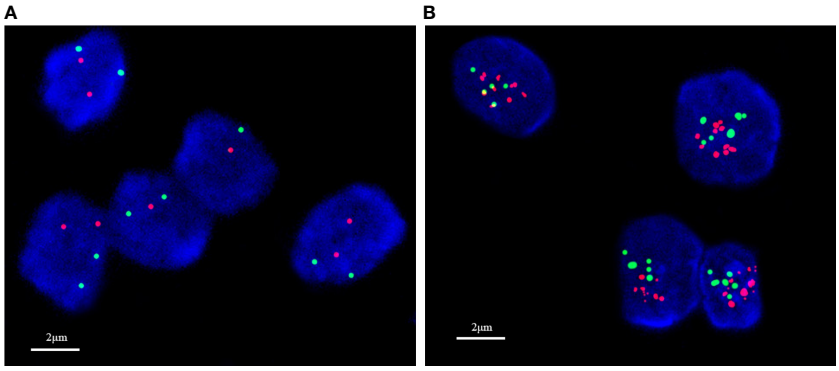

FIGURE 3
In situ hybridization detection of HER2 status in breast cancer. **(A)** No amplification; **(B)** amplification.

positive cases and 79 AR negative cases. The positive rate of AR was 43.5% in the HER2-low group and 11.1% in the HER2-zero group. HER2-low showed a higher AR positive rate than HER2-zero, and the difference was statistically significant ($p < 0.01$).

In the TNBC cohort (n=73), the AR positive rate was 31.5%, and the incidence of HER2-low was higher in AR positive breast cancer than in AR negative (86.96% VS 52.00%).

Clinicopathological features of low HER2 expression

In the HER2-negative group of the non-pCR cohort, compared with the HER2-zero cases, the cases with low HER2 expression had statistical differences in RCB grade, MP grade and the number of metastatic lymph nodes, and the pathological remission rate was lower; and the number of metastatic lymph nodes was more (Table 6).

Among HER2-negative cases, the clinicopathological characteristics of consistent cases (including: HER2-zero and HER2-low) and differential cases (including: HER2-zero to HER2-low and HER2-low to HER2-zero cases) were analyzed (Table 7). There were differences in histological type, Ki-67, RCB grade, and the number of lymph node metastasis among the four groups, and the difference was statistically significant ($p<0.05$).

Discussion

Neoadjuvant therapy combined with anti- HER2 therapy is an effective treatment option for HER2-positive breast cancer (based on IHC defined as HER2-amplified IHC 3+ or IHC 2+ and ISH amplification). The heterogeneity of HER2 expression before and after neoadjuvant therapy for breast cancer is an area of interest for clinicians and pathologists. HER2-low breast cancer is emerging as a new entity, leading to biological and clinical complexity. Currently, the evolution of HER2-low expression from primary breast cancer to residual breast cancer after neoadjuvant therapy was assessed in a cohort by including the HER2-low category in the characterization of primary and post-neoadjuvant residual tumours.

In a cohort of 775 patients with pathological non-pCR breast cancer after neoadjuvant therapy, HER2-low expressing breast cancers accounted for almost more than half (59.61%) of the entire HER2-negative cohort, which is consistent with available

TABLE 2 HER2 expression distribution according to breast cancer subtype in the HER2-negative primary and residual breast cancer cohort.

	HER2 expression n (%)		
	0	Low	*p*
Primary breast cancer n (%)			
HR-positive/HER2-negative	104 (17.57)	423 (71.45)	0.000*
Triple-negative	26 (4.39)	39 (6.59)	
Total	130	462	
Residual breast cancer n (%)			
HR-positive/HER2-negative	129 (22.47)	383 (66.72)	0.000*
Triple-negative	29 (5.05)	33 (5.75)	
Total	158	416	

*, P<0.01.

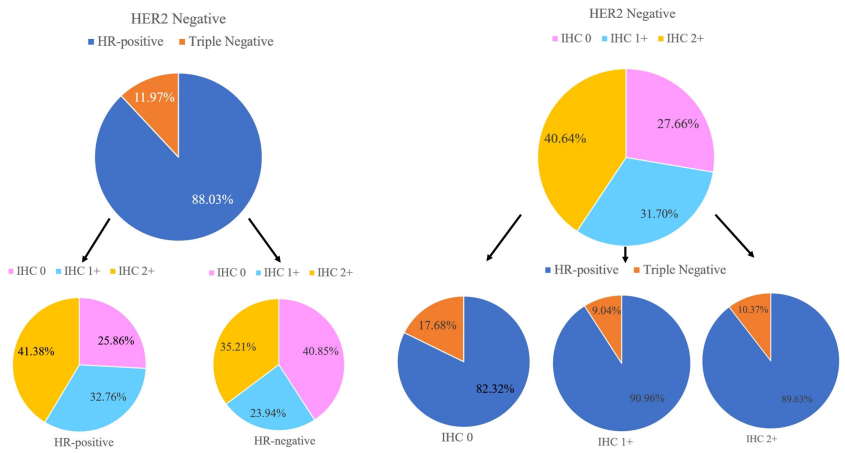

FIGURE 4
Hormone receptor (HR) status, HER2-low status, and IHC scores distributions within the HER2-negative population.

research data (8). Furthermore, in this cohort, the proportion of HER2-low cases in breast cancer samples with residual tumours after neoadjuvant therapy was lower than in breast cancer primaries, and the decrease in HER2-low cases in residual tumours after neoadjuvant therapy compared with breast cancer primaries was mainly due to the fact that HER2-low cases switched to HER2-zero with treatment.

The study showed an association between HR status and HER2-low. HER2-low expression consisted of 80.27% and 60.00% in the HR-positive/HER2-negative cohort and triple-negative cohort for primary breast cancer, respectively, and 74.14% and 53.23% in the HR-positive/HER2-negative cohort and triple-negative cohort for residual breast cancer after NAT, respectively. HER2-low cases were more common in the HR-positive/HER2-negative breast cancer cohort, while HER2-zero cases were more common in the TNBC cohort. This result is consistent with those in previous studies (9, 10) and Schettini et al (9) reported a higher incidence of HR-positive/HER2-negative phenotype than triple-negative phenotype in HER2-low breast cancer. ER levels were higher in the HR-positive/HER2-negative subgroup than in the HER2-low cohort. In conclusion, HR status is a key determinant of the underlying biology of HER2-low breast cancer. The complexity between HER2 and HR pathways may play a key role in biologically defining the HER2-low phenotype (11, 12) However, whether HER2-low can be considered as a separate subtype needs to be further validated in future studies.

Our main objective was to study the evolution of HER2-low from primary breast cancer to residual breast cancer after neoadjuvant therapy. In the whole cohort, the HER2 noncompliance rate was 21.41%, mainly due to the switch from HER2-low to HER2-zero cases. In particular, approximately 17% of patients with HER2-low primary breast cancer exhibited conversion to HER2-zero after neoadjuvant therapy, whereas about 38% of patients with HER2-zero in the primary tumour switched to HER2-low, further confirming the instability of HER2-low expression. The great instability of HER2-low breast cancer was shown in the conversion from HER2-zero phenotype to HER2-low phenotype or from HER2-low phenotype to HER2-zero phenotype and with the use of ADC analogues (13). Therefore, re-testing for HER2 should be recommended for patients with breast cancer after undergoing neoadjuvant therapy. In addition, inconsistent HER2

TABLE 3 Distribution of HER2 expression by IHC according to tumor phenotype in the HER2-low cohort.

	HER2 expression n (%)		
	1+	2+	*p*
Primary breast cancer n (%)			
HR-positive/HER2-negative	141 (30.52)	282 (61.04)	0.435
Triple-negative	14 (3.03)	25 (5.41)	
Total	155	307	
Residual breast cancer n (%)			
HR-positive/HER2-negative	136 (32.69)	247 (59.38)	0.067
Triple-negative	17 (4.09)	16 (3.85)	
Total	153	263	

TABLE 4 HER2 expression evolution from primary breast cancer to residual according to tumor phenotype in the HER2-low cohort.

Primary cancer	HER2 expression on residual breast cancer n (%)			
HR-Pos	HER2-zero	HER2-low	HER2-pos	Total
HER2-zero	62 (11.76)	41 (7.78)	1 (0.19)	104 (19.73)
HER2-low	67 (12.71)	342 (64.90)	14 (2.66)	423 (80.27)
Total	129 (24.48)	383 (72.68)	15 (2.85)	527 (100)
HR-Neg				
HER2-zero	17 (26.15)	9 (13.85)	0 (0)	26 (40.00)
HER2-low	12 (18.46)	24 (36.92)	3 (4.62)	39 (60.00)
Total	29 (44.62)	33 (50.77)	3 (4.62)	65 (100)

low expression is primarily driven by the TNBC subgroup, which shows a higher conversion rate compared to the HR-positive/HER2-negative subgroup, especially when considering the conversion of TNBC to the HER2-low phenotype. It should be considered that these patients have exhausted their primary treatment options, including hormonal strategies and chemotherapy after neoadjuvant therapy, but may still benefit from additional therapy. In such cases, those who exhibit low HER2 expression may be ideal candidates for inclusion in ongoing clinical trials of anti-HER2 ADCs. In contrast, although HER2-low expression was observed less frequently in triple-negative cohorts than in HR-positive cohorts, approximately 50% of TNBC patients exhibited an HER2-low status. This result opens up new treatment decisions and opportunities for patients with TNBC.

In general, our findings emphasise the importance of re-testing for HER2 in breast cancer patients after neoadjuvant therapy. Indeed, low HER2 expression can be detected in breast cancer patients with primary HER2-zero after neoadjuvant therapy, thus expanding the treatment options for patients. However, it is unclear whether patients with HER2-low breast cancer who exhibit complete deletion of HER2 expression during disease evolution can still benefit from these new treatment strategies.

In addition, we analysed the pathological remission rates after neoadjuvant therapy in HER2-zero versus HER2-low cases to detect

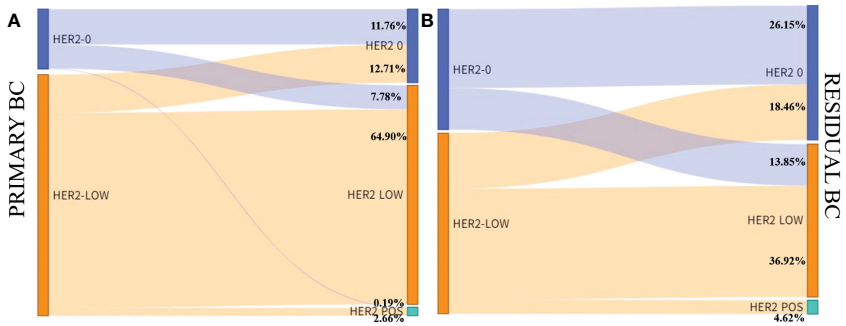

FIGURE 5
HER2 expression evolution from primary breast cancer to residual according to tumor phenotype in the HER2-low cohort. **(A)** Hormone receptor positive; **(B)** Hormone receptor negative.

TABLE 5 Distribution of breast cancer patients with HER2-negative in different AR states.

	HER2 expression n (%)		
	0	Low	*p*
Residual breast cancer n (%)			
AR-positive	80 (15.27)	261 (49.81)	0.002*
AR-negative	67 (12.78)	116 (22.14)	
Total	147 (28.05)	377 (71.95)	

*, P<0.01.

TABLE 6 Baseline patient characteristics stratified by breast residual HER2 status (HER2 0 vs. HER2-low).

Demographics	Total (n=593)	HER2-zero (n=164)	HER2-low (n=429)	$\chi 2$	P Value*
Age					
<70 years	583	160 (97.56%)	423 (98.60%)	0.775	0.379
≥70 years	10	4 (2.44%)	6 (1.40%)		
Menopausal status					
Pre/peri-	401	115 (70.12%)	286 (66.67%)	0.647	0.421
Post-	192	49 (29.88%)	143 (33.33%)		
Histology					
Invasive ductal	524	145 (88.41%)	379 (88.34%)	3.414	0.181
Mucinous adenocarcinoma	7	4 (2.44%)	3 (0.70%)		
Other	62	15 (9.15%)	47 (10.96%)		
Maximum diameter after treatment					
<2	268	77 (46.95%)	191 (44.52%)	0.283	0.595
≥2	325	87 (53.05%)	238 (55.48%)		
Ki-67					
≤20%	414	107 (65.24%)	307 (71.56%)	2.247	0.134
>20%	179	57 (34.76%)	122 (28.44%)		
Miller-Payne (MP)					
1	10	1 (0.61%)	10 (2.33%)	12.277	0.015*
2	71	19 (11.59%)	52 (12.12%)		
3	445	116 (70.73%)	328 (76.46%)		
4	54	20 (12.19%)	34 (7.92%)		
5	13	8 (4.87%)	5 (1.16%)		
Residual cancer burden					
I	63	25 (15.24%)	38 (8.86%)	6.589	0.037*
II	172	51 (31.10%)	121 (28.20%)		
III	358	88 (53.66%)	270 (62.94%)		
Number of metastatic sites					
<3	316	92 (56.09%)	194 (45.22%)	5.621	0.018*
≥3	277	72 (43.91%)	235 (54.78%)		

*, P<0.05.

the difference between these two types. The main finding of our study was that HER2-zero and HER2-low expressing tumours are different biological subtypes with distinct clinicopathological features, including differences in HR-positive tumours and in pathological remission rates. Compared to HER2-zero cases, HER2-low cases had statistically different RCB grading, MP grading, and number of metastatic lymph nodes; the pathological remission rates were lower, and the number of metastatic lymph nodes was higher. We also analysed the clinicopathological characteristics of concordant cases (including HER2-zero and HER2-low cases) versus discrepant cases (including HER2-zero to HER2-low, and HER2-low to HER2-zero cases) in the HER2-negative cohort. There were differences in histological staging, Ki-67 index, MP grading, RCB grading, and number of lymph node metastases among the four groups of cases; the differences were statistically significant ($p < 0.05$).

The biological staging of breast cancer has always been based on HR status (HER2-negative and HER2-positive) (14). Our study not only confirmed the correlation between HER2 low and HR status, but also closely correlated with AR status. In addition, in order to confirm the correlation between low HER2 expression and AR positivity, we excluded the influence of HR status and conducted the study on the TNBC cohort. The incidence of HER2 low expression in AR positive cohort was significantly higher than

TABLE 7 Baseline patient characteristics stratified by HER2 status evolution (Differential vs. Consistent).

Demographics	Total (n=574)	Differential (n=129)		Consistent (n=445)		χ2	P Value*
		HER2 0-low	HER2 low-0	HER2 0	HER2 low		
		N=50	N=79	N=79	N=366		
Age							
<70 years	548	49 (98%)	78 (98.73%)	76 (96.20%)	361 (98.63%)	2.376	0.498
≥70 years	26	1 (2%)	1 (1.27%)	3 (3.80%)	5 (1.37%)		
Menopausal status							
Pre/peri-	393	32 (64%)	51 (64.56%)	59 (74.68%)	251 (68.58%)	2.438	0.487
Post-	181	18 (36%)	28 (35.44%)	20 (25.32%)	115 (31.42%)		
Histology							
Invasive ductal	505	46 (92%)	74 (93.67%)	65 (82.28%)	320 (87.43%)	14.693	0.023*
Mucinous adenocarcinoma	7	0 (0%)	0 (0%)	4 (5.06%)	3 (0.82%)		
Other	62	4 (8%)	5 (6.33%)	10 (12.66%)	43 (11.75%)		
Maximum diameter after treatment							
<2	265	18 (36%)	35 (44.30%)	40 (50.63%)	172 (46.99%)	2.925	0.403
≥2	309	32 (64%)	44 (55.70%)	39 (49.37%)	194 (53.01%)		
Ki-67							
≤20%	401	30 (60%)	45 (56.96%)	56 (70.89%)	270 (73.77%)	11.248	0.010*
>20%	173	20 (40%)	34 (43.04%)	23 (29.11%)	96 (26.23%)		
Miller-Payne (MP)							
1	9	1 (2%)	0 (0%)	0 (0%)	8 (2.19%)	23.137	0.027*
2	71	3 (6%)	6 (7.59%)	12 (15.19%)	54 (14.75%)		
3	425	41 (82%)	59 (74.68%)	52 (65.82%)	273 (74.59%)		
4	53	5 (10%)	10 (12.67%)	11 (13.93%)	27 (7.38%)		
5	12	0 (0%)	4 (5.06%)	4 (5.06%)	4 (1.09%)		
Residual cancer burden							
I	59	9 (18%)	10 (12.66%)	11 (13.92%)	29 (7.92%)	13.684	0.033*
II	159	10 (20%)	21 (26.58%)	30 (37.97%)	98 (26.78%)		
III	356	31 (62%)	48 (60.76%)	38 (48.11%)	239 (65.30%)		
Number of metastatic sites							
<3	276	21 (42%)	56 (70.89%)	35 (44.30%)	164 (44.81%)	19.221	0.000*
≥3	298	29 (58%)	23 (29.11%)	44 (55.70%)	202 (55.19%)		

*, P<0.05.

that in AR negative cohort. This has not been shown in other studies.In breast cancer, these new subtypes can be distinguished by the standardized pathological assessment of HRs and HER2, especially in HER2-low breast cancer. This will lead to more complex breast cancer subtypes and provide new targeted therapeutic options to improve breast cancer prognosis.

This study also has certain limitations, because the collected cases were recent breast cancer patients, whose prognostic information was not obtained. Therefore, some biological characteristics of HER2-low breast cancer were not studied.

Conclusion

HER2-low breast cancer is highly unstable during disease evolution and has certain biological characteristics, and breast cancer with HER2-low positivity has certain biological characteristics, which are correlated with positive HR and positive AR. Whether HER2-low breast cancer can be regarded as a new subtype still needs to be confirmed by more studies. At the same time, re-detection of HER2 in breast cancer after neoadjuvant therapy may bring new treatment opportunities for a certain proportion of 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 the Ethics Committee of the Fourth Hospital of Hebei Medical University: 2022KS023. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conception and design: YL. Administrative support: YL. Provision of study materials or patients: JS. Collection and assembly of data: JS. Data analysis and interpretation: JS. Manuscript writing: All authors. Final approval of manuscript: All authors. All authors contributed to the article and approved the submitted version.

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. de Moura Leite L, Cesca MG, Tavares MC, Santana DM, Saldanha EF, Guimarães PT, et al. HER2–low status and response to neoadjuvant chemotherapy in HER2 negative early breast cancer. *Breast Cancer Res TR* (2021) 190(1):155–63. doi: 10.1007/s10549-021-06365-7

2. Tarantino P, Hamilton E, Tolaney SM, Cortes J, Morganti S, Ferraro E, et al. HER2-low breast cancer: Pathological and clinical landscape. *Clin Oncol* (2020) 38:1951–62. doi: 10.1200/JCO.19.02488

3. Marchiò C, Annaratone L, Marques A, Casorzo L, Berrino E, Sapino A, et al. Evolving concepts in HER2 evaluation in breast cancer: Heterogeneity, HER2-low carcinomas and beyond. *Semin Cancer Biol* (2020) 72:123–5. doi: 10.1016/j.semcancer.2020.02.016

4. Riecke K, Witzel I. Targeting the human epidermal growth factor receptor family in breast cancer beyond HER2. *Breast Care (Basel)* (2020) 15:579–85. doi: 10.1159/000510998

5. Nakada T, Sugihara K, Jikoh T, Abe Y, Agatsuma T. The latest research and development into the antibody-drug conjugate, [Fam-] trastuzumab deruxtecan (DS-8201a), for HER2 cancer therapy. *Chem Pharm Bull (Tokyo)* (2019) 67:173–85. doi: 10.1248/cpb.c18-00744

6. Banerji U, van Herpen CML, Saura C, Thistlethwaite F, Lord S, Moreno V, et al. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: A phase 1 dose-escalation and dose-expansion study. *Lancet Oncol* (2019) 20:1124–35. doi: 10.1016/S1470-2045(19)30328-6

7. Tarantino P, Hamilton E, Tolaney SM, Cortes J, Morganti S, Ferraro E, et al. HER2-low breast cancer: Pathological and clinical landscape. *J Clin Oncol Off J Am Soc Clin Oncol* (2020) 38(17):1951–62. doi: 10.1200/JCO.19.02488

8. Schalper KA, Kumar S, Hui P, Gershkovich P. A retrospective population-based comparison of HER2 immunohistochemistry andfluorescence *in situ* hybridization in breast carcinomas: Impact of 2007 American society of clinical Oncology/College of American pathologists criteria. *Arch Pathol Lab Med* (2014) 138:213–9. doi: 10.5858/arpa.2012-0617-OA

9. Schettini F, Chic N, Brasó-Maristany F, Paré L, Pascual T, Conte B, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. *NPJ Breast Cancer* (2021) 7:1-020–00208-2. doi: 10.1038/s41523-020-00208-2

10. Ballard M, Jalikis F, Krings G, Schmidt RA, Chen YY, Rendi MH, et al. 'Non-classical'HER2 FISH results in breast cancer: A multi-institutional study. *Mod Pathol* (2017) 30:227–35. doi: 10.1038/modpathol.2016.175

11. Tarantino P, Nicolò E, Zagami P, Giugliano F, Trillo AP, Marra A, et al. Evolution of low HER2 expressions between primary and metastatic breast cancer. *Ann Oncol* (2020) 31:S15–41. doi: 10.1016/j.annonc.2020.03.185

12. Miglietta F, Griguolo G, Bottosso M, Giarratano T, Lo Mele M, Fassan M, et al. Evolution of HER2-low expression from primary to recurrent breast cancer. *NPJ Breast Cancer* (2021) 7(1):137. doi: 10.1038/s41523-021-00343-4

13. Modi S, Modi S, Park H, Iwata H, Tamura K, Tsurutani J, et al. Antitumor activity and safety of trastuzumab deruxtecan in patients with HER2-low-expressing advanced breast cancer: Results from a phase ib study. *Clin Oncol* (2020) 38:1887–96. doi: 10.1200/JCO.19.02318

14. Denkert C, Seither F, Schneeweiss A, Link T, Blohmer JU, Just M, et al. . Clinical and molecular characteristics of HER2-low-positive breast cancer: Pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. *The Lancet Oncology* (2021) (2021) 22(8):1151–61. doi: 10.1016/S1470-2045(21)00301-6

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Single progesterone receptor-positive phenotype has the similar clinicopathological features and outcome as triple-negative subtype in metastatic breast cancer

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Objective: The same clinicopathological features and prognosis have been reported between single progesterone receptor-positive (sPR-positive) and triple-negative phenotype in early-stage breast cancer, but such similarity has not been studied in metastatic breast cancer (MBC). Therefore, the purpose of this study was to estimate the difference between sPR-positive phenotype and other phenotypes in MBC.

Methods: Patients with HER-2-negative MBC were selected from the Surveillance, Epidemiology and End Results database. Pearson's $\chi 2$ test was used to compare the difference of clinicopathologic factors between sPR-positive phenotype and other phenotypes. Univariate and multivariate analyses were performed to evaluate the effects of hormone receptor (HoR) phenotypes and other clinicopathologic factors on the cancer-specific survival (CSS) and overall survival (OS).

Results: Overall, 10877 patients including 7060 patients (64.9%) with double HoR-positive (dHoR-positive), 1533 patients (14.1%) with single estrogen receptor-positive (sER-positive), 126 patients (1.2%) with sPR-positive and 2158 patients (19.8%) with double HoR-negative (dHoR-negative) were analyzed. The patients with sPR-positive or dHoR-negative were more likely to be younger, higher grade and tumor stage, visceral and brain metastasis than ER-positive phenotypes (P<0.001). MBC with sPR-positive had the similar CSS (HR: 1.135, 95% CI: 0.909-1.417, P=2.623) and OS (HR: 1.141, 95%CI: 0.921-1.413, P=0.229) as dHoR-negative, but worse outcome than ER-positive phenotypes. Chemotherapy significantly improved the survival for MBC, especially for sPR-positive MBC (CSS, HR: 0.39, 95%CI: 0.213-0.714, P=0.002; OS, HR: 0.366, 95% CI: 0.203-0.662, P=0.001).

Conclusions: Patients with sPR-positive and triple-negative have similar biological behavior and prognosis in MBC. Chemotherapy may be a preferred recommendation for MBC with sPR-positive.

KEYWORDS

metastatic breast cancer, single progesterone receptor-positive, endocrine therapy, chemotherapy, outcome

Introduction

Breast cancer is the most common malignant tumor in women and seriously threatens their health and lives (1). Fortunately, after the finding of hormone receptors (HoR) including estrogen receptor (ER) and progesterone receptor (PR), endocrine therapy was gradually becoming the standard treatment for patients with HoR-positive breast cancer and significantly improved the survival for those patients (2). With the development of endocrine therapy, many traditional endocrine therapy regimens including tamoxifen, ovarian function suppression, aromatase inhibitor and fulvestrant have contributed greatly to the survival of patients with HoR-positive breast cancer (2–5). In addition, the combination of cyclin-dependent kinase 4/6 inhibitors and the above endocrine drugs becomes a better choice for patients with HoR-positive breast cancer, especially for metastatic breast cancer (MBC) (6).

More than 80% of breast cancers are HoR-positive (7), and the National Comprehensive Cancer Network (NCCN) guidelines recommend endocrine therapy for patients with ER-positive (ER+) and/or PR-positive (PR+). Actually, there are four HoR phenotypes including double HoR-positive phenotype (ER+/PR+, dHoR-positive), single ER-positive phenotype (ER+/PR-, sER-positive), single PR-positive phenotype (ER-/PR+, sPR-positive) and double HoR-negative (ER-/PR-, dHoR-negative). Many experts have suspected the existence of sPR-positive phenotype and thought it resulted from technical artifacts (8–10), but more and more evidence has justified the existence of this phenotype both in biology and clinic (11, 12). Many previous studies have explored the causes of sPR-positive breast cancer and demonstrated that the major mechanism is the secondary loss of ER (13–15). Furthermore, multiple studies have demonstrated that the breast cancer with sPR-positive and HER-2-negative phenotype has the same clinicopathological characteristics as triple-negative subtype and is also not sensitive to endocrine therapy (11, 16–19). However, those studies included the patients with stage I-III breast cancer but not MBC. Although the most recent study included patients with MBC, the proportion of MBC in the statistical analysis was very small (20). Therefore, we used the stage IV breast cancer with HER-2-negative at the initial diagnosis from the Surveillance, Epidemiology and End Results (SEER) database to analyze the clinicopathological difference between sPR-positive phenotype and other HoR phenotypes.

Material and methods

Data source and patient selection

Retrospective study was performed by using the National Cancer Institute's SEER database which covers approximately 28% of the United States population. Because the SEER database began collecting the HER-2 status and distant metastatic sites from 2010, our study employed the data of SEER database from 2010 to 2018. SEER*Stat version 8.3.8 (http://seer.cancer.gov/seerstat) was used to identify the eligible patients based on the following inclusion criteria: breast cancer, definite distant metastasis, HER-2-negative status, years of diagnosis from 2010 to 2018, one primary cancer only, available HoR status and other clinicopathological information (Figure 1, flowchart). Finally, 10877 patients were enrolled in our study and their information including sex, age, race, marital status, histology type, grade, tumor and lymph node stage, ER and PR status, metastatic sites, therapeutic methods and survival months were collected and analyzed. Because the personally identifiable information about patients could not be obtained from the SEER database, our study was approved to be exempt from ethical review by ethics Committee of our institution.

Statistical analysis

The enrolled patients were divided into four cohorts including ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR- according to HoR status. Then, Pearson's $\chi 2$ test was used to estimate the clinicopathologic difference among these four cohorts. The cancer-specific survival (CSS) and overall survival (OS) were the endpoints of our study. CSS was defined as the interval from the diagnosis of breast cancer to death caused by breast cancer or the final follow-up in censored cases, and OS was defined as the interval from diagnosis of breast cancer to death from all causes or the last follow-up in censored cases. Survival differences were assessed through Kaplan-Meier analysis, followed by a log–rank test. Then, the multivariable Cox proportional hazards model was used and hazard ratios (HR) with the corresponding 95% confidence intervals (CI) were subsequently calculated to estimate the independent prognostic factors. STATA software (Version 13; Stata Corporation) was applied for all statistical analyses. The forest plot was generated by Microsoft Office Excel (Version 2021;

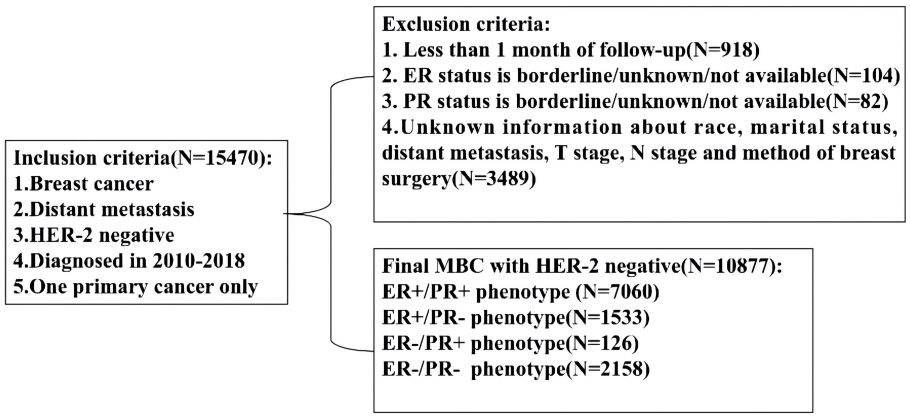

FIGURE 1
Flowchart for patient selection from the Surveillance, Epidemiology and End Results (SEER) database. MBC, metastatic breast cancer; ER, estrogen receptor; PR, progesterone receptor.

Microsoft Corporation). All tests were two sided and p value <0.05 were considered statistically significant.

Results

Patient characteristics

A total of 10877 patients were diagnosed with HER-2-negative MBC at initial presentation between 2010 and 2018 and were included in this study. Among them, 7060 patients (64.9%) were dHoR-positive, 1533 patients (14.1%) were sER-positive, 126 patients (1.2%) were sPR-positive and 2158 patients (19.8%) were dHoR-negative (Table 1). The patients with sPR-positive or dHoR-negative were more likely to be younger and higher percentage of black race when compared with dHoR-positive or sER-positive (P<0.001). A lower proportion of patients with sPR-positive or dHoR-negative presented invasive lobular carcinoma than patients with dHoR-positive or sER-positive (P<0.001). Furthermore, the patients with sPR-positive or dHoR-negative presented higher histological grade and tumor stage than patients with dHoR-positive or sER-positive (P<0.001). Less bone metastasis occurred to patients with sPR-positive (40.5%) or dHoR-negative (42.8%) than patients with dHoR-positive (76%) or sER-positive (68%), but more visceral and brain metastasis happened to patients with sPR-positive or dHoR-negative than patients with dHoR-positive or sER-positive (P<0.001). More patients with sPR-positive (42.9%) or dHoR-negative (40.5%) got surgery of the breast than patients with dHoR-positive (30.1%) or sER-positive (30.9%). Also, more patients with sPR-positive (71.4%) or dHoR-negative (80.6%) accepted chemotherapy than patients with dHoR-positive (52.8%) or sER-positive (58.1%).

Univariate survival analysis

The follow-up time ranged from 1 to 106 months, with a median of 19 months. Finally, death occurred to 6381 patients including 3633 patients with dHoR-positive, 1006 patients with sER-positive, 89 patients with sPR-positive and 1653 patients with dHoR-negative. As shown in Figure 2, the patients with sPR-positive had the same CSS as patients with dHoR-negative (median CSS: 12 and 14 months, respectively, P=0.345), but both had significantly worse CSS than patients with dHoR-positive (median CSS: 44 months, P<0.001). Also, the patients with sPR-positive had the same OS as patients with dHoR-negative (median OS: 11 and 13 months, respectively, P=0.348), but both had worse OS than patients with dHoR-positive (median OS: 40 months, P<0.001). In addition to HoR status, other clinicopathologic factors could also have impacts on the survival of patients with MBC. As shown in Table 2, worse CSS and OS were seen in those patients who were older, black race, unmarried status, higher histological grade (III-IV), higher tumor stage (T_{3-4}), visceral and brain metastasis. Anti-tumor treatments including radiation, chemotherapy and especially surgery of the breast could significantly extend the survival for patients with MBC.

Multivariate survival analysis

When multivariate survival analysis was performed (Table 3), better outcomes were seen in patients with ER-positive. Especially in patients with dHoR-positive, multivariate survival analysis shown significant better CSS (HR: 0.366, 95%CI: 0.293-0.458, P<0.001) and OS (HR: 0.382, 95%CI: 0.309-0.474, P<0.001) compared with patients of sPR-positive. Also, the patients with sER-positive exhibited better CSS (HR:0.624, 95%CI: 0.497-0.784, P<0.001) and OS (HR:0.625, 95%CI: 0.501-0.778, P<0.001) than patients with sPR-positive. However, patients with dHoR-negative had the same CSS (HR: 1.135, 95%CI: 0.909-1.417, P=0.263) and OS (HR: 1.141, 95%CI: 0.921-1.413, P=0.229) compared with patients of sPR-positive. Then, the older age, black race, unmarried status, invasive lobular carcinoma, higher histological grade (III–IV), higher tumor stage (T_{3-4}), visceral (lung and liver) and brain metastasis were independent risk factors for OS and CSS. Surgery

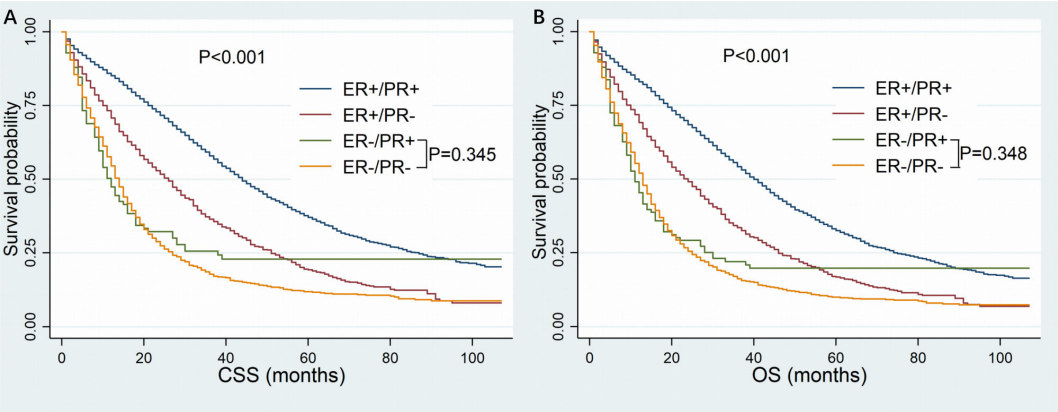

FIGURE 2
Kaplan-Meier curves of cancer-specific survival **(A)** and overall survival **(B)** based on hormone receptor status for patients with HER-2-negative metastatic breast cancer. CSS, cancer-specific survival; OS, overall survival; ER, estrogen receptor; PR, progesterone receptor.

TABLE 1 The clinicopathological features of patients with HER-2-negative MBC in different hormone receptor status.

Variables	N (%)	ER+/PR+, N (%)	ER+/PR-, N (%)	ER-/PR+, N (%)	ER-/PR-, N (%)	P value
Total	10877 (100)	7060 (64.9)	1533 (14.1)	126 (1.2)	2158 (19.8)	
Age at diagnosis						0.001
≤60	5271 (48.5)	3395 (48.1)	674 (44)	68 (54)	1134 (52.5)	
>60	5606 (51.5)	3665 (51.9)	859 (56)	58 (46)	1024 (47.5)	
Sex						0.001
Female	10726 (98.6)	6939 (98.3)	1518 (99)	125 (99.2)	2144 (99.4)	
Male	151 (1.4)	121 (1.7)	15 (1)	1 (0.8)	14 (0.6)	
Race						<0.001
White	8111 (74.6)	5442 (77.1)	1136 (74.1)	77 (61.1)	1456 (67.4)	
Black	1866 (17.1)	989 (14)	283 (18.5)	36 (28.6)	558 (25.9)	
Others	900 (8.3)	629 (8.9)	114 (7.4)	13 (10.3)	144 (6.7)	
Marital status						0.474
Married	5000 (46)	3281 (46.5)	699 (45.6)	55 (43.7)	965 (44.7)	
Unmarried	5877 (54)	3779 (53.5)	834 (54.4)	71 (56.3)	1193 (55.3)	
Histological type						<0.001
IDC	7714 (70.9)	4904 (69.5)	1018 (66.4)	96 (76.2)	1696 (78.6)	
ILC	1275 (11.7)	992 (14.1)	229 (14.9)	6 (4.8)	48 (2.2)	
IDC and ILC	494 (4.6)	378 (5.3)	66 (4.3)	2 (1.6)	48 (2.2)	
Others	1394 (12.8)	786 (11.1)	220 (14.4)	22 (17.4)	366 (17)	
Grade						<0.001
I-II	4987 (45.8)	3964 (56.1)	653 (42.6)	15 (11.9)	355 (16.5)	
III-IV	4263 (39.2)	2025 (28.7)	604 (39.4)	95 (75.4)	1539 (71.3)	
Unknown	1627 (15)	1071 (15.2)	276 (18)	16 (12.7)	264 (12.2)	
Tumor stage						<0.001

(Continued)

TABLE 1 Continued

Variables	N (%)	ER+/PR+, N (%)	ER+/PR-, N (%)	ER-/PR+, N (%)	ER-/PR-, N (%)	P value
T_{0-2}	5134 (47.2)	3504 (49.6)	716 (46.7)	50 (39.7)	864 (40)	
T_{3-4}	5743 (52.8)	3556 (50.4)	817 (53.3)	76 (60.3)	1294 (60)	
Lymph node stage						<0.001
N_0	3858 (35.5)	2585 (36.6)	562 (36.7)	42 (33.3)	669 (31)	
N_{1-2}	5137 (47.2)	3427 (48.5)	686 (44.7)	56 (44.5)	968 (44.9)	
N_3	1882 (17.3)	1048 (14.9)	285 (18.6)	28 (22.2)	521 (24.1)	
Bone metastasis						<0.001
No	3497 (32.2)	1697 (24)	490 (32)	75 (59.5)	1235 (57.2)	
Yes	7380 (67.8)	5363 (76)	1043 (68)	51 (40.5)	923 (42.8)	
Lung metastasis						<0.001
No	7660 (70.4)	5155 (73)	1136 (74.1)	75 (59.5)	1294 (60)	
Yes	3217 (29.6)	1905 (27)	397 (25.9)	51 (40.5)	864 (40)	
Liver metastasis						<0.001
No	8713 (80.1)	5846 (82.8)	1194 (77.9)	92 (73)	1581 (73.3)	
Yes	2164 (19.9)	1214 (17.2)	339 (22.1)	34 (27)	577 (26.7)	
Brain metastasis						<0.001
No	10236 (94.1)	6753 (95.7)	1423 (92.8)	113 (89.7)	1947 (90.2)	
Yes	641 (5.9)	307 (4.3)	110 (7.2)	13 (10.3)	211 (9.8)	
Chemotherapy						<0.001
Yes	6450 (59.3)	3730 (52.8)	891 (58.1)	90 (71.4)	1739 (80.6)	
No	4427 (40.7)	3330 (47.2)	642 (41.9)	36 (28.6)	419 (19.4)	
Radiation						0.079
Yes	3909 (35.9)	2548 (36.1)	583 (38)	40 (31.7)	738 (34.2)	
No	6968 (64.1)	4512 (63.9)	950 (62)	86 (68.3)	1420 (65.8)	
Surgery						<0.001
Yes	3525 (32.4)	2123 (30.1)	473 (30.9)	54 (42.9)	875 (40.5)	
No	7352 (67.6)	4937 (69.6)	1060 (69.1)	72 (57.1)	1283 (59.5)	

MBC, metastatic breast cancer; ER, estrogen receptor; PR, progesterone receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

of the breast and chemotherapy obviously increased the survival for MBC. Furthermore, the subgroup survival analysis shown that chemotherapy significantly improved the CSS (HR: 0.39, 95%CI: 0.213-0.714, P=0.002) and OS (HR: 0.366, 95%CI: 0.203-0.662, P=0.001) for patients with sPR-positive (Figure 3).

Discussion

Detection of hormone receptors can provide prognostic information for breast cancer patients (21, 22), and also endocrine therapy can significantly improve the survival for patients with HoR-positive (2). Thus, the accuracy of hormone receptors testing becomes very critical for breast cancer patients. In 2010, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) published the guideline for ER/PR immunohistochemical (IHC) detection, which clearly proposed that the expression level of ER/PR should be detected in all newly diagnosed breast cancer patients, and emphasized the basic operating procedures, quality control and result interpretation criteria of ER/PR detection (23). This guideline defined 1% as the threshold for positive ER/PR expression in IHC, and recommended that the percentage of positive cells and the intensity of positive staining should be noted in the report. Double HoR-positive phenotype occurs in the majority of patients with breast cancer and has better outcome than single HoR-positive phenotypes including sER-positive phenotype and sPR-positive phenotype (11, 16–18, 24, 25). There was a controversy that whether sPR-positive phenotype is an error or entity. Some experts attributed the sPR-positive phenotype to artifacts arising from the preparation or

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TABLE 2 Unadjusted CSS and OS for patients with HER-2-negative MBC.

