

Development and validation of a pathological model predicting the efficacy of neoadjuvant therapy for breast cancer based on RCB scoring

Keywords

Breast cancer, Pathomics, Residual Cancer Burden, Neoadjuvant therapy

Abstract

Introduction

Breast cancer has become the most prevalent malignant tumor among women globally, posing a serious threat to women's life and health. Neoadjuvant therapy (NAT) has emerged as one of the standard treatment approaches for breast cancer patients. However, due to varying responses to NAT among different patients, significant differences in treatment effectiveness occur, impacting the timely alteration of treatment strategies for patients.

Material and methods

Based on clinical and pathological characteristics along with the RCB scoring, we utilized a support vector machine (SVM) algorithm to construct a Breast Cancer Pathological Characteristics (PBCS) prediction model. We thoroughly validated the PBCS and compared it to a Pathomics Signatures (PS) prediction model.

Results

we developed a pathological prediction label, named PS. Subsequently, through univariate and multivariate analysis, we discovered a significant correlation between HER2 and the patients' RCB scores. Integrating HER2 into PS, we constructed a breast cancer pathological prediction model, named PBCS. PBCS exhibits good performance in predicting the effectiveness of postoperative therapy (RCB 0~1) in both the training sets (AUC 0.86 [95% CI 0.7988-0.9173]) and validation sets (AUC 0.83 [95% CI 0.7219-0.9382]). In the validation set, PBCS significantly outperforms the PS (AUC 0.65 [95% CI 0.5121-0.7886]). Calibration curves and clinical decision curves also strongly support PBCS's ability to effectively predict the efficacy of therapy (RCB 0~1).

Conclusions

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PBCS can assist clinical and pathological physicians in accurately predicting patients' post-treatment RCB grading before initiating NAT. This offers a new approach to forecast breast cancer patients' responsiveness to NAT, aiding in devising personalized treatment strategies for patients.

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Materials and methods

This study included a total of 201 breast cancer patients who completed NAT, divided into a training group of 140 cases and a validation group of 61 cases. Based on clinical and pathological characteristics along with the **Residual Cancer Burden (RCB