Variables	Number (%)	Cancer-specific survival (CSS)		Over survival (OS)	
		Median CSS (months)	P value	Median OS (months)	P value
Total	10877 (100)				
Age at diagnosis					
≤60	5271 (48.5)	36	<0.001	34	<0.001
>60	5606 (51.5)	31		27	
Sex					
Female	10726 (98.6)	33	0.968	30	0.787
Male	151 (1.4)	36		29	
Race					
White	8111 (74.6)	35	<0.001	32	<0.001
Black	1866 (17.1)	23		29	
Others	900 (8.3)	38		35	
Marital status					
Married	5000 (46)	38	<0.001	36	<0.001
Unmarried	5877 (54)	29		26	
Histological type					
IDC	7714 (70.9)	33	<0.001	30	<0.001
ILC	1275 (11.7)	40		36	
IDC and ILC	494 (4.6)	41		37	
Others	1394 (12.8)	26		23	
Grade					
I-II	4987 (45.8)	46	<0.001	41	<0.001
III-IV	4263 (39.2)	23		21	
Unknown	1627 (15)	29		27	
HoR status					
ER+/PR+	7060 (64.9)	44	<0.001	40	<0.001
ER+/PR-	1533 (14.1)	26		24	
ER-/PR+	126 (1.2)	12		11	
ER-/PR-	2158 (19.8)	14		13	
Tumor stage					
T_{0-2}	5134 (47.2)	40	<0.001	37	<0.001
T_{3-4}	5743 (52.8)	28		26	
Lymph node stage					
N_0	3858 (35.5)	35	0.009	31	0.056
N_{1-2}	5137 (47.2)	34		30	
N_3	1882 (17.3)	30		27	
Bone metastasis					
No	3497 (32.2)	28	0.002	25	0.001

(Continued)

TABLE 2 Continued

Variables	Number (%)	Cancer-specific survival (CSS)		Over survival (OS)	
		Median CSS (months)	P value	Median OS (months)	P value
Yes	7380 (67.8)	35		32	
Lung metastasis					
No	7660 (70.4)	37	<0.001	34	<0.001
Yes	3217 (29.6)	24		21	
Liver metastasis					
No	8713 (80.1)	38	<0.001	34	<0.001
Yes	2164 (19.9)	19		18	
Brain metastasis					
Yes	10236 (94.1)	35	<0.001	32	<0.001
No	641 (5.9)	12		12	
Radiation					
Yes	3909 (35.9)	36	<0.001	33	<0.001
No	6968 (64.1)	32		29	
Surgery					
Yes	3525 (32.4)	46	<0.001	42	<0.001
No	7352 (67.6)	29		26	
Chemotherapy					
Yes	6450 (59.3)	34	<0.001	32	<0.001
No	4427 (40.7)	33		28	

CSS, cancer-specific survival; OS, overall survival; MBC, metastatic breast cancer; HoR, hormone receptor; ER, estrogen receptor; PR, progesterone receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

assay of the sample, such as inadequate tissue fixation or technique failure of the IHC assay (10, 26). However, some studies confirmed its existence through IHC (11, 12, 27). Besides, subsequent researches justified its presence through analyzing PAM50 expression signature and mRNA level of ESR1, which also revealed that 53-65% of patients with sPR-positive phenotype were basal-like and didn't respond well to endocrine therapy (28, 29). Recent studies shown that the sPR-positive phenotype has the same characteristics as dHoR-negative phenotype and may not well respond to endocrine therapy (11, 16–18, 20). But those studies didn't include or included a small percentage of patients with MBC which clearly differs from early-stage breast cancer. Therefore, we estimated the eligible patients from SEER database to figure out if the biological behavior of MBC with sPR-positive is the same as early-stage breast cancer reported by previous researches.

Consistent with previous studies (11, 12, 16, 17, 26), the patients with sPR-positive accounted for 1.2% of the whole cohort in our study. Also, our study exhibited the same clinicopathological features between sPR-positive phenotype and dHoR-negative phenotype, such as, younger age, less proportion of invasive lobular carcinoma, higher histologic grade, later tumor stage and more lymph nodes involved. What has not been reported is the difference of metastatic sites between breast cancer with sPR-positive phenotype and other phenotypes. Our study shown the metastatic tendency of sPR-positive phenotype kept with dHoR-negative phenotype and it was more likely to be visceral and brain metastasis for sPR-positive phenotype compared with ER-positive phenotypes. This finding further sheds light on the similar aggressive biological behavior between sPR-positive phenotype and dHoR-negative phenotype in MBC.

Compared with sPR-positive phenotype, the patients with dHoR-positive or sER-positive phenotype significantly exhibited better outcomes. While, the same prognosis between sPR-positive phenotype and dHoR-negative phenotype was seen in our study. The difference of prognosis among these four cohorts keeps with previous studies (11, 16, 17). Multiple studies (30, 31) have reported that surgery of the breast can improve the survival for stage IV breast cancer, which was also proved in our study. Then, chemotherapy as the main treatment to delay the progression of MBC can also significantly improve the survival for MBC, especially for such patients with sPR-positive phenotype as shown in the forest plot. This interesting finding was also reported in a previous study which used propensity score matching cohorts to show the significant benefit from chemotherapy for sPR-positive phenotype (17). The remarkable effect of chemotherapy on MBC with sPR-positive phenotype may be due to the insensitivity of this phenotype to endocrine therapy.

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TABLE 3 Multivariable Cox regression for CSS and OS among patients with HER-2-negative MBC.

Variables	Cancer-specific survival (CSS)			Overall survival (OS)		
	HR	95%CI	P value	HR	95%CI	P value
Age at diagnosis						
≤60	Ref			Ref		
>60	1.195	1.132-1.262	<0.001	1.247	1.184-1.314	<0.001
Sex						
Female	Ref			Ref		
Male	1.229	0.982-1.539	0.072	1.230	0.995-1.520	0.055
Race						
White	Ref			Ref		
Black	1.198	1.119-1.282	<0.001	1.221	1.144-1.302	<0.001
Others	0.902	0.815-0.998	0.045	0.923	0.839-1.016	0.102
Marital status						
Married	Ref			Ref		
Unmarried	1.221	1.158-1.289	<0.001	1.254	1.192-1.320	<0.001
Histological type						
IDC	Ref			Ref		
ILC	1.155	1.056-1.263	0.002	1.134	1.042-1.234	0.004
IDC and ILC	1.151	1.013-1.309	0.031	1.144	1.013-1.292	0.03
Others	1.073	0.990-1.162	0.085	1.087	1.008-1.172	0.031
Grade						
I-II	Ref			Ref		
III-IV	1.477	1.386-1.575	<0.001	1.428	1.343-1.517	<0.001
Unknown	1.174	1.082-1.273	<0.001	1.135	1.051-1.227	0.001
HoR status						
ER-/PR+	Ref			Ref		
ER+/PR+	0.366	0.293-0.458	<0.001	0.382	0.309-0.474	<0.001
ER+/PR-	0.624	0.497-0.784	<0.001	0.625	0.501-0.778	<0.001
ER-/PR-	1.135	0.909-1.417	0.263	1.141	0.921-1.413	0.229
Tumor stage						
T0-2	Ref			Ref		
T3-4	1.243	1.177-1.312	<0.001	1.240	1.178-1.306	<0.001
Lymph node stage						
N0	Ref			Ref		
N1-2	0.996	0.940-1.056	0.906	0.989	0.935-1.045	0.69
N3	1.061	0.983-1.144	0.13	1.047	0.973-1.126	0.219
Bone metastasis						
No	Ref			Ref		
Yes	1.274	1.200-1.354	<0.001	1.241	1.172-1.315	<0.001

(Continued)

TABLE 3 Continued

Variables	Cancer-specific survival (CSS)			Overall survival (OS)		
	HR	95%CI	P value	HR	95%CI	P value
Lung metastasis						
No	Ref			Ref		
Yes	1.245	1.175-1.319	<0.001	1.227	1.161-1.296	<0.001
Liver metastasis						
No	Ref			Ref		
Yes	1.754	1.649-1.866	<0.001	1.710	1.611-1.815	<0.001
Brain metastasis						
No	Ref			Ref		
Yes	1.811	1.639-2.001	<0.001	1.788	1.624-1.968	<0.001
Surgery						
Yes	Ref			Ref		
No	1.698	1.596-1.808	<0.001	1.693	1.595-1.796	<0.001
Radiation						
Yes	Ref			Ref		
No	1.021	0.964-1.082	0.484	1.037	0.982-1.096	0.194
Chemotherapy						
Yes	Ref			Ref		
No	1.412	1.332-1.497	<0.001	1.467	1.388-1.550	<0.001

CSS, cancer-specific survival, OS, overall survival; HR, hazard ratios; CI, confidence intervals; MBC, metastatic breast cancer; HoR, hormone receptor; ER, estrogen receptor; PR, progesterone receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

Unfortunately, the information about endocrine therapy can't be acquired from SEER database. Although the explicit endocrine therapy information can't be obtained, most of the patients with ER-positive and/or PR-positive would have received appropriate endocrine therapy for the wide use of NCCN guidelines. Bardou, et al (18) performed a retrospective study including patients from two large breast cancer databases to evaluate whether progesterone receptor status provided prediction of benefit from endocrine treatment. One of the cohorts including 1688 patients of endocrine therapy shown that sPR-positive phenotype had the same outcome compared with dHoR-negative phenotype, and another cohort containing 10444 patients of endocrine therapy also demonstrated that result. In addition, a large meta-analysis including 20 trails shown that 1236 patients with sPR-positive phenotype didn't benefit from adjuvant tamoxifen therapy (Rate ration=0.9, 95CI%: 0.73-1.12, P=0.35) (19). Actually, previous studies have revealed that only 20-30% of patients with sPR-positive breast cancer are luminal-like and the majority are basal-like (28, 29, 32), which explained why patients with sPR-positive didn't significantly benefit from endocrine therapy. The right treatments are crucial for MBC because the noneffective therapeutic regimens may lead to tumor progression and finally worsen the outcome. Therefore, the MBC with sPR-positive should

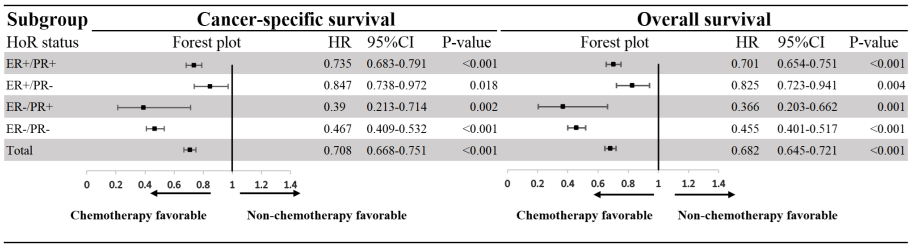

FIGURE 3
Effect of chemotherapy on patients with HER-2-negative metastatic breast cancer according to hormone receptor status. HoR, hormone receptor; ER, estrogen receptor; PR, progesterone receptor; HR, hazard rations; CI, confidence intervals.

be dealt with seriously and chemotherapy can be the most crucial treatment for such group for its outstanding effect on improving survival as shown above. Meanwhile, the gene expression measurement should be performed to find the minority patients of sPR-positive phenotype belonging to luminal-like and endocrine therapy should also be used to palliate the progression of MBC.

Several limitations of this study must be elucidated. Firstly, some bias is inevitable due to retrospective nature of this study. Secondly, endocrine therapy information is not available from the SEER database and we can't directly analysis the effect of endocrine therapy on MBC with sPR-positive. Finally, the number of patients with sPR-positive is not very large, so the conclusion of our study must be further justified by larger population. However, our study is the first one that used the MBC with HER-2-negative to analysis the difference between sPR-positive phenotype and other phenotypes in clinicopathological features and survival. And it further confirms the similar biological behavior between sPR-positive phenotype and triple-negative phenotype in MBC, which can guide the clinicians to make better treatment strategies when facing with this rare phenotype.

Conclusions

MBC with sPR-positive and HER-2-negative has the similar biological behavior to triple-negative MBC, such as younger age, higher histological grade, larger tumor burden and predisposition to visceral and brain metastasis. The MBC with sPR-positive and HER-2-negative has the similar prognosis to MBC of triple-negative but worse prognosis than ER-positive phenotype. Chemotherapy may be a preferred recommendation for patients with sPR-positive phenotype because it significantly improves their survival.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: the Surveillance, Epidemiology, and End Results (SEER) database (https://seer.cancer.gov).

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YL, XZ, and LH conceived and designed this study. YL, HP, FL, and JC collected and analyzed the data. YL, SQ, and HP organized the manuscript. YL, XZ, and LH reviewed the paper and revised the manuscript. All authors contributed to the article and approved the submitted version.

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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. 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. Baum M, Drinkley DM, Dossett JA, Mcpherson K, Patterson JS, Rubens RD, et al. Controlled trial of tamoxifen as adjuvant agent in management of early breast cancer. interim analysis at four years by nolvadex adjuvant trial organisation. *Lancet* (1983) 1 (8319):257–61.

3. Baum M, Budzar AU, Cuzick J, Forbes J, Houghton JH, Klijn JG, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. *Lancet* (2002) 359(9324):2131–9. doi: 10.1016/s0140-6736(02)09088-8

4. Francis PA, Regan MM, Fleming GF, Lang I, Ciruelos E, Bellet M, et al. Adjuvant ovarian suppression in premenopausal breast cancer. *N Engl J Med* (2015) 372(5):436–46. doi: 10.1056/NEJMoa1412379

5. Robertson JFR, Bondarenko IM, Trishkina E, Dvorkin M, Panasci L, Manikhas A, et al. Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. *Lancet* (2016) 388(10063):2997–3005. doi: 10.1016/S0140-6736(16)32389-3

6. Gao JJ, Cheng J, Bloomquist E, Sanchez J, Wedam SB, Singh H, et al. CDK4/6 inhibitor treatment for patients with hormone receptor-positive, HER2-negative, advanced or metastatic breast cancer: a US food and drug administration pooled analysis. *Lancet Oncol* (2020) 21(2):250–60. doi: 10.1016/S1470-2045(19)30804-6

7. Kohler BA, Sherman RL, Howlader N, Jemal A, Ryerson AB, Henry KA, et al. Annual report to the nation on the status of cancer, 1975-2011, featuring incidence of breast cancer subtypes by Race/Ethnicity, poverty, and state. *J Natl Cancer Inst* (2015) 107(6):djv048. doi: 10.1093/jnci/djv048

8. De Maeyer L, Van Limbergen E, De Nys K, Moerman P, Pochet N, Hendrickx W, et al. Does estrogen receptor negative/progesterone receptor positive breast carcinoma exist? *J Clin Oncol* (2008) 26(2):335–6. doi: 10.1200/JCO.2007.14.8411

9. Maleki Z, Shariat S, Mokri M, Atri M. ER-negative /PR-positive breast carcinomas or technical artifacts in immunohistochemistry? *Arch Iran Med* (2012) 15(6):366–9.

10. Nadji M, Gomez-Fernandez C, Ganjei-Azar P, Morales AR. Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. *Am J Clin pathology.* (2005) 123(1):21–7. doi: 10.1309/4WV79N2GHJ3X1841

11. Ahmed SS, Thike AA, Zhang K, Lim JC, Tan PH. Clinicopathological characteristics of oestrogen receptor negative, progesterone receptor positive breast cancers: re-evaluating subsets within this group. *J Clin Pathol* (2017) 70(4):320–6. doi: 10.1136/jclinpath-2016-203847

12. Ng CH, Pathy NB, Taib NA, Mun KS, Rhodes A, Yip CH. The estrogen receptor negative-progesterone receptor positive breast carcinoma is a biological entity and not a technical artifact. *Asian Pac J Cancer Prev* (2012) 13(4):1111–3. doi: 10.7314/APJCP.2012.13.4.1111

13. Borras M, Lacroix M, Legros N, Leclercq G. Estrogen receptor-negative/progesterone receptor-positive evsa-T mammary tumor cells: a model for assessing the biological property of this peculiar phenotype of breast cancers. *Cancer Lett* (1997) 120(1):23–30. doi: 10.1016/S0304-3835(97)00285-1

14. Koehorst SG, Jacobs HM, Thijssen JH, Blankenstein MA. Wild type and alternatively spliced estrogen receptor messenger RNA in human meningioma tissue and MCF7 breast cancer cells. *J Steroid Biochem Mol Biol* (1993) 45(4):227–33. doi: 10.1016/0960-0760(93)90336-U

15. Martin MB, Saceda M, Lindsey RK. Regulation of estrogen receptor expression in breast cancer. *Adv Exp Med Biol* (1993) 330:143–53. doi: 10.1007/978-1-4615-2926-2_11

16. Bae SY, Kim S, Lee JH, Lee HC, Lee SK, Kil WH, et al. Poor prognosis of single hormone receptor- positive breast cancer: Similar outcome as triple-negative breast cancer. *BMC Cancer.* (2015) 15:138. doi: 10.1186/s12885-015-1121-4

17. Zheng H, Ge C, Lin H, Wu L, Wang Q, Zhou S, et al. Estrogen receptor-negative/progesterone receptor-positive and her-2-negative breast cancer might no longer be classified as hormone receptor-positive breast cancer. *Int J Clin Oncol* (2022) 27:1145–53. doi: 10.1007/s10147-022-02158-0

18. Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J Clin Oncol* (2003) 21(10):1973–9. doi: 10.1200/JCO.2003.09.099

19. Early Breast Cancer Trialists' Collaborative G, Davies C, Godwin J, Gray R, Clarke M, Cutter D, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* (2011) 378(9793):771–84. doi: 10.1016/S0140-6736(11)60993-8

20. Xiao Y, Li J, Wu Z, Zhang X, Ming J. Influence of progesterone receptor on metastasis and prognosis in breast cancer patients with negative HER-2. *Gland Surg* (2022) 11(1):77–90. doi: 10.21037/gs-21-677

21. Hilsenbeck SG, Ravdin PM, de Moor CA, Chamness GC, Osborne CK, Clark GM. Time-dependence of hazard ratios for prognostic factors in primary breast cancer. *Breast Cancer Res Treat* (1998) 52(1-3):227–37. doi: 10.1023/A:1006133418245

22. Martin M. Molecular biology of breast cancer. *Clin Transl Oncol* (2006) 8(1):7–14. doi: 10.1007/s12094-006-0089-6

23. 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

24. Lv M, Mao Y, Song Y, Wang Y, Liu X, Wang X, et al. Clinical features and survival of single hormone receptor-positive breast cancer: A population-based study of 531,605 patients. *Clin Breast Cancer.* (2020) 20(5):e589–e99. doi: 10.1016/j.clbc.2020.04.010

25. Li Y, Yang D, Yin X, Zhang X, Huang J, Wu Y, et al. Clinicopathological characteristics and breast cancer-specific survival of patients with single hormone receptor-positive breast cancer. *JAMA Netw Open* (2020) 3(1):e1918160. doi: 10.1001/jamanetworkopen.2019.18160

26. Rakha EA, El-Sayed ME, Green AR, Paish EC, Powe DG, Gee J, et al. Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. *J Clin Oncol* (2007) 25(30):4772–8. doi: 10.1200/JCO.2007.12.2747

27. Kuroda H, Muroi N, Hayashi M, Harada O, Hoshi K, Fukuma E, et al. Oestrogen receptor-negative/progesterone receptor-positive phenotype of invasive breast carcinoma in Japan: re-evaluated using immunohistochemical staining. *Breast Cancer.* (2019) 26(2):249–54. doi: 10.1007/s12282-018-0898-9

28. Itoh M, Iwamoto T, Matsuoka J, Nogami T, Motoki T, Shien T, et al. Estrogen receptor (ER) mRNA expression and molecular subtype distribution in ER-negative/progesterone receptor-positive breast cancers. *Breast Cancer Res Treat* (2014) 143(2):403–9. doi: 10.1007/s10549-013-2763-z

29. Schroth W, Winter S, Buttner F, Goletz S, Faisst S, Brinkmann F, et al. Clinical outcome and global gene expression data support the existence of the estrogen receptor-negative/progesterone receptor-positive invasive breast cancer phenotype. *Breast Cancer Res Treat* (2016) 155(1):85–97. doi: 10.1007/s10549-015-3651-5

30. Xiong Z, Deng G, Wang J, Li X, Xie X, Shuang Z, et al. Could local surgery improve survival in *de novo* stage IV breast cancer? *BMC Cancer* (2018) 18(1):885. doi: 10.1186/s12885-018-4767-x

31. Li X, Huang R, Ma L, Liu S, Zong X. Locoregional surgical treatment improves the prognosis in primary metastatic breast cancer patients with a single distant metastasis except for brain metastasis. *Breast* (2019) 45:104–12. doi: 10.1016/j.breast.2019.03.006

32. Yu KD, Jiang YZ, Hao S, Shao ZM. Molecular essence and endocrine responsiveness of estrogen receptor-negative, progesterone receptor-positive, and HER2-negative breast cancer. *BMC Med* (2015) 13:254. doi: 10.1186/s12916-015-0496-z

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The utility of diffusion-weighted imaging for differentiation of phyllodes tumor from fibroadenoma and breast cancer

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Objective: To evaluate the utility of apparent diffusion coefficient (ADC) values for differentiating breast tumors.

Methods: The medical records of 17 patients with phyllodes tumor [PT; circular regions of interest (ROI-cs) n = 171], 74 patients with fibroadenomas (FAs; ROI-cs, n = 94), and 57 patients with breast cancers (BCs; ROI-cs, n = 104) confirmed by surgical pathology were retrospectively reviewed.

Results: There were significant differences between PTs, FAs, and BCs in ADCmean, ADCmax, and ADCmin values. The cutoff ADCmean for differentiating PTs from FAs was 1.435×10^{-3} mm^2/s, PTs from BCs was 1.100×10^{-3} mm^2/s, and FAs from BCs was 0.925×10^{-3} mm^2/s. There were significant differences between benign PTs, borderline PTs, and malignant PTs in ADCmean, ADCmax, and ADCmin values. The cutoff ADCmean for differentiating benign PTs from borderline PTs was 1.215×10^{-3} mm^2/s, and borderline PTs from malignant PTs was 1.665×10^{-3} mm^2/s.

Conclusion: DWI provides quantitative information that can help distinguish breast tumors.

KEYWORDS

diffusion-weighted image (DWI), apparent diffusion coefficient (ADC), value, magnetic resonance imaging (MRI), breast tumors, phyllodes tumors

Introduction

Phyllodes tumor (PT), first introduced by Muller in 1838, is a fibroepithelial neoplasm that is histologically similar to a fibroadenoma (FA). PTs are rare, accounting for 2% to 4.4% of all diagnosed FAs in one institution (1). Breast magnetic resonance imaging (MRI) has an overall sensitivity of 90% and a specificity of 72% for detecting breast lesions (2, 3).

From 2004 to 2019, there were only a few reports describing the characteristics of PTs on MRI (1, 4–8). PTs may be detected on MRI and classified according to the American College of Radiology Breast Imaging-Reporting and Data System (BI-RADS). The BI-RADS evaluates the benign and malignant nature of lesions according to morphological characteristics and kinetic curve assessments. PTs are classified as benign, borderline, or malignant based on semi-quantitative histological features (mitotic phase, degree of stromal dysplasia, and margin) (9). PTs that do not have typical malignant signs but sufficient suspicious manifestations should be classified as BI-RADS IV. PTs exhibit different time-signal intensity curve (TIC) types (10, 11). The TICs exhibited by benign PTs may be similar to FAs, while the TICs exhibited by borderline and malignant PTs may be similar to breast cancers (BCs). TIC type does not correlate with the histologic grade of PTs (5). PTs and FAs may have a contrast enhancement pattern suggestive of malignancy in up to one-third of cases, and some potentially benign lesions cannot be differentiated from BCs (4).

Diffusion-weighted imaging (DWI) has become clinically relevant (12, 13). DWI is a non-invasive MRI technique that can measure the diffusion of water molecules across tissues, *in vivo*. The motion of water molecules in tissues depends on tissue cellularity and the integrity of cell membranes. Differences in the motion of water molecules between tissues cause signal attenuation. To date, DWI for breast tumor applications has relied on the mono-exponential model with b-values of 0 and 800 s/mm^2 (14–16). Other studies adopted b = 0/1000 s/mm^2 (17, 18). This assumes an exponential decay in signal intensity with the product of the b value and apparent diffusion coefficient (ADC). ADC values reflect the slope of the best fit straight line to the log signal as a function of the b-value (19). When the b-value is >1000 s/mm^2, signal intensity corresponds to the anatomical and physiological characteristics of breast tissue and, thus, deviates from the single exponential model. In this case, a bi-exponential model is necessary to measure diffusion and microperfusion parameters. ADC values may be determined in three different types of tumor regions of interest (ROIs), including a circular ROI (ROI-c), single-slice ROI (ROI-s), and whole-tumor ROI (ROI-w) (13). ADC values can provide objective and accurate quantitative information (20–24). ADC values are impacted by ROI selection (8). A smaller ROI placed over the most hypointense ADC area may provide better discrimination performance by reflecting the worst pathology within a heterogeneous lesion, but whole tumor measurement may allow better reproducibility (13). The objective of this study was to evaluate the utility of ADC values to differentiate between PTs, FAs, and BCs, and to classify PTs.

Materials and methods

Study subjects

The medical records of female patients diagnosed with breast tumors between 1 January 2017 and 5 April 2022 were retrospectively reviewed. This retrospective analysis of breast MRI data was approved by the Institutional Research Ethics Board of our institute (Approval No. 20220509). The requirement for informed consent was waived. Inclusion criteria were: 1) unilateral or bilateral solid breast tumor, 2) no history of surgery, 3) no history of other tumors or systemic diseases, and 4) surgical pathology provided a definitive diagnosis. All patients underwent MRI examination 3–7 days prior to surgery. Patients were divided into three groups based on pathological findings: Group A, PT; Group B, FA; and Group C, BC.

MRI protocols

Patients were scanned using a 3.0-T (Ingenia, Philips Medical systems, Netherlands) superconducting MRI scanner. DWI sequences were obtained with b-values of 0 and 800 s/mm^2. DWI parameters: FOV (mm): RL × AP × FH, 340 × 196 × 150; voxel (mm): 3.04 × 1.87 × 3; REC voxel MPS (mm): 1.06 × 1.06 × 1.06; slice thickness (mm): 3; slice gap (mm): 0; matrix (slices): 112 × 105 × 50; REC matrix: 320; NSA: 2; scan percentage (%): 163.2; total scan duration (min): 3:07; SNR: 1.027; TR (ms): 12500; min.TR (ms): 11007; TE (ms): 82; EPI factor: 93; BW in EPI freq.dir (HZ): 2129.8; WFS (pix)/BW (hz): 24.817/17.5; fold-over suppression: oversampling; P (mm): 153; A (mm): 73; stacks: 1; type: parallel; slices: 50; slice gap: 0; slice orientation: transverse; fold-over direction: AP; fat shift direction: P; packages: 1; local torso SAR: <64%; whole body SAR/level: <1.7 W/kg/normal; SED: <0.3 kj/kg; coilpower: 51%; maxB1 + rms: 1.67 uT. ADC maps were processed using the post-processing software (Philips Intellispace Portal). Two radiologists placed an ROI-c (10–300 mm^2) on a 2D single-slice of each breast tumor. The area of the ROI-cs (mm^2), ADCmean, ADCmax, ADCmin, and standard deviation (SD) were calculated.

Statistical analysis

Statistical analysis was conducted using SPSS v28.0.1. Descriptive statistics, including mean and standard deviation, were summarized for each ADC parameter. Normality of ADC values was evaluated with the single-sample Shapiro-Wilk test. Normally distributed data with homogeneity of variance were compared with ANOVA. Non-normally distributed data with heterogeneous variance were compared with the non-parametric Kruskal-Wallis H test. Pairwise comparison was made with the Mann-Whitney *U* test. The area under the curve (AUC) of receiver operating characteristic (ROC) curves was used to assess the diagnostic performance of ADC parameters for breast tumors. $p < 0.05$ was considered statistically significant.

Results

The medical records of 148 patients with breast tumors were retrospectively reviewed, including 17 patients with PTs [eight benign PTs (Figure 1), six borderline PTs (Figure 2), and three malignant PTs (Figure 3)], 74 patients with FAs, and 57 patients

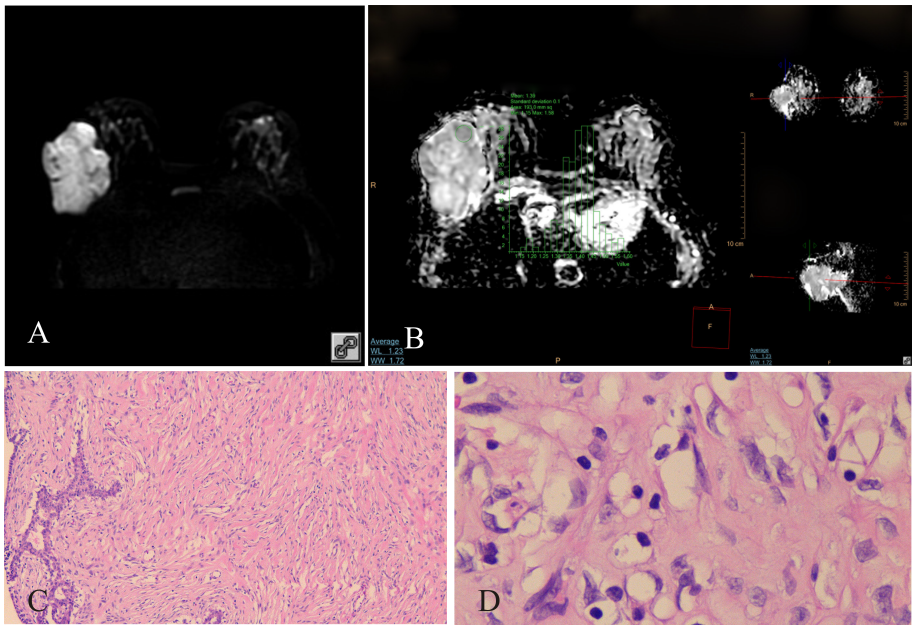

FIGURE 1
Benign PT of the right breast, female, age 46 years. **(A)** DWI (b800s/mm^2): mixed hyperintense signal; **(B)** ADC map: isointense–hyperintense mixed signal, ADCmean = 1.39 × 10^{-3} mm^2/s; **(C)** HE ×100: tumor stromal cells were dispersed; **(D)** HE ×400: no nuclear division was observed, tumor cells were loosely arranged.

with BCs. A total of 369 ROI-cs were evaluated, including 171 ROI-cs for PTs, 94 ROI-cs for FAs, and 104 ROI-cs for BCs. Patients' mean (SD) age was 49.17 ± 2.95 years (range, 19–74 years old), and time since diagnosis ranged from 3 weeks to 2 months; 88 patients underwent surgical resection, and 60 patients underwent excisional biopsy.

ADCmean, ADCmax, and ADCmin of PTs were 1.6083 (0.83–2.16) ± 0.26015 × 10^{-3} mm^2/s, 1.8112 (0.94–2.44) ± 0.28428 × 10^{-3} mm^2/s, and 1.4113 (0.69–2.05) ± 0.28392 × 10^{-3} mm^2/s, respectively, which were higher than the ADCmean, ADCmax, and ADCmin of FAs and BCs. Multiple group comparisons conducted with the Kruskal-Wallis H test and Mann-Whitney

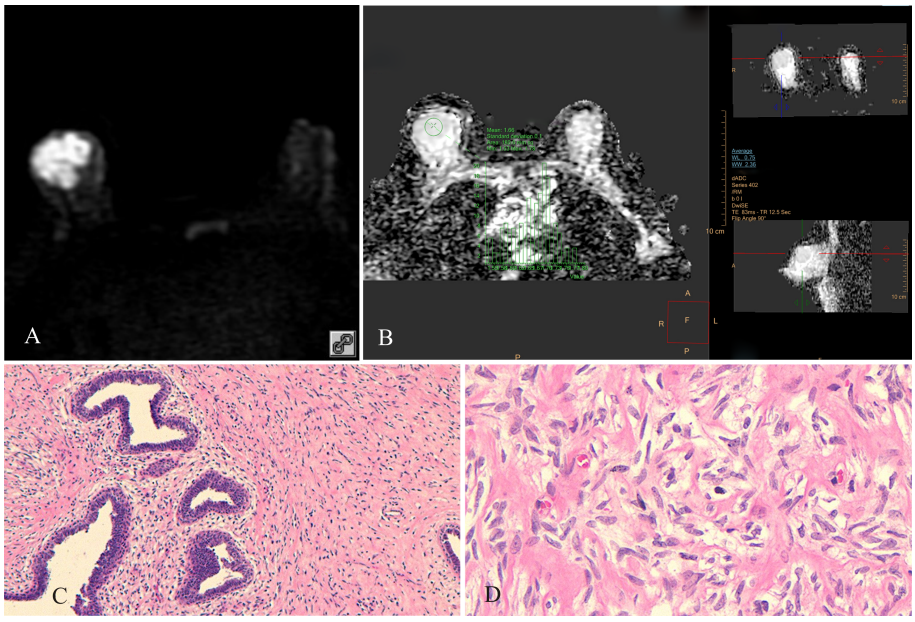

FIGURE 2
Borderline PT of the right breast, female, age 45 years. **(A)** DWI (b800 s/mm^2): mixed hyperintense signal; **(B)** ADC map: isointense signal, ADC mean = 1.66×10^{-3} mm^2/s; **(C)** HE ×100: uneven distribution of tumor stromal cells, high cell density in some areas, low cell density in other areas; **(D)** HE ×400: tumor stromal cells had "tadpole-like" nuclei, cells were closely packed.

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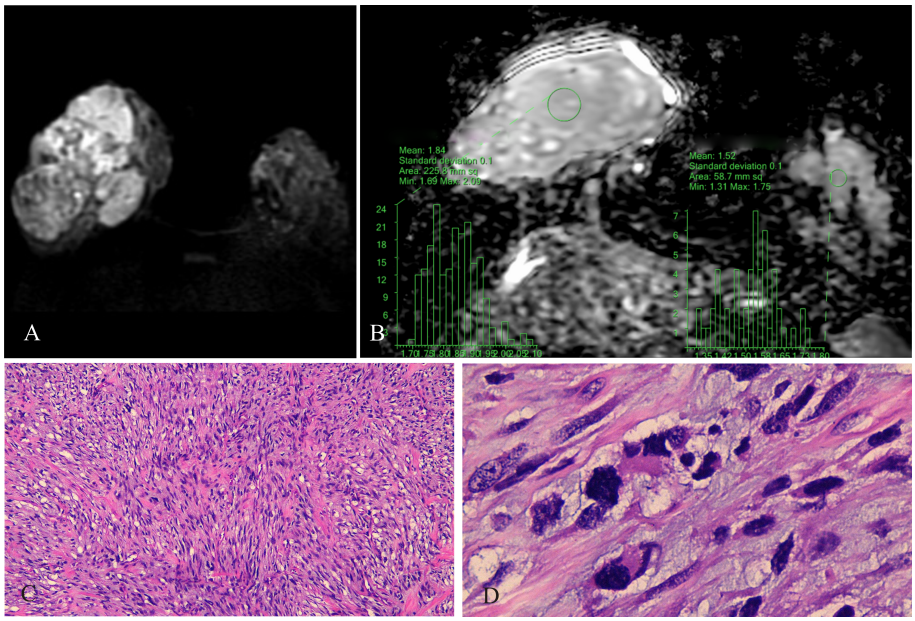

FIGURE 3
Malignant PT of the right breast, female, age 29 years. **(A)** DWI (b800 s/mm^2): mixed hyperintense signal; **(B)** ADC map: isointense-hyperintense mixed signal, ADCmean = 1.84 × 10^{-3} mm^2/s; ADCmean (left normal breast) = 1.52 × 10^{-3} mm^2/s; **(C)** HE ×100: tumor stromal cells were closely packed; **(D)** HE ×400: nuclear fission, interstitial edema was insignificant.

U test showed significant differences ($p < 0.001$) (Supplementary Tables 1–4). ROC curve analysis and the Youden index were used to determine optimum cutoff values for ADC parameters for differentiating PTs, FAs, and BCs (Supplementary Figure 1 and Table 1). ADCmean had the largest AUC among ADC mean, ADCmax, and ADCmin. For PTs vs. FAs, the AUC of ADCmean was 0.823 (95% CI 0.764–0.881). For PTs vs. BCs, the AUC of ADCmean was 0.987 (95% CI 0.977–0.996). For FAs vs. BCs, the AUC of ADCmean was 0.906 (95% CI 0.8677–0.946). The cutoff ADCmean for differentiating PTs from FAs was 1.435 × 10^{-3} mm^2/s, PTs from BCs was 1.100 × 10^{-3} mm^2/s, and FAs from BCs was 0.925×10^{-3} mm^2/s.

The ADCmeans of benign PTs, borderline PTs, and malignant PTs were 1.5619 (1.25–1.92) ± 0.14886 × 10^{-3} mm^2/s, 1.3098 (0.83–1.68) ± 0.25017 × 10^{-3} mm^2/s, and 1.7962 (1.45–2.16) ± 0.13255 × 10^{-3} mm^2/s, respectively (Supplementary Table 5). ADCmean, ADCmax, and ADCmin of benign PTs, borderline PTs, and malignant PTs were significantly different (Supplementary Tables 6–9). For benign PTs vs. borderline PTs, the AUC of ADCmean was 0.771 (95% CI 0.672–0.870). For borderline PTs vs. malignant PTs, the AUC of ADCmean was 0.982 (95% CI 0.964–0.999). The cutoff ADCmean for differentiating benign PTs from borderline PTs was 1.215 × 10^{-3} mm^2/s and borderline PTs from malignant PTs was 1.665 × 10^{-3} mm^2/s. For benign PTs vs. malignant PTs, the ADCmin had the largest AUC among ADCmean, ADCmax, ADCmin; which was 0.905 (95% CI 0.848–0.961); the cutoff ADCmin was 1.465 × 10^{-3} mm^2/s (Supplementary Figure 2 and Table 2).

TABLE 1 Diagnostic performance of ADC parameters for differentiating between PTs, FAs, and BCs.

Parameter	Comparison group	AUC	Cutoff	Sensitivity	Specificity	Youden Index	95% CI	p-Value
ADCmean (×10^{-3} mm^2/s)	a	0.823	1.435	0.813	0.819	0.632	0.764–0.881	<0.0001
	b	0.987	1.100	0.947	0.942	0.890	0.977–0.996	<0.0001
	c	0.906	0.925	0.947	0.729	0.676	0.867–0.946	<0.0001
ADCmax (×10^{-3} mm^2/s)	a	0.802	1.575	0.836	0.681	0.517	0.743–0.862	<0.0001
	b	0.952	1.545	0.848	0.962	0.809	0.929–0.976	<0.0001
	c	0.777	1.390	0.585	0.860	0.445	0.714–0.840	<0.0001
ADCmin (×10^{-3} mm^2/s)	a	0.810	1.245	0.784	0.787	0.571	0.753–0.868	<0.0001
	b	0.986	0.965	0.906	0.971	0.878	0.977–0.996	<0.0001
	c	0.894	0.760	0.915	0.776	0.691	0.850–0.938	<0.0001

a, PT vs. FA; b, PT vs. BC; c, FA vs. BC (AUC of ROI-c and SD was small and were not included in further analyses).

TABLE 2 Diagnostic performance of ADC parameters for classifying PTs.

Parameter	Comparison Group	AUC	Cutoff	Sensitivity	Specificity	Youden Index	95% CI	p-Value
ADCmean ($\times10^{-3}$ mm^2/s)	a	0.771	1.215	1.000	0.488	0.488	0.672~0.870	<0.0001
	b	0.879	1.625	0.692	0.949	0.641	0.816~0.943	<0.0001
	c	0.982	1.665	0.976	0.885	0.860	0.964~0.999	<0.0001
ADCmax ($\times10^{-3}$ mm^2/s)	a	0.702	1.520	0.923	0.463	0.386	0.595~0.809	0.0009
	b	0.772	1.750	0.577	0.962	0.538	0.684~0.861	<0.0001
	c	0.940	1.785	0.854	0.897	0.751	0.896~0.984	<0.0001
ADCmin ($\times10^{-3}$ mm^2/s)	a	0.749	1.080	1.000	0.561	0.561	0.641~0.857	<0.0001
	b	0.905	1.465	0.942	0.833	0.776	0.848~0.961	<0.0001
	c	0.951	1.460	0.951	0.833	0.785	0.916~0.985	<0.0001

a, benign PTs vs. borderline PTs; b, benign PTs vs. malignant PTs; c, borderline PTs vs. malignant PTs.

Discussion

BI-RADS is a comprehensive guideline used by radiologists for breast tumor classification. Conventional MRI sequences are a complementary approach to assessing breast tumors. DWI technology is not included in the BI-RADS system, but the use of ADC values to evaluate breast tumors has become a research hotspot in recent years (14, 17, 23, 25, 26). The multiparameter MRI model with dynamic contrast-enhanced (DCE)-MRI, DWI, and synthetic MRI is a robust tool for evaluating malignancies in BI-RADS IV lesions. Including clinical features may further improve the diagnostic performance of this model (10). PTs are rare breast tumors that have not been widely recognized by clinicians. Reports on the use of ADC values to analyze PTs are scarce (5, 7, 8). Due to the large size of PTs (7), ROI-cs can be used to obtain ADC values that reflect tumor heterogeneity (14, 15).

Clinically, PTs are usually managed surgically. Benign and borderline PTs require wide excision. Malignant PTs >10 cm or PTs with rapid progression in a short period require whole mastectomy. PTs are likely to recur after surgery, but only malignant PTs develop distant metastases (27). PTs and FAs are difficult to distinguish on breast imaging modalities. On mammography, PTs usually present as rounded, oval, or lobulated masses with well-rounded edges, similar to FAs. On ultrasound, PTs present as well-defined solid, low-echo areas, almost identical to FAs. The sensitivity of fine needle aspiration biopsy for diagnosis of PT is only 40%, and has a high false-negative rate (28). Coarse needle biopsy has a slightly higher sensitivity (approximately 63%) (29), but histopathological examination of the whole tumor is generally required for diagnosis.

In this study, conventional MRI showed that the imaging characteristics of benign, borderline, and malignant PTs overlap, and benign PTs could not be precisely differentiated from other BCs. In previous reports, MRI findings for eight cases of benign PTs identified some characteristics of large benign PTs (>3 cm), but distinguishing small PTs from small FAs was difficult (1); MRI of 24 PTs (n = 1 malignant; n = 23 benign) showed PTs had benign morphological features, administration of contrast material suggested malignancy in 33% of cases, and PTs and FAs could not be precisely differentiated (4); a retrospective review of dynamic MRI findings for 30 cases of PTs (n = 19 benign; n = 6 borderline; n = 5 malignant) showed no significant association between TIC patterns (persistent, plateau, washout) and histopathological findings (5).

According to the results of this study, the ADCmeans of PTs, FAs, and BCs were 1.6083 (0.83–2.16) ± 0.26015 × 10^{-3} mm^2/s, 1.2711 (0.81–2.20) ± 0.31678 × 10^{-3} mm^2/s, and 0.8496 (0.60–1.26) ± 0.14857 × 10^{-3}mm^2/s, respectively. The ADCmeans of PTs was significantly higher than those of FAs and BCs ($p < 0.001$). ADCmean had the best efficacy to discriminate between PTs, FAs, and BCs compared to ADCmax and ADCmin, and had the highest specificity. The specificity of ADCmean for differentiating between PTs and FAs or PTs and BCs was 81.90% and 94.2%, respectively. These findings suggest ADCmean has potential as a clinically useful technology. In 2020, Jelena et al. (26) reported that DWI is a clinically useful tool for the differentiation of malignant from benign lesions based on mean ADC values. To the authors' knowledge, the present study is the first published report comparing the ADC values of PTs, FAs, and BCs.

The ADCmeans of benign PTs, borderline PTs, and malignant PTs were 1.5619 (1.25–1.92) ± 0.14886 × 10^{-3} mm^2/s, 1.3098 (0.83–1.68) ± 0.25017 × 10^{-3} mm^2/s, and 1.7962 (1.45–2.16) ± 0.13255 × 10^{-3} mm^2/s, respectively, and were significantly different. ADC values of malignant PTs at b0/1000 s/mm^2 have been reported as 1.37 ± 0.03 (10^{-3} mm^2/s) (5), 1.03 ± 0.03 (10^{-3} mm^2/s), and 1.45 ± 0.03 (10^{-3} mm^2/s) (7). DWI is performed using motion-sensitizing gradients applied during MR image acquisition to probe local diffusion characteristics. The resulting diffusion-weighted MRI signal is reduced in intensity proportional to water mobility, and is commonly described by the monoexponential equation: SD=S0 e^{-b*}ADC (13). Theoretically, as the b value increases, the corresponding ADC value should gradually decrease. Therefore, ADC values obtained in this study at b0/800 s/mm^2 should be greater than those reported at b1000s/mm^2. This was not always the case, likely due to the heterogeneity of breast tumors (14, 15).

The motion of water molecules in tissues depends on tissue cellularity and the integrity of cell membranes (30, 31). Consequently, PT cellularity should correlate with ADC values. Previous reports show an association between the ADC values of BCs and some histological features (32), and malignant tumors had lower ADC values than benign tumors due to high cellularity in the tumors (33). In the present study, ADC values reflected pathological findings, which showed that malignant and borderline PTs had high cell densities, while tumor cells of benign PTs were more dispersed. However, the ADCmean of malignant PTs was higher than benign or borderline PTs. This may be because the ADCmean of malignant PTs was not only related to tumor cell density, but also to the necrosis, cystic degeneration, and edema occurring inside the tumor. Extensive necrosis and interstitial edema allow water protons to move freely, which strongly influence the ADC value. The cutoff ADCmean has important clinical application. Correct diagnosis of PT grade is required before breast surgery. In our study, PTs were benign at ADCmean > 1.215×10^{-3} mm^2/s or malignant with internal liquefaction, necrosis, and cystic degeneration at ADCmean >1.665×10^{-3} mm^2/s. ADCmin had clinical application for the differentiation of benign and malignant PTs, and PTs were considered malignant at ADCmin >1.465×10^{-3} mm^2/s.

Limitations of the study

This study was associated with several limitations. First, it was a retrospective study, and the clinical value of ADC values for discriminating between breast tumors should be verified in prospective studies. Second, the sample size was small, and there may have been interobserver variability with regard to ROI-c selection, which may have introduced bias. Third, DWI sequences included b-values of 0 and 800 s/mm^2; further research should include multi-b-value DWI. Fourth, distortion and deformation often occur at high b-values, which may disturb ADC parameters.

Conclusion

Breast DWI acquiring b0 and 800 s/mm^2 images took 3 minutes. This enabled us to obtain satisfactory ADC values to evaluate the histological characteristics of a tumor. ADCmean differentiated PTs, FAs, and BCs, and benign PTs from borderline PTs and borderline PTs from malignant PTs. ADCmin helped differentiate benign PTs from malignant PTs. Overall, ADC values provided quantitative information that has potential to distinguish between PTs, FAs, and BCs, and classify PTs.

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 People's Hospital of Longhua. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Image evaluation, JF, JY, and RWL; Quality control, YZ and RWL; Literature retrieval, RFL; Data and statistics, LL and RWL; MR scanning, ZH, and LZ; Manuscript writing, RWL. All authors contributed to the article and approved the submitted version.

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.2023.938189/full#supplementary-material

References

1. Kinoshita T, Fukutomi T, Kubochi K. Magnetic resonance imaging of benign phyllodes tumors of the breast. *Breast J* (2004) 10(3):232–6. doi: 10.1111/j.1075-122X.2004.21316.x

2. Moy L, Elias K, Patel V, Lee J, Babb JS, Toth HK, et al. Is breast MRI helpful in the evaluation of inconclusive mammographic findings? *AJR Am J Roentgenol* (2009) 193 (4):986–93. doi: 10.2214/ajr.08.1229

3. Peters NH, Borel Rinkes IH, Zuithoff NP, Mali WP, Moons KG, Peeters PH. Meta-analysis of MR imaging in the diagnosis of breast lesions. *Radiology* (2008) 246 (1):116–24. doi: 10.1148/radiol.2461061298

4. Wurdinger S, Herzog AB, Fischer DR, Marx C, Raabe G, Schneider A, et al. Differentiation of phyllodes breast tumors from fibroadenomas on MRI. *AJR Am J Roentgenol* (2005) 185(5):1317–21. doi: 10.2214/ajr.04.1620

5. Yabuuchi H, Soeda H, Matsuo Y, Okafuji T, Eguchi T, Sakai S, et al. Phyllodes tumor of the breast: Correlation between MR findings and histologic grade. *Radiology* (2006) 241(3):702–9. doi: 10.1148/radiol.2413051470

6. Balaji R, Ramachandran KN. Magnetic resonance imaging of a benign phyllodes tumor of the breast. *Breast Care (Basel)* (2009) 4(3):189–91. doi: 10.1159/000220604

7. Alhabshi SM, Rahmat K, Abu Hassan H, Westerhout CJ, Chandran PA. Advanced MRI applications and findings of malignant phyllodes tumour: Review of two cases. *Jpn J Radiol* (2013) 31(5):342–8. doi: 10.1007/s11604-013-0183-y

8. Guo Y, Tang WJ, Kong QC, Liang YY, Han XR, Zheng BJ, et al. Can whole-tumor apparent diffusion coefficient histogram analysis be helpful to evaluate breast phyllode tumor grades? *Eur J Radiol* (2019) 114:25–31. doi: 10.1016/j.ejrad.2019.02.035

9. Tavassoli FA, Devilee P. *World health organization classification of tumors: Pathology and genetics tumours of the breast and female genital organs*. Lyon: IARC Press (2003) p. 99–103.

10. Sun SY, Ding Y, Li Z, Nie L, Liao C, Liu Y, et al. Multiparameter MRI model with DCE-MRI, DWI, and synthetic MRI improves the diagnostic performance of BI-RADS 4 lesions. *Front Oncol* (2021) 11:699127. doi: 10.3389/fonc.2021.699127

11. Kuhl CK, Schild HH, Morakkabati N. Dynamic bilateral contrast-enhanced MR imaging of the breast: trade-off between spatial and temporal resolution. *Radiology* (2005) 236(3):789–800. doi: 10.1148/radiol.2363040811

12. Koh DM, Collins DJ. Diffusion-weighted MRI in the body: Applications and challenges in oncology. *AJR Am J Roentgenol* (2007) 188(6):1622–35. doi: 10.2214/ajr.06.1403

13. Partridge SC, Amornsiripanitch N. DWI in the assessment of breast lesions. *Top Magn Reson Imaging* (2017) 26(5):201–09. doi: 10.1097/rmr.0000000000000137

14. Ao F, Yan Y, Zhang ZL, Li S, Li WJ, Chen GB. The value of dynamic contrast-enhanced magnetic resonance imaging combined with apparent diffusion coefficient in the differentiation of benign and malignant diseases of the breast. *Acta Radiol* (2022) 63 (7):891–900. doi: 10.1177/02841851211024002

15. Niu S, Wang X, Zhao N, Liu G, Kan Y, Dong Y, et al. Radiomic evaluations of the diagnostic performance of DM, DBT, DCE MRI, DWI, and their combination for the diagnosisof breast cancer. *Front Oncol* (2021) 11:725922. doi: 10.3389/fonc.2021.725922

16. Geng X, Zhang D, Suo S, Chen J, Cheng F, Zhang K, et al. Using the apparent diffusion coefficient histogram analysis to predict response to neoadjuvant chemotherapy in patients with breast cancer: Comparison among three region of interest selection methods. *Ann Transl Med* (2022) 10(6):323. doi: 10.21037/atm-22-1078

17. Kunimatsu N, Kunimatsu A, Uchida Y, Mori I, Kiryu S. Whole-lesion histogram analysis of apparent diffusion coefficient for the assessment of non-mass enhancement lesions on breast MRI. *J Clin Imaging Sci* (2022) 12:12. doi: 10.25259/jcis_201_2021

18. Kim JY, Kim JJ, Lee JW, Lee NK, Lee G, Kang T, et al. Risk stratification of ductal carcinoma *in situ* using whole-lesion histogram analysis of the apparent diffusion coefficient. *Eur Radiol* (2019) 29(2):485–93. doi: 10.1007/s00330-018-5666-x

19. White NS, McDonald C, Farid N, Kuperman J, Karow D, Schenker-Ahmed NM, et al. Diffusion-weighted imaging in cancer: physical foundations and applications of restriction spectrum imaging. *Cancer Res* (2014) 74(17):4638–52. doi: 10.1158/0008-5472.can-13-3534

20. Schob S, Meyer HJ, Pazaitis N, Schramm D, Bremicker K, Exner M, et al. ADC Histogram analysis of cervical cancer aids detecting lymphatic metastases-a preliminary study. *Mol Imaging Biol* (2017) 19(6):953–62. doi: 10.1007/s11307-017-1073-y

21. Kang Y, Choi SH, Kim YJ, Kim KG, Sohn CH, Kim JH, et al. Gliomas: Histogram analysis of apparent diffusion coefficient maps with standard- or high-b-value diffusion-weighted MR imaging–correlation with tumor grade. *Radiology* (2011) 261(3):882–90. doi: 10.1148/radiol.11110686

22. Zhang YD, Wang Q, Wu CJ, Wang XN, Zhang J, Liu H, et al. The histogram analysis of diffusion-weighted intravoxel incoherent motion (IVIM) imaging for differentiating the gleason grade of prostate cancer. *Eur Radiol* (2015) 25(4):994–1004. doi: 10.1007/s00330-014-3511-4

23. Jin YN, Zhang Y, Cheng JL, Zhang XP, Hu Y, Shao XN. The role of histogram analysis in diffusion-weighted imaging in the differential diagnosis of benign and malignant breast lesions. *BMC Med Inform Decis Mak* (2020) 20(1):239. doi: 10.1186/s12911-020-01257-0

24. Kim EJ, Kim SH, Park GE, Kang BJ, Song BJ, Kim YJ, et al. Histogram analysis of apparent diffusion coefficient at 3.0t: Correlation with prognostic factors and subtypes of invasive ductal carcinoma. *J Magn Reson Imaging* (2015) 42(6):1666–78. doi: 10.1002/jmri.24934

25. 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

26. Maric J, Boban J, Ivkovic-Kapicl T, Djilas D, Vucaj-Cirilovic V, Bogdanovic-Stojanovic D. Differentiation of breast lesions and distinguishing their histological subtypes using diffusion-weighted imaging and ADC values. *Front Oncol* (2020) 10:332. doi: 10.3389/fonc.2020.00332

27. Sevinç A, Aksoy S, Güray Durak M, Balcı P. Is the extent of surgical resection important in patient outcome in benign and borderline phyllodes tumors of the breast? *Turk J Med Sci* (2018) 48(1):28–33. doi: 10.3906/sag-1704-47

28. El Hag IA, Aodah A, Kollur SM, Attallah A, Mohamed AA, Al-Hussaini H. Cytological clues in the distinction between phyllodes tumor and fibroadenoma. *Cancer Cytopathol* (2010) 118(1):33–40. doi: 10.1002/cncy.20057

29. Lee AH. Recent developments in the histological diagnosis of spindle cell carcinoma, fibromatosis and phyllodes tumour of the breast. *Histopathology* (2008) 52(1):45–57. doi: 10.1111/j.1365-2559.2007.02893.x

30. Guo Y, Cai YQ, Cai ZL, Gao YG, An NY, Ma L, et al. Differentiation of clinically benign and malignant breast lesions using diffusion-weighted imaging. *J Magn Reson Imaging* (2002) 16(2):172–8. doi: 10.1002/jmri.10140

31. Gauvain KM, McKinstry RC, Mukherjee P, Perry A, Neil JJ, Kaufman BA, et al. Evaluating pediatric brain tumor cellularity with diffusion-tensor imaging. *AJR Am J Roentgenol* (2001) 177(2):449–54. doi: 10.2214/ajr.177.2.1770449

32. Tuan Linh L, Minh Duc N, Minh Duc N, Tra My TT, Viet Bang L, Cong Tien N, et al. Correlations between apparent diffusion coefficient values and histopathologic factors in breast cancer. *Clin Ter* (2021) 172(3):218–24. doi: 10.7417/ct.2021.2318

33. Partridge SC, Mullins CD, Kurland BF, Allain MD, DeMartini WB, Eby PR, et al. Apparent diffusion coefficient values for discriminating benign and malignant breast MRI lesions: Effects of lesion type and size. *AJR Am J Roentgenol* (2010) 194 (6):1664–73. doi: 10.2214/ajr.09.3534

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Clinical characteristics and overall survival prognostic nomogram for metaplastic breast cancer

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Background: Metaplastic breast cancer (MBC) is a rare breast tumor and the prognostic factors for survival in patients still remain controversial. This study aims to develop and validate a nomogram to predict the overall survival (OS) of patients with MBC.

Methods: We searched the Surveillance, Epidemiology, and End Results (SEER) database for data about patients including metaplastic breast cancer and infiltrating ductal carcinoma (IDC) from 2010 to 2018. The survival outcomes of patients between MBC and IDC were analyzed and compared with the Kaplan-Meier (KM) method. MBC patients were randomly allocated to the training set and validation I set by a ratio of eight to two. Meanwhile, the performance of this model was validated again by the validation II set, which consisted of MBC patients from the Union Hospital of Fujian Medical University between 2010 and 2018. The independent prognostic factors were selected by univariate and multivariate Cox regression analyses. The nomogram was constructed to predict individual survival outcomes for MBC patients. The discriminative power, calibration, and clinical effectiveness of the nomogram were evaluated by the concordance index (C-index), the receiver operating characteristic (ROC) curve, and the decision curve analysis (DCA).

Results: MBC had a significantly higher T stage (T2 and above accounting for 75.1% vs 39.9%), fewer infiltrated lymph nodes (N0 accounted for 76.2% vs 67.7%), a lower proportion of ER (22.2% vs 81.2%), PR (13.6% vs 71.4%), and HER-2(6.7% vs 17.7%) positive, radiotherapy(51.6% vs 58.0%) but more chemotherapy(67.5% vs 44.7%), and a higher rate of mastectomy(53.2% vs 36.8%), which was discovered when comparing the clinical baseline data between MBC and IDC. Age at diagnosis, T, N, and M stage, as well as surgery and radiation treatment, were all significant independent prognostic factors for overall survival (OS). In the validation I cohort, the nomogram's C-index (0.769 95% CI 0.710 -0.828) was indicated to be considerably higher than the standard AJCC model's (0.700 95% CI 0.644 -0.756). Nomogram's great predictive capability capacity further was

supported by the comparatively high C-index of the validation II sets (0.728 95% CI 0.588-0.869).

Conclusions: Metaplastic breast cancer is more aggressive, with a worse clinical prognosis than IDC. This nomogram is recommended for patients with MBC, both American and Chinese, which can help clinicians make more accurate individualized survival analyses.

KEYWORDS

SEER, metaplastic breast cancer, nomogram, overall survival (OS), prognosis

Background

Female breast cancer has overtaken others as the most commonly diagnosed malignancy, with an expected 2.3 million new cases in 2020, based on data from the International Agency for Research on Cancer (1). Metaplastic breast cancer (MBC) is a group of rare and heterogeneous invasive carcinomas, characterized by cell differentiation of the tumor epithelium towards squamous and/or mesenchymal-like components such as spindle cells, chondrocytes, and osteoblasts, accounting for only 0.2-5% of all breast cancer (2). MBC has been considered more aggressive, with poor clinical outcomes and a large unmet demand for treatment, compared to invasive ductal breast carcinoma(IDC). Due to the rarity of MBC, limitations of tailored understanding of the clinical characteristics and prognosis exist in previous reports. The majority of MBC's local and system-optimally regulated treatment approaches are deduced from IDC's treatment practice and have not been rigorously confirmed in MBC patients. The American Joint Committee on Cancer Staging (AJCC) system is the most commonly used to assess a patient's prognosis for breast cancer (3). However, disregard for other parameters (such as age), limited precision, and poor performance in forecasting individual survival risk are some of its main disadvantages. Patients with MBC, therefore, require a tailor-made prediction model. Nomogram is confirmed as a reliable and alternative prognosis assessment tool in many carcinomas and is even thought to be a new emerging standard (4). Based on clinical, immunological, and pathological data from the Surveillance, Epidemiology, and End Results (SEER) database, we intend to develop a maneuverable, definitive, and high-exactness nomogram to foresee MBC patient individual survival endings (5–7).

Abbreviations: MBC, metaplastic breast cancer; IDC, infiltrating ductal carcinoma; TNBC, triple negative breast cancer; PARPi, poly(adenosine diphosphate-ribose) polymerase inhibitors; BRCA1, DNA repair associated; BRCA2, DNA repair associated; 502=upper-inner quadrant of the breast; 503=lower-inner quadrant of the breast; 504=upper-outer quadrant of the breast; 505=lower-outer quadrant of the breast; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2.

Materials and methods

Data source and study population

The Surveillance, Epidemiology, and End Results (SEER) Program provides information on cancer statistics in an effort to reduce the cancer burden, and no ethics committee review approval was needed. We included patients diagnosed with confirmed MBC by extracting and screening data from the SEER database, which included persons from 18 areas (1975-2018) and was released on August 20, 2021. And patients diagnosed with confirmed MBC from the Union Hospital of Fujian Medical University between 2010 and 2018, also were included in this study. The including and excluding criteria of patients with MBC were as follows.

Inclusion criteria:

(1) the years of diagnosis spanned from 2010 to 2018.
(2) the primary site of the tumor was the breast.
(3) according to ICD-0-3, histological types were restricted to 8500/3 (IDC) and 8052/3, 8070/3-8072/3, 8074/3, 8560/3, 8571/3,8572/3, 8575/3, 8980/3 (MBC) (8).

Exclusion criteria:

(1) patients with missing information of age at diagnosis, marital status, PR status, ER status, HER2 status, surgery, or other important clinicopathological data.
(2) patients under the age of 18 years old.
(3) the patients have other cancer other than breast cancer.
(4) patients who have survived or followed up less than one month since the initial diagnosis.
(5) diagnosis of MBC patients obtained from autopsy or death.

The demographic parameters included age at diagnosis is distributed into <50 years, 50-64 years, 65-79 years, and 80+ years, gender is divided into women and males, race (white, black, and others), marital status is classified into married, single and divorced (separated, widowed and divorced). The clinicopathologic parameters included laterality of primary is divided into right and left, site of the

tumor is distributed into 502, 503, 504,505 and others, AJCC stage is divided into I, II, III, and IV, T stage is divided intoT1, T2, T3, and T4, N stage is divided into N0, N1, N2, and N3), M stage is divided intoM0 and M1, ER status is distributed into negative or positive), PR status is distributed into negative or positive, HER2 status is distributed into negative or positive, the subtype of breast cancer is distributed into HR +/HER2-, HR+/HER2+, HR-/HER2+, and HR-/HER2-, surgery type is classified into no surgery, breast-conserving, and mastectomy, radiotherapy is divided into yes and no, and chemotherapy is divided into yes and no. The primary clinical outcome for this series was overall survival (OS), which was defined as the time from the date of diagnosis to the date of death owing to any cause or the final follow-up.

Statistical analysis

The chi-square test or Fisher exact test was performed to evaluate the clinical and pathological characteristics of the different cohorts. Kaplan-Meier analysis was utilized to construct the survival curve. The discrepancy in the survival of each group was evaluated using the log-rank test. The life table approach was performed to figure out overall survival over three and five years. The patients with MBC were split into the training sets and validation sets with an 8:2 ratio, using the "createDataPartition" function of R software to guarantee that result events were distributed randomly. The Cox regression model, hazard ratios (HRs), and 95% confidence intervals (CIs) were utilized to confirm prognostic factors in the training set. Univariate Cox regression analyses were conducted for all variables, followed by multivariate Cox regression for variables with $p < 0.1$ in univariate Cox regression. Finally, variables with $p < 0.05$ in multivariate Cox backward stepwise regression were determined as independent risk factors. To prevent multicollinearity, in the multivariate analysis, T, N, and M stage variables were utilized instead of AJCC stage variables. Based on the findings of the multivariate Cox regression, the nomogram model was generated utilizing the RMS package in the R program, and further verified by the validation sets. The C-index, the receiver operating characteristic (ROC) curve, and the decision curve analysis (DCA) were used to evaluate the predictive accuracy, discrimination ability, as well as clinical effectiveness and benefit of the nomogram model respectively (9, 10).

The SEERStat software, version 8.3.9, was applied to extract the data. R software version 3.5.3 and IBM SPSS Statistics 26 were utilized to conduct statistical analyses. For all of the analyses, a two-tailed p-value of <0.05 was deemed statistically significant.

Results

Clinical and pathological characteristics

A total of 225,548 eligible patients were included in this study, based on data from the SEER database. The median age of 223943 (99.3%) IDC patients was 59 years old, whereas 1605 MBC patients had a median age of 61 years old. The proportion of MBC patients over 65 years old was greater than the proportion of IDC patients (p <0.001). The proportion of black patients with MBC is larger (p <0.001). When it comes to marital status, MBC patients have a higher number of divorced patients than IDC patients, but a lower proportion of married patients. Compared to patients with IDC, those with MBC had considerably significantly larger primary tumors. Furthermore, MBC patients exhibited a greater T stage than IDC patients (p <0.001), with T2 (47.9% vs 30.9%), T3 (16.9% vs 5.1%), and T4 (10.3% vs 3.9%), but a lower axillary lymph node involvement rate (76.2% vs. 67.7%, p <0.001), as well as no significant difference in the proportion of distant metastasis(4.9% vs 4.0%, p=0.066). meanwhile, the majority of MBC patients are "triple-negative", with HR-/HER2- (68.3% vs 12.4% p<0.001), a meaning lower expression of the ER (22.2% vs 81.2%), PR (13.6% vs 71.4%), and HER-2(6.7% vs 17.7%) receptors (p<0.001). MBC patients received less radiotherapy but more chemotherapy. Patients with MBC were more likely to have a mastectomy (53.2% vs 36.8%), whereas those with IDC were more likely to have breast-conserving (41.9% vs 57.4%)surgery(p<0.001) (Table 1). There were no statistically significant differences between the training and verification I sets of MBC patients with 17 variables (Supplementary Table 1).

For the validation II cohort, 49 Chinese MBC patients, who were diagnosed in the Union Hospital of Fujian Medical University between 2010 and 2018, were included in this study. Among these patients, the median age was 50 years old, and the median follow-up time was 79 months (3-139 months). When it comes to marital status, Chinese MBC patients have a higher number of married patients. Compared to American MBC patients, a higher proportion of Chinese MBC patients were under 65 years old, with particularly less than 50 years old(20.8% vs 51.0%). Furthermore, a higher percentage of mastectomy (95.9%) was performed on Chinese MBC patients. And in comparison with American patients, a larger proportion of Chinese patients with MBC receive chemotherapy (67.0% vs 95.9%) and less radiotherapy (50.6% vs 38.8%). There were no statistically significant differences between American patients and Chinese patients with MBC on other variables (Supplementary Table 2).

Survival analysis

The median follow-up period of MBC was 53 months (1-107 months). According to the KM analysis, MBC patients' survival was considerably shortened than that of IDC patients (p < 0.001). The three-year and five-year overall survival rates of MBC were 74.5 and 67.4%, respectively. Likewise, IDC's three-year and five-year overall survival rates were 91.6 and 86.5%, respectively (Figure 1). The histological category of MBC was found to be a poorer prognosis element for breast carcinoma by univariate Cox regression analysis

Prognostic factors in MBC

The patients with MBC were split into the training sets (n =1284) and validation I sets (n = 321) with an 8:2 ratio, using the "createDataPartition" function of R software to guarantee that result events were distributed randomly.

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TABLE 1 The characteristics of 225,548 breast cancer patients.

Characteristic	MBC, N (%)	IDC, N (%)	N (%)	*P*-value
	1605(0.7%)	223943(99.3%)	225548(100%)	
Age(years)				< 0.001
<50	349(21.7%)	54559(24.4%)	54908(24.3%)	
50-64	586(36.5%)	88196(39.3%)	88782(39.4%)	
65-79	471(29.4%)	64271(28.7%)	64742(28.7%)	
80+	199(12.4%)	16917(7.6%)	17116(7.6%)	
Sex				0.137
Female	1598(99.6%)	222244(99.2%)	223842(99.2%)	
Male	7(0.4%)	1699(0.8%)	1706(0.8%)	
Race				< 0.001
White	1218(75.9%)	175687(78.5%)	176905(78.4%)	
Black	265(16.5%)	24573(10.9%)	24838(11.0%)	
Others	122(7.6%)	23683(10.6%)	23805(10.6%)	
Marital				< 0.001
Married	843(52.5%)	132731(59.3%)	133574(59.2%)	
Single	305(19.0%)	36779(16.4%)	37084(16.5%)	
Divorced	457(28.5%)	54433(24.3%)	54890(24.3%)	
Laterality				0.745
Right	799(49.8%)	110570(49.4%)	111369(49.4%)	
Left	806(50.2%)	113373(50.6%)	114179(50.6%)	
Site				0.461
others	625(38.9%)	85240(38.1%)	85865(38.1%)	
502	210(13.1%)	29356(13.1%)	29566(13.1%)	
503	95(5.9%)	12800(5.7%)	12895(5.7%)	
504	538(33.5%)	79353(35.4%)	79891(35.4%)	
505	137(8.5%)	17194(7.7%)	17331(7.7%)	
AJCC stage				< 0.001
I	359(22.4%)	115640(51.6%)	115999(51.4%)	
II	922(57.4%)	76361(34.1%)	77283(34.3%)	
III	246(15.3%)	23071(10.3%)	23317(10.3%)	
IV	78(4.9%)	8871(4.0%)	8949(4.0%)	
T stage				< 0.001
T1	399(24.9%)	134484(60.1%)	134883(59.8%)	
T2	770(47.9%)	69194(30.9%)	69964(31.0%)	
T3	271(16.9%)	11600(5.1%)	11871(5.3%)	
T4	165(10.3%)	8665(3.9%)	8830(3.9%)	
N stage				< 0.001
N0	1223(76.2%)	151690(67.7%)	152913(67.8%)	
N1	276(17.2%)	54122(24.2%)	54398(24.1%)	

(Continued)

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TABLE 1 Continued

Characteristic	MBC, N (%)	IDC, N (%)	N (%)	*P*-value
N2	71(4.4%)	11592(5.2%)	11663(5.2%)	
N3	35(2.2%)	6539(2.9%)	6574(2.9%)	
M stage				0.066
M0	1527(95.1%)	215072(96.0%)	216599(96.0%)	
M1	78(4.9%)	8871(4.0%)	8949(4.0%)	
ER status				< 0.001
Negative	1248(77.8%)	42078(18.8%)	43326(19.2%)	
Positive	357(22.2%)	181865(81.2%)	182222(80.8%)	
PR status				< 0.001
Negative	1387(86.4%)	64097(28.6%)	65484(29.0%)	
Positive	218(13.6%)	159846(71.4%)	160064(71.0%)	
HER-2 status				< 0.001
Negative	1497(93.3%)	184336(82.3%)	185833(82.4%)	
Positive	108(6.7%)	39607(17.7%)	39715(17.6%)	
Subtype				< 0.001
HR+/HER2-	401(25.0%)	156663(70.0%)	157064(69.6%)	
HR+/HER2+	37(2.3%)	27758(12.4%)	27795(12.3%)	
HR-/HER2+	71(4.4%)	11849(5.3%)	11920(5.3%)	
HR-/HER2-	1096(68.3%)	27673(12.4%)	28769(12.8%)	
Surgery				< 0.001
no surgery	78(4.9%)	12886(5.8%)	12964(5.8%)	
breast-conserving	673(41.9%)	128573(57.4%)	129246(57.3%)	
mastectomy	854(53.2%)	82484(36.8%)	83338(36.9%)	
Chemotherapy				< 0.001
No	521(32.5%)	123815(55.3%)	124336(55.1%)	
Yes	1084(67.5%)	100128(44.7%)	101212(44.9%)	
Radiotherapy				< 0.001
No	777(48.4%)	93995(42.0%)	94772(42.0%)	
Yes	828(51.6%)	129948(58.0%)	130776(58.0%)	

MBC Metaplastic breast carcinoma, IDC Infiltrating ductal carcinoma, 502 Upper-inner quadrant of breast, 503 Lower-inner quadrant of breast, 504 Upper-outer quadrant of breast, 505 Lower-outer quadrant of breast, ER, Estrogen receptor; PR, Progesterone receptor; HER-2, Human epidermal growth factor receptor 2.

In the training set, the Cox regression model was utilized to find the variables that influence MBC prognosis. Age, marital status, tumor site, AJCC stage, T stage, N stage, M stage, surgery, radiotherapy, and chemotherapy all demonstrated statistically significant variations in survival prognostic variables, in the univariate analysis, but the sex (p=0.700), race (p=0.131), PR status (p=0.312), ER status (p=0.296), HER-2 status(p=0.518) and subtype (p=0.913). Finally, followed by multivariate Cox regression, age, T stage, N stage, M stage, surgery, and radiotherapy were determined as independent prognostic factors for patients with MBC (Table 2). Kaplan-Meier survival curves of each independent prognostic factor were shown in Figure 2.

Construction and validation of a nomogram

The independent prognostic factors (age, T stage, N stage, M stage, surgery, and radiotherapy), which were found by the Cox regression, were utilized to develop a nomogram model to assess the

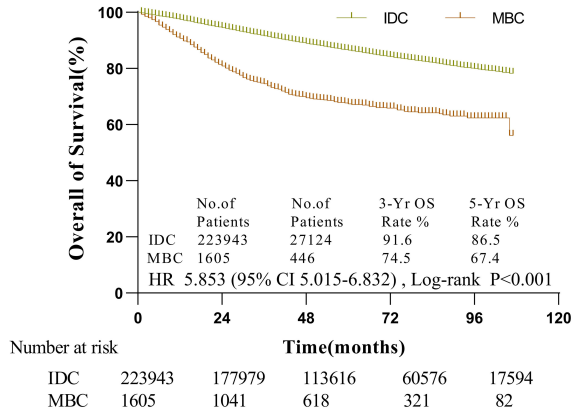

FIGURE 1
The survival of patients with MBC and IDC by Kaplan-Meier analysis. Patients with MBC had worse survival (HR = 5.853, 95% CI, 5.015-6.832,p < 0.001) with 3- and 5-year OS rates of 74.5 and 67.4% vs. 91.6 and 86.5% in IDC patients, respectively.

overall survival of MBC (Figure 3). The nomogram model showed that T stage had the greatest impact on prognosis, and the smallest is radiotherapy. Scores are awarded to all subtypes of all factors (Table 3).

The nomogram model has been verified internally and externally. The internal verification revealed that the C-index estimated by overall survival for the training sets was 0.794 (95% CI 0.771-0.816). The C-index indicated by the overall survival of the externally confirmed was 0.769 (95% CI 0.710-0.828), according to the validation I sets. In the training and validation I sets, the calibration plots revealed high uniformity between the nomogram prognostication and the actual observation (Figure 4). The ROC of the training and verification I sets is depicted in (Figure 5). In the verification I sets, the C-index of the overall survival predicted by the nomogram was 0.769 (95% CI 0.710 -0.828), which was greater than the C-index of the AJCC staging system (C-index=0.700 95% CI 0.644 -0.756). The DCA was applied to make comparisons of the availability and advantages between the nomogram model and the AJCC staging system. In the validation I sets, the nomogram has a greater overall advantage over a number of death hazards than the AJCC staging system, which was revealed by the 3-year and 5-year DCA curves, (Figure 6).

According to the validation II sets, the C-index indicated by the overall survival of the externally confirmed was 0.728 (95% CI 0.588-0.869). The calibration plots in the training and validation II sets indicated a comparatively high uniformity between the nomogram prognostication and the actual observation (Figure 7). The training and verification II sets' ROC is provided in Figure 8. In the validation II sets, the 3-year and 5-year DCA curves also indicated that the nomogram had a bigger overall advantage over the availability than the AJCC staging scheme (Figure 9).

Discussion

Metaplastic breast cancer is rare and generally highly aggressive invasive carcinoma, accounting for 0.2-5% of all breast cancers, characterized by differentiation of the neoplastic epithelium to squamous and/or mesenchymal components (11). The histologic structure of MBC is diversified, consisting of both neoplastic cells and metaplastic cancer tissue, or just metaplastic neoplastic tissue (12), which was further divided into several subgroups: low-grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, squamous cell carcinoma, metaplastic carcinoma with mesenchymal differentiation, mixed metaplastic carcinoma, according to The World Health Organization (WHO) (2, 11, 13).

Traditionally, for assessing prognosis, diagnosing cancer patients, and selecting the most beneficial treatment modalities, the American Joint Committee on Cancer (AJCC) staging guideline has emerged as the gold standard (3). Given that it ignored other biological factors that impact cancer prognosis, at the level of individual treatment, the decisive status of the AJCC staging system has aroused suspicion. Actually, biological markers and other factors may also play a part. In our study, this nomogram demonstrates that, in addition to the T stage, N stage, and M stage, the age of diagnosis, surgery, and whether radiation is administered have a larger influence on prognosis.

According to earlier research, MBC typically affects women over the age of 50 (2, 11). In our study, age is divided into <50 years old, 50-64 years old, 65-80 years old, and 80+ years old. The univariate and multivariate analysis revealed that age is an independent prognostic factor for patients with MBC. The nomogram model showed that age had a pretty great impact on prognosis, in which the 80+ years old subtype of age is assigned a rather high score.

Currently, ER status, PR status, and HER-2 status are three core indicators in medical decision-making, according to ASCO and NCCN recommendations (14–16). However, in our study, ER,PR, and HER-2 status were not the independent prognostic factor for patients with MBC in either multifactorial or univariate Cox analysis. In this study, the positive rate for these three markers is relatively low. Weigelt et al. also demonstrate that more than 90% of MBC patients have a triple-negative phenotype (13), which is consistent with the findings of this study. Even if HR or HER2 status is positive, the efficacy of endocrine treatment and targeted therapy for MBC patients needs to be further investigated.

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TABLE 2 Univariate and multivariate Cox regression analysis of overall survival (MBC Training Cohort).

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Age (years)		**<0.001**		<0.001
<50	Reference		Reference	
50-64	1.37 (1.00-1.91)	0.069	1.44 (1.02-2.02)	0.036
65-79	1.70 (1.21-2.38)	0.002	1.94 (1.38-2.74)	<0.001
80+	4.06 (2.87-5.74)	<0.001	4.26 (2.96-6.15)	<0.001
Sex		0.700		
Female	Reference			
Male	0.68 (0.10-4.85)	0.700		
Race		0.131		
White	Reference			
Black	1.23 (0.95-1.60)	0.120		
Others	0.78 (0.50-1.22)	0.279		
Marital		**<0.001**		
Married	Reference			
Single	1.57 (1.19-2.06)	0.001		
Divorced	1.71 (1.35-2.16)	<0.001		
Laterality		**0.054**		
Right	Reference			
Left	1.23 (1.00-1.51)	0.054		
Site		**0.002**		
others	Reference			
502	0.59 (0.41-0.86)	0.006		
503	0.51 (0.30-0.87)	0.013		
504	0.69 (0.54-0.88)	0.002		
505	0.87 (0.61-1.27)	0.480		
AJCC stage		**<0.001**		
I	Reference			
II	3.29 (2.13-5.07)	<0.001		
III	8.94 (5.67-14.10)	<0.001		
IV	29.35 (18.00-47.92)	<0.001		
T stage		**<0.001**		<0.001
T1	Reference		Reference	
T2	2.28 (1.55-3.35)	<0.001	1.98 (1.34-2.92)	0.001
T3	6.54 (4.40-9.72)	<0.001	4.97 (3.29-7.52)	<0.001
T4	11.16 (7.41-16.81)	<0.001	5.19 (3.29-8.17)	<0.001
N stage		**<0.001**		0.003
N0	Reference		Reference	
N1	2.09 (1.64-2.67)	<0.001	1.37 (1.05-1.79)	0.019

(Continued)

TABLE 2 Continued

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
N2	2.73 (1.83-4.07)	<0.001	1.41 (0.92-2.16)	0.110
N3	3.58 (2.19-5.87)	<0.001	2.42 (1.42-4.09)	0.001
M stage		**<0.001**		<0.001
M0	Reference		Reference	
M1	8.46 (6.32-11.32)	<0.001	3.12 (2.21-4.40)	<0.001
ER status		0.296		
Negative	Reference			
Positive	1.14 (0.89-1.45)	0.296		
PR status		0.312		
Negative	Reference			
Positive	0.85 (0.62-1.16)	0.312		
HER-2 status		0.518		
Negative	Reference			
Positive	0.87 (0.56-1.34)	0.518		
Subtype		0.913		
HR+/HER2-	Reference			
HR+/HER2+	0.89 (0.45-1.77)	0.742		
HR-/HER2+	0.81 (0.45-1.48)	0.499		
HR-/HER2-	0.97 (0.76-1.23)	0.792		
Surgery		**<0.001**		<0.001
no surgery	Reference		Reference	
breast-conserving	0.16 (0.11-0.23)	<0.001	0.35 (0.23-0.54)	<0.001
mastectomy	0.34 (0.25-0.51)	<0.001	0.50 (0.35-0.72)	<0.001
Chemotherapy		**<0.001**		
No	Reference			
Yes	0.58 (0.47-0.71)	<0.001		
Radiotherapy		**<0.001**		0.002
No	Reference		Reference	
Yes	0.57 (0.46-0.71)	<0.001	0.69 (0.54-0.87)	0.002

MBC Metaplastic breast carcinoma, 502 Upper-inner quadrant of breast, 503 Lower-inner quadrant of breast, 504 Upper-outer quadrant of breast, 505 Lower-outer quadrant of breast, ER, Estrogen receptor; PR, Progesterone receptor; HER-2, Human epidermal growth factor receptor 2. For all of the analyses, variables with p<0.1 were deemed statistically significant in univariate Cox regression, and variables with $p < 0.05$ were deemed statistically significant in multivariate Cox.

MBC manifests as a rapidly increasing palpable breast mass, appearing as an ill-defined phyma on imaging without unique radiological signs (12, 17). In this study, patients with MBC had primary tumors that were noticeably larger than those with IDC. Most MBC patients (75.1%) arrived with tumors that were T2 and above, whereas most IDC patients presented with tumors smaller than 20mm (i.e.T1). Further, T3 and greater stage accounted for 27.2% of MBC, and only 9.2% of IDC ($p < 0.001$). Interestingly, patients with MBC presented a lower rate of lymph node metastasis (2). In this study, only 23.8% of 1605 patients with MBC demonstrate lymph node involvement. Although lymph node involvement was less frequent, more commonly stage II and above (MBC 77.6% vs. IDC 48.4%, $p < 0.0001$) were seen in MBC patients. MBC patients were also more likely to have stage III (16.9%) or stage IV disease (10.3%), in comparison to IDC patients (10.3% and 4.0%, respectively). The outcomes of the appeal were also corroborated by single-center data from the Union Hospital of Fujian Medical University.

It was not until 2000 that MBC was officially recognized as a distinct pathologic phenotype (12), which results in lacking randomized controlled studies that evaluate treatment modalities

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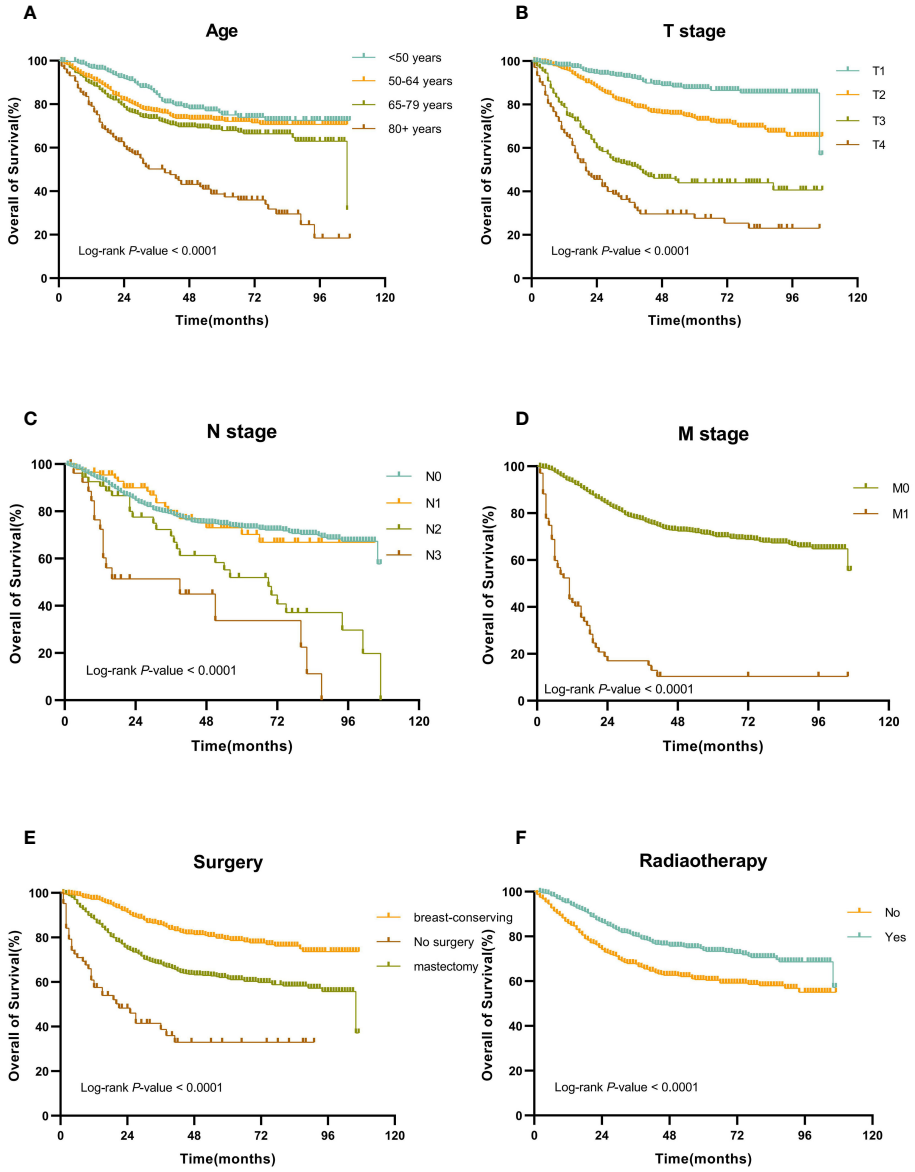

FIGURE 2
Kaplan-Meier OS curves for patients with MBC according to different independent prognostic factors. **(A-F)** Kaplan-Meier OS curves for patients with MBC according to **(A)** age, **(B)** T stage, **(C)** N stage, **(D)** M stage, **(E)** surgery, and **(F)** surgery.

and the prognosis in patients with MBC. And that for patients with MBC, endocrine therapy and targeted therapy, which target ER, PR, and HER2 respectively, have limited benefits. Although poor prognosis indicates the limitations of the existing therapeutic alternatives, like IDC, surgery, radiotherapy, and chemotherapy are still the mainstays of treatment for MBC. No association of surgery type with survival was concluded by Haque W and colleagues, examining patients with MBC from Surveillance, Epidemiology, and End Results (SEER) data from 1988 to 2006 (18). Whereas, in this study, surgery was proven to be an independent prognostic factor for MBC in both univariate and multifactorial analyses. Ninety-four point two percent of MBC and IDC patients were treated surgically, but patients with MBC most frequently underwent mastectomy (53.2%), whereas those with IDC most frequently underwent BCS (57.4%). This discrepancy was attributable to a larger primary tumor of the MBC patients, with 16.9% of MBC primary tumors measuring more than 5cm in size as opposed to just 5.1% of IDC primary tumors. Compared to mastectomy, breast-conserving surgery has a better prognosis, according to Kaplan-Meier overall survival curves for patients with MBC, which may be caused by the effects of receiving radiotherapy following breast-conserving surgery (19), with is consistent with the study of Onitilo and colleagues (20). Meanwhile, the effects of a mastectomy on a patient's physical appearance, quality of life, and psychological health cannot be denied, which may lead to a poorer prognosis (21).

In addition to surgery, radiotherapy (HR=0.57, p<0.001) was found to be also an independent prognostic factor for MBC in both univariate and multivariate Cox regression analysis. In contrast to pT1-2 N0 instances, radiotherapy was associated with OS

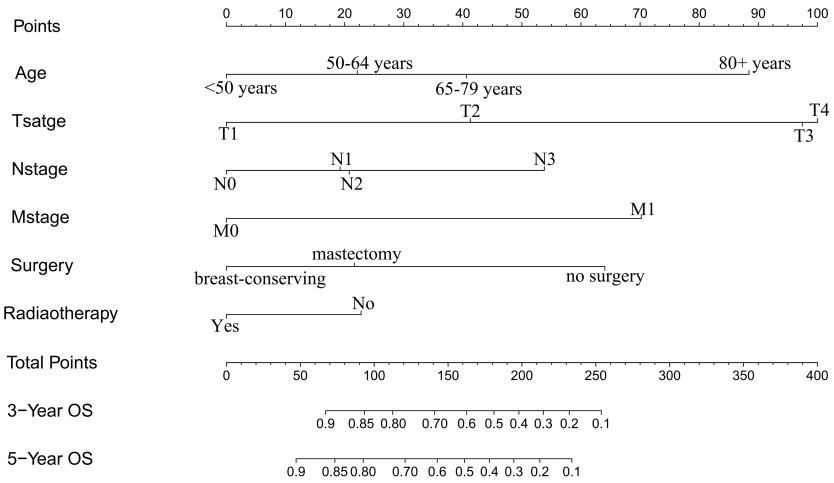

FIGURE 3
Nomogram predicted 3- and 5-year overall survival for patients with MBC. The nomogram is used by summing the points identified on the top scale for each independent covariate. The total points projected to the bottom scale indicated the % probability of the 3- and 5-year OS.

TABLE 3 Point assignment and prognostic score in the nomogram (MBC Training Cohort).

Variable	Score
Age(years)	
<50	0
50-64	22
65-79	41
80+	88
T stage	
T1	0
T2	41
T3	97
T4	100
N stage	
N0	0
N1	19
N2	21
N3	54
M stage	
M0	0
M1	70
Surgery	
no surgery	64
breast-conserving	0
mastectomy	22
Radiotherapy	
No	23
Yes	0

improvements in pT3-4/N+ patients (8, 18), according to a report from the National Cancer Data Base (NCDB) (22). And Warren H et al. report that patients at "high risk" who have tumors greater than 5cm in size or more than four metastatic axillary lymph nodes are the ones who can benefit from radiotherapy (8, 22, 23). The majority of the MBC patients in this study have primary tumors that were large enough to benefit from radiation. However, there is a dearth of evidence on the effectiveness of chemotherapy in MBC patients. In this multivariate Cox regression analysis, chemotherapy was proven not to be an independent prognostic factor for MBC patients. The application of chemotherapy is an extension of more prevalent histologic subtypes of breast cancer (24). And retrospective studies by D. Rayson have demonstrated that MBC patients benefit less from conventional chemotherapy regimens than do IDC patients (2).

Notably, studies have found evidence that patients with triple negative breast cancer(TNBC) who have BRCA mutations may benefit from poly(adenosine diphosphate-ribose) polymerase inhibitors (PARPi) and platinum salts treatment. For homologous recombination DNA repair, BRCA1/2 encode proteins are indispensable. And breast cancers with BRCA mutations exist a deficiency in homologous recombination repair. Utilizing the principle of synthetic lethality, the poly(adenosine diphosphate-ribose) polymerase inhibitors (PARPi) could target and kill tumor cells with a deficiency in homologous recombination repair. Therefore, Olaparib is the first PARPi to have received approval for the treatment of breast cancer as a result. On the other hand, BRCA mutations make cancer cells more sensitive to the platinum compound, which is also connected to a defective homologous recombination system. Some findings suggest BRCA mutation carriers had longer disease-free intervals and survival following platinum salt therapy (25–31). Thus, BRCA sequencing could be a suitable biomarker for predicting patient response to PARPi and platinum salts in TNBC. And the majority of MBC patients are "triple-negative"(i.e., negative for human epidermal growth factor receptor 2 and estrogen and progesterone receptors), who may benefit from PARPi and platinum salts treatment.

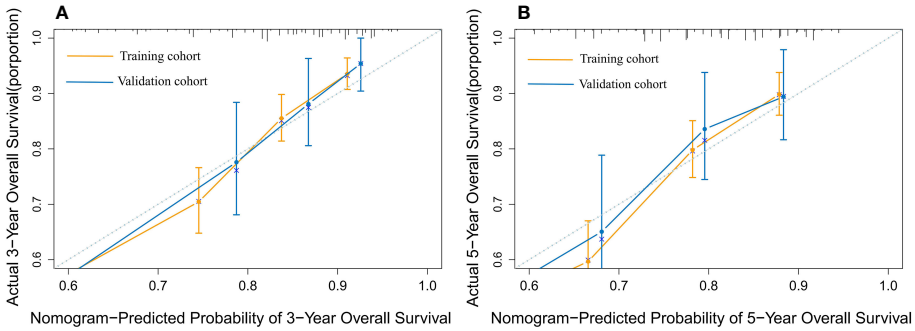

FIGURE 4
The calibration plot for predicting 3- and 5-year overall survival for patients with MBC. Calibration plot of nomogram prediction of **(A)** 3-year and **(B)** 5-year OS of patients with MBC in the training and validation I sets.

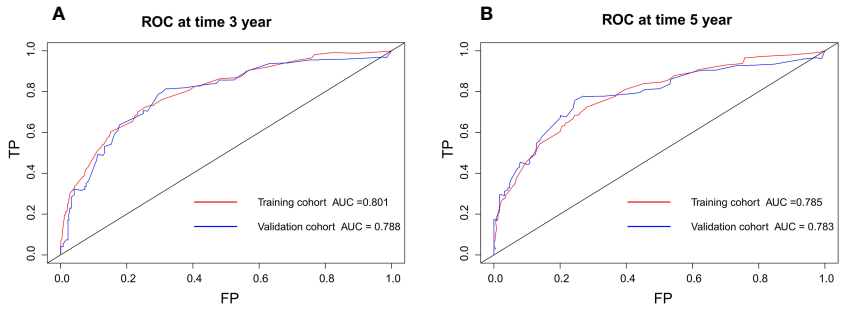

FIGURE 5
Discriminatory accuracy for predicting OS examined by ROC analysis calculating AUC. There-year OS in the training and validation I sets **(A)**. Five-year OS in the training and validation I sets **(B)**.

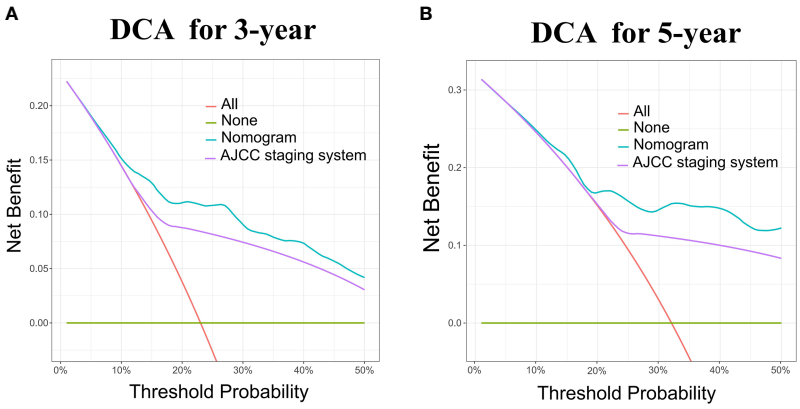

FIGURE 6
DCA for the Nomogram and AJCC staging system in the validation cohort. DCA in the prediction of patients at 3-year **(A)** and 5-year **(B)** in the training and validation I sets.

In contrast to IDC, MBC is a relatively chemorefractory malignancy with a significant unmet demand. Some clinical trials for MBC are now conducted to find more effective treatments. For instance, an isolated study from 2018 discovered a durable response to therapy with a P13K inhibitor (buparlisib) for MBC (32). And a 2018 study observing the response of these MBCs to inhibition of mTOR with Afinitor (everolimus) or Toris (temsirolimus) drugs found that patients with triple-negative MBC treated with mTOR inhibitors warranted further exploration (33, 34). Sylvia Adams et al. also found no additional safety issues in MBC patients treated with the combination of ibritumomab and nabumab, and achieved an

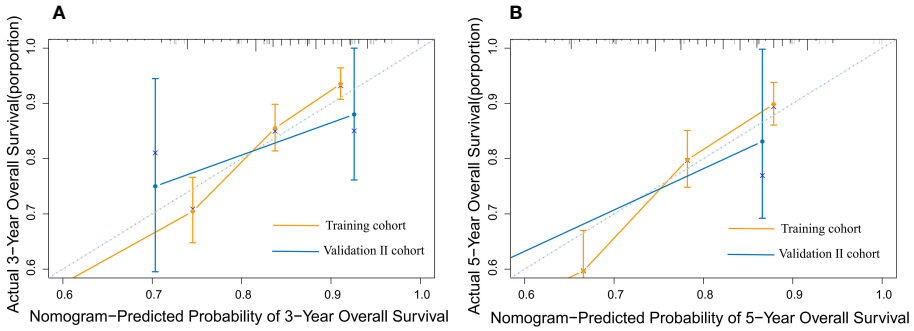

FIGURE 7
The calibration plot for predicting 3- and 5-year overall survival for patients with MBC. Calibration plot of nomogram prediction of **(A)** 3-year and **(B)** 5-year OS of patients with MBC in the training and validation II sets.

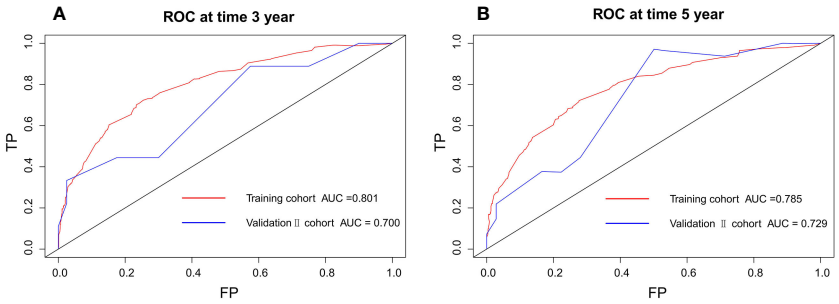

FIGURE 8
Discriminatory accuracy for predicting OS examined by ROC analysis calculating AUC. There-year OS in the training and validation II sets **(A)**. Five-year OS in the training and validation II sets **(B)**.

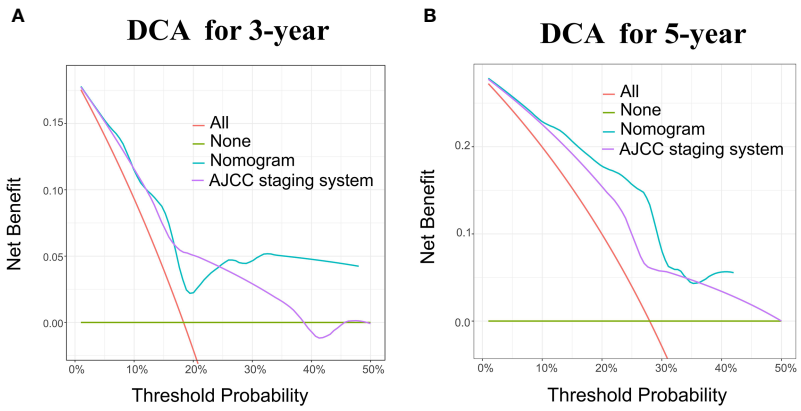

FIGURE 9
DCA for the Nomogram and AJCC staging system in the validation cohort. DCA in the prediction of patients at 3-year **(A)** and 5-year **(B)** in the training and validation II sets.

objective remission rate of 18% for the primary endpoint (35). Meanwhile, in examining the levels of tumor-infiltrating lymphocytes (TILs) and survival data of patients with MBC, Kalaw et al. found the clinical significance and prognostic value of FOXP3, PD-1/PD-L1 and tumor-infiltrating lymphocytes in MBC and confirmed that immunotherapy may be a potential treatment for part patients with MBC (36).

The nomogram model has been validated internally and externally in multiple ways. A relatively higher C-index, relatively high uniformity of the calibration plots, a great receiver operating

characteristic(ROC) curve, and decision curve analysis(DCA), prove that the nomogram model has higher predictive accuracy, stronger discriminative ability, greater clinical effectiveness, and benefit respectively. And the nomogram model had a bigger overall advantage over the availability than the AJCC staging model.

In this study, the nomogram model could also be applied to the Chinese MBC patients, which was confirmed by the verification II sets. According to the data of 49 MBC patients from the Union Hospital of Fujian Medical University, Chinese MBC patients more received a mastectomy, with a larger proportion of chemotherapy and less radiotherapy. Part of the reason is that patients with MBC have a younger age composition in China. Young breast cancer patients have distinctive biological behavior, a more aggressive type of pathology, and are more likely to accept a mastectomy and chemotherapy, which was also proved by Partridge et al. (37).

Limitations of this study include that we failed to explore the characteristics and prognosis of several subgroups of MBC separately. Second, SEER data lacks information about BRCA, FOXP3, PD-1/PD-L1, chemotherapy regimens, and genomic profiling. Additionally, there is a dearth of more data from Chinese research centers to verify the nomogram. Finally, prospective research on therapy options and prognosis is critical, although MBC is relatively rare. However, our investigation provided fresh insight into the clinicopathological features and prognosis of MBC patients.

Conclusions

MBC patients have larger primary tumors, less lymph node invasion, mostly triple-negative phenotype, and relatively chemorefractory tumors with a high unmet need. Patients with MBC have a much poorer prognosis than those with IDC. This nomogram is recommended for patients with MBC, both American and Chinese, which can help clinicians make more accurate individualized survival analyses.

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 Union Hospital of Fujian Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conception and design: CZ and CX; Development of methodology: CZ, CF, YW, JL, SL, HH, ZH, and CX; Acquisition of data, analysis, and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): CZ, CF, ZH, and CX; Writing, review and/or revision of the manuscript: CZ, CF, ZH, and CX; Study supervision: SL and HH; Revising: CZ, CF, ZH, and CX. All authors contributed to the article and approved the submitted version.

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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1030124/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) 71:209–49. doi: 10.3322/caac.21660

2. Tray N, Taff J, Adams S. Therapeutic landscape of metaplastic breast cancer. *Cancer Treat Rev* (2019) 79:101888. doi: 10.1016/j.ctrv.2019.08.004

3. 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) 67:93–9. doi: 10.3322/caac.21388

4. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP. Nomograms in oncology: More than meets the eye. *Lancet Oncol* (2015) 16:e173–80. doi: 10.1016/S1470-2045(14)71116-7

5. Zhang F, Zheng W, Ying L, Wu J, Wu S, Ma S, et al. A nomogram to predict brain metastases of resected non-small cell lung cancer patients. *Ann Surg Oncol* (2016) 23:3033–9. doi: 10.1245/s10434-016-5206-3

6. Shao C, Feng X, Yu J, Meng Y, Liu F, Zhang H, et al. A nomogram for predicting pancreatic mucinous cystic neoplasm and serous cystic neoplasm. *Abdom Radiol (NY)* (2021) 46:3963–73. doi: 10.1007/s00261-021-03038-3

7. Karakiewicz PI, Benayoun S, Kattan MW, Perrotte P, Valiquette L, Scardino PT, et al. Development and validation of a nomogram predicting the outcome of prostate biopsy based on patient age, digital rectal examination and serum prostate specific antigen. *J Urol* (2005) 173:1930–4. doi: 10.1097/01.ju.0000158039.94467.5d

8. Hu J, Tan J, Dong F, Zhang X, Ming J, Huang T. The effect of post-mastectomy radiotherapy in patients with metaplastic breast cancer: A propensity score-matched analysis of the SEER database. *Front Oncol* (2021) 11:593121. doi: 10.3389/fonc.2021.593121

9. Vickers AJ, Elkin EB. Decision curve analysis: A novel method for evaluating prediction models. *Med Decis Making* (2006) 26:565–74. doi: 10.1177/0272989X06295361

10. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. *J Thorac Oncol* (2010) 5(9):1315–6. doi: 10.1097/JTO.0b013e3181ec173d

11. Reddy TP, Rosato RR, Li X, Moulder S, Piwnica-Worms H, Chang JC. A comprehensive overview of metaplastic breast cancer: Clinical features and molecular aberrations. *Breast Cancer Res* (2020) 22:121. doi: 10.1186/s13058-020-01353-z

12. Pezzi CM, Patel-Parekh L, Cole K, Franko J, Klimberg VS, Bland K. Characteristics and treatment of metaplastic breast cancer: Analysis of 892 cases from the national cancer data base. *Ann Surg Oncol* (2007) 14:166–73. doi: 10.1245/s10434-006-9124-7

13. Weigelt B, Eberle C, Cowell CF, Ng CKY, Reis-Filho JS. Metaplastic breast carcinoma: More than a special type. *Nat Rev Cancer* (2014) 14:147–8. doi: 10.1038/nrc3637

14. Nicolini A, Ferrari P, Duffy MJ. Prognostic and predictive biomarkers in breast cancer: Past, present and future. *Semin Cancer Biol* (2018) 52:56–73. doi: 10.1016/j.semcancer.2017.08.010

15. Paul Wright G, Davis AT, Koehler TJ, Melnik MK, Chung MH. Hormone receptor status does not affect prognosis in metaplastic breast cancer: A population-based analysis with comparison to infiltrating ductal and lobular carcinomas. *Ann Surg Oncol* (2014) 21:3497–503. doi: 10.1245/s10434-014-3782-7

16. Zhang Y, Lv F, Yang Y, Qian X, Lang R, Fan Y, et al. Clinicopathological features and prognosis of metaplastic breast carcinoma: Experience of a major Chinese cancer center. *PloS One* (2015) 10:e0131409. doi: 10.1371/journal.pone.0131409

17. Bian T, Lin Q, Wu Z, Cui C, Qi C, Li L, et al. Metaplastic carcinoma of the breast: Imaging and pathological features. *Oncol Lett* (2016) 12:3975–80. doi: 10.3892/ol.2016.5177

18. Haque W, Verma V, Naik N, Butler EB, Teh BS. Metaplastic breast cancer: Practice patterns, outcomes, and the role of radiotherapy. *Ann Surg Oncol* (2018) 25:928–36. doi: 10.1245/s10434-017-6316-2

19. Guidolin K, Lock M, Vogt K, McClure JA, Winick-Ng J, Vinden C, et al. Recurrence and mortality after breast-conserving surgery without radiation. *Curr Oncol* (2019) 26:380–8. doi: 10.3747/co.26.5225

20. Onitilo AA, Engel JM, Stankowski RV, Doi SA. Survival comparisons for breast conserving surgery and mastectomy revisited: Community experience and the role of radiation therapy. *Clin Med Res* (2015) 13:65–73. doi: 10.3121/cmr.2014.1245

21. Weber WP, Soysal SD, Fulco I, Barandun M, Babst D, Kalbermatten D, et al. Standardization of oncoplastic breast conserving surgery. *Eur J Surg Oncol* (2017) 43:1236–43. doi: 10.1016/j.ejso.2017.01.006

22. Xia LY, Xu WY, Hu QL. The different outcomes between breast-conserving surgery plus radiotherapy and mastectomy in metaplastic breast cancer: A population-based study. *PloS One* (2021) 16:e0256893. doi: 10.1371/journal.pone.0256893

23. Tseng WH, Martinez SR. Metaplastic breast cancer: to radiate or not to radiate? *Ann Surg Oncol* (2011) 18:94–103. doi: 10.1245/s10434-010-1198-6

24. Tadros AB, Sevilimedu V, Giri DD, Zabor EC, Morrow M, Plitas G. Survival outcomes for metaplastic breast cancer differ by histologic subtype. *Ann Surg Oncol* (2021) 28:4245–53. doi: 10.1245/s10434-020-09430-5

25. Pellegrino B, Herencia-Ropero A, Llop-Guevara A, Pedretti F, Moles-Fernández A, Viaplana C, et al. Preclinical *In vivo* validation of the RAD51 test for identification of homologous recombination-deficient tumors and patient stratification. *Cancer Res* (2022) 82:1646–57. doi: 10.1158/0008-5472.CAN-21-2409

26. Cruz C, Castroviejo-Bermejo M, Gutierrez-Enriquez S, Llop-Guevara A, Ibrahim YH, Gris-Oliver A, et al. RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. *Ann Oncol* (2018) 29:1203–10. doi: 10.1093/annonc/mdy099

27. Tung NM, Garber JE. BRCA1/2 testing: Therapeutic implications for breast cancer management. *Br J Cancer* (2018) 119:141–52. doi: 10.1038/s41416-018-0127-5

28. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al. And investigators, adjuvant olaparib for patients with BRCA1- or BRCA2-mutated breast cancer. *N Engl J Med* (2021) 384:2394–405. doi: 10.1056/NEJMoa2105215

29. Loibl S, O'Shaughnessy J, Untch M, Sikov WM, Rugo HS, McKee MD, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): A randomised, phase 3 trial. *Lancet Oncol* (2018) 19:497–509. doi: 10.1016/S1470-2045(18)30111-6

30. Llop-Guevara A, Vladimirova V, Schneeweiss A, Villacampa G, Karn T, Zahm DM, et al. 2O association of RAD51 with homologous recombination deficiency (HRD) and clinical outcomes in untreated triple-negative breast cancer (TNBC): Analysis of the GeparSixto randomized clinical trial. *Ann Oncol* (2021) 32(12):1590–1596. doi: 10.1016/j.annonc.2021.03.016

31. Faraoni I, Graziani G. Role of BRCA mutations in cancer treatment with Poly (ADP-ribose) polymerase (PARP) inhibitors. *Cancers (Basel)* (2018) 10(12):487. doi: 10.3390/cancers10120487

32. Moulder S, Helgason T, Janku F, Wheler J, Moroney J, Booser D, et al. Inhibition of the phosphoinositide 3-kinase pathway for the treatment of patients with metastatic metaplastic breast cancer. *Ann Oncol* (2015) 26:1346–52. doi: 10.1093/annonc/mdv163

33. Basho RK, Yam C, Gilcrease M, Murthy RK, Helgason T, Karp DD, et al. Comparative effectiveness of an mTOR-based systemic therapy regimen in advanced, metaplastic and nonmetaplastic triple-negative breast cancer. *Oncologist* (2018) 23:1300–9. doi: 10.1634/theoncologist.2017-0498

34. Yang MH, Chen IC, Lu YS. PI3K inhibitor provides durable response in metastatic metaplastic carcinoma of the breast: A hidden gem in the BELLE-4 study. *J Formos Med Assoc* (2019) 118:1333–8. doi: 10.1016/j.jfma.2018.12.004

35. Adams S, Othus M, Patel SP, Miller KD, Chugh R, Schuetze SM, et al. A multicenter phase II trial of ipilimumab and nivolumab in unresectable or metastatic metaplastic breast cancer: Cohort 36 of dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors (DART, SWOG S1609). *Clin Cancer Res* (2022) 28:271–8. doi: 10.1158/1078-0432.CCR-21-2182

36. Martin EE, Huang W, Anwar T, Arellano-Garcia C, Burman B, Guan JL, et al. MMTV-cre;Ccn6 knockout mice develop tumors recapitulating human metaplastic breast carcinomas. *Oncogene* (2016) 36:2275–85. doi: 10.1038/onc.2016.381

37. Azim HA, Partridge AH. Biology of breast cancer in young women. *Breast Cancer Res* (2014) 16(4):427. doi: 10.1186/s13058-014-0427-5

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Bilateral metachronous breast malignancies: Malignant phylloides and invasive breast carcinoma—a case report

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A 57-year-old Malay nullipara initially presented with a right breast lump that was increasing in size but defaulted follow-up. Two years later, she developed a contralateral breast lump. She only returned to the hospital when the right breast lump had become painful, 4 years from its onset. The biopsy of the right breast lump was a phylloides tumor and that of the left breast lump was a carcinoma. She had bilateral palpable axillary lymph nodes. She underwent bilateral mastectomy and axillary dissection. The pathology report confirmed the right breast lesion to be a malignant phylloides and the left breast lesion to be a carcinoma (pT3N2). She declined adjuvant treatment. A year after the surgical operation of the metachronous lesions, she had a right chest wall recurrence with widespread pulmonary metastases. She was given palliative chemotherapy but succumbed several months later.

KEYWORDS

metachronous, malignant phylloides, breast carcinoma, mastectomy, recurrence

Introduction

The occurrence of bilateral breast cancer is uncommon; the incidence is 3% of all breast cancers. The second cancer may be synchronous or metachronous. Synchronous breast cancer is defined as breast cancer occurring within 1 year of the earlier cancer. Metachronous cancers develop more than a year from the initial cancer (1). There have been rare reports of synchronous phylloides tumors (PTs) with contralateral invasive carcinoma (2) and metachronous bilateral breast cancers of different histopathology (3–5).

Case report

Madam R was a Malay nullipara, a banker, with no known medical illness. She had menarche and menopause at the age of 12 and 55 years, respectively. She had no history of oral contraceptive pill usage or hormonal replacement therapy. There was no family history of breast or ovarian malignancy.

At 57 years old, she complained of a right breast lump. A mammogram was done at the National Cancer Society Center. She was informed that there were mammogram abnormalities in the right breast. However, she defaulted follow-up and sought alternative treatment instead.

Two years later, she presented to another hospital. The right breast lump had increased in size and she was found to have a contralateral breast lump. A mammogram with complementary breast and axillary ultrasound showed bilateral BI-RADS 5 lesions. A core-needle biopsy showed the right breast lump to be a PT, but the left breast lump was reported to be benign. As the left breast biopsy may have missed the lesion because it was discordant with the imaging findings, she was advised to undergo a repeat biopsy but she declined. Again, she defaulted follow-up.

At 61 years old, the patient came to our hospital due to pain in the right breast lump. On clinical examination, there was a hard bosselated mass occupying the whole right breast measuring 12 cm × 13 m with a right axillary lymph node measuring 1.5 cm. On the contralateral breast, there was an upper inner quadrant mass measuring 4.5 cm × 6.8 cm with a left axillary lymph node measuring 1 cm.

A mammogram of the left breast showed a lobulated mass with clustered microcalcifications at the upper inner quadrant with associated architectural distortion. Core-needle biopsy was repeated for the left breast lump and reported to be a left invasive carcinoma, no special type, ER positive >95%, PR positive 30%, and HER2 negative 1+.

Computed tomography (CT) of the thorax, abdomen, and pelvis was done to stage the disease. The CT scan revealed a well-defined lobulated heterogeneously enhancing mass at the retroareolar and outer half of the right breast measuring 7.9 × 13.3 × 10.5 cm with a necrotic center and foci of calcifications within. The right nipple was retracted and the skin was thickened. The mass was abutting the right pectoralis muscles with no clear fat plane at the central region (Figure 1A). A lobulated heterogeneously enhancing mass at the upper inner quadrant of the left breast extended to the retroareolar region, measuring 2.4 × 5.4 × 4.2 cm with calcifications and a necrotic center within (Figure 1B). There were bilateral enlarged axillary lymph nodes.

The patient underwent bilateral mastectomy and axillary dissection successfully (Figures 2A-C). Intraoperatively, the masses were not attached to the underlying pectoralis muscle and were easily removed from the pectoralis fascia. The right mastectomy specimen weighed 1.2 kg with a maximal diameter of 16 cm and was a malignant phylloides (Figure 3). A right axillary dissection was performed as the lymph nodes were palpable, but all 18 lymph nodes were clear of malignancy. The left mastectomy specimen weighed 400 g. The lump had a diameter of 6 cm. It was an invasive carcinoma of no special type (ER positive 60%, PR positive 50%, and HER2 negative) (Figure 4). The left axillary dissection revealed 7 positive out of a total of 13 lymph nodes. Microscopically, the closest surgical margin for each tumor was the deep margin measuring 1 mm.

Unfortunately, the patient declined all adjuvant therapy despite repeated advice. A year after the operation, she developed an enlarging right chest wall lump. When she came, it measured 10 cm which was biopsied and confirmed to be a recurrent malignant PT. There were also right palpable axillary lymph nodes. A CT scan of the thorax demonstrated a large right chest wall mass, right axillary lymph nodes measuring 3.1 × 4.9 × 6.8 cm, and multiple lung nodules of varying sizes.

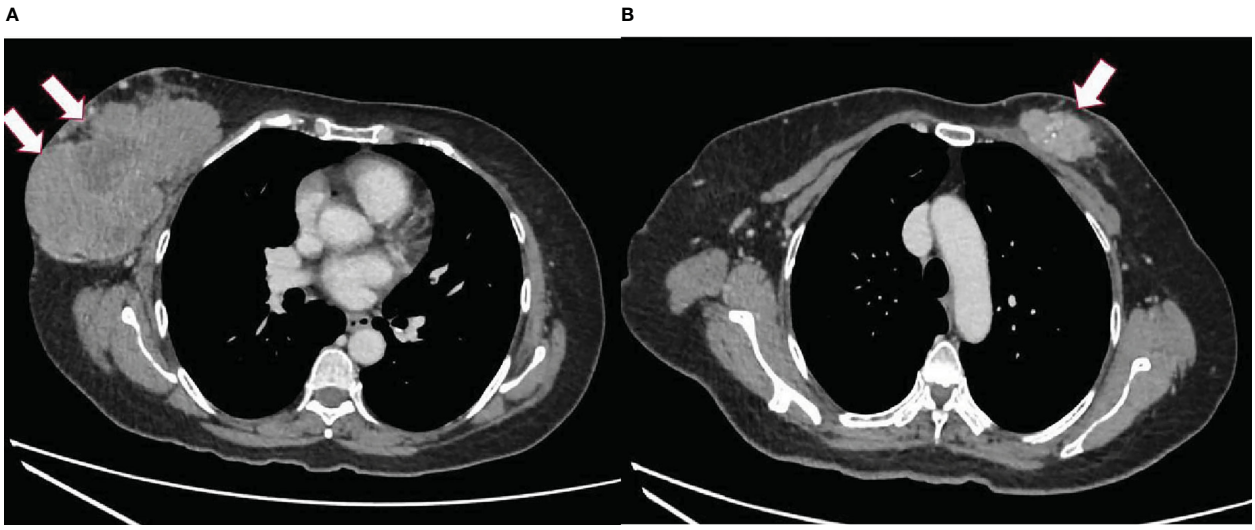

FIGURE 1
CT scan of the thorax. **(A)** Right breast mass, arrowed. **(B)** Left breast mass, arrowed.

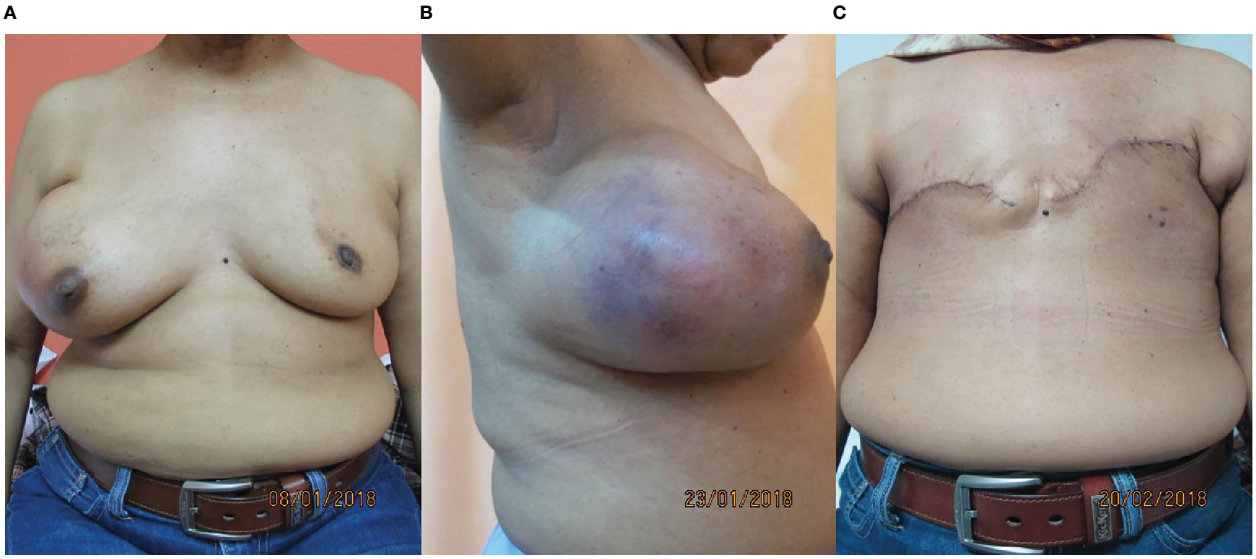

FIGURE 2
Photos of the patient. **(A, B)** Preoperatively, front and right lateral views. **(C)** Postoperatively, front.

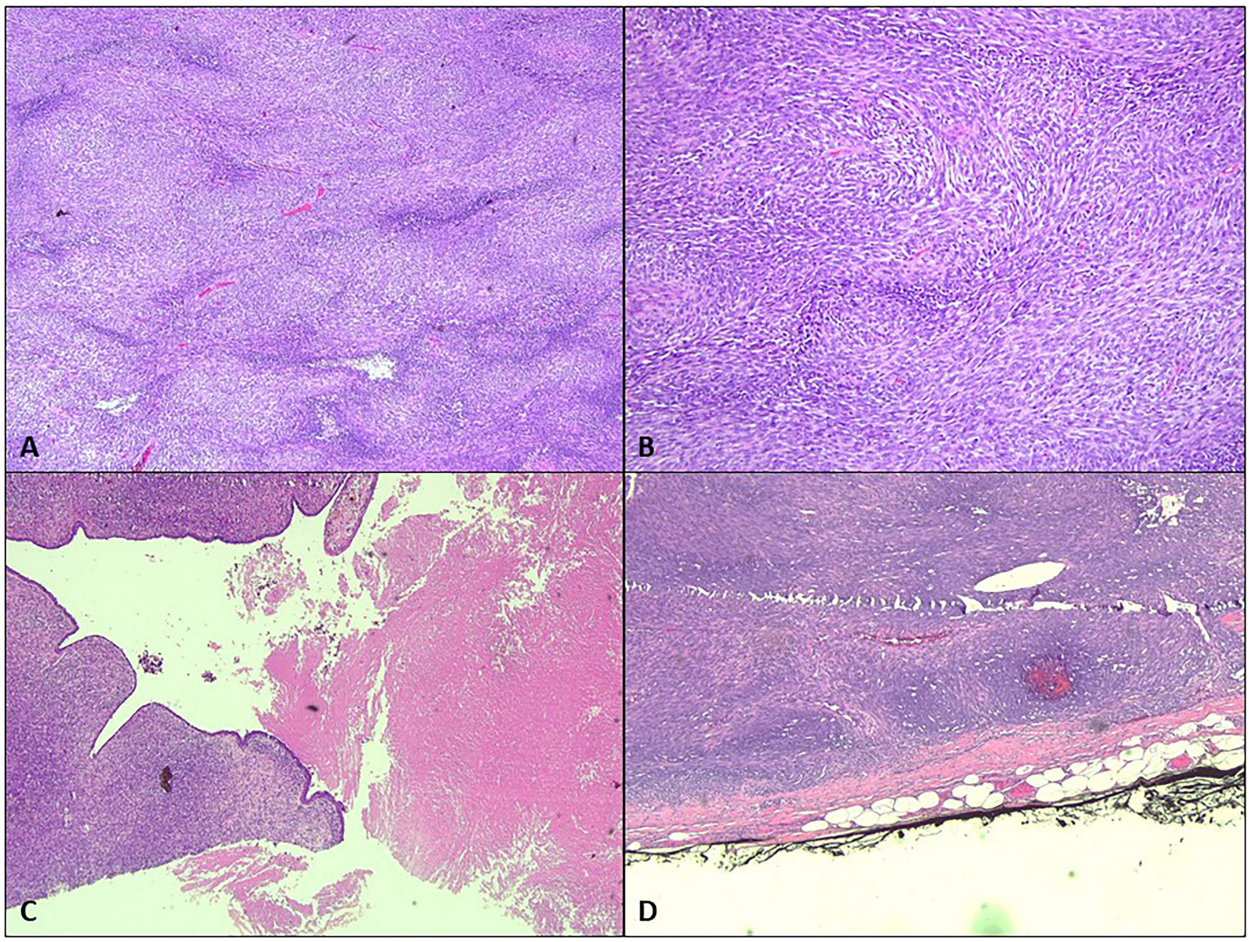

FIGURE 3
Microscopic analysis of the right breast. **(A)** The right breast tumor shows hypercellularity [hematoxylin–eosin (H&E) staining, ×4]. **(B)** The spindle-shaped cells are arranged in fascicles in a herringbone pattern (H&E, ×20). **(C)** Focal leaf-like pattern typical of phylloides tumor with adjacent necrosis (H&E, ×4). **(D)** The tumor is seen less than 1 mm from the inked deep margin (H&E, ×4).

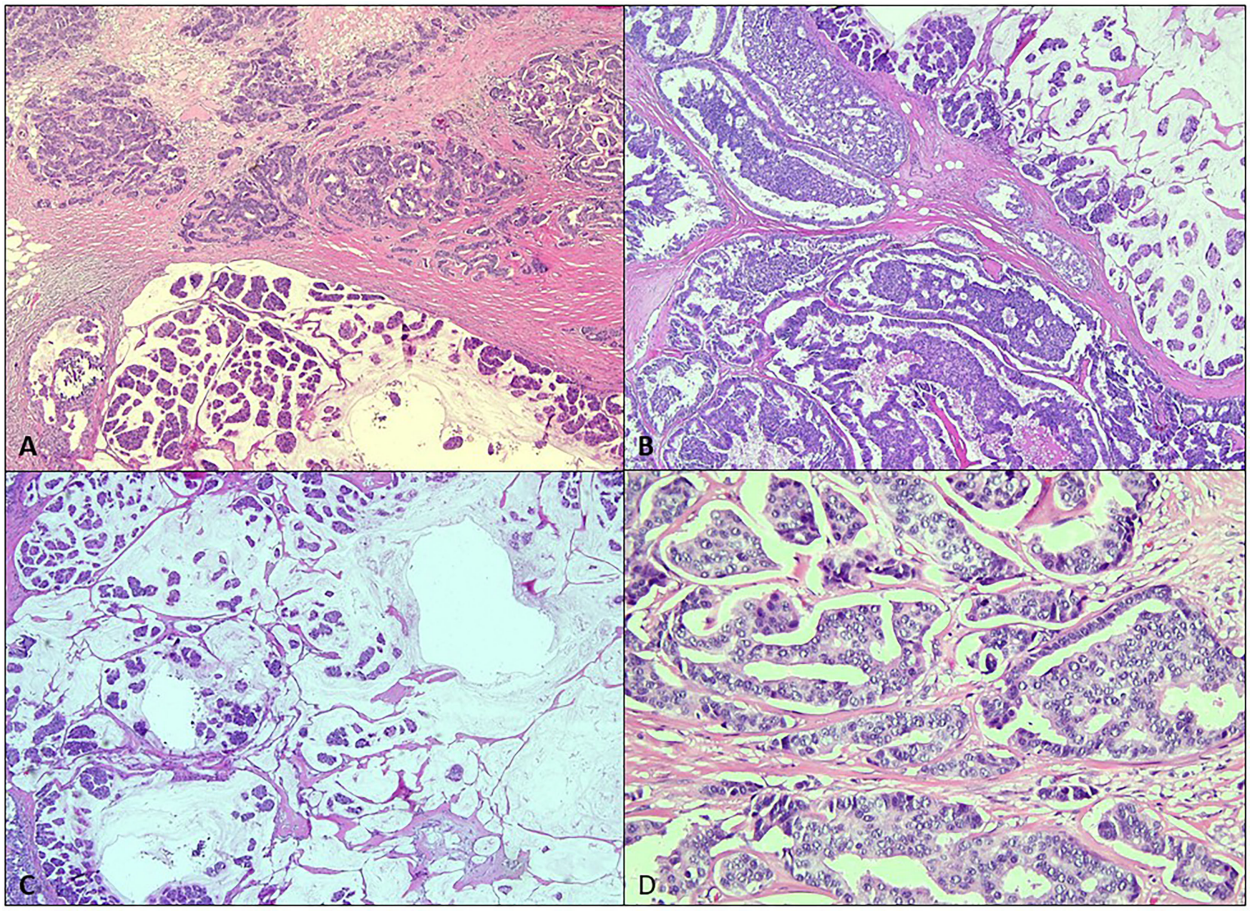

FIGURE 4
Microscopic analysis of the left breast. **(A, B)** The left breast tumor shows variable morphology of invasive carcinoma comprised of infiltrating small ducts, cords, and cribriform glands with a micropapillary pattern (H&E, ×4). **(C)** Clusters of cells floating in mucin pools (H&E, ×4). **(D)** The malignant cells display pleomorphic vesicular nuclei with inconspicuous nucleoli (H&E, ×20).

She underwent several cycles of palliative chemotherapy (paclitaxel) but had neutropenic sepsis with obvious disease progression. She declined further chemotherapy and was just given oral endocrine treatment (letrozole) for several weeks. She succumbed soon after due to widespread pulmonary metastases.

Discussion

PTs, originally known as cystosarcoma phylloides, are rare fibroepithelial breast tumors. They are characterized by the proliferation of epithelial and stromal components. They constitute less than 1% of breast neoplasms (6) and are more common in the fifth decade of life, as in this patient. They are characterized by rapidly growing breast lumps with a median symptom duration of 2 months (7). The WHO has classified PTs into benign, borderline (low-grade malignant), and malignant (high-grade malignant) based on histology, and they constitute 52.3%–74.6%, 11.1%–16.1%, and 9.3%–20% of cases, respectively (6).

PTs appear as well-circumscribed round or oval lobulated masses on mammogram. They occasionally contain calcifications. While on ultrasound, they appear as well-circumscribed round or oval lobulated hypoechoic, solid masses. Scattered cystic components may be seen at times. There are no distinct imaging characteristics to reliably distinguish benign from malignant PTs. However, features that may raise a suspicion of malignancy include branching, segmental or pleomorphic-type microcalcifications and spiculated masses on mammogram, and increased height–width ratio of irregular or ill-defined lesions on ultrasound (8).

In our patient, her initial right breast biopsy showed epithelial and connective tissue hyperplasia with no evidence of malignancy, with features favoring a benign PT. However, 2 years later, the right breast (mastectomy specimen) had transformed into a malignant PT with fibrosarcomatous elements.

Malignant transformation usually arises from the stromal components (6). For this patient, the right breast tumor showed malignant features such are hypercellularity, composed of spindle-shaped cells arranged in fascicles in a herringbone and a focal leaf-like pattern typical of PT with adjacent necrosis. The left breast tumor showed the morphology of invasive carcinoma comprised of infiltrating small ducts, cords, and cribriform glands with a micropapillary pattern and clusters of cells floating in mucin pools.

There were several reports of synchronous PTs with contralateral invasive carcinoma but only one case was reported

to be metachronous (2, 7). We believe this is only the second of such a case reported in the literature.

Treatment of PTs is generally wide local excision, or mastectomy, with a minimum margin of 1 cm. Adequate clear margins can prevent a recurrence, especially in borderline and malignant PTs (8). PTs typically behave more like sarcomas and do not metastasize to regional lymph nodes (9). In this case, for PT in the right breast, mastectomy and axillary lymph node dissection were performed, due to the size of the tumor which occupied the whole breast and the palpable ipsilateral right axillary lymph nodes. Mastectomy and axillary dissection were performed on the contralateral breast (invasive carcinoma) with palpable lymph nodes. She would have benefitted from prompt adjuvant chemotherapy, radiotherapy, and endocrine therapy to treat the left breast carcinoma with high nodal involvement. Adjuvant chemotherapy is usually reserved for metastatic malignant phylloides. Radiotherapy has a role in the treatment of malignant phylloides and carcinoma when there are involved margins, or margins less than 1 cm for malignant phylloides (10). As the two malignancies respond differently to the various treatment modalities, each lesion has to be dealt with individually, as stated above.

Postoperatively, the risk of local recurrence for PTs is reported as 8% in 10 years with adequate excised margin (2). Unfortunately, for this patient, both tumors had close surgical margins. This made the local recurrence risk of the PT to be high as the surgical margin was less than 1 cm. Hence, regular clinical and annual mammograms, in cases of breast-conserving surgery, are essential to detect early signs of local recurrence.

It is reported that distant metastases (brain and lungs) occur in 10% of malignant PTs. Once metastases have occurred, they indicate a poor prognosis as they respond to systemic therapy poorly (8). The survival outcomes for those with metastatic disease from a breast carcinoma are better than those from a malignant PT. There has been no single publication comparing survival data head-to-head between breast carcinoma and malignant phylloides. For those with stage 4 breast carcinoma, the 5-year survival has been found to vary from 15% to 27% (11). However, the median overall survival of those with metastatic malignant PT has been reported to be only 10.7 months (12).

The coexistence of invasive breast carcinoma and PTs in the ipsilateral breast has been described in the literature; it is hypothesized that this could be due to the invasive breast carcinoma cells arising from the adjacent tissue or within the phylloides tissues. Mathias et al. (2) postulated that the coexistence of contralateral invasive breast carcinoma and PTs could be associated with germline mutations (*PTEN* and *PARP4* genes) and exposure to radiotherapy. Both of these factors increase the risks of metachronous and synchronous malignancies. However, our patient was not known to have any germline mutation and was neither exposed to prior radiotherapy.

Similar to this patient, there is a small but significant percentage of the Malay population in Malaysia who tend to favor alternative treatment and present themselves late to the hospital which results in a poor clinical outcome (13) despite ongoing public education efforts by the government and non-governmental agencies.

Conclusion

Metachronous and synchronous contralateral malignant phylloides and invasive breast carcinoma are rare. However, clinicians and pathologists need to be aware of the possibility of two different pathologies existing when dealing with bilateral breast disease, as in this case, as the required treatment options and eventual outcomes differ. More studies need to be done in order to understand the histopathological events better.

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

Written consent was obtained from the patient for publication of this case report.

Author contributions

NA was responsible for the final editing, provided the patient's photos, and acted as the main clinician. IHR reported the images and edited the draft. JG wrote the initial draft. QL co-wrote the initial draft. NMI and SMP prepared and reported the pathology slides. All authors contributed to the article and approved the submitted version.

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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.

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References

1. Al-Jurf AS, Jochimsen PR, Urdaneta LF, Scott DH. Factors influencing survival in bilateral breast cancer. *J Surg Onc* (1981) 16(4):343–8. doi: 10.1002/jso.2930160407

2. Mathias M, Joshi D, Kishen Prasad HL, Sajitha K, Lobo L, Hidangmayum A. Synchronous phyllodes tumor and invasive breast carcinoma in contralateral breasts- a rare case report. *Int J Res Med Sci* (2021) 9(2):623–6. doi: 10.18203/2320-6012.ijrms20210455

3. Powell CM, Rosen PP. Adipose differentiation in cystosarcoma phyllodes: a study of 14 cases. *Am J Surg Pathol* (1994) 18(7):720–7. doi: 10.1097/00000478-199407000-00008

4. Gebrim LH, Bernardes Júnior JR, Nazário AC, Kemp C, Lima GR. Malignant phyllodes tumor in the right breast and invasive lobular carcinoma within fibroadenoma in the other: case report. *Sao Paulo Med J* (2000) 118(2):46–8. doi: 10.1590/S1516-31802000000200004

5. Merck B, Martínez PC, Ramos MP, Banac NM. Infiltrating ductal carcinoma and synchronous malignant phyllodes tumour. diagnostic and therapeutic approaches. *Clin Transl Oncol* (2006) 8(11):830–2. doi: 10.1007/s12094-006-0140-7

6. Panko N, Jebran AA, Gomberawalla A, Connolly M. Invasive ductal carcinoma within a benign phyllodes tumor. *Am J Case Rep* (2017) 18:813–6. doi: 10.12659/ajcr.903774

7. Sato T, Muto I, Sakai T. Coexistence of malignant phyllodes tumor and Her2-positive locally advanced breast cancer in distinct breasts: A case report. *Int J Surg Case Rep* (2016) 19:163–. doi: 10.1016/j.ijscr.2015.12.043

8. Tan PH, Jayabaskar T, Chuah KL, Lee HY, Tan Y, Hilmy M, et al. Phyllodes tumors of the breast: the role of pathologic parameters. *Am J Clin Pathol* (2005) 123(4):529–40. doi: 10.1309/U6DVBFM81MLJC1FN

9. Chaney AW, Pollack A, McNeese MD, Zagars GK, Pisters PWT, Pollock RE, et al. Primary treatment of cystosarcoma phyllodes of the breast. *Cancer* (2000) 89(7):1502–11. doi: 10.1002/1097-0142(20001001)89:7<1502::AID-CNCR13>3.0.CO;2-P

10. Mitus JW, Blecharz P, Jakubowicz J, Reinfuss M ,Walasek T, Wysocki W. Phyllodes tumors of the breast. the treatment results for 340 patients from a single cancer centre. *Breast* (2019) 43:85–90. doi: 10.1016/j.breast.2018.11.009

11. Khale R. Optimisation of breast cancer management in low-resource and middle-resource countries: executive summary of the breast health global initiative consensus, 2010. *Lancet Oncol* (2011) 4:387–98. doi: 10.1016/S1470-2045(11)70031-6

12. Parkes A, Wang WL, Patel S, Leung CH, Lin H, Conley AP, et al. Outcomes of systemic therapy in metastatic phyllodes tumor of the breast. *Breast Cancer Res Treat* (2021) 186:871–82. doi: 10.1007/s10549-021-06116-8

13. Abdullah N, Mohamed N. Influence of cultural practices on breast cancer risks, stage at presentation and outcome in a multi-ethnic developing country. *Oncol Lett* (2021) 22(5):806. doi: 10.3892/ol.2021.13067

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Development and validation of nomograms to predict the survival probability and occurrence of a second primary malignancy of male breast cancer patients: a population-based analysis

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Background: Male breast cancer (MBC) is rare, which has restricted prospective research among MBC patients. With effective treatments, the prognosis of MBC patients has improved and developing a second primary malignancy (SPM) has become a life-threatening event for MBC survivors. However, few studies have focused on the prognosis of MBC patients and looked into the SPM issue in MBC survivors.

Method: We reviewed MBC patients diagnosed between 1990 and 2016 from the latest Surveillance, Epidemiology, and End Results (SEER) Plus database. Competing risk models and nomograms were conducted for predicting the risk of cancer-specific death and SPM occurrence. C-indexes, calibration curves, ROC curves, and decision curve analysis (DCA) curves were applied for validation.

Result: A total of 1,843 MBC patients with complete information were finally enrolled and 60 (3.26%) had developed an SPM. Prostate cancer (40%) was the most common SPM. The median OS of all the enrolled patients was 102.41 months, while the median latency from the initial MBC diagnosis to the subsequent diagnosis of SPM was 67.2 months. The patients who suffered from an SPM shared a longer OS than those patients with only one MBC (p = 0.027). The patients were randomly divided into the development cohort and the validation cohort (at a ratio of 7:3). The Fine and Gray competing risk model was used to identify the risk factors. Two nomograms were constructed and validated to predict the 5-year, 8-year, and 10-year survival probability of MBC patients,

both of which had good performance in the C-index, ROC curves, calibration plots, and DCA curves, showing the ideal discrimination capability and predictive value clinically. Furthermore, we, for the first time, constructed a nomogram based on the competing risk model to predict the 5-year, 8-year, and 10-year probability of developing an SPM in MBC survivors, which also showed good discrimination, calibration, and clinical effectiveness.

Conclusion: We, for the first time, included treatment information and clinical parameters to construct a nomogram to predict not only the survival probability of MBC patients but also the probability of developing an SPM in MBC survivors, which were helpful in individual risk estimation, patient follow-up, and counseling in MBC patients.

KEYWORDS

male breast cancer, second primary malignancy, prognosis, survival probability, nomogram

Introduction

Breast cancer is relatively uncommon in men. Approximately 2,000 men are diagnosed with breast cancer annually in the USA, accounting for 1% of all new breast cancer patients and 0.03% of all new malignant diseases in men (1). Male breast cancer (MBC) has a similar mortality rate to female breast cancer at 17% (2). Mortality rates in Europe remained fairly stable, but the USA indicated an increase in incidence (3, 4). This trend could result from an increase in longevity in the population, since age is the major determinant of risk for most solid tumors. The incidence of MBC had a similar increasing rate with that of female breast cancer, which is probably related to the popularity of mammography screening (5, 6). However, it was shown that the prognosis of MBC patients was worse than that of female breast cancer patients (7–9). Similar to female breast cancer, the incidence of MBC also has regional differences, which is higher in North America and Europe and lower in Asia (10). The majority of MBCs do not have specific risk factors, and some small-sample studies showed that a high level of estrogen and an imbalance of hormones may contribute to the development of MBC (11–13). Genetic factors may also have a possible connection to MBC, and BRCA2 mutations appear to be the strongest risk factor for breast cancer in men with a lifetime risk of 7%, which is approximately 80 times more than the general population (14).

The rarity of MBC has restricted prospective studies on it. Principles of treatments of MBC are derived largely from randomized trials carried out in women (15, 16). Ninety percent of MBCs are estrogen-receptor-positive; tamoxifen is the standard adjuvant therapy, and some individuals could also benefit from chemotherapy. Hormonal therapy is the main treatment for metastatic disease (17), while chemotherapy can also provide palliation (10). In addition, advances in early screening and treatments have caused a considerable proportion of MBC survivors. For some survivors, second primary malignancy (SPM) is one of the most potentially life-threatening outcomes (18). At present, no research has focused on the SPM in MBC survivors, and the prediction models of developing an SPM in MBC patients have not been provided. In this study, we developed two nomogram models to predict the survival probability of MBC patients using the competing risk method. Furthermore, we built an additional nomogram to predict the probability of an MBC survivor developing an SPM.

Method

Data sources and population selection

The data of the present research were obtained from the latest Surveillance, Epidemiology, and End Results (SEER) Plus database (SEER 9 Registries data, with additional treatment information, Nov 2021 sub). The SEER database is an authoritative source of information on cancer, covering approximately 34.6% of the population in the USA. The records of male patients diagnosed with breast carcinoma between 1990 and 2016 were extracted using the SEER*Stat software (version 8.4.1), ensuring long-term follow-up of at least 5 years to estimate the risk of developing a second primary cancer. The International Classification of Diseases for Oncology third edition (ICD-O-3) was used to identify breast malignancy by site code C50 (including C50.1 to C50.9). The three key variables “year of diagnosis”, “sequence number”, and “total number of in situ/malignant tumors for patient” of the SEER Plus database were used to determine the status of SPM. Cases that were diagnosed as synchronous cancers occurring as SPM within 2 months after initial diagnosis or those in which the breast malignancy was not the patients’ first primary malignancy were excluded. The inclusion criteria were as follows: (1) male breast malignancy was the only or the first primary malignancy; (2) histological diagnosis confirming the existence of breast

malignancy; and (3) under treatment and the follow-up data were available. The exclusion criteria were as follows: (1) incomplete cases with missing information on important variables; (2) the SPM (if any) data were incomplete; (3) initially diagnosed with distant metastasis; and (4) synchronous cancers. The flowchart of case selection is shown in Supplementary Figure 1.

Variable declaration and outcome

A total of 1,843 MBC patients were involved in this study. Variables such as age, race, marital status, year of diagnosis, sequence number, total number of in *situ*/malignant tumors for patient, histological type, tumor grade, TMN stage, surgery performance, radiotherapy performance, chemotherapy performance, months from diagnosis to treatment, the hormone receptor (HR) status, HER2 status, survival time, and cause of death were extracted. Age was regrouped into six groups (<45, 45–55, 55–65, 65–75, 75–85, and 85+). Race was regrouped into white, black, and other. Marital status included married, single, and divorced. Histological type was divided into infiltrating duct, adenocarcinoma, and other by the SEER Plus database. The HR status was classified as HR positive [estrogen receptor (ER) and/or progesterone receptor (PR) was positive] and HR negative (both ER and PR were negative). TMN stage was adjusted to the 6th AJCC staging edition by the SEER Plus database in the additional analysis. The site and the diagnosis time of the SPM were recorded. Overall survival (OS) refers to the time from the initial cancer diagnosis to cancer-specific death.

Study design and methods

The cumulative incidence of cancer-specific death and the occurrence of SPM were calculated based on the Fine and Gray competing risk model. The Kaplan–Meier method was constructed to estimate the difference in OS between MBC survivors with and without an SPM. The entire cohort was randomly divided into a development cohort (70%) and a validation cohort (30%) for the development and validation for the competing risk nomogram. Standardized mean differences (SMDs) were used to assess distributional differences in the baseline variables between the development and validation cohorts. As HER2 status is known to be tested after 2010, and HER2 status should be routinely diagnosed clearly in breast cancer patients nowadays, sensitivity analyses were carried out excluding those MBC patients whose HER2 was unknown or whose diagnosis was made prior to 2010.

Variable selection

The univariate and multivariate Cox regression analyses were firstly performed to identify variables that significantly affected the breast cancer-specific survival and occurrence of SPM. However, applying only univariate and multivariate Cox regression analyses was inadequate, because aside from the primary tumor, there were other factors that might threaten the patients' lives, such as accidents and infectious or other serious diseases. As a result, death due to other causes acted as a competing risk event to death due to a specific cancer. Hence, the Cox proportional hazards model might overestimate the incidence rate of the outcome with the passage of time. Similarly, death due to primary breast cancer or other causes also acted as a competing event for the MBC patients to develop an SPM—only those cured from MBC could have the probability of developing an SPM during their long survival time. In this study, the additional Fine and Gray competing risk analysis was applied to compare the association among different causes of death with a competing risk framework: death due to breast cancer or death due to other causes. Then, as for the occurrence of SPM in MBC survivors, the Fine and Gray method was also applied: death due to primary breast cancer or other causes was the competing event in the development of an SPM.

Competing risk nomogram construction and evaluation

In order to help clinicians predict the survival probability of MBC patients and their individual probability to develop an SPM, nomograms were established based on the multivariate competing risk models. Next, we identified low-risk and high-risk survivors by calculating the 50th quantiles of total points of the nomograms and compared the difference of their survival time. Validation of these nomograms was performed by calculating the concordance index (C-index) and plotting calibration curves by a bootstrapping method with 1,000 resamples. Furthermore, the receiver operating characteristic (ROC) curves were drawn to estimate the predictive value by calculating the area under the ROC curves (AUCs). Meanwhile, decision curve analyses (DCAs) were conducted to show the clinical effectiveness of the nomogram models.

Statistical analyses

All analyses were performed using R software (version 4.21, https://www.r-project.org/). Significance level was set as $p < 0.05$.

Results

Patient characteristics

A total of 1,843 MBC patients, who were initially diagnosed between 1990 and 2016, were finally enrolled in the present study. Among these MBC patients, 60 (3.26%) developed at least one SPM. A total of 339 (18.39%) patients died from MBC, and 707 (38.4%) patients died from other causes. Among those survivors who suffered from an SPM, prostate cancer represented 24 (40%) of all SPMs, followed by lung and bronchus cancer at 6 (10.0%), melanoma of the skin at 5 (8.3%), secondary breast cancer at 4 (6.7%), liver cancer at 3 (5.0%), urinary bladder cancer at 3 (5.0%),

kidney and renal pelvis cancer at 2 (3.3%), NHL at 2 (3.3%), pancreas cancer at 2 (3.3%), rectal cancer at 2 (3.3%), and stomach cancer at 2 (3.3%). The SPM details of these MBC survivors are shown in Figure 1. The median OS of all the enrolled patients was 102.41 months. The median latency from diagnosis of initial breast primary cancer to subsequent diagnosis of the SPM was 67.2 months. The detailed information of these MBC patients is summarized in Tables 1, 2.

Kaplan–Meier analysis

As is shown in Figure 2A, there was no significant difference in OS between the development and validation cohorts ($p = 0.83$). The OS of MBC patients who did not suffer from an SPM was 101.87 ± 68.17 months, while the OS of those who suffered from an SPM was 118.63 ± 75.76 months. Those who developed an SPM have a significantly longer OS (Figure 2B, $p = 0.027$).

Univariate and multivariate Cox regression analysis with cancer-specific death

Univariate and multivariate Cox regression were applied in the development cohort to select the predictive variables for the prediction models of cancer-specific death. MBC patients whose HER2 was unknown or diagnosed prior to 2010 were excluded in the following sensitivity analyses as mentioned above. As is shown in Table 3, tumor grade, TMN stage, surgery, and chemotherapy were related to OS in the univariate analysis, while in the multivariate Cox regression, chemotherapy failed to show a significant relation with OS.

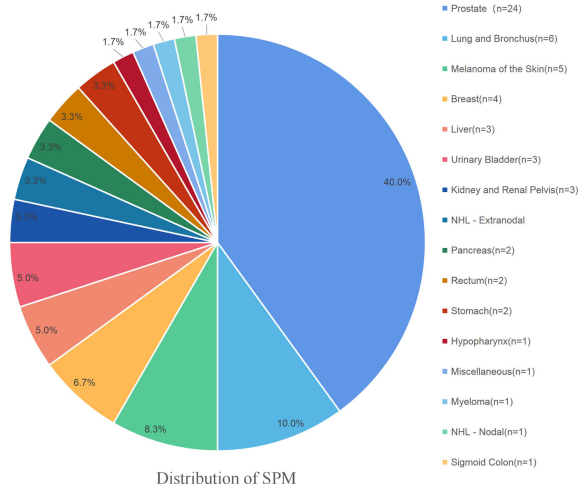

FIGURE 1
The detailed distribution of the SPMs among MBC survivors. Prostate cancer represented 24 (40%) of all SPMs, followed by lung and bronchus at 6 (10.0%), melanoma of the skin at 5 (8.3%), the secondary breast cancer at 4 (6.7%), liver at 3 (5.0%), urinary bladder at 3 (5.0%), kidney and renal pelvis at 2 (3.3%), NHL at 2 (3.3%), pancreas at 2 (3.3%), rectum at 2 (3.3%), and stomach at 2 (3.3%).

Univariate and multivariate Cox regression analysis with the occurrence of SPM

Univariate and multivariate Cox regression were also applied to select the predictive variables for the occurrence of SPM. As is shown in Table 4, marital status showed a significant relation with the occurrence of SPM in the univariate analysis. Moreover, in the multivariate Cox regression, age, race, tumor differentiated grade, histological type, TMN stage, chemotherapy, and the waiting time from diagnosis to begin treatment were significant.

Fine and Gray competing risk models

The Fine and Gray method was used to estimate the risk predictors for cancer-specific death and the occurrence of SPM. The results of the characteristics are provided in Table 5. Age, race, marital status, histological type, TMN stage, therapy, the waiting time from diagnosis to begin treatment, HR status, and HER2 status were the significant risk factors for both cancer-specific death and the development of an SPM.

Nomogram construction and validation

The first two nomograms were established based on the previously mentioned risk factors to predict the survival probability of MBC patients. Age, race, marital status, tumor differentiated grade, histology, TMN stage, surgery, radiotherapy, chemotherapy, duration to begin treatment, HR status, and HER2 status, which were selected by the Fine and Gray method, were enrolled in nomogram model 1 to predict the 5-year, 8-year, and 10-year survival probability of MBC patients (Figure 3A). Meanwhile, age, tumor differentiated grade, TMN stage, and surgery, which were selected by the multivariate Cox regression, were included in nomogram model 2 (Figure 3B) to predict the same survival probability above. The C-index of model 1 was 0.710 in the development cohort and 0.703 in the validation cohort, while model 2 had a C-index at 0.728 in the development cohort and 0.718 at the validation cohort. Both model 1 (AUC = 0.713) and model 2 (AUC = 0.757) achieved a better predictive value than the AJCC TMN staging system (AUC = 0.689) in the ROC analysis shown in Figure 4A. The integrated discrimination improvement (IDI) and net reclassification improvement (NRI) between model 1 and TMN stage were 0.610 (95% CI 0.490–0.258) and 0.333 (95% CI 0.182–0.508), respectively. Meanwhile, The IDI and NRI between model 2 and TMN stage were 0.059 (95% CI 0.036–0.193) and 0.290 (95% CI 0.154–0.513), respectively. The calibration curves show that both model 1 and model 2 had good agreement between predicted probability and the observed outcome (Figures 4B, C). The DCA also showed that model 1 (Figures 4D, E) and model 2 (Figures 4F, G) had a good discrimination in both the development and validation cohorts. We divided the patients into a low-risk group and a high-risk group at the 50th percentile of nomogram total points and compared the difference of the survival time among these subgroups. Figures 5A, B show that there were significant

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TABLE 1 Patient characteristics and clinicopathological variables.

Variables	Total	Cohort		*p*-value
		Development	Validation	
N	**1,843**	1,291	552	
Survival months		102.44 ± 68.48	102.37 ± 68.52	0.985
Age		66.86 ± 12.57	66.89 ± 12.52	0.994
Age group				0.962
<45	80	58 (4.49%)	22 (3.99%)	
45–55	226	154 (11.93%)	72 (13.04%)	
55–65	458	323 (25.02%)	135 (24.46%)	
65–75	527	366 (28.35%)	161 (29.17%)	
75–85	425	302 (23.39%)	123 (22.28%)	
85+	127	88 (6.82%)	39 (7.07%)	
Race				0.681
White	1,573	1,106 (85.67%)	467 (84.60%)	
Black	164	110 (8.52%)	54 (9.78%)	
Other	106	75 (5.81%)	31 (5.62%)	
Marital status				0.352
Married	1,331	937 (72.58%)	394 (71.38%)	
Single	389	263 (20.37%)	126 (22.83%)	
Divorced	123	91 (7.05%)	32 (5.80%)	
Tumor grade				0.207
Grade I	242	163 (12.63%)	79 (14.31%)	
Grade II	944	649 (50.27%)	295 (53.44%)	
Grade III	636	465 (36.02%)	171 (30.98%)	
Grade IV	21	14 (1.08%)	7 (1.27%)	
Histological type				
Infiltrating duct	1,634	1,148 (88.92%)	486 (88.04%)	0.763
Adenocarcinoma	112	75 (5.81%)	37 (6.70%)	
Other	97	68 (5.27%)	29 (5.25%)	
TMN stage				0.952
0	2	2 (0.15%)	0 (0.00%)	
I	645	446 (34.55%)	199 (36.05%)	
IIA	596	419 (32.46%)	177 (32.07%)	
IIB	270	189 (14.64%)	81 (14.67%)	
IIIA	153	111 (8.60%)	42 (7.61%)	
IIIC	92	64 (4.96%)	28 (5.07%)	
IIIB	85	60 (4.65%)	25 (4.53%)	
Surgery performed				0.847
Yes	1,821	1,276 (98.84%)	545 (98.73%)	
No	22	15 (1.16%)	7 (1.27%)	

(Continued)

TABLE 1 Continued

Variables	Total	Cohort		*p*-value
		Development	Validation	
Radiotherapy performed				0.976
No	1,273	892 (69.09%)	381 (69.02%)	
Yes	570	399 (30.91%)	171 (30.98%)	
Chemotherapy performed				0.930
No	1,158	812 (62.90%)	346 (62.68%)	
Yes	685	479 (37.10%)	206 (37.32%)	
Duration to begin treatment				0.281
Less than 1 month	1,060	753 (58.33%)	307 (55.62%)	
More than 1 month	783	538 (41.67%)	245 (44.38%)	
HR status				0.932
Positive	1,792	1,255 (97.21%)	537 (97.28%)	
Negative	51	36 (2.79%)	15 (2.72%)	
HER2 status				0.616
Positive	69	52 (4.03%)	17 (3.08%)	
Negative	535	373 (28.89%)	162 (29.35%)	
Borderline/Unknown	1,239	866 (67.08%)	373 (67.57%)	
SPM occurrence				
No	1,783	1,255 (97.21%)	528 (95.65%)	0.084
Yes	60	36 (2.79%)	24 (4.35%)	

*Statistically significant ($p < 0.05$).

TABLE 2 Patient characteristics (with or without SPM).

Variables	Total	Occurrence of SPM		*p*-value
		No	Yes	
N	**1,843**	1,783	60	
Survival months		101.87 ± 68.17	118.63 ± 75.76	**0.027***
Age		66.84 ± 12.63	67.53 ± 10.02	0.817
Age group				0.160
<45	80	80 (4.49%)	0 (0.00%)	
45–55	226	218 (12.23%)	8 (13.33%)	
55–65	458	444 (24.90%)	14 (23.33%)	
65–75	527	502 (28.15%)	25 (41.67%)	
75–85	425	415 (23.28%)	10 (16.67%)	
85+	127	124 (6.95%)	3 (5.00%)	
Race				0.637
White	1,573	1,524 (85.47%)	49 (81.67%)	
Black	164	158 (8.86%)	6 (10.00%)	

(Continued)

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TABLE 2 Continued

Variables	Total	Occurrence of SPM		*p*-value
		No	Yes	
Other	106	101 (5.66%)	5 (8.33%)	
Marital status				0.172
Married	1,331	1,294 (72.57%)	37 (61.67%)	
Single	389	372 (20.86%)	17 (28.33%)	
Divorced	123	117 (6.56%)	6 (10.00%)	
Tumor grade				0.202
Grade I	242	229 (12.84%)	13 (21.67%)	
Grade II	944	915 (51.32%)	29 (48.33%)	
Grade III	636	618 (34.66%)	18 (30.00%)	
Grade IV	21	21 (1.18%)	0 (0.00%)	
Histological type				
Infiltrating duct	1,634	1,583 (88.78%)	51 (85.00%)	0.538
Adenocarcinoma	112	108 (6.06%)	4 (6.67%)	
Other	97	92 (5.16%)	5 (8.33%)	
TMN stage				0.952
0	2	2 (0.11%)	0 (0.00%)	
I	645	624 (35.00%)	21 (35.00%)	
IIA	596	577 (32.36%)	19 (31.67%)	
IIB	270	261 (14.64%)	9 (15.00%)	
IIIA	153	146 (8.19%)	7 (11.67%)	
IIIC	92	91 (5.10%)	1 (1.67%)	
IIIB	85	82 (4.60%)	3 (5.00%)	
Surgery performed				0.732
Yes	1,821	1,762 (98.82%)	59 (98.33%)	
No	22	21 (1.18%)	1 (1.67%)	
Radiotherapy performed				0.488
No	1,273	1,234 (69.21%)	39 (65.00%)	
Yes	570	549 (30.79%)	21 (35.00%)	
Chemotherapy performed				0.370
No	1,158	1,117 (62.65%)	41 (68.33%)	
Yes	685	666 (37.35%)	19 (31.67%)	
Duration to begin treatment				0.233
Less than 1 month	1,060	1,021 (57.26%)	39 (65.00%)	
More than 1 month	783	762 (42.74%)	21 (35.00%)	
HR status				0.597
Positive	1,792	1,733 (97.20%)	59 (98.33%)	
Negative	51	50 (2.80%)	1 (1.67%)	
HER2 status				0.616

(Continued)

TABLE 2 Continued

Variables	Total	Occurrence of SPM		*p*-value
		No	Yes	
Positive	69	69 (3.87%)	0 (0.00%)	
Negative	535	524 (29.39%)	11 (18.33%)	
Borderline/Unknown	1,239	1,190 (66.74%)	49 (81.67%)	

*Statistically significant ($p < 0.05$).

TABLE 3 Univariate and multivariate analysis of cancer-specific death.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age group				
65–75	1		1	
55–65	0.78 (0.37, 1.66)	0.5205	0.5 (0.2, 1.4)	0.182
75–85	0.84 (0.40, 1.77)	0.6561	1.8 (0.6, 5.6)	0.288
45–55	0.73 (0.26, 2.03)	0.5438	0.2 (0.0, 1.6)	0.135
85+	0.88 (0.28, 2.73)	0.8249	2.06 (0.68, 6.26)	0.2024
<45	1.56 (0.49, 5.01) 0.4551	0.4551	0.4 (0.0, 6.3)	0.481
Race				
White	1		1	
Black	1.53 (0.71, 3.29)	0.2745	1.5 (0.7, 3.3)	0.355
Other	0.84 (0.25, 2.83)	0.7745	0.6 (0.2, 1.9)	0.360
Marital status				
Married	1		1	
Single	0.80 (0.40, 1.60)	0.5307	0.8 (0.4, 1.6)	0.523
Divorced	1.42 (0.53, 3.85)	0.4870	1.0 (0.3, 2.7)	0.928
Tumor grade				
Grade II	1		1	
Grade III	2.17 (1.22, 3.87)	**0.0083***	1.9 (1.1, 3.4)	**0.029***
Grade I	0.66 (0.22, 1.98)	0.4601	1.0 (0.3, 3.1)	0.954
Grade IV	0.00 (0.00, Inf)	0.9891	0.0 (0.0, Inf)	0.998
Histological type				
Infiltrating duct	1		1	
Adenocarcinoma	0.73 (0.17, 3.18)	0.6791	1.2 (0.3, 5.2)	0.814
Other	0.51 (0.12, 2.18)	0.3625	0.5 (0.1, 2.0)	0.316
TMN stage				
Stage 0+I	1		1	
Stage II	2.57 (1.15, 5.74)	**0.0213***	2.2 (1.0, 5.0)	**0.061***
Stage III	6.33 (2.68, 14.98)	**<0.0001***	5.6 (2.2, 14.6)	**<0.001***
Surgery performed				

(Continued)

TABLE 3 Continued

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Yes	1		1	
No	5.16 (1.70, 15.68)	**0.0038***	3.8 (1.4, 10.6)	**0.009***
Radiotherapy performed				
No	1		1	
Yes	1.51 (0.87, 2.63)	0.1474	0.9 (0.5, 1.8)	0.870
Chemotherapy performed				
No	1		1	
Yes	2.56 (1.47, 4.48)	**0.0009***	1.3 (0.6, 2.6)	0.520
Duration to begin treatment				
Less than 1 month	1		1	
More than 1 month	0.85 (0.49, 1.46)	0.5494	1.1 (0.6, 1.9)	0.765
HR status				
Positive	1		1	
Negative	0.96 (0.12, 7.63)	0.9684	0.7 (0.1, 5.8)	0.764
HER2 status				
Negative	1		1	
Positive	11.52 (0.71, 3.26)	0.2793	1.2 (0.6, 2.7)	0.603

*Statistically significant ($p < 0.05$).

differences in survival time between different risk groups in both the development cohort (p = 0.0022) and the validation cohort (p = 0.002), based on nomogram model 1 (Figure 3A). Meanwhile, Figures 5C, D also show the survival time difference between different risk groups in the development cohort (p = 0.001) and the validation cohort (p = 2e-04), based on nomogram model 2 (Figure 3B), which indicated that both of these nomogram models had a good discrimination capability for the survival probability of the MBC patients. The details of these two nomograms are shown in Supplementary Tables 1, 2.

An additional nomogram model 3 was established to predict the probability of MBC survivors developing an SPM within 10 years after the initial diagnosis. All of the risk factors selected by the Fine and Gray method were included in model 3 (Figure 6A). The C-index of model 3 was 0.909 in the development cohort and 0.494 in the validation cohort. The AUC of the ROC curve in model 3 is 0.934 (Figure 6B). The calibration curve is shown in Figure 6C. The DCA curve is shown in Figure 6D in the development cohort and in Figure 6E in the validation cohort. The details of these risk factors are shown in Supplementary Table 3.

Discussion

MBC is a rare disease whose causes remain incompletely characterized and understood. Because of the limitation of large-scale randomized prospective research, MBC treatment largely follows the guidelines of female breast cancer (19). By applying sufficient therapies, such as surgery, chemotherapy, radiotherapy, endocrine therapy, targeted therapy, and immunotherapy, the prognosis of MBC survivors has improved in the past 25 years (20). With the longevity of the MBC survivors, SPM has become a life-threatening event. In the present study, we enrolled 1,843 MBC patients who were randomly divided into a development and a validation group at a ratio of 7:3. No difference was found between these two groups (Table 1). At present, a few studies focused on the prognosis of MBC patients. Wang et al. developed a nomogram to predict distant metastasis in MBC patients, based on univariate and multivariate logistic regression analyses, but did not focus on the probability of survival and the development of an SPM (21). Chen et al. constructed a nomogram to predict the prognosis of MBC patients based on univariate and multivariate Cox regression (22). Similar research was published by Zhang et al. (23). However, as we mentioned above, applying only Cox regression analysis was inadequate and would overestimate the risk of cancer-specific death, because aside from the primary tumor, there were other factors that might threaten their life (24), and death due to other causes actually acted as a competing event to death caused by MBC. In this study, two nomograms were constructed to predict the survival probability of MBC patients based on the Fine and Gray competing risk analysis and multivariate Cox regression, respectively, to correct this bias. Sun et al. performed a competing

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TABLE 4 Univariate and multivariate analysis of the occurrence of SPM.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age group				
65–75	1.0		1.0	
55–65	0.62 (0.15, 2.54)	0.5103	0.1 (0.0, 0.6)	**0.009***
75–85	0.20 (0.02, 1.71)	0.1429	17,323.8 (1570.8, 191059.2)	**<0.001***
45–55	0.47 (0.05, 3.94)	0.4831	0.0 (0.0, 0.0)	**<0.001***
85+	0.00 (0.00, Inf)	0.9921	0.7 (0.0, Inf)	1.000
<45	0.00 (0.00, Inf)	0.9939	0.6 (0.0, Inf)	1.000
Race				
White	1.0		1.0	
Black	1.64 (0.35, 7.77)	0.5310	Inf (Inf, Inf)	**<0.001***
Other	0.00 (0.00, Inf)	0.9928	349.3 (0.0, Inf)	1.000
Marital status				
Married	1.0		1.0	
Single	3.10 (0.77, 12.57)	0.1127	45.9 (10.8, 195.3)	**<0.001***
Divorced	9.04 (1.95, 42.02)	**0.0050***	0.0 (0.0, 0.0)	**<0.001***
Tumor grade				
Grade II	1.0		1.0	
Grade III	0.40 (0.08, 1.95)	0.2575	Inf (Inf, Inf)	**<0.001***
Grade I	1.07 (0.22, 5.24)	0.9355	Inf (Inf, Inf)	**<0.001***
Grade IV	0.00 (0.00, Inf)	0.9935	1.4 (0.0, Inf)	1.000
Histological type				
Infiltrating duct	1.0		1.0	
Adenocarcinoma	2.55 (0.31, 21.18)	0.3853	0.0 (0.0, 0.0)	**<0.001***
Other	3.69 (0.76, 18.01)	0.1068	Inf (4,969,507.8, Inf)	**<0.001***
TMN stage				
I	1.0		1.0	
IIA	0.51 (0.05, 5.64)	0.5811	Inf (Inf, Inf)	**<0.001***
IIB	4.95 (0.94, 25.96)	0.0585	1957392.5 (318,862.9, Inf)	**<0.001***
IIIA	5.56 (0.76, 40.72)	0.0915	1.5 (0.1, 16.9)	0.727
IIIC	0.00 (0.00, Inf)	0.9906	1.4 (0.0, Inf)	1.000
IIIB	3.57 (0.31, 40.68)	0.3051	0.3 (0.0, 4.9)	0.404
0	0.00 (0.00, Inf)	0.9983	0.8 (0.0, Inf)	1.000
Surgery performed				
Yes	1.0		1.0	
No	0.00 (0.00, Inf)	0.9931	0.7 (0.0, Inf)	1.000
Radiotherapy performed				
No	1.0		1.0	

(Continued)

TABLE 4 Continued

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Yes	1.14 (0.33, 3.94)	0.8360	0.0 (0.0, 0.0)	**<0.001***
Chemotherapy performed				
No	1.0		1.0	
Yes	1.92 (0.58, 6.37)	0.2856	2.8 (0.5, 15.1)	0.239
Duration to begin treatment				
Less than 1 month	1.0		1.0	
More than 1 month	3.30 (0.71, 15.42)	0.1286	6,062,810.1 (987,643.0, Inf)	**<0.001***
HR status				
Positive	1.0		1.0	
Negative	0.00 (0.00, Inf)	0.9909	0.5 (0.0, Inf)	1.000
HER2 status				
Negative	1.0		1.0	
Positive	0.00 (0.00, Inf)	0.9903	1.0 (0.0, Inf)	1.000

*Statistically significant ($p < 0.05$).

TABLE 5 Risk factors associated with cancer-specific death and occurrence of SPM.

Variables	Cancer-specific death		Occurrence of SPM	
	SHR	*p*-value	SHR	*p*-value
Age group				
65–75	ref		ref	
55–65	1.0137	**<0.0001***	0.7516	**<0.0001***
75–85	2.3679	**<0.0001***	0.7861	**<0.0001***
45–55	1.8132	**<0.0001***	0.8458	**<0.0001***
85+	0.547	**<0.0001***	0.8998	**<0.0001***
<45	6.5540	**<0.0001***	0.9159	**<0.0001***
Race				
White	ref		ref	
Black	1.7756	**<0.0001***	1.0583	**<0.0001***
Other	0.5697	**<0.0001***	0.9682	**<0.0001***
Marital status				
Married	ref		ref	
Single	0.5560	**<0.0001***	0.8393	**<0.0001***
Divorced	0.5677	**<0.0001***	1.0487	**<0.0001***
Tumor grade				
Grade II	ref		ref	
Grade III	1.5597	**<0.0001***	1.0574	**<0.0001***
Grade I	0.1559	**<0.0001***	0.8183	**<0.0001***

(Continued)

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TABLE 5 Continued

Variables	Cancer-specific death		Occurrence of SPM	
	SHR	*p*-value	SHR	*p*-value
Grade IV	0.0153	**<0.0001***	0.6641	**<0.0001***
Histological type				
Infiltrating duct	ref		ref	
Adenocarcinoma	0.0486	**<0.0001***	1.5327	**<0.0001***
Other	0.3203	**<0.0001***	1.0089	**<0.0001***
TMN stage				
Stage 0+I	ref		ref	
Stage II	0.0912	**<0.0001***	0.6253	**<0.0001***
Stage III	4.0035	**<0.0001***	0.6135	**<0.0001***
Surgery performed				
Yes	ref		ref	
No	2.9478	**<0.0001***	1.1238	**<0.0001***
Radiotherapy performed				
No	ref		ref	
Yes	0.6601	**<0.0001***	1.1854	**<0.0001***
Chemotherapy performed				
No	ref		ref	
Yes	1.9436	**<0.0001***	0.9004	**<0.0001***
Duration to begin treatment				
Less than 1 month	ref		ref	
More than 1 month	0.7742	**<0.0001***	0.9765	**<0.0001***
HR status				
Positive	ref		ref	
Negative	0.0939	**<0.0001***	0.9360	**<0.0001***
HER2 status				
Negative	ref		ref	
Positive	1.5371	**<0.0001***	1.1042	**<0.0001***

*Statistically significant ($p < 0.05$).

risk analysis in MBC patients but failed to include treatment information (25). As is shown in the present study, treatments influenced cancer-specific death and the occurrence of SPM. Different clinical circumstances with different treatment strategies might lead to different outcomes.

A few studies estimated the effect of initial treatment on the development of SPM in female breast cancer patients (26, 27), but no research has focused on the development of SPM in MBC survivors. To our knowledge, this is the first available nomogram for developing an SPM in MBC survivors in the presence of competing events.

In this study, 60 survivors developed an SPM. Prostate cancer was the most common SPM. Interestingly, previous research showed that prostate cancer was also the most common SPM in colon cancer survivors treated with colectomy (28). However, prostate cancer had a bigger portion in SPM patients than in the whole population (29). The efficiency of endocrine therapy, along with the high proportion of HR-positive status in MBC patients (17), warrants further study to clarify whether the endocrine status is related to the occurrence of the SPM. It is also worth noting that patients who suffered from an SPM shared a longer OS than those patients with only one MBC (Figure 2, $p = 0.027$), which indicated that the cumulative incidence of developing an SPM increased with the prolonged survival time.

Univariate and multivariate Cox regression analyses were insufficient, and in this study, we applied additional Fine and Gray competing risk analysis to show the differences among the risk factors

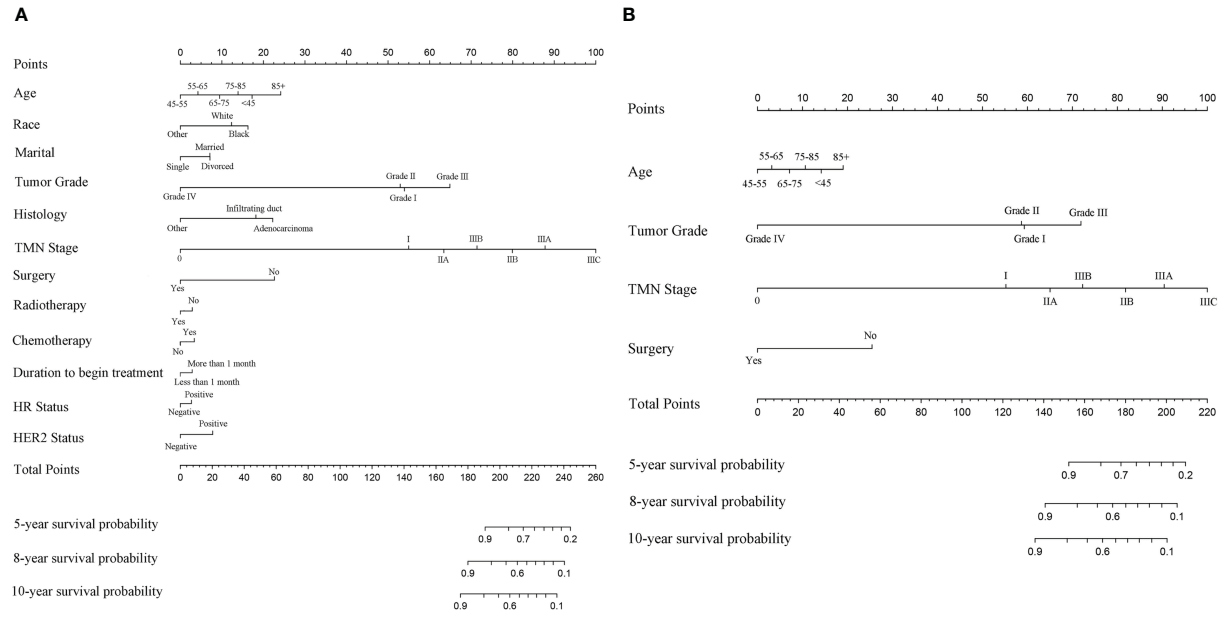

FIGURE 3
(A) The nomogram model 1 to predict the 5-year, 8-year, and 10-year survival probability of MBC patients based on the Fine and Gray method. **(B)** The nomogram model 2 to predict the same survival probability of MBC patients based on the multivariate Cox regression.

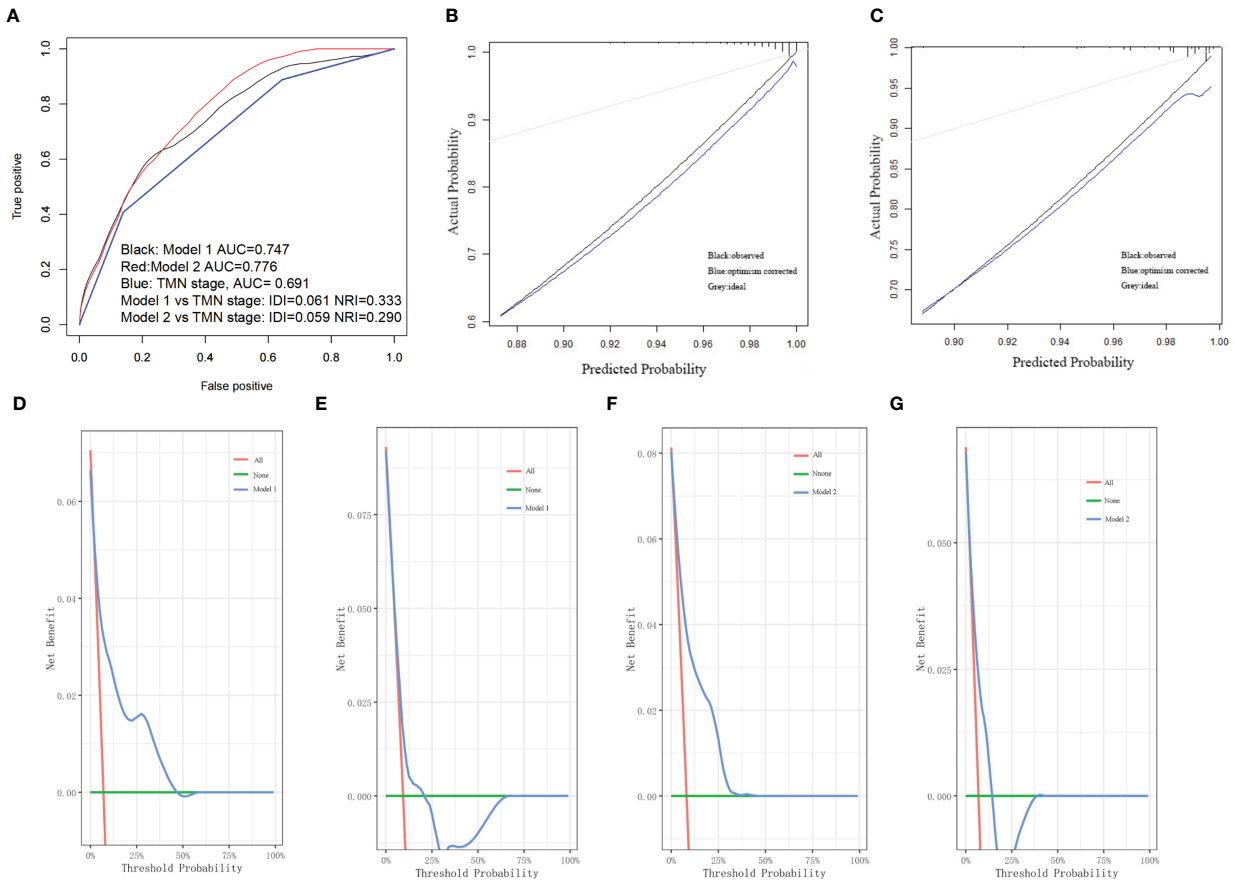

FIGURE 4
(A) Both model 1 and model 2 showed better predictive value than TMN stage in the ROC analyses. **(B)** The calibration curve of model 1. **(C)** The calibration curve of model 2. **(D)** The DCA of model 1 in the development cohort. **(E)** The DCA of model 1 in the validation cohort. **(F)** The DCA of model 2 in the development cohort. **(G)** The DCA of model 2 in the validation cohort.

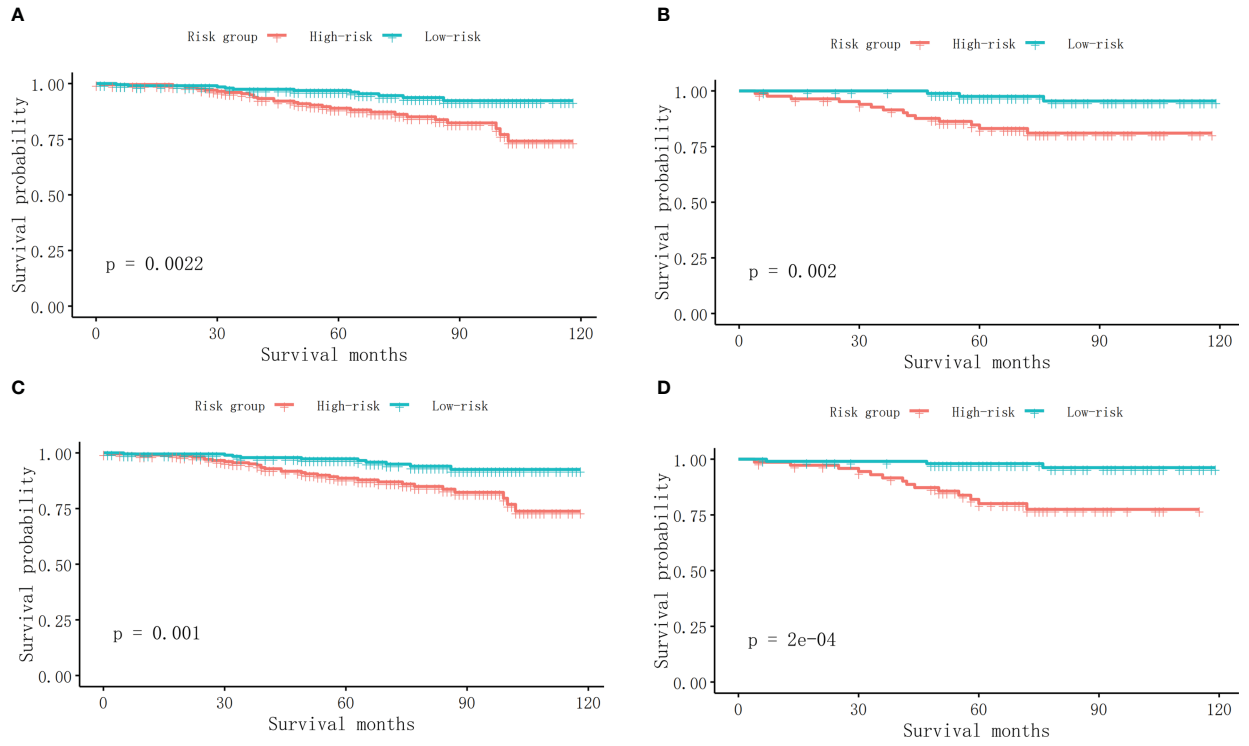

FIGURE 5
(A) The survival curves between different risk groups in the development cohort in model 1. **(B)** The survival curves between different risk groups in the validation cohort in model 1. **(C)** The survival curves between different risk groups in the development cohort in model 2. **(D)** The survival curves between different risk groups in the validation cohort in model 2.

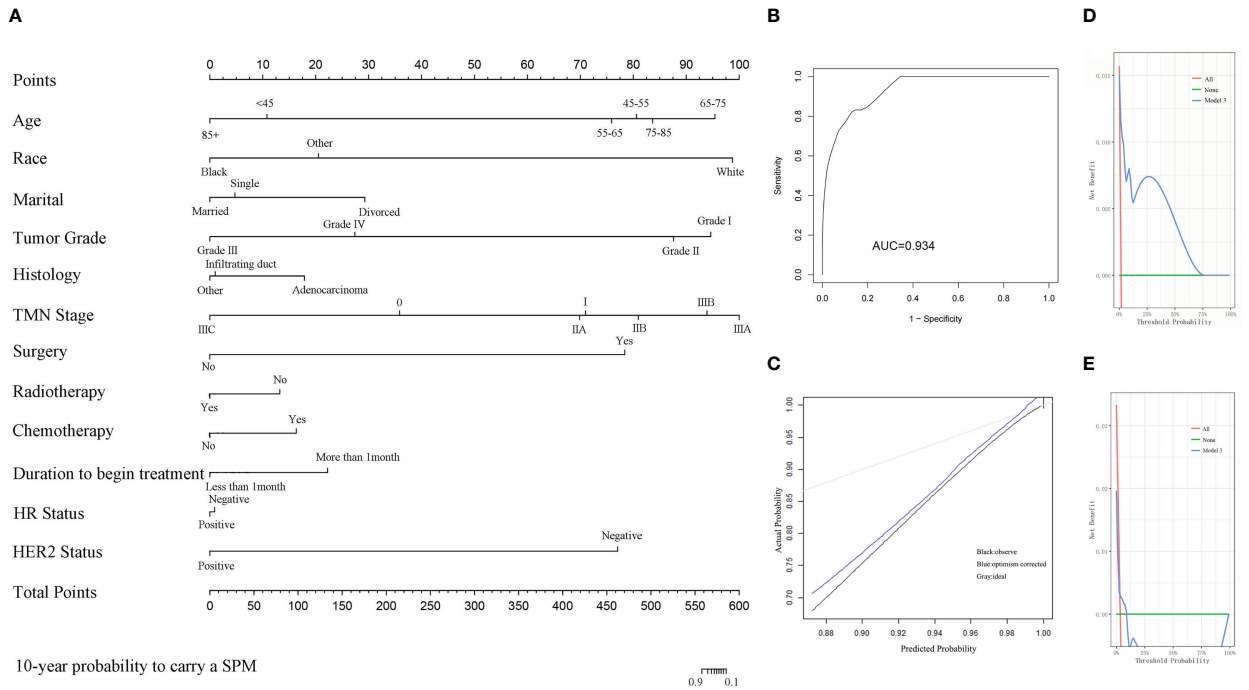

FIGURE 6
(A) The nomogram of model 3 for predicting the 10-year probability of MBC survivors who suffer from an SPM. **(B)** The ROC curve of model 3. **(C)** The calibration curve of model 3. **(D)** The DCA of model 3 in the development cohort. **(E)** The DCA of model 3 in the validation cohort.

associated with OS and the occurrence of SPM. We have constructed two nomogram models to predict the OS of the MBC patients: model 1 based on the risk factors selected by the Fine and Gray method, and model 2 based on the multivariate analysis. Both of these nomogram models achieved good C-index. Model 2 had an even better predictive value than model 1 and the TMN stage in the combined ROC analysis (Figure 4A). The calibration plots, the DCA curves, and the survival curves of different risk groups altogether showed that both of these models had an ideal discrimination capability and predictive value. Model 1 included more clinical details while model 2 was more simplified. According to our study, higher age at diagnosis, higher TMN stage, absence of surgery and radiotherapy, more than 1 month waiting time to begin treatment, and being HR and HER2 positive contributed to a poorer prognosis in MBC patients.

An additional nomogram model 3 was constructed based on the Fine and Gray method to predict the probability of the occurrence of an SPM. Li et al. focused on the SPM on female breast cancer patients and constructed a nomogram to predict the SPM probability of female breast cancer patients (30). A similar study was published by Bao et al. on female breast cancer patients (31). Mellemkjær et al. investigated whether pregnancy near the time of the initial female breast cancer diagnosis would increase the risk of an SPM and obtained a negative result (32). Chen et al. found that germline pathogenic variants in BRCA1, BRCA2, and ERCC2 increased the risk for female breast cancer patients of developing an SPM (33). Nevertheless, no similar research had been published in MBC patients and few studies had focused on the SPM issue in MBC patients. Satram-Hoang et al. found that there is a general tendency towards higher risks of SPM among younger men compared to older men but did not provide a predictive model (34). Hung et al. found that the risk of SPM was significantly higher for both male and female breast cancer patients compared with the general population (35). In this study, we constructed an available nomogram to predict the SPM probability of MBC patients. There were 36 SPM patients in the development cohort and 24 in the validation (Table 1). Nomogram model 3 achieved good performance in the C-index and DCA curve in the development cohort and attained an ordinary score in the validation cohort, which was attributed to the rarity of MBC and the small number of the enrolled SPM patients. However, the present study is still the first research to look into the SPM of MBC patients, and achieved an AUC at 0.934 (Figure 6B), which indicated a good predictive value of the predictive model.

A nomogram had been widely used for the prediction of certain clinical outcomes because it is convenient and reliable. In this study, we, for the first time, constructed competing risk nomograms including both the treatment information and the clinicopathological parameters to predict the prognosis of MBC patients and, for the first time, developed a competing risk nomogram to predict the probability of developing an SPM in MBC patients, which was thought to be helpful for both clinicians and the patients to estimate the risk and manage their strategies about treatment and follow-up.

There are some limitations in our study. First, this study was a population-based retrospective study using the SEER Plus database, which had missed some important variables of some of the patients, leading to more than 1,000 MBC patients being excluded because of the incomplete information. Second, some important risk factors for SPM that were rapidly developing or widely used in clinical practice nowadays, such as diet and lifestyle, family history of cancer, oncogene test, radiotherapy or chemotherapy protocols, and the performance of endocrine therapy, targeted therapy, or immunotherapy, were not included in the SEER Plus database. Additionally, MBC is a rare disease, and the SEER Plus database did not involve a larger population worldwide, which had restricted the scale of the present study and might lead to bias. An additional larger study is needed to determine the mechanism of SPM in MBC patients.

Conclusion

Our study for the first time included the treatment information and clinical parameters needed to construct an external validation competing risk nomogram to predict the survival probability of MBC patients, according to which higher age at diagnosis, higher TMN stage, absence of surgery and radiotherapy, more than 1 month waiting time to begin treatment, and being HR and HER2 positive contributed to a poorer prognosis in MBC patients. This study also, for the first time, constructed a nomogram to predict the probability of developing an SPM in MBC survivors, which was helpful in individual risk estimation, patient follow-up, and counseling in MBC patients.

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 Committee of Guangzhou Red Cross Hospital of Jinan University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

HH, ZL, ZH, and LH performed the study, analyzed the data, prepared figures and/or tables, and authored or reviewed drafts of the paper. WL, GL, and YM conceived and designed the study, performed the study, authored or reviewed drafts of the paper, and approved the final draft. All authors contributed to the article and approved the submitted version.

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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.2023.1076997/full#supplementary-material

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* (2016) 66 (1):7–30. doi: 10.3322/caac.21332

2. Expert Panel on Breast Imaging, Niell BL, Lourenco AP, Moy L, Baron P, Didwania AD, et al. ACR appropriateness criteria(®) evaluation of the symptomatic Male breast. *J Am Coll Radiol* (2018) 15(11s):S313–s320. doi: 10.1016/j.jacr.2018.09.017

3. Chichura A, Attai DJ, Kuchta K, Nicholson K, Kopkash K, Pesce C, et al. Male Breast cancer patient and surgeon experience: The Male WhySurg study. *Ann Surg Oncol* (2022) 29(10):6115–31. doi: 10.1245/s10434-022-12135-6

4. Giordano SH, Cohen DS, Buzdar AU, Perkins G, Hortobagyi GN. Breast carcinoma in men: a population-based study. *Cancer* (2004) 101(1):51–7. doi: 10.1002/cncr.20312

5. Anderson WF, Jatoi I, Tse J, Rosenberg PS. Male Breast cancer: a population-based comparison with female breast cancer. *J Clin Oncol* (2010) 28(2):232–9. doi: 10.1200/JCO.2009.23.8162

6. Kreiter E, Richardson A, Potter J, Yasui Y. Breast cancer: trends in international incidence in men and women. *Br J Cancer* (2014) 110(7):1891–7. doi: 10.1038/bjc.2014.66

7. Fentiman IS. Prognostic difficulties of men with breast cancer. *Breast J* (2021) 27 (12):877–82. doi: 10.1111/tbj.14297

8. Fox S, Speirs V, Shaaban AM. Male Breast cancer: an update. *Virchows Arch* (2022) 480(1):85–93. doi: 10.1007/s00428-021-03190-7

9. Iorfida M, Bagnardi V, Rotmensz N, Munzone E, Bonanni B, Viale G, et al. Outcome of male breast cancer: a matched single-institution series. *Clin Breast Cancer* (2014) 14(5):371–7. doi: 10.1016/j.clbc.2014.02.008

10. Fentiman IS, Fourquet A, Hortobagyi GN. Male Breast cancer. *Lancet* (2006) 367(9510):595–604. doi: 10.1016/S0140-6736(06)68226-3

11. Clarke CN, Cortina CS, Fayanju OM, Dossett LA, Johnston FM, Wong SL. Breast cancer risk and screening in transgender persons: A call for inclusive care. *Ann Surg Oncol* (2022) 29(4):2176–80. doi: 10.1245/s10434-021-10217-5

12. Ewertz M, Holmberg L, Tretli S, Pedersen BV, Kristensen A. Risk factors for male breast cancer–a case-control study from Scandinavia. *Acta Oncol* (2001) 40 (4):467–71. doi: 10.1080/028418601750288181

13. Anderson WF, Devesa SS. Breast carcinoma in men. *Cancer* (2005) 103(2):432–3. doi: 10.1002/cncr.20797

14. Johansen Taber KA, Morisy LR, Osbahr AJ 3rd, Dickinson BD. Male Breast cancer: risk factors, diagnosis, and management (Review). *Oncol Rep* (2010) 24 (5):1115–20. doi: 10.3892/or_00000962

15. Yadav S, Sangaralingham L, Payne SR, Giridhar KV, Hieken TJ, Boughey JC, et al. Surveillance mammography after treatment for male breast cancer. *Breast Cancer Res Treat* (2022) 194(3):693–8. doi: 10.1007/s10549-022-06645-w

16. Healy NA, Parag Y, Wallis MG, Tanner J, Kilburn-Toppin F. Outcomes of male patients attending the symptomatic breast unit: adherence to local and national imaging guidelines and effectiveness of clinical examination and imaging in detecting male breast cancer. *Clin Radiol* (2022) 77(1):e64–74. doi: 10.1016/j.crad.2021.09.018

17. Zattarin E, Ligorio F, Nichetti F, Bianchi G, Capri G, de Braud F. Prolonged benefit from palbociclib plus letrozole in heavily pretreated advanced male breast cancer: case report. *Tumori* (2021) 107(6):Np15–np19. doi: 10.1177/0300891620976981

18. Wood ME, Vogel V, Ng A, Foxhall L, Goodwin P, Travis LB. Second malignant neoplasms: assessment and strategies for risk reduction. *J Clin Oncol* (2012) 30 (30):3734–45. doi: 10.1200/JCO.2012.41.8681

19. Ly D, Forman D, Ferlay J, Brinton LA, Cook MB. An international comparison of male and female breast cancer incidence rates. *Int J Cancer* (2013) 132(8):1918–26. doi: 10.1002/ijc.27841

20. O'Malley CD, Prehn AW, Shema SJ, Glaser SL. Racial/ethnic differences in survival rates in a population-based series of men with breast carcinoma. *Cancer* (2002) 94(11):2836–43. doi: 10.1002/cncr.10521

21. Wang D, Yang L, Yang Y, Chen M, Yang H. Nomogram for predicting distant metastasis of male breast cancer: A SEER population-based study. *Med (Baltimore)* (2022) 101(39):e30978. doi: 10.1097/MD.0000000000030978

22. Chen S, Liu Y, Yang J, Liu Q, You H, Dong Y, et al. Development and validation of a nomogram for predicting survival in Male patients with breast cancer. *Front Oncol* (2019) 9:361. doi: 10.3389/fonc.2019.00361

23. Zhang LP, Lin H, Wang AJ. Development and validation of a nomogram to predict survival for advanced male breast cancer. *Andrologia* (2022) 54(8):e14479. doi: 10.1111/and.14479

24. Li Z, Wu X, Huang H, Xu F, Liang G, Lin C, et al. MTHFR C677T polymorphism and cerebrovascular lesions in elderly patients with CSVD: A correlation analysis. *Front Genet* (2022) 13:987519. doi: 10.3389/fgene.2022.987519

25. Sun W, Cheng M, Zhou H, Huang W, Qiu Z. Nomogram predicting cause-specific mortality in nonmetastatic Male breast cancer: A competing risk analysis. *J Cancer* (2019) 10(3):583–93. doi: 10.7150/jca.28991

26. Molina-Montes E, Requena M, Sánchez-Cantalejo E, Fernández MF, Arroyo-Morales M, Espín J, et al. Risk of second cancers cancer after a first primary breast cancer: a systematic review and meta-analysis. *Gynecol Oncol* (2015) 136(1):158–71. doi: 10.1016/j.ygyno.2014.10.029

27. Rubino C, de Vathaire F, Diallo I, Shamsaldin A, Lê MG. Increased risk of second cancers following breast cancer: role of the initial treatment. *Breast Cancer Res Treat* (2000) 61(3):183–95. doi: 10.1023/A:1006489918700

28. Zhang B, Guo K, Zheng X, Sun L, Shen M, Ruan S. Risk of second primary malignancies in colon cancer patients treated with colectomy. *Front Oncol* (2020) 10:1154. doi: 10.3389/fonc.2020.01154

29. Miller KD, Ortiz AP, Pinheiro PS, Bandi P, Minihan A, Fuchs HE, et al. Cancer statistics for the US Hispanic/Latino population, 2021. *CA Cancer J Clin* (2021) 71 (6):466–87. doi: 10.3322/caac.21695

30. Li D, Weng S, Zhong C, Tang X, Zhu N, Cheng Y, et al. Risk of second primary cancers among long-term survivors of breast cancer. *Front Oncol* (2019) 9:1426. doi: 10.3389/fonc.2019.01426

31. Bao S, Jiang M, Wang X, Hua Y, Zeng T, Yang Y, et al. Nonmetastatic breast cancer patients subsequently developing second primary malignancy: A population-based study. *Cancer Med* (2021) 10(23):8662–72. doi: 10.1002/cam4.4351

32. Mellemkjær L, Eibye S, Albieri V, Kjær SK, Boice JD Jr. Pregnancy-associated cancer and the risk of second primary cancer. *Cancer Causes Control* (2022) 33(1):63–71. doi: 10.1007/s10552-021-01500-7

33. Chen F, Park SL, Wilkens LR, Wan P, Hart SN, Hu C, et al. Genetic risk of second primary cancer in breast cancer survivors: The multiethnic cohort study. *Cancer Res* (2022) 82(18):3201–8. doi: 10.1158/0008-5472.CAN-21-4461

34. Satram-Hoang S, Ziogas A, Anton-Culver H. Risk of second primary cancer in men with breast cancer. *Breast Cancer Res* (2007) 9(1):R10. doi: 10.1186/bcr1643

35. Hung MH, Liu CJ, Teng CJ, Hu YW, Yeh CM, Chen SC, et al. Risk of second non-breast primary cancer in Male and female breast cancer patients: A population-based cohort study. *PloS One* (2016) 11(2):e0148597. doi: 10.1371/journal.pone.0148597

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Survival analysis and prognosis of patients with breast cancer with pleural metastasis

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Background: Breast cancer (BC) is the most common malignant cancer. The prognosis of patients differs according to the location of distant metastasis, with pleura being a common metastatic site in BC. Nonetheless, clinical data of patients with pleural metastasis (PM) as the only distant metastatic site at initial diagnosis of metastatic BC (MBC) are limited.

Patient cohort and methods: The medical records of patients who were hospitalized in Shandong Cancer Hospital between January 1, 2012 and December 31, 2021 were reviewed, and patients eligible for the study were selected. Survival analysis was conducted using Kaplan–Meier (KM) method. Univariate and multivariate Cox proportional-hazards models were used to identify prognostic factors. Finally, based on these selected factors, a nomogram was constructed and validated.

Results: In total, 182 patients were included; 58 (group A), 81 (group B), and 43 (group C) patients presented with only PM, only lung metastasis (LM), and PM combined with LM, respectively. The KM curves revealed no significant difference in overall survival (OS) among the three groups. However, in terms of survival after distant metastasis (M-OS), the difference was significant: patients with only PM exhibited the best prognosis, whereas those with PM combined with LM exhibited the worst prognosis (median M-OS: 65.9, 40.5, and 32.4 months, respectively; $P = 0.0067$). For patients with LM in groups A and C, those with malignant pleural effusion (MPE) exhibited significantly worse M-OS than those without MPE. Univariate and multivariate analyses indicated that primary cancer site, T stage, N stage, location of PM, and MPE were independent prognostic factors for patients with PM without other distant metastasis. A nomogram prediction model incorporating these variables was created. According to the C-index (0.776), the AUC values of the 3-, 5-, and 8-year M-OS (0.86, 0.86, and 0.90, respectively), and calibration curves, the predicted and actual M-OS were in good agreement.

Conclusion: BC patients with PM only at the first diagnosis of MBC exhibited a better prognosis than those with LM only or PM combined with LM. We identified

five independent prognostic factors associated with M-OS in this subset of patients, and a nomogram model with good predictive efficacy was established.

KEYWORDS

breast cancer, metastatic breast cancer, first metastatic site, pleural metastasis, survival, prognostic factors, nomogram model

1 Introduction

Breast cancer (BC) is the cancer with the highest prevalence worldwide (1) and the leading cause of cancer-related deaths among females (2). BC has a tendency for distant metastasis (3), and the majority of BC-related deaths are due to metastasis (4). BC exhibits heterogeneity in metastasis and prognosis (1). Even though patients with distant metastases are all defined as MBC (5), different sites of metastasis have variable impacts on clinical outcomes (6), and the prognosis varies greatly. The metastatic sites should be taken into consideration when assessing prognosis and making therapeutic strategies for patients with MBC (5).

The lung and pleura are among the most common metastatic sites of BC (7). Cummings MC et al. performed an autopsy examination of women who died of BC and found that the most common organs involved were lung/pleura, followed by bone, liver and non-axillary lymph nodes (8). The lung is generally accepted as one of the primary target visceral organs of BC metastasis (1), and is anatomically related to pleura. Lung metastasis (LM) is the most common accompanied organ metastasis site of pleural metastasis (PM) in BC. Some studies on MBC did not distinguish between LM and PM (8–11). Thus when interpreting these data, it must be noted that LM are referred to as either including or excluding PM (12). The site of first distant metastasis correlates with the survival of patients with BC (13, 14). And lung metastasis (LM) is of particular attention because of its high morbidity and association with high mortality of patients (15). PM usually manifested as pleural nodulations or pleural thickening (16), with or without malignant pleural effusion (MPE) (17). However, PM often goes unnoticed until the appearance of MPE. There is little evidence regarding the prognosis of patients with MBC when pleura is the first recurrence site. In the present study, we wanted to explore the prognostic differences between pleural and lung when serving as the first site of distant metastasis after radical surgery for primary BC, which could help to supplement the vacancy of current data.

MPE is a common manifestation of PM (18) and a frequent complication during the course of MBC (19). Approximately 11% of the patients with BC eventually present with symptomatic pleural effusions; at autopsy, 36%–65% of patients retrospectively suffered from this condition (20, 21). Although MPE is rarely the initial manifestation of cancer (20), it carries a significant symptom burden (22) and is considered to be associated with a dismal prognosis (23). However, not all PM was accompanied by MPE (24), especially when the initial diagnosis of MBC. Therefore, we hypothesized that among BC patients with PM, the presence or absence of MPE would lead to different prognoses. The diversity in prognosis of BC patients is caused by the combined effect of multiple pathological factors. An understanding of prognostic factors is imperative for individualization of prognosis in patients with BC. However, the prognostic factors in BC patients with PM are unclear, particularly when no other distant metastases exist. In addition, the prognosis plays a central role for patients with BC and oncologists to choose optimally treatment in this era of individualized therapy (25). Therefore, an accurate prediction model is needed for this subset of patients.

Over the past few years, nomograms have been widely recognized as a predictive method for several diseases, including BC (26). Nomograms can generate an individual probability of a clinical event by integrating diverse determinant variables and meet the requirement for biologically and clinically integrated models (27). Evidence-based guidelines suggest using conservative treatments in patients with limited life expectancy, whereas they suggest offering more aggressive treatment modalities for patients with better prognosis. Real-world data can inform the outcome comparisons (28). Our study aimed to investigate whether patients with BC in whom PM was the primary event of recurrence exhibit a prognosis different than those with LM. Furthermore, we explored the prognostic factors and created a nomogram model, which can aid physicians in better evaluating the patient's prognosis and selecting patients for different treatment tactics.

2 Patients and methods

2.1 Study population and variables

This was a single-center retrospective cohort study. Patients with MBC confirmed by pathology who were consecutively hospitalized at Shandong Cancer Hospital between January 1, 2012 and December 31, 2021, were included in this study. The inclusion criteria according to primary BC were as follows: (1) female patients who had undergone radical surgery for BC; (2) T stage was 1–3; and (3) unilateral BC. Patients with other malignancies or diseases that severely affected the patient's survival and prognosis were eliminated. These diseases include acute myocardial infarction/congestive heart failure, acute

Abbreviations: BC, breast cancer; MBC, Metastatic BC; PM, pleural metastasis; MPE, malignant pleural effusion; LM, lung metastasis; M-OS, survival after diagnosis of distant metastasis.

cerebrovascular disease, chronic obstructive pulmonary disease, irreversible severe renal/hepatic impairment (such as severe hepatitis, cirrhosis…), serious mental illness, diabetes mellitus with severe complications. Tracing patient's clinical data, and the metastatic sites at the first diagnosis of MBC after surgery were determined. Only those with PM or LM at first MBC diagnosis were further screened, 182 patients were finally included in this study and pertinent data were updated retrospectively using current tumor classification criteria. The Flow chart of patient selection was shown in Figure 1.

The diagnosis of PM was based on pleural biopsy results, imaging, pleural fluid analysis, and medical thoracoscopy. Cancer staging of the primary cancer was based on the TNM staging system by the 8th American Joint Committee on Cancer (AJCC). HER2 was determined locally by IHC/FISH and determined positive by 3+ staining or FISH positivity (29). Cancers with estrogen receptor-positive (ER+) and/or progesterone receptor-positive (PR+) were considered hormone receptor-positive (HR+), while ER-negative (ER-) and PR-negative (PR-) were considered HR-negative (HR-). Distant metastasis-free interval was defined as the period after radical surgery till the first diagnosis of MBC. Medical attention due to symptoms refers to the diagnosis of MBC was because of symptoms such as chest pain, dyspnea and thoracic pressure, rather than regular follow-up examinations. OS and survival time after distant metastasis (M-OS) were defined as the time from the diagnosis of BC or distant metastasis to death, respectively. The follow-up cut-off was July 31, 2022. If the patient was alive at the last censored follow-up, we considered her to have not reached the study endpoint. Our study was approved by the Shandong Cancer Hospital Ethical Committee.

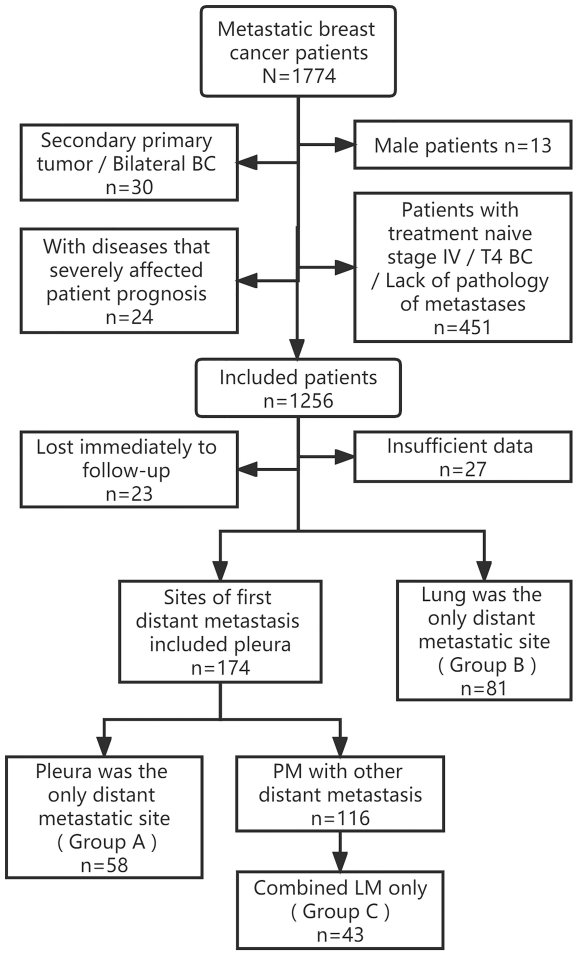

FIGURE 1
Flow chart of patient selection. BC, Breast cancer; PM, Pleural metastasis; LM, Lung metastasis.

2.2 Statistical analysis and model construction

Chi-square tests were used to compare the clinicopathological characteristics among groups. Comparisons of continuous variables were performed using ANOVA. The Kaplan–Meier (KM) method was used to calculate the survival end-points (OS and M-OS), and the log-rank test was conducted to assess the differences among subgroups. The factors independently associated with M-OS of patients with PM were assessed using univariate and multivariate Cox regression analyses, and hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. A two-sided P value < 0.05 was considered significant. A prediction nomogram based on the results of multivariate logistic regression analysis was developed using the "rms" package. The concordance index (C-index) was generated to measure the predictive accuracy and discrimination capabilities. Receiver operating characteristic (ROC) curves were depicted and the predictive accuracy was examined with the area under the curve (AUC). A calibration curve was plotted to test the association between the expected probabilities and observed outcome frequencies.

3 Results

3.1 Patient baseline

We obtained the clinical data of these 182 patients and followed them up. PM was the primary event at first MBC diagnosis in 58 (31.9%) patients (group A), 81 (44.5%) patients (group B) had LM without other distant metastases, and 43 (23.6%) patients had LM and PM (group C). The baseline features of these individuals according to metastatic sites are given in Table 1. The median age of patients at initial BC diagnosis was 42 years (range, 23–71 years) and most patients were premenopausal (73.1%). In terms of therapy, the majority of patients (91.2%) did not receive neoadjuvant chemotherapy and 151 (83.0%) patients underwent a mastectomy. Most patients were at histopathological grade II (58.2%) or T2 stage (56.6%). Moreover, 23.1% of patients were at the N0 stage, 36.8% at N1, 20.3% at N2, and 19.8% at N3. Luminal B was the most common molecular subtype (33.5%), followed by luminal A (26.9%), triple-negative (23.6%), and HER-2-enriched type (16.0%). After surgery, 180 (98.9%) patients received chemotherapy and 79 (43.4%) received radiotherapy. There was no significant difference in the distribution of the described variables among the three groups (Table 1).

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TABLE 1 Baseline characteristics of the entire cohort.

Characteristic	Total (N=182)	Group A (N=58)	Group B (N=81)	Group C (N=43)	P Value
Age at BC diagnosis (years)					0.154
Median (range)	42 (23-71)	43 (26-71)	41 (28-65)	44 (23-70)	
Mean (SD)	44 (9.4)	46 (10.3)	43 (8.2)	42 (10.0)	
Menstrual status					0.820
premenopause	133 (73.1)	42 (72.4)	58 (71.6)	33 (76.7)	
menopause	49 (26.9)	16 (27.6)	23 (28.4)	10 (23.3)	
Neoadjuvant Chemotherapy					0.862
Received	16 (8.8)	5 (8.6)	8 (9.9)	3 (7.0)	
Not received	166 (91.2)	53 (91.4)	73 (90.1)	40 (93.0)	
Surgery type					0.812
Mastectomy	151 (83.0)	48 (82.8)	66 (81.5)	37 (86.0)	
Lumpectomy	31 (17.0)	10 (17.2)	15 (18.5)	6 (14.0)	
Molecular Subtype					0.539
Luminal A	49 (26.9)	16 (27.6)	21 (25.9)	12 (27.9)	
Luminal B	61 (33.5)	23 (39.7)	23 (28.4)	15 (34.9)	
Triple negative	43 (23.6)	9 (15.5)	25 (30.9)	9 (20.9)	
HER2 enriched	29 (16.0)	10 (17.2)	12 (14.8)	7 (16.3)	
Histopathological grading					0.863
I	24 (13.2)	7 (12.1)	11 (13.6)	6 (14.0)	
II	106 (58.2)	35 (60.3)	44 (54.3)	27 (62.8)	
III	52 (28.6)	16 (27.6)	26 (32.1)	10 (23.3)	
T category					0.549
T1	61 (33.5)	15 (25.9)	29 (35.8)	17 (39.5)	
T2	103 (56.6)	38 (65.5)	43 (53.1)	22 (51.2)	
T3	18 (9.9)	5 (8.6)	9 (11.1)	4 (9.3)	
N category					0.821
N0	42 (23.1)	14 (24.1)	15 (18.5)	13 (30.2)	
N1	67 (36.8)	19 (32.8)	33 (40.7)	15 (34.9)	
N2	37 (20.3)	13 (22.4)	16 (19.8)	8 (18.6)	
N3	36 (19.8)	12 (20.7)	17 (21.0)	7 (16.3)	
Adjuvant Chemotherapy					0.238
Done	180 (98.9)	58 (100)	79 (97.5)	43 (100)	
Not done	2 (1.1)	0 (0)	2 (2.5)	0 (0)	
Radiotherapy					0.956
Done	79 (43.4)	26 (44.8)	35 (43.2)	18 (41.9)	
Not done	103 (56.6)	32 (55.2)	46 (56.8)	25 (58.1)	

Group A, PM without other distant metastases; Group B, LM without other distant metastases; Group C, LM and PM without other distant metastases. Data are presented as No. (%) or median (range), unless otherwise indicated. SD, standard deviation.

3.2 Survival analysis

At the end of the follow-up period, 158 (86.8%) patients died. Meanwhile, 13 (22.4%), 7 (8.6%), and 4 (9.3%) patients were alive in groups A, B, and C, respectively. The 3-, 5-, and 8-year cumulative M-OS rates of patients in groups A, B, and C, respectively, were 79.3%, 61.7%, and 48.8%; 53.4%, 23.5%, and 30.2%; and 20.7%, 12.3%, and 4.6%. The prognosis of patients with only PM (group A) was significantly better than that of patients with only LM (group B) or LM with PM (group C) in terms of M-OS (median M-OS: 65.9 vs. 40.5 vs. 32.4 months, P = 0.0067; Figure 2A); however, the difference in their OS was not significant (median OS: 119.8 vs. 111.2 vs. 108.2 months, P = 0.3638; Figure 2B). The M-OS was significantly prolonged in group A compared with that in groups B (median M-OS: 65.9 vs 40.5 months, P = 0.0060; Supplemental Figure 1A) or C (median M-OS: 65.9 vs 32.4 months, P = 0.0077; Supplemental Figure 1B). There was no significant difference in M-OS between groups B and C (median M-OS: 40.5 vs 32.4 months, P = 0.3789; Supplemental Figure 1C). Additionally, there was no significant difference in OS between groups A and B, groups A and C, and groups B and C (median OS: 119.8 vs. 111.2 months, P = 0.1223; 119.8 vs. 108.2 months, P = 0.5760; 111.2 vs. 108.2 months, P = 0.6102, respectively; Supplemental Figures 1D–F).

Given the high incidence of MPE in patients with PM (81.0% in group A and 76.7% in group C), we compared the M-OS between patients with and without MPE within the two groups. We observed a significant difference in M-OS (median M-OS: 55.4 vs. 89.3 months in group A, P = 0.0035; 24.8 vs. 64.0 months in group C, P = 0.0241) between patients with and without MPE (Figures 3A, B).

3.3 Characteristics of PM patients without other distant metastasis

Then, we analyzed the clinicopathological features of PM patients without other distant metastasis. Overall, 35 of the 58 patients (60.3%) were < 45 years at initial BC diagnosis. A total of 48.3% and 51.7% of the cancers were lateralized to the left and right, respectively, and most were located in the inner quadrant of the breast (46.6%). As for local treatment, mastectomy was performed in 82.8% of patients. At initial BC diagnosis, these patients with a high proportion of AJCC stage III, T2 stage, and pathohistological grade II, corresponding to 48.3%, 65.5%, and 60.3%, respectively. The majority of cancers were HR-positive and HER2-negative (HR+HER2-) (67.2%), with the highest frequency in the luminal B subtype (39.7%). Overall, 55.2%, 77.6%, and 15.5% of patients received radiotherapy, endocrine therapy, and

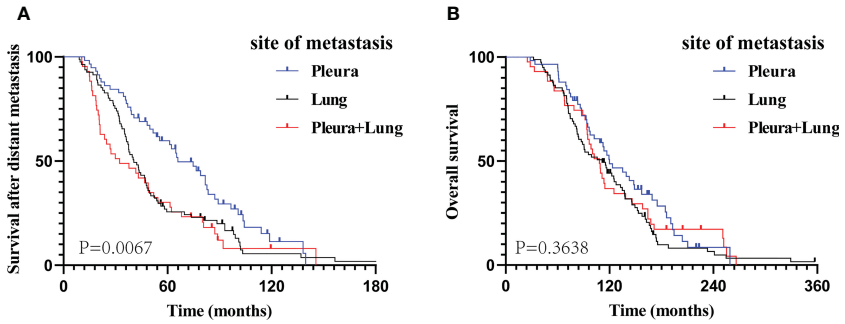

FIGURE 2
The Kaplan–Meier curve analysis of study cohorts. M-OS **(A)** and OS **(B)** curves according to different metastatic sites at the time of diagnosed of metastatic breast cancer. M-OS, survival after distant metastasis; OS, overall survival.

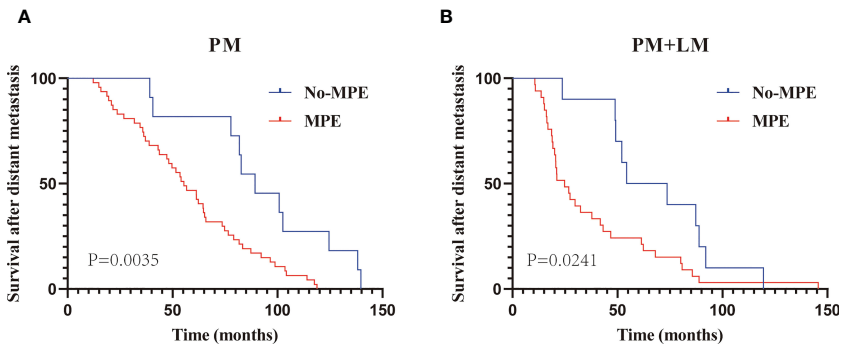

FIGURE 3
The Kaplan–Meier curve analysis of M-OS in PM with or without MPE. Patients with only PM **(A)**; patients with PM and LM **(B)**. PM, Pleural metastasis; MPE, Malignant pleural effusion; LM, Lung metastasis.

anti-HER2 therapy, respectively. In total, 40 (69.0%) patients were diagnosed with distant metastasis within 5 years of radical surgery. Overall, medical attention due to symptoms was recorded in 17 (29.3%) patients, and 14 (24.1%) patients had a chest wall recurrence. Most PM (79.3%) were located ipsilateral to the primary BC, and 47 (81.0%) patients presented with MPE. Supraclavicular lymph node metastasis was observed in 20 (34.5%) patients (6 patients were identified at the time of surgery for the primary BC, and 14 were diagnosed concomitantly with the PM). Detailed patient characteristics are given in Table 2.

TABLE 2 Characteristics of patients with PM as the only site of distant metastasis at first MBC diagnosis.

Variable	Number	Percent
Age at initial BC diagnosis (years)		
<45	35	60.3%
≥45	23	39.7%
Laterality		
Left	28	48.3%
Right	30	51.7%
Primary tumor site		
Outer quadrant	18	31.0%
Inner quadrant	27	46.6%
The areolar region/central axis	13	22.4%
Surgery type		
Mastectomy	48	82.8%
Lumpectomy	10	17.2%
Two diameter ratio		
<1.4	28	48.3%
≥1.4	30	51.7%
AJCC stage at initial BC diagnosis		
I	7	12.1%
II	23	39.6%
III	28	48.3%
T category of primary BC		
T1	15	25.9%
T2	38	65.5%
T3	5	8.6%
N category of primary BC		
N0	14	24.1%
N1	19	32.8%
N2	13	22.4%
N3	12	20.7%
Histopathological Grade of primary BC		
I	7	12.1%
II	35	60.3%
III	16	27.6%
Molecular Subtype of metastases		
Luminal A	16	27.6%
Luminal B	23	39.7%
Triple negative	9	15.5%
HER2 enriched (HR +/HR -)	10	17.2%
Subtype		
HR+Her2+	6	10.3%
HR+Her2-	39	67.2%
HR-Her2+	4	6.9%
HR-Her2-	9	15.5%
ER		
Negative	13	22.4%
Positive	45	77.6%
PR		
Negative	19	32.8%
Positive	39	67.2%
Ki-67		
≤20%	36	62.1%
>20%	22	37.9%
Radiotherapy		
Received	32	55.2%
Not received	26	44.8%
Endocrine therapy		
Received	45	77.6%
Not received	13	22.4%
Anti-HER2 therapy		
Received	9	15.5%
Not received	49	84.5%
Distant metastasis free interval		
≤ 5 yrs.	40	69.0%
> 5 yrs.	18	31.0%
Chest wall recurrence		
Yes	14	24.1%
No	44	75.9%

(Continued)

(Continued)

TABLE 2 Continued

Variable	Number	Percent
Age at diagnosis of MBC (years)		
<50	29	50.0%
≥50	29	50.0%
Female hormone levels		
Premenopausal status	27	46.6%
Menopausal status	31	53.4%
hether the patient came to medical attention because of symptoms		
Yes	17	29.3%
No	41	70.7%
Location of PM		
Ipsilateral	46	79.3%
Contralateral/Bilateral	12	20.7%
MPE		
Yes	47	81.0%
No	11	19.0%
Supraclavicular lymph node metastasis		
Yes	20	34.5%
No	38	65.5%

BC, breast cancer; PM, pleural metastasis; MPE, malignant pleural effusion; MBC, metastatic breast cancer; HR+, hormone receptor-positive; HR-, hormone receptor-negative.

3.4 Screening of prognostic variables

The prognostic factors of patients with only PM at first MBC diagnosis assessed using Cox regression analyses are presented in Table 3. It is worth mentioning that the AJCC stage, to some extent, corresponds to the T and N stage categorization. Thus, to avoid repetition, only T and N stage classifications were included in our univariate analysis. Six variables (primary cancer site, T stage, N stage, molecular subtype, location of PM, and MPE) that were significantly associated with M-OS ($P < 0.05$) in univariate analysis were further included in the multi-factor Cox regression model. Based on the multivariate analysis, we ultimately ascertained that primary cancer in inner quadrant (vs. outer quadrant; HR: 3.65; 95% CI: 1.52–8.79; P = 0.004), T2/3 stage (vs. T1 stage; HR: 2.68; 95% CI: 1.11–6.43; P = 0.028), N3 stage (vs. N0 stage; HR: 5.30; 95% CI: 1.40–19.99; P = 0.014), PM located contralateral/bilateral to the primary BC (vs. ipsilateral; HR: 3.41; 95% CI: 1.42–8.19; P = 0.006), and MPE (vs. without MPE; HR: 4.42; 95% CI = 1.39–14.05; P = 0.012) were significantly correlated with poor M-OS of patients with PM (Table 3). Additionally, the KM curves confirmed the above statistical findings. Patients whose primary cancer was located in the inner quadrant were more likely to survive for a shorter time than those whose primary cancer was located in the outer quadrant (P = 0.0160; Figure 4A). Survival rates declined with high T stage (T2/3 vs. T1 stage, P = 0.0031; Figure 4B) and N stage (N3 vs. N0 stage, P = 0.0024; Figure 4C). Patients whose PM was located ipsilateral to the primary BC and without MPE tended to have a high survival probability (location of PM: P = 0.0287, Figure 4D; MPE: P = 0.0035, Figure 3A). In summary, primary cancer site, T stage, N stage, location of PM, and MPE were significant factors that were associated with M-OS.

3.5 Construction and validation of a 3-, 5-, and 8-year M-OS predicting nomogram

The screened five factors were used to develop a nomogram for patients with only PM at first MBC diagnosis (Figure 5A), and all the predictors were integrated to predict the 3-, 5-, and 8-year M-OS of patients. The nomogram exhibited favorable accuracy in predicting the M-OS with a C-index of 0.776 (95% CI = 0.740–0.812). The above outcomes corresponded with the ROC curves and AUC values (Figure 5B). The AUC values of 3-, 5-, and 8-year M-OS were 0.86, 0.86, and 0.90, respectively, which were > 0.70, indicating that the constructed nomogram has good predictive efficiency for M-OS. The calibration curves revealed that the predictive outcomes were in good accordance with the actual 3-, 5-, and 8-year M-OS (Figures 5C–E).

4 Discussion

Tumor metastasis contributes to high cancer mortality (30), BC has variable aggressiveness and a high propensity to develop distant metastases (31). Extensive studies have proven that BC exhibits metastatic heterogeneity with distinct metastatic precedence to various organs, leading to differences in responses to therapy and prognoses (1). Recent studies have revealed that BC subtypes differ not only in primary tumor characteristics but also in their metastatic behavior (32). In our study, although there was no significant difference in the molecular subtype of primary cancer among the three groups, PM, LM, and PM combined with LM mainly originated from luminal B (39.7%), triple negative (30.9%), and luminal B (34.9%) types, respectively.

The first site of distant metastasis is associated with the prognosis of BC patients (6). Although pleura is a common metastatic site of BC (7), PM has rarely been reported as the first metastatic site in patients with BC. The proportion of such patients may be underestimated because of the time lag in follow-up examinations or the lack of accurate and effective means of examination. In our research, 29.3% of patients did not visit the hospital until presenting with symptoms related to PM. The prognosis of BC patients with single-site metastasis was significantly better than that of patients with multiple metastatic sites (33). In addition, the presence of visceral metastases has a significant negative prognostic impact on patients (28). Schröder J et al. revealed that patients with bone-only metastasis showed better survival than visceral with or without bone metastases (34). Our results indicated that the prognosis of patients with PM not complicated by other distant sites is indeed better than that of patients combined with LM or whose lung serve as the single distant metastatic site. Despite no significant advantage was observed in M-

TABLE 3 Cox analysis of prognostic factors in patients with PM as the only site of distant metastasis at first diagnosis of MBC.

Variable	Univariable analysis		Multivariable analysis	
	HR* (95% CI)	p value	HR *(95% CI)	p value
Age at initial BC diagnosis (yrs.) (≥45 vs. <45)	1.47 (0.79-2.74)	0.223	–	–
Primary tumor site (vs. Outer quadrant)				0.014
Inner quadrant	2.59 (1.18-5.65)	0.017	3.65 (1.52-8.79)	0.004
The areolar region/central axis	3.35 (1.35-8.29)	0.009	2.07 (0.70-6.13)	0.189
Surgery (Breast-conserving surgery vs. Mastectomy)	0.76 (0.35-1.66)	0.489	–	–
Two diameter ratio (≥1.4 vs. <1.4)	1.42 (0.78-2.61)	0.254	–	–
T Stage of primary BC (T2/3 vs. T1)	2.94 (1.40-6.20)	0.005	2.68 (1.11-6.43)	0.028
N Stage of primary BC (vs. N0)				0.078
N1	2.49 (1.06-5.88)	0.037	1.82 (0.58-5.76)	0.307
N2	4.35 (1.67-11.36)	0.003	3.14 (0.86-11.49)	0.084
N3	4.02 (1.65-9.82)	0.002	5.30 (1.40-19.99)	0.014
Histopathological Grade of primary BC (vs. I)				
II	1.60 (0.61-4.22)	0.342	–	–
III	2.24 (0.78-6.43)	0.134	–	–
Molecular Subtype of metastases (vs. Luminal A)				0.644
Luminal B	3.86 (1.74-8.58)	0.001	1.50 (0.52-4.32)	0.449
HER2 enriched (HR +/HR -)	1.68 (0.63-4.45)	0.300	1.05 (0.30-3.68)	0.938
Triple negative	2.25 (0.78-6.50)	0.134	0.75 (0.24-2.38)	0.625
ER (P vs. N)	1.51 (0.67-3.40)	0.323	–	–
PR (P vs. N)	0.99 (0.51-1.95)	0.990	–	–
HER-2 (P vs. N)	1.17 (0.48-2.81)	0.732	–	–
Radiotherapy (Not done vs. Done)	1.24 (0.68-2.28)	0.484	–	–
Distant metastasis free interval (> 5 yrs. vs. ≤ 5 yrs.)	0.56 (0.28-1.11)	0.098	–	–
Chest wall recurrence (Yes vs. ≤ No)	0.91 (0.45-1.84)	0.784	–	–
Age at diagnosis of MBC (yrs.) (≥50 vs. <50)	1.10 (0.60-2.02)	0.749	–	–
Female hormone levels (Menopausal status vs. Premenopausal status)	1.21 (0.66-2.21)	0.537	–	–
Whether the patient came to medical attention because of symptoms (Yes vs. No)	1.61 (0.82-3.14)	0.166	–	–
Location of PM (Contralateral/Bilateral vs. Ipsilateral)	2.15 (1.07-4.33)	0.033	3.41 (1.42-8.19)	0.006
MPE (Yes vs. No)	3.01 (1.24-7.28)	0.015	4.42 (1.39-14.05)	0.012
Supraclavicular lymph node metastasis (Yes vs. No)	1.55 (0.80-2.99)	0.192	–	–

-, negative; HR*, hazard ratio; BC, breast cancer; MBC, metastatic breast cancer; HR+, hormone receptor-positive; HR-, hormone receptor-negative; PM, pleural metastasis; MPE, malignant pleural effusion.

OS for patients with only LM compared with patients with combined PM, the survival rates at 3-, 5- years were all superior. Despite improvements in treatment, MBC has a poor prognosis and an overall 5-year survival rate of only 27% for patients in the United States (35). However, LM has a relatively good prognosis in visceral metastasis as the first distant metastasis of BC (6, 13). Redig AJ et al. tested the relationship between site of metastasis and outcome, and the best prognosis was observed among patients with lung as first anatomic site of distant metastasis, followed by those with first metastatic involvement of bone, liver and central nervous system (6). Combined the existing data, it might be inferred that PM has a better prognosis than visceral metastasis. However, further validation with clinical data is required and the underlying mechanism should be elucidated.

PM most commonly originates from metastatic lung carcinomas and breast carcinomas (36); the mechanisms include hematogenous spread, direct invasion from a neighboring cancer, and retrograde lymphatic spread from the mediastinum (37). Breast

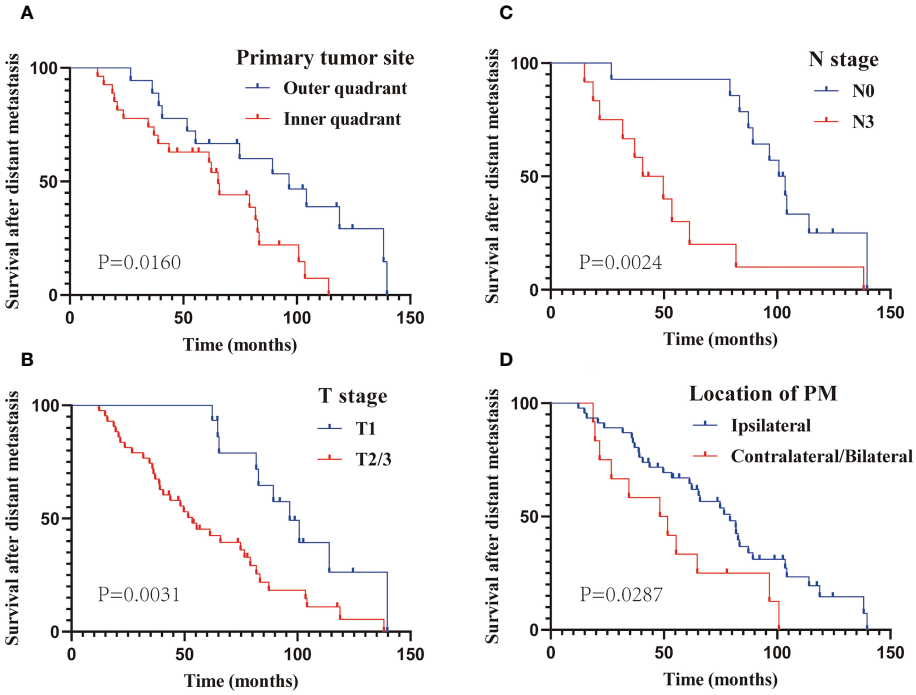

FIGURE 4
The Kaplan–Meier curve analysis of M-OS in subgroups based on multivariate analysis. Subgroup of primary site **(A)**; T stage **(B)**; N stage **(C)**; location of PM **(D)**. PM, Pleural metastasis.

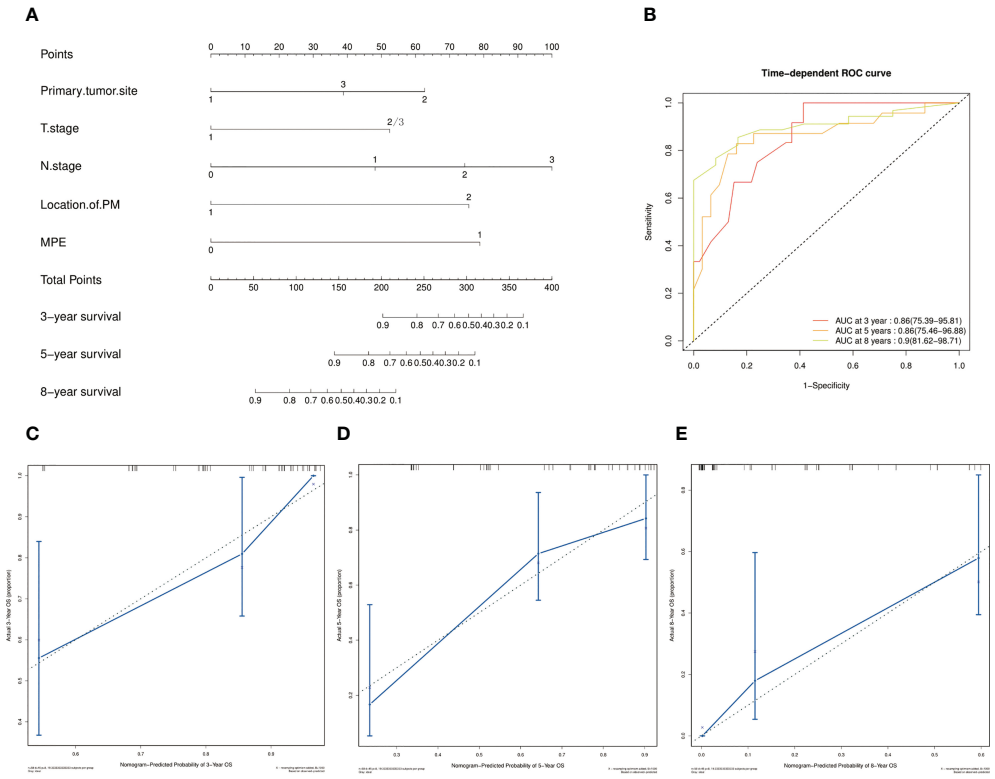

FIGURE 5
Prognostic nomograms of 3-, 5-, and 8-year M-OS in patients with only PM at first MBC diagnosis. Points are defined based on the prognostic contribution of the factors. Points summing the contribution of Primary tumor site, T Stage, N Stage, Location of PM, and MPE are translated to the survival probability at 3, 5 and 8 years **(A)**; ROC curve with AUC for 3-, 5-, and 8-year M-OS rate in patients with solitary PM at first MBC diagnosis **(B)**; Calibration curves of the nomogram for 3-, 5-, and 8-year M-OS prediction **(C–E)**.

carcinoma is the most common metastatic malignancy identified in pleural effusion specimens from women (38). PM is often accompanied by MPE (39), but not all tumors metastasizing to the pleura cause MPE (40). In our results, the incidence of MPE in patients with PM (81.0% in group A and 76.7% in group C) was high. On the one hand, MPE is consider an unfavorable complication that restricts life quality (41) and related to poor prognosis (42). Consistently, our study reported that in BC, MPE was an independent risk factor for patients with PM. On the other hand, Poe RH et al. reported that the median survival of BC patients in whom MPE was the initial and only recurrent site was 48 months, compared with 12 months for patients associated with other metastatic diseases (43). The MPE is more commonly unilateral and ipsilateral to the primary BC (18), Poe RH et al. suggested that this indicated that MPE was a regional rather than systemic disease, probably accounting for the better outlook in patients with effusion alone (43). Similarly, our data showed that the majority of initial PM was located ipsilateral and had a better prognosis. PM located contralateral/bilateral to the primary BC is a factor that worsens the prognosis. Differently, the patients with MPE without other distant metastases at the initial diagnosis of MBC exhibited a better prognosis compared with LM patients with or without PM, but without significant difference (Supplemental Figure 2). Whereas the M-OS of PM patients without MPE was substantially longer (median M-OS:89.3 months in group A; 64.0 months in group C). Thomas et al. (44) speculated that in BC, the laterality of PM is because of lymphatic dissemination. Similarly, Agalioti T et al. (39) stated that BC may invade the pleura because of local proximity rather than through the bloodstream. This may be one of the reasons for its better prognosis than other distant metastases. Moreover, pleura is of itself innocuous and once thought to be biologically inert (45). Oncogene signals and/or transcription factor activation in tumor cells determine paracrine gene expression. The balance between vasoactive mediators and possible protective molecules in the pleural space dictates the occurrence of vasoactive signaling with subsequent MPE development. In turn, this signal cocktail exert a multitude of effects on tumor cells (46). To some extent, tumor colonization of the pleura but not causing MPE may be a manifestation of its poor malignant biological behavior. This is also reflected by other clinical features of these patients. Patients with ipsilateral PM without MPE as their only evidence of distant metastasis may could to be staged as limited disease. However, our data are limited and potentially biased. More clinical data and the specific mechanism investigation are needed in the future for further elucidation.

Our study classified patients into three groups according to primary cancer location: outer quadrant, inner quadrant, and areolar area/central axis. Pokieser W et al. (47) reported that invasive ductal carcinomas located in the inner quadrants were significantly associated with increased pleural effusion as the first site of metastasis, which may be associated with a higher rate of internal mammary lymph node metastasis. Similarly, our study reported that 46.6% of patients had primary cancers located in the inner quadrant. Furthermore, our results indicated that primary cancer location in the inner quadrant is a poor prognostic factor for patients. Some studies demonstrated that BC situated in inner quadrants have a worse prognosis (48–51), which may cause by the anatomical accessibility of the tumor to the internal mammary lymph node (49, 52). Additionally, growing evidence suggests differences in metastatic spread among BC biologic subtypes (6). Smid M et al. suggested that the majority of pleural relapse occurred in both luminal subtypes (53), which is consistent with our findings. Prognosis of metastatic breast is confirmed to be affected by a combination of factors such as molecular features (54). The prognostic role exerted by pathological factors varies in different disease contexts. Although we observed significant differences in M-OS among the four molecular subtypes of BC (Supplemental Figures 3A, B), multivariate Cox results revealed that it was not an independent prognostic factor for patients with only PM. Similarly, Yang Y et al. suggested that the prognosis of patients with cancer with MPE was independent of histology (41). This may be caused by the particularities of the studied patients or by data bias. In addition, BC is highly heterogeneous, and patients with the same molecular subtype also have distinct molecular features, responses to treatment, and prognosis (55, 56). Global burden of molecular mutations into primary tumor and metastatic samples seemed to be independent of the molecular subtype of primary tumor and metastatic sites in the study of Callens C et al. (54). By contrast, one study by Schrijver et al. showed different molecular mutational signatures for different metastatic sites (57). This may be one of the reasons why molecular subtypes did not appear as a predictor of survival in PM patients without other distant metastases, and the mechanisms remain to be further investigated and elucidated. Furthermore, the lymph node status and tumor size were independent predictors of death due to BC (58). Several studies have reported that the higher the T/N stage, the worse the prognosis of patients with BC (59), which was consistent with our results.

Evidence-based guidelines suggest the use of conservative treatments for patients with limited life expectancy, whereas they suggest offering more aggressive treatment modalities for patients with better prognoses. In this study, we focused on analyzing the survival of patients with PM without other distant metastasis at the time of first MBC diagnosis and identifying the prognostic factors. Identifying these characteristics and understanding their prognostic value in diseases could enable customized treatments for this patient group. The nomogram model constructed in this study included all the independent risk factors that we screened, and it provided a visual and user-friendly tool for risk evaluation and prognostic prediction of patients with BC with only PM, facilitating tailored management strategy for these patients.

However, inevitable the study has some limitations. (1) Our study was a single-center retrospective analysis with a limited number of cases, which may have caused some restrictions and biases in the results. (2) Although the nomogram achieved ideal prediction efficacy; it lacked external validation to further enforce the reliability.

5 Conclusion

BC with PM without additional distant metastasis at the time of first MBC diagnosis exhibited a better prognosis than those with

combined LM or LM alone. For patients with PM, the prognosis of patients with MPE was worse. Primary cancer site, T stage, N stage, location of PM, and MPE were identified as independent prognostic factors for predicting M-OS in patients with PM as the only distant metastatic site. The nomogram provided a quantitative method for predicting individual survival in this subset of patients.

Data availability statement

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

Author contributions

SL, ZY, and CL contributed to the conception and design of the study. SL collected data, performed the statistical analysis, and wrote manuscript. CL, WS, XL, and LS wrote sections of the manuscript. ZY reviewed and revised the manuscript, and acts as guarantor. All authors listed have read the final manuscript and agree to its publication.

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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.2023.1104246/full#supplementary-material

References

1. Liang Y, Zhang H, Song X, Yang Q. Metastatic heterogeneity of breast cancer: molecular mechanism and potential therapeutic targets. *Semin Cancer Biol* (2020) 60:14–27. doi: 10.1016/j.semcancer.2019.08.012

2. Kabil MF, Mahmoud MY, Bakr AF, Zaafar D, El-Sherbiny IM. Switching indication of PEGylated lipid nanocapsules-loaded with rolapitant and deferasirox against breast cancer: enhanced *in-vitro* and *in-vivo* cytotoxicity. *Life Sci* (2022) 305:120731. doi: 10.1016/j.lfs.2022.120731

3. Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. *J Clin Oncol Off J Am Soc Clin Oncol* (2010) 28(20):3271–7. doi: 10.1200/JCO.2009.25.9820

4. Yousefi M, Nosrati R, Salmaninejad A, Dehghani S, Shahryari A, Saberi A. Organ-specific metastasis of breast cancer: molecular and cellular mechanisms underlying lung metastasis. *Cell Oncol (Dordrecht)* (2018) 41(2):123–40. doi: 10.1007/s13402-018-0376-6

5. 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 canc* (2019) 19(1):1091. doi: 10.1186/s12885-019-6311-z

6. Gerratana L, Fanotto V, Bonotto M, Bolzonello S, Minisini AM, Fasola G, et al. Pattern of metastasis and outcome in patients with breast cancer. *Clin Exp metastasis* (2015) 32(2):125–33. doi: 10.1007/s10585-015-9697-2

7. Takeda Y, Tsuta K, Shibuki Y, Hoshino T, Tochigi N, Maeshima AM, et al. Analysis of expression patterns of breast cancer-specific markers (mammaglobin and gross cystic disease fluid protein 15) in lung and pleural tumors. *Arch Pathol Lab Med* (2008) 132(2):239–43. doi: 10.5858/2008-132-239-AOEPOB

8. Cummings MC, Simpson PT, Reid LE, Jayanthan J, Skerman J, Song S, et al. Metastatic progression of breast cancer: insights from 50 years of autopsies. *J pathology* (2014) 232(1):23–31. doi: 10.1002/path.4288

9. Emi Y, Kitamura K, Shikada Y, Kakeji Y, Takahashi I, Tsutsui S. Metastatic breast cancer with HER2/neu-positive cells tends to have a morbid prognosis. *Surgery.* (2002) 131(1 Suppl):S217–21. doi: 10.1067/msy.2002.119580

10. Lin S, Mo H, Li Y, Guan X, Chen Y, Wang Z, et al. Clinicopathological characteristics and survival outcomes in patients with synchronous lung metastases upon initial metastatic breast cancer diagnosis in han population. *BMC canc* (2021) 21(1):1330. doi: 10.1186/s12885-021-09038-2

11. Viot J, Bachour M, Meurisse A, Pivot X, Fiteni F. Follow-up of patients with localized breast cancer and first indicators of advanced breast cancer recurrence: a retrospective study. *Breast (Edinburgh Scotland)* (2017) 34:53–7. doi: 10.1016/j.breast.2017.05.005

12. Klebe M, Fremd C, Kriegsmann M, Kriegsmann K, Albrecht T, Thewes V, et al. Frequent molecular subtype switching and gene expression alterations in lung and pleural metastasis from luminal a-type breast cancer. *JCO Precis Oncol* (2020) 4. doi: 10.1200/PO.19.00337

13. Chen MT, Sun HF, Zhao Y, Fu WY, Yang LP, Gao SP, et al. Comparison of patterns and prognosis among distant metastatic breast cancer patients by age groups: a SEER population-based analysis. *Sci Rep* (2017) 7(1):9254. doi: 10.1038/s41598-017-10166-8

14. Tseng LM, Hsu NC, Chen SC, Lu YS, Lin CH, Chang DY, et al. Distant metastasis in triple-negative breast cancer. *Neoplasma.* (2013) 60(3):290–4. doi: 10.4149/neo_2013_038

15. Medeiros B, Allan AL. Molecular mechanisms of breast cancer metastasis to the lung: clinical and experimental perspectives. *Int J Mol Sci* (2019) 20(9):2272. doi: 10.3390/ijms20092272

16. Cagle PT, Allen TC. Pathology of the pleura: what the pulmonologists need to know. *Respirology (Carlton Vic)* (2011) 16(3):430–8. doi: 10.1111/j.1440-1843.2011.01957.x

17. Jung JI, Kim HH, Park SH, Song SW, Chung MH, Kim HS, et al. Thoracic manifestations of breast cancer and its therapy. *Radiographics Rev Publ Radiological Soc North America Inc* (2004) 24(5):1269–85. doi: 10.1148/rg.245035062

18. Connolly JEJr, Erasmus JJ, Patz EFJr. Thoracic manifestations of breast carcinoma: metastatic disease and complications of treatment. *Clin radiology* (1999) 54(8):487–94. doi: 10.1016/S0009-9260(99)90844-9

19. Changchien CY, Chang HH, Dai MS, Tsai WC, Tsai HC, Wang CY, et al. Distinct JNK/VEGFR signaling on angiogenesis of breast cancer-associated pleural fluid based on hormone receptor status. *Cancer science* (2021) 112(2):781–91. doi: 10.1111/cas.14772

20. Molina S, Martinez-Zayas G, Sainz PV, Leung CH, Li L, Grosu HB, et al. Breast and lung effusion survival score models: improving survival prediction in patients with malignant pleural effusion and metastasis. *Chest.* (2021) 160(3):1075–94. doi: 10.1016/j.chest.2021.03.059

21. Schrijver W, Schuurman K, van Rossum A, Droog M, Jeronimo C, Salta S, et al. FOXA1 levels are decreased in pleural breast cancer metastases after adjuvant endocrine therapy, and this is associated with poor outcome. *Mol Oncol* (2018) 12 (11):1884–94. doi: 10.1002/1878-0261.12353

22. Holling N, Patole S, Medford ARL, Maskell NA, Bibby AC. Is systemic anticancer therapy associated with higher rates of malignant pleural effusion control in people with pharmacologically sensitive tumors?: a retrospective analysis of prospectively collected data. *Chest.* (2021) 160(5):1915–24. doi: 10.1016/j.chest.2021.05.027

23. Karpathiou G, Benli J, Désage AL, Jacob M, Tiffet O, Peoc'h M, et al. Prognostic role of immune microenvironment in pleural metastases from breast and lung adenocarcinomas. *Ann Trans Med* (2022) 10(8):430. doi: 10.21037/atm-21-6326

24. Rodrîguez-Panadero F, Borderas Naranjo F, López Mejîas J. Pleural metastatic tumours and effusions. frequency and pathogenic mechanisms in a post-mortem series. *Eur Respir J* (1989) 2(4):366–9.

25. Xi G, Guo W, Kang D, Ma J, Fu F, Qiu L, et al. Large-Scale tumor-associated collagen signatures identify high-risk breast cancer patients. *Theranostics.* (2021) 11 (7):3229–43. doi: 10.7150/thno.55921

26. Kim SY, Cho N, Choi Y, Lee SH, Ha SM, Kim ES, et al. Factors affecting pathologic complete response following neoadjuvant chemotherapy in breast cancer: development and validation of a predictive nomogram. *Radiology.* (2021) 299(2):290–300. doi: 10.1148/radiol.2021203871

27. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP. Nomograms in oncology: more than meets the eye. *Lancet Oncol* (2015) 16(4):e173–80. doi: 10.1016/S1470-2045(14)71116-7

28. Deluche E, Antoine A, Bachelot T, Lardy-Cleaud A, Dieras V, Brain E, et al. Contemporary outcomes of metastatic breast cancer among 22,000 women from the multicentre ESME cohort 2008-2016. *Eur J Cancer (Oxford Engl 1990)* (2020) 129:60–70. doi: 10.1016/j.ejca.2020.01.016

29. Göker E, Hendriks MP, van Tilburg M, Barcaru A, Mittempergher L, van Egmond A, et al. Treatment response and 5-year distant metastasis-free survival outcome in breast cancer patients after the use of MammaPrint and BluePrint to guide preoperative systemic treatment decisions. *Eur J Cancer (Oxford Engl 1990)* (2022) 167:92–102. doi: 10.1016/j.ejca.2022.03.003

30. Deng Y, Tan C, Huang S, Sun H, Li Z, Li J, et al. Site-specific polyplex on CCR7 down-regulation and T cell elevation for lymphatic metastasis blocking on breast cancer. *Advanced healthcare materials* (2022) 11(22):e2201166. doi: 10.1002/adhm.202201166

31. da Luz FAC, Araújo BJ, de Araújo RA. The current staging and classification systems of breast cancer and their pitfalls: is it possible to integrate the complexity of this neoplasm into a unified staging system? *Crit Rev oncology/hematology* (2022) 178:103781. doi: 10.1016/j.critrevonc.2022.103781

32. Wei S, Siegal GP. Metastatic organotropism: an intrinsic property of breast cancer molecular subtypes. *Adv anatomic pathology* (2017) 24(2):78–81. doi: 10.1097/PAP.0000000000000140

33. Yamamura J, Kamigaki S, Fujita J, Osato H, Manabe H, Tanaka Y, et al. New insights into patterns of first metastatic sites influencing survival of patients with hormone receptor-positive, HER2-negative breast cancer: a multicenter study of 271 patients. *BMC canc* (2021) 21(1):476. doi: 10.1186/s12885-021-08219-3

34. Schröder J, Fietz T, Köhler A, Petersen V, Tesch H, Spring L, et al. Treatment and pattern of bone metastases in 1094 patients with advanced breast cancer - results from the prospective German tumour registry breast cancer cohort study. *Eur J Cancer (Oxford Engl 1990)* (2017) 79:139–48. doi: 10.1016/j.ejca.2017.03.031

35. Redig AJ, McAllister SS. Breast cancer as a systemic disease: a view of metastasis. *J Internal Med* (2013) 274(2):113–26. doi: 10.1111/joim.12084

36. Ciampa A, Fanger G, Khan A, Rock KL, Xu B. Mammaglobin and CRxA-01 in pleural effusion cytology: potential utility of distinguishing metastatic breast carcinomas from other cytokeratin 7-positive/cytokeratin 20-negative carcinomas. *Cancer.* (2004) 102(6):368–72. doi: 10.1002/cncr.20627

37. Shroff GS, Benveniste MF, Carter BW, de Groot PM, Wu CC, Viswanathan C, et al. Imaging of metastases in the chest: mechanisms of spread and potential pitfalls. *Semin ultrasound CT MR.* (2017) 38(6):594–603. doi: 10.1053/j.sult.2017.07.007

38. Shield PW, Papadimos DJ, Walsh MD. GATA3: a promising marker for metastatic breast carcinoma in serous effusion specimens. *Cancer cytopathology* (2014) 122(4):307–12. doi: 10.1002/cncy.21393

39. Agalioti T, Giannou AD, Stathopoulos GT. Pleural involvement in lung cancer. *J Thorac disease* (2015) 7(6):1021–30. doi: 10.3978/j.issn.2072-1439.2015.04.23

40. Egan AM, McPhillips D, Sarkar S, Breen DP. Malignant pleural effusion. *QJM monthly J Assoc Physicians* (2014) 107(3):179–84. doi: 10.1093/qjmed/hct245

41. Yang Y, Du J, Wang YS, Kang HY, Zhai K, Shi HZ. Prognostic impact of pleural effusion in patients with malignancy: a systematic review and meta-analysis. *Clin Trans science* (2022) 15(6):1340–54. doi: 10.1111/cts.13260

42. Tiran V, Stanzer S, Heitzer E, Meilinger M, Rossmann C, Lax S, et al. Genetic profiling of putative breast cancer stem cells from malignant pleural effusions. *PloS One* (2017) 12(4):e0175223. doi: 10.1371/journal.pone.0175223

43. Poe RH, Qazi R, Israel RH, Wicks CM, Rubins JM. Survival of patient with pleural involvement by breast carcinoma. *Am J Clin Oncol* (1983) 6(5):523–7. doi: 10.1097/00000421-198310000-00002

44. Thomas JM, Redding WH, Sloane JP. The spread of breast cancer: importance of the intrathoracic lymphatic route and its relevance to treatment. *Br J canc* (1979) 40 (4):540–7. doi: 10.1038/bjc.1979.219

45. Assis LV, Isoldi MC. Overview of the biochemical and genetic processes in malignant mesothelioma. *Jornal brasileiro pneumologia publicacao oficial da Sociedade Bras Pneumologia e Tisilogia* (2014) 40(4):429–42. doi: 10.1590/S1806-37132014000400012

46. Stathopoulos GT, Kalomenidis I. Malignant pleural effusion: tumor-host interactions unleashed. *Am J Respir Crit Care Med* (2012) 186(6):487–92. doi: 10.1164/rccm.201203-0465PP

47. Pokieser W, Cassik P, Fischer G, Vesely M, Ulrich W, Peters-Engl C. Malignant pleural and pericardial effusion in invasive breast cancer: impact of the site of the primary tumor. *Breast Cancer Res Treat* (2004) 83(2):139–42. doi: 10.1023/B:BREA.0000010706.24181.b6

48. Sarp S, Fioretta G, Verkooijen HM, Vlastos G, Rapiti E, Schubert H, et al. Tumor location of the lower-inner quadrant is associated with an impaired survival for women with early-stage breast cancer. *Ann Surg Oncol* (2007) 14(3):1031–9. doi: 10.1245/s10434-006-9231-5

49. Lim ST, Choi JE, Kim SJ, Kim HA, Kim JY, Park HK, et al. Prognostic implication of the tumor location according to molecular subtypes in axillary lymph node-positive invasive ductal cancer in a Korean population. *Breast Cancer Res Treat* (2016) 156(3):473–83. doi: 10.1007/s10549-016-3771-6

50. Gaffney DK, Tsodikov A, Wiggins CL. Diminished survival in patients with inner versus outer quadrant breast cancers. *J Clin Oncol.* (2003) 21(3):467–72. doi: 10.1200/JCO.2003.12.047

51. Zucali R, Mariani L, Marubini E, Kenda R, Lozza L, Rilke F, et al. Early breast cancer: evaluation of the prognostic role of the site of the primary tumor. *J Clin Oncol Off J Am Soc Clin Oncol* (1998) 16(4):1363–6. doi: 10.1200/JCO.1998.16.4.1363

52. Hwang KT, Kim J, Kim EK, Jung SH, Sohn G, Kim SI, et al. Poor prognosis of lower inner quadrant in lymph node-negative breast cancer patients who received no chemotherapy: a study based on nationwide Korean breast cancer registry database. *Clin Breast canc* (2017) 17(4):e169–e84. doi: 10.1016/j.clbc.2016.12.011

53. Smid M, Wang Y, Zhang Y, Sieuwerts AM, Yu J, Klijn JG, et al. Subtypes of breast cancer show preferential site of relapse. *Cancer Res* (2008) 68(9):3108–14. doi: 10.1158/0008-5472.CAN-07-5644

54. Callens C, Driouch K, Boulai A, Tariq Z, Comte A, Berger F, et al. Molecular features of untreated breast cancer and initial metastatic event inform clinical decision-making and predict outcome: long-term results of ESOPE, a single-arm prospective multicenter study. *Genome Med* (2021) 13(1):44. doi: 10.1186/s13073-021-00862-6

55. Knott SRV, Wagenblast E, Khan S, Kim SY, Soto M, Wagner M, et al. Asparagine bioavailability governs metastasis in a model of breast cancer. *Nature.* (2018) 554(7692):378–81. doi: 10.1038/nature25465

56. Pareja F, Weigelt B, Reis-Filho JS. Problematic breast tumors reassessed in light of novel molecular data. *Modern Pathol an Off J United States Can Acad Pathology Inc* (2021) 34(Suppl 1):38–47. doi: 10.1038/s41379-020-00693-7

57. Schrijver W, Selenica P, Lee JY, Ng CKY, Burke KA, Piscuoglio S, et al. Mutation profiling of key cancer genes in primary breast cancers and their distant metastases. *Cancer Res* (2018) 78(12):3112–21. doi: 10.1158/0008-5472.CAN-17-2310

58. Johansson ALV, Trewin CB, Fredriksson I, Reinertsen KV, Russnes H, Ursin G. In modern times, how important are breast cancer stage, grade and receptor subtype for survival: a population-based cohort study. *Breast Cancer Res BCR* (2021) 23(1):17. doi: 10.1186/s13058-021-01393-z

59. Phung MT, Tin Tin S, Elwood JM. Prognostic models for breast cancer: a systematic review. *BMC canc* (2019) 19(1):230. doi: 10.1186/s12885-019-5442-6

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A nomogram for predicting the risk of male breast cancer for overall survival

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Background: Male breast cancer (MBC) is a rare disease, accounting for <1% of all male carcinomas. Lack of prospective data, the current therapy for MBC is based on retrospective analysis or information that is extrapolated from studies of female patients. We constructed a nomogram model for predicting the overall survival (OS) of MBC patients and verify its feasibility using data from China.

Methods: Constructed a predictive model using 1224 MBC patients from the Surveillance, Epidemiology and End Results (SEER) registry between 2010 and 2015. The performance of the model was externally validated between 2002 to 2021 using 44 MBC patients from the Fujian Medical University Union Hospital. The independent prognostic factors were selected by univariate and multivariate Cox regression analyses. The nomogram was constructed to predict individual survival outcomes for MBC patients. The discriminative power, calibration, and clinical effectiveness of the nomogram were evaluated by the receiver operating characteristic (ROC) curve, and the decision curve analysis (DCA).

Results: A total of 1224 male breast cancer patients were in the training cohort and 44 in the validation cohort. T status (p<0.001), age at diagnosis (p<0.001), histologic grade (p=0.008), M status (p<0.001), ER status (p=0.001), Her2 status (p=0.019), chemotherapy (p=0.015) were independently associated with OS. The diagnostic performance of this model was evaluated and validated using ROC curves on the training and validation datasets. In the training cohort, the nomogram-predicted AUC value was 0.786 for 3-year OS and 0.767 for 5-year OS. In the validation cohort, the nomogram-predicted AUC value was 0.893 for 3-year OS and 0.895 for 5-year OS. Decision curve analysis demonstrated that the nomogram was more benefit than the AJCC stage.

Conclusions: We developed a nomogram that predicts 3-year and 5-year survival in MBC patients. Validation using bootstrap sampling revealed optimal discrimination and calibration, suggesting that the nomogram may have clinical utility. The results remain reproducible in the validation cohort which included Chinese data. The model was superior to the AJCC stage system as shown in the decision curve analysis (DCA).

KEYWORDS

male breast cancer, nomogram, predictive model, risk factors, SEER

Introduction

Breast cancer is one of the most common malignancies worldwide for women. However, male breast cancer (MBC) is a rare disease, accounting for <1% of all male carcinomas (1). Due to the lack of data on risk factors, prognostic value, and treatment options related to MBC, the therapeutic patterns for male breast cancer that clinicians recommended are based on female breast cancer (2, 3).

However, whether the management of female breast cancer (FBC) can be used as a reference for MBC is still controversial. Some studies have concluded that MBC and FBC are two completely different types with different biological behaviors and should be treated differently (4, 5).

Therefore, a personalized prediction model is required for patients with male breast cancer. A nomogram is a simplified numerical model for statistical predictions that combines different independent factors (6–8). However, can the model built using the Surveillance, Epidemiology, and End Results (SEER) database be applicable to the Chinese? Few articles have been published on this subject.

In our study, a nomogram model was constructed by the SEER database for predicting the overall survival (OS) of MBC patients. Further, it was investigated whether the model was also applicable to the Chinese population.

Materials and methods

Patient selection and data collection

Data were acquired from the open-access, authoritative database of the SEER Program. Launched in 1973 by the United States Centers for Disease Control and Prevention and National Cancer Institute, the SEER database includes information on patients with endocrine, respiratory, digestive system, and other tumors, and covers approximately 34.6% of the population in the United States. The training cohort data used in this study came from a public, anonymous database and did not require ethics committee approval or informed consent. The validation cohort data were approved, and informed consent was obtained from the ethics committee of Fujian Medical University Union Hospital.

Training cohort data of MBC patients from 2010 to 2015 in the SEER database were extracted and screened by SEER Stat version 8.3.5 software. Validation cohort data from 2002 to 2021 in Fujian Medical University Union Hospital were extracted. Inclusion criteria were 1) pathologically diagnosed patients with breast cancer, based on the malignant behavior of International Classification of Diseases (ICD)-O-3 SEER site/histology validation code 8500/3, 2) male, and 3) complete clinicopathological and follow-up data. Exclusion criteria were 1) unknown important date, 2) with history of other types of cancer, 3) with less than 1 month of survival, and 4) diagnosis depends on biopsy/autopsy. According to the inclusion and exclusion criteria, cases meeting the criteria were gradually screened out, and 1,224 MBC patients were finally included in the training cohort. A total of 44 patients were included in the validation cohort. The study was not subject to review by the Institutional Review Board because we used unidentified, previously collected, and publicly available data. The flowchart of the male breast cancer selection is shown in Figure 1.

The clinicopathological information of patients in Fujian Medical University Union Hospital and the SEER database, including age, marital status, radiotherapy, chemotherapy, surgery, stage, grade, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, and subtype, was compared. Also, variables such as survival state and time were compared. Data from 1,224 patients extracted from the SEER database were used as the training cohort to analyze the independent influencing factors of MBC prognosis and establish a prediction model. The validation of the model was further demonstrated using the data of 44 patients from Fujian Medical University Union Hospital as the validation cohort.

Statistical analysis

Demographic and clinical characteristics were summarized using descriptive statistics. Categorical variables were reported as whole numbers and proportions, and continuous variables were

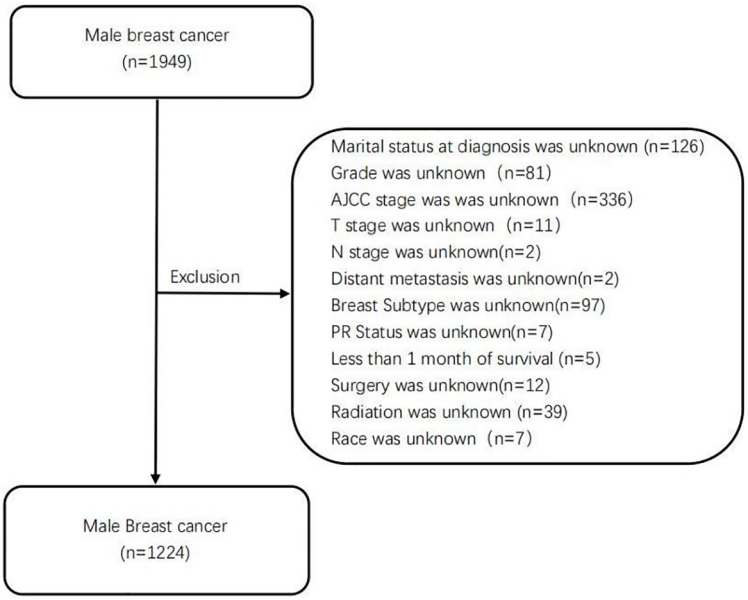

FIGURE 1
The flowchart of the selection for male breast patients in SEER database. SEER, Surveillance, Epidemiology, and End Results.

reported as medians with standard deviation (SD). Pearson's χ^2 test and Fisher's exact test were used for categorical variables, and the Mann–Whitney U test was used for rank variables to compare the baseline characteristics of the training cohort and the validation cohort. The Kaplan–Meier method was used to describe the OS curve, and the log-rank test was used to evaluate the survival differences of distinct subgroups of each variable. The cutoff age for male breast cancer was determined by the X-tile procedure at 64 to 80 years (Figure 2). Patients were divided into three groups for further analysis (age ≤ 64, 65–80, and >80 years). Significant variables were screened by Cox univariate analysis, and variables with $p < 0.1$ in univariate analysis were included in the multivariate Cox proportional hazards model. The above statistical analyses were performed with IBM SPSS Statistics 26.

The prediction performance of the nomogram was internally verified by 1,000 resampling using the bootstrap method. The discrimination degree of the model was evaluated by the consistency index (concordance index (C-index)), receiver operating characteristic (ROC) curve, and area under the curve (AUC), and the model was detected by drawing the calibration curve. Degree of calibration was performed to ensure that the model is accurate and reliable. Decision curve analysis (DCA) was used to evaluate the overall survival of the nomogram compared with American Joint Committee on Cancer (AJCC) staging. Test level $\alpha = 0.05$ (two-tailed). The above statistical analyses were performed with R 4.1.0 software.

Results

Patient characteristics

Table 1 depicts the baseline characteristics of patients including training cohort (n = 1224) and validation cohort (n = 44). Pearson's

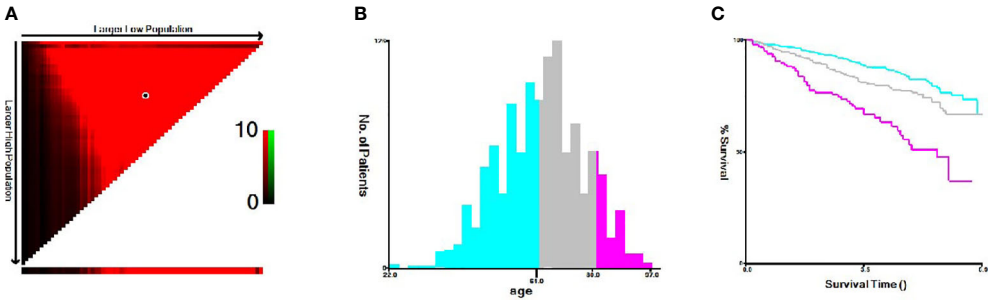

FIGURE 2
X-tile analysis of optimal cutoffs for age. **(A)** X-tile plot of the age training cohort. **(B)** Cutoffs are highlighted with histograms of the entire cohort. **(C)** Different prognoses determined by cutoffs are shown with Kaplan–Meier plots (age ≤ 64 years = blue, age 65–80 years = gray, and age >80 years = magenta).

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TABLE 1 Demographics and clinical characteristics of male breast cancer in training cohort and validation cohort.

Characteristic	Training Cohort, N (%)	Validation Cohort, N (%)	X^2	P-value
age	65.35±12.24	58.57±11.38		0.001
T status				0.003
T1	544 (44.4%)	30 (68.2%)		
T2	533 (43.5%)	11 (25.0%)		
T3	40 (3.3%)	2 (4.5%)		
T4	107 (8.7%)	1 (2.3%)		
N status				0.271
N0	667 (54.5%)	28 (63.6%)		
N1	384 (31.4%)	11 (25.0%)		
N2	109 (8.9%)	2 (4.5%)		
N3	64 (5.2%)	3 (6.8%)		
M status				0.016
M0	1144 (93.5%)	37 (84.1%)		
M1	80 (6.5%)	7 (15.9%)		
Stage				0.495
I	361 (29.5%)	19 (43.2%)		
II	578 (47.2%)	14 (31.8%)		
III	205 (16.7%)	1 (2.3%)		
IV	80 (6.5%)	10 (22.7%)		
Grade				0.001
Grade I、II	764 (62.4%)	33 (79.5%)		
Grade III	460 (37.6%)	9 (20.5%)		
ER status			24.252	<0.001
Positive	1191 (97.3%)	37 (84.1%)		
Negative	33 (2.7%)	7 (15.9%)		
PR status			3.386	0.066
Positive	1120 (91.5%)	35 (79.5%)		
Negative	104 (8.5%)	9 (20.5%)		
HER2 status			0.004	0.951
Positive	163 (13.3%)	6 (13.6%)		
Negative	1061 (86.7%)	38 (86.4%)		
Subtype			17.504	<0.001
HR+/HER2-	1040 (85.0%)	32 (72.7%)		
HR+/HER2+	154 (12.6%)	5 (11.4%)		
HR-/HER2+	9 (0.7%)	2 (4.5%)		
HR-/HER2-	21 (1.7%)	5 (11.4%)		
Surgery			2.143	0.143
Yes	1151 (94%)	39 (88.6%)		
No	73 (6.0%)	5 (11.4%)		

(Continued)

TABLE 1 Continued

Characteristic	Training Cohort, N (%)	Validation Cohort, N (%)	X^2	P-value
Chemotherapy			0.045	0.833
Yes	509 (41.6%)	19 (43.2%)		
No	715 (58.4%)	25 (56.8%)		
Radiotherapy			15.901	<0.001
Yes	368 (30.1%)	1 (2.3%)		
No	856 (69.9%)	43 (97.8%)		

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor.

TABLE 2 Univariable and multivariable Cox regression analyses for OS in patients with MBC.

Variable	Univariable		Multivariable	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age		<0.001		<0.001
≤64 years	Ref		Ref	
65–80 years	1.53 (1.13–2.08)	0.006	1.93 (1.40–2.68)	<0.001
>80 years	3.32 (2.32–4.75)	<0.001	3.70 (2.45–5.60)	<0.001
Grade		0.001		0.008
I	Ref		Ref	
II	1.08 (0.64–1.81)	0.773	0.90 (0.53–1.52)	0.683
III	1.80 (1.08–3.00)	0.024	1.42 (0.84–2.40)	0.197
T status		<0.001		<0.001
T1	Ref		Ref	
T2	1.99 (1.44–2.76)	<0.001	1.70 (1.21–2.39)	0.002
T3	4.69 (2.69–8.17)	<0.001	3.03 (1.67–5.51)	<0.001
T4	5.02 (3.42–7.38)	<0.001	2.91 (1.87–4.55)	<0.001
N status		<0.001		0.107
N0	Ref			
N1	1.60 (1.18–2.16)	0.002		
N2	2.34 (1.57–3.51)	<0.001		
N3	1.99 (1.18–3.34)	0.009		
M status		<0.001		<0.001
M0	Ref		Ref	
M1	6.16 (4.47–8.47)		3.00 (1.86–4.84)	
Stage		<0.001		–
I	Ref		–	
II	1.47 (0.10–2.17)	0.052	–	–
III	2.71 (1.78–4.14)	<0.001	–	–
IV	9.42 (6.14–14.46)	<0.001	–	–
ER status		<0.001		0.001
Negative	Ref		Ref	

(Continued)

TABLE 2 Continued

Variable	Univariable		Multivariable	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Positive	0.32 (0.17–0.59)		0.32 (0.17–0.62)	
PR status		0.019		0.556
Negative	Ref			
Positive	0.60 (0.39–0.92)			
HER2 status		0.002		0.019
Negative	Ref		Ref	
Positive	1.70 (1.22–2.36)		1.53 (1.07–219)	
Subtype		<0.001	–	–
HR+/HER2 –	Ref		–	–
HR+/HER2+	1.75 (1.25–2.46)	0.001	–	–
HR–/HER2+	2.31 (0.57–9.31)	0.24	–	–
HR–/HER2–	5.84 (2.97–11.47)	<0.001	–	–
Surgery type		<0.001		0.001
No surgery	Ref		Ref	
PM	0.19 (0.11–0.31)	<0.001	0.51 (0.28–0.93)	0.028
TM	0.14 (0.10–0.20)	<0.001	0.40 (0.25–0.63)	<0.001
Radiotherapy	0.90 (0.67–1.21)	0.476		
Chemotherapy	0.74 (0.56–0.98)	0.036	0.67(0.49–0.93)	0.015

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TM, total mastectomy; PM, partial mastectomy; OS, overall survival; MBC, male breast cancer.

χ^2 test, Fisher's exact test, and Mann–Whitney U test were used to compare the baseline characteristics of the training cohort and validation cohort. Marital status ($p = 0.010$), T status ($p = 0.003$), M status ($p = 0.016$), grade ($p = 0.001$), ER status ($p < 0.001$), subtype ($p < 0.001$), and radiotherapy ($p < 0.001$) were significantly different between the training and validation cohorts, which might be attributed to the difference of race. The average age of patients in the training cohort and validation cohort was 65.35 ± 12.24 and 58.57 ± 11.38 years, respectively, and the difference was statistically significant.

Univariable analysis and multivariable analysis

On the univariable analysis (Table 2), age ($p < 0.001$), histologic grade ($p < 0.001$), T status ($p < 0.001$), N status ($p < 0.001$), M status ($p < 0.001$), AJCC staging ($p < 0.001$), ER status ($p < 0.001$), PR status ($p = 0.019$), HER2 status ($p = 0.002$), tumor subtype ($p < 0.001$), receipt of chemotherapy ($p = 0.036$), and surgery type ($p < 0.001$) were significantly associated with survival outcomes (all $p < 0.05$). On the multivariable analysis (Table 2) that included age ($p < 0.001$), histologic grade ($p = 0.008$), T status ($p < 0.001$), M status ($p < 0.001$), ER status ($p = 0.001$), HER2 status ($p = 0.019$), receipt of chemotherapy ($p = 0.015$), and surgery type ($p = 0.001$) were independently associated with survival outcomes (all $p < 0.05$). According to multivariable analysis, the Kaplan–Meier plot was used to show the differences in OS among these clinical benefits (Figure 3).

Nomogram construction and validation

Multivariate-derived coefficients were used to develop a novel nomogram to predict male breast cancer 3-year overall survival and 5-year overall survival (Figure 4).

According to the results, the nomogram contains age, histologic grade, T status, M status, ER status, HER2 status, receipt of chemotherapy, and surgery type. The nomogram illustrates that the ER status accounted for a vast majority of the proportion compared with other clinical features. The calibration curve of the nomogram showed high consistencies between the predicted and observed survival probability in both the training and validation cohorts (Figure 5). Perfectly calibrated models are indicated by dashed lines, and the results all show a good fit to the actual probabilities of the predicted probabilities. The ROC curves of the 3-year OS nomogram and 5-year OS nomogram for both the training and validation cohorts are shown in Figure 6. In Figure 6A, the 3-year OS AUC value was 0.786 in the training cohort and 0.893 in the validation cohort. In Figure 6B, the 5-year

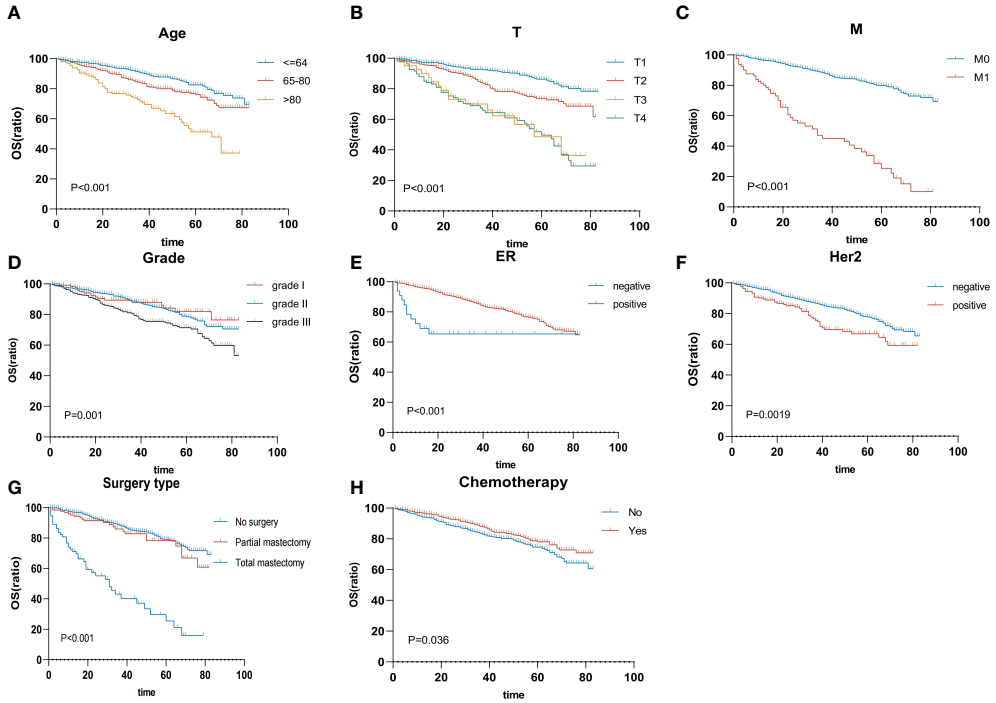

FIGURE 3
Overall survival rates are stratified by patient characteristics. Kaplan–Meier overall survival curves of the training cohort ($p < 0.05$) according to **(A)** age, **(B)** T status, **(C)** M status, **(D)** grade, **(E)** ER status, **(F)** HER2 status, **(G)** surgery type, and **(H)** chemotherapy. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

OS AUC value was 0.767 in the training cohort and 0.895 in the validation cohort.

DCA curves showed that the nomogram could better predict the 3-and 5-year OS, as it added more clinical benefits compared with AJCC staging for all threshold probabilities in the training cohorts (Figure 7).

Discussion

Breast cancer has become the most common malignancy in women worldwide, but breast cancer in men is still very rare. Due to its rarity, many clinical decisions have been informed and developed by the practice of female patients (9). However, MBC

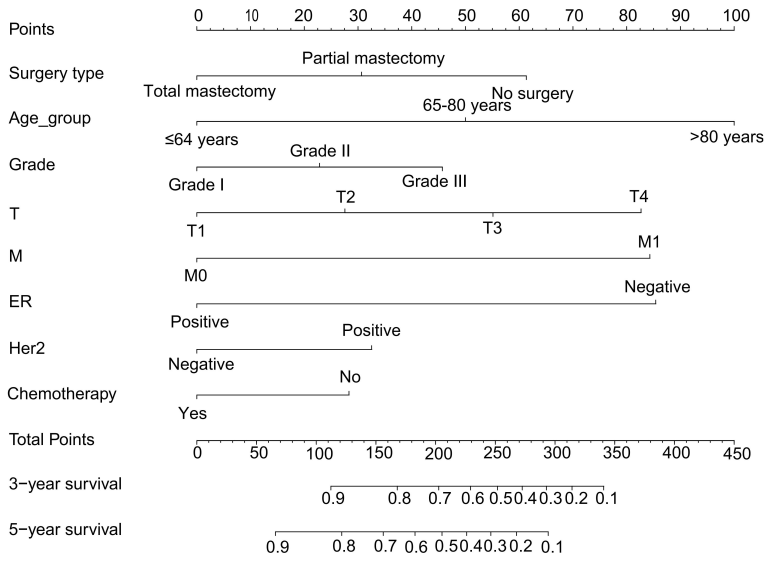

FIGURE 4
Nomogram predicting the 3-year and 5-year overall survival of MBC patients. Survival rates were determined by summing all scores and drawing a vertical line between the total score and the probability of survival scale. MBC, male breast cancer.

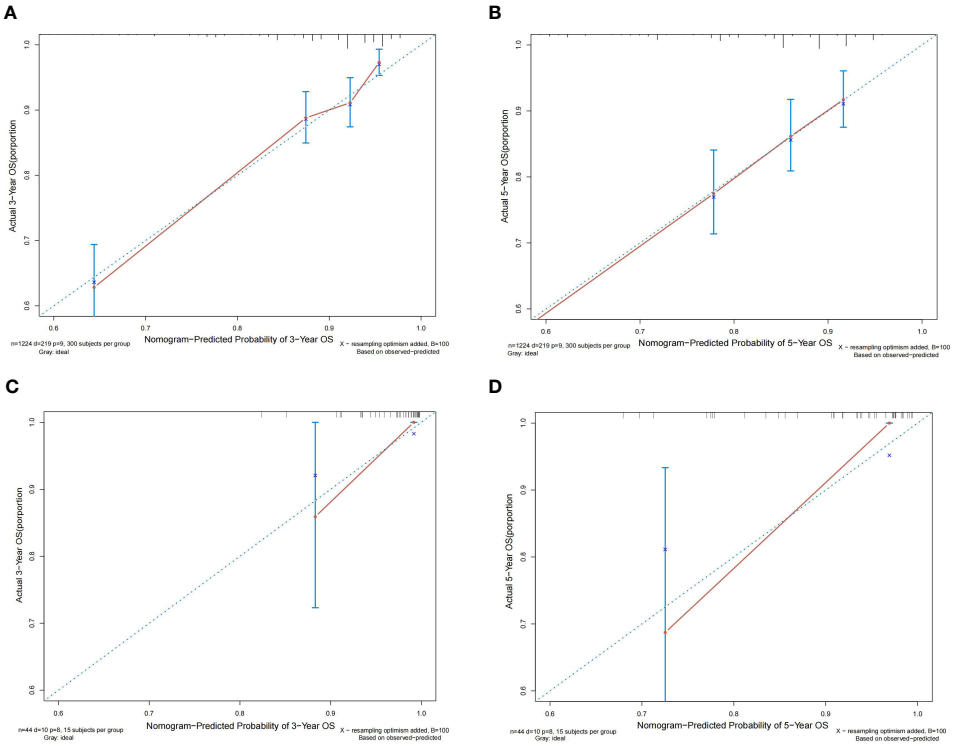

FIGURE 5
Calibration plots of the relationship between predicted probabilities and actual values based on nomograms. **(A, B)** Calibration curves for 3-year and 5-year overall survival in the training cohort. **(C, D)** Calibration curves for 3-year and 5-year overall survival in the validation cohort.

is considered to be a disease with distinct characteristics from FBC (5, 10). Meanwhile, an analysis from the National Cancer Database showed that overall survival rates for MBC remained lower than for FBC after adjusting for age, race, clinical, and treatment issues (11). Therefore, clinical characteristics and overall survival of MBC need to be further investigated.

From the baseline characteristics of MBC, the median age at the time of diagnosis of MBC is 65.35 ± 12.24 years, similar to a previous study (12). The majority of patients present with grade I or grade II disease (62.4%), ER-positive (97.3%), and less distant metastases (93.5%), compared with previous female studies (13, 14).

Traditionally, AJCC staging is the most general tool used to assess prognosis. It indicates the objective tumor load and metastasis status. However, the prognosis of tumors is composed of multiple biological and clinical factors. Current National Comprehensive Cancer Network (NCCN) and American Society of Clinical Oncology (ASCO) guidelines recommend the use of ER, PR, HER2, and Ki-67 status also as important prognostic factors in

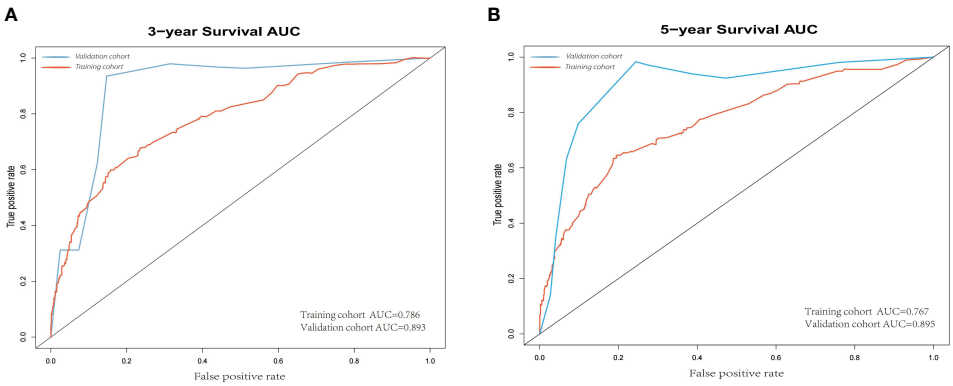

FIGURE 6
(A) 3-year ROC of OS nomogram using training and validation cohorts. **(B)** 5-year ROC of OS nomogram using training and validation cohorts. ROC, receiver operating characteristic; OS, overall survival.

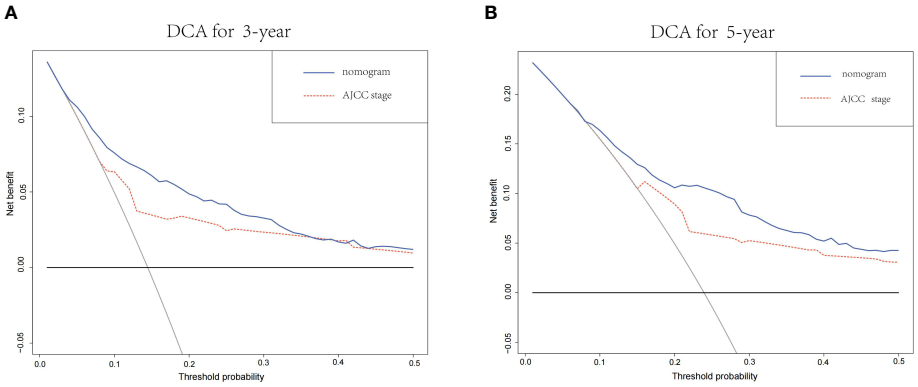

FIGURE 7
(A) The DCA of the nomogram and the AJCC stage system for 3-year OS in the training cohort. **(B)** The DCA of the nomogram and the AJCC TNM staging system for 5-year OS in the training cohort. DCA, decision curve analysis; AJCC, American Joint Committee on Cancer; OS, overall survival.

medical decision making. In addition to T, N, M, ER, PR, and HER2 status, in our Cox analysis, age, histologic grade, and whether or not to perform surgery and chemotherapy were also associated with OS. Therefore, it is necessary to establish a more comprehensive model to predict OS in MBC.

Previous research attempted to use predictive models for FBC on male breast cancer patients (15), but it was found that the predictive factors were not the same, possibly due to differences in the biological determinants of male and female breast cancer. Therefore, it is necessary for us to establish an independent predictive model based on data from male breast cancer.

In our study, in addition to surgery type, age, T status, M status, and histological grade, the expression status of ER and HER2, as well as the use of chemotherapy, also play important roles in the prognosis of MBC. It is noteworthy that N status was found to be significant in our univariate analysis but lost its significance in the multivariate analysis when considering multiple factors. This finding deviates from previous research results (16, 17). It is possible that the lack of significance of the N stage in the multivariate analysis could be due to a small sample size of male breast cancer cases included in our study.

It is worth noting that radiotherapy does not improve OS in MBC (p = 0.476). In previous studies of FBC, radiotherapy did improve local relapse in breast cancer patients, but whether radiotherapy improves OS remains controversial (18, 19). There are still few relevant studies of MBC. According to Kaplan–Meier survival analysis, our research findings indicate that there was no statistically significant difference in survival rates between male breast cancer patients who underwent total mastectomy and those who underwent partial mastectomy. This is consistent with previous research (20), suggesting that surgical procedures may not significantly impact survival outcomes in male breast cancer. However, adjuvant radiotherapy after partial breast resection may have mitigated potential survival differences. Further research with larger sample sizes and controlled confounding factors are needed for confirmation.

China has the highest number of breast cancer cases, accounting for approximately 18.4% of global breast cancer cases (21). In our study, the median age of diagnosis in China showed different patterns from the United States: the median age of diagnosis in China was almost 7 years earlier than that in the United States. This gap is smaller than in previous studies of FBC (22). Additionally, other different MBC features were demonstrated in our results, such as a higher proportion of T1 status patients, a higher proportion of grade I and II patients, a lower ER positive proportion, and a lower proportion of radiotherapy. There are differences in follow-up duration and basic patient characteristics between the training and validation cohorts. However, based on the ROC curves, it can be observed that the model achieved good validation performance across different baselines. Nevertheless, it cannot be denied that the bias in validation results may be influenced by different baselines. Therefore, further validation on multiple datasets is necessary.

In this model, the DCA curve indicates that this nomogram model has better predictions when compared to the AJCC staging. A higher C-index and a relatively high uniformity of the calibration plots were also shown in the model. In addition, we validated it with single-center data in China. Although there are more differences between the validation cohort and the training cohort, it also shows better validation results when external validation is performed. As far as we know, this is the first study to build and verify a nomogram in MBC with the SEER database and China single-center data.

Inevitably, our study has some limitations. First, this is a retrospective study, in which selection bias is inevitable. Second, some important confounding prognostic factors were not available in the SEER database, which include the Ki-67 index (23) and BRCA1- and BRCA2-related mutations (24, 25). Third, due to the data being derived from a single center, there is a need for additional validation using data from multiple centers to further assess the model's reliability and generalizability.

Conclusion

Male breast cancer has been neglected due to its rarity, resulting in fewer studies related to treatment and prognosis. In this study, we developed a clinical prognostic model that combines the prognostic

characteristics of male breast cancer and validated it with Chinese male breast cancer data. The results showed that the prediction model is applicable to different ethnic groups.

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

Conception and design: YW and CX. Development of methodology: YW, JL, CZ, SL, HH, JB, and CX. Acquisition of data, analysis, and interpretation of data (e.g., statistical analysis, biostatistics, and computational analysis): YW, JB, and CX. Writing, review, and/or revision of the manuscript: YW and CX. Study supervision: SL and HH. Manuscript revision: YW, JL, and CX. All of the authors reviewed and approved the final 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. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* (2022) 72(5):409–36. doi: 10.3322/caac.21731

2. Hassett MJ, Somerfield MR, Baker ER, Cardoso F, Kansal KJ, Kwait DC, et al. Management of male breast cancer: ASCO guideline. *J Clin Oncol* (2020) 38(16):1849–63. doi: 10.1200/JCO.19.03120

3. Losurdo A, Rota S, Gullo G, Masci G, Torrisi R, Bottai G, et al. Controversies in clinicopathological characteristics and treatment strategies of male breast cancer: A review of the literature. *Crit Rev Oncol Hematol* (2017) 113:283–91. doi: 10.1016/j.critrevonc.2017.03.013

4. Scomersi S, Giudici F, Cacciatore G, Losurdo P, Fracon S, Cortinovis S, et al. Comparison between male and female breast cancer survival using propensity score matching analysis. *Sci Rep* (2021) 11(1):11639. doi: 10.1038/s41598-021-91131-4

5. Silva SN, Gomes BC, André S, Félix A, Rodrigues AS, Rueff J. Male and female breast cancer: the two faces of the same genetic susceptibility coin. *Breast Cancer Res Treat* (2021) 188(1):295–305. doi: 10.1007/s10549-021-06159-x

6. Iasonos A, Schrag D, Raj GV, Panageas KS. How to build and interpret a nomogram for cancer prognosis. *J Clin Oncol* (2008) 26(8):1364–70. doi: 10.1200/JCO.2007.12.9791

7. Wu J, Zhang H, Li L, Hu M, Chen L, Xu B, et al. A nomogram for predicting overall survival in patients with low-grade endometrial stromal sarcoma: A population-based analysis. *Cancer Commun (Lond)* (2020) 40(7):301–12. doi: 10.1002/cac2.12067

8. Jin C, Cao J, Cai Y, Wang L, Liu K, Shen W, et al. A nomogram for predicting the risk of invasive pulmonary adenocarcinoma for patients with solitary peripheral subsolid nodules. *J Thorac Cardiovasc Surg* (2017) 153(2):462-469.e1. doi: 10.1016/j.jtcvs.2016.10.019

9. Xie X, Wang J, Shi D, Zou Y, Xiong Z, Li X, et al. Identification of a 4-mRNA metastasis-related prognostic signature for patients with breast cancer. *J Cell Mol Med* (2019) 23(2):1439–47. doi: 10.1111/jcmm.14049

10. Kornegoor R, van Diest PJ, Buerger H, Korsching E. Tracing differences between male and female breast cancer: both diseases own a different biology. *Histopathology* (2015) 67(6):888–97. doi: 10.1111/his.12727

11. Wang F, Shu X, Meszoely I, Pal T, Mayer IA, Yu Z, et al. Overall mortality after diagnosis of breast cancer in men vs women. *JAMA Oncol* (2019) 5(11):1589–96. doi: 10.1001/jamaoncol.2019.2803

12. Yadav S, Karam D, Bin Riaz I, Xie H, Durani U, Duma N, et al. Male breast cancer in the United States: Treatment patterns and prognostic factors in the 21st century. *Cancer* (2020) 126(1):26–36. doi: 10.1002/cncr.32472

13. Lin S, Liu C, Tao Z, Zhang J, Hu X. Clinicopathological characteristics and survival outcomes in breast carcinosarcoma: A SEER population-based study. *Breast (Edinburgh Scotland)* (2020) 49:157–64. doi: 10.1016/j.breast.2019.11.008

14. Liu J, Zheng X, Han Z, Lin S, Han H, Xu C. Clinical characteristics and overall survival prognostic nomogram for invasive cribriform carcinoma of breast: a SEER population-based analysis. *BMC Cancer* (2021) 21(1):168. doi: 10.1186/s12885-021-07895-5

15. Vaysse C, Sroussi J, Mallon P, Feron JG, Rivain AL, Ngo C, et al. Prediction of axillary lymph node status in male breast carcinoma. *Ann Oncol* (2013) 24(2):370–6. doi: 10.1093/annonc/mds283

16. 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

17. Soliman AA, Denewer AT, El-Sadda W, Abdel-Aty AH, Refky B. A retrospective analysis of survival and prognostic factors of male breast cancer from a single center. *BMC Cancer* (2014) 14:227. doi: 10.1186/1471-2407-14-227

18. Rogowski P, Schönecker S, Pazos M, Reitz D, Braun M, Pölcher M, et al. Pattern of care of adjuvant radiotherapy in male breast cancer patients in clinical practice: an observational study. *Strahlenther Onkol* (2019) 195(4):289–96. doi: 10.1007/s00066-018-1337-8

19. Matuschek C, Bölke E, Haussmann J, Mohrmann S, Nestle-Krämling C, Gerber PA, et al. The benefit of adjuvant radiotherapy after breast conserving surgery in older patients with low risk breast cancer- a meta-analysis of randomized trials. *Radiat Oncol (London England)* (2017) 12(1):60. doi: 10.1186/s13014-017-0796-x

20. Lin AP, Huang TW, Tam KW. Treatment of male breast cancer: meta-analysis of real-world evidence. *Br J Surg* (2021) 108(9):1034–42. doi: 10.1093/bjs/znab279

21. Lei S, Zheng R, Zhang S, Wang S, Chen R, Sun K, et al. Global patterns of breast cancer incidence and mortality: A population-based cancer registry data analysis from 2000 to 2020. *Cancer Commun (Lond)* (2021) 41(11):1183–94. doi: 10.1002/cac2.12207

22. Chen C, Sun S, Yuan J-P, Wang Y-H, Cao T-Z, Zheng H-M, et al. Characteristics of breast cancer in Central China, literature review and comparison with USA. *Breast (Edinburgh Scotland)* (2016) 30:208–13. doi: 10.1016/j.breast.2016.01.004

23. Liang Q, Ma D, Gao R-F, Yu K-D. Effect of ki-67 expression levels and histological grade on breast cancer early relapse in patients with different immunohistochemical-based subtypes. *Sci Rep* (2020) 10(1):7648. doi: 10.1038/s41598-020-64523-1

24. Ibrahim M, Yadav S, Ogunleye F, Zakalik D. Male BRCA mutation carriers: clinical characteristics and cancer spectrum. *BMC Cancer* (2018) 18(1):179. doi: 10.1186/s12885-018-4098-y

25. Silvestri V, Barrowdale D, Mulligan AM, Neuhausen SL, Fox S, Karlan BY, et al. Male breast cancer in BRCA1 and BRCA2 mutation carriers: pathology data from the Consortium of Investigators of Modifiers of BRCA1/2. *Breast Cancer Res* (2016) 18(1):15. doi: 10.1186/s13058-016-0671-y

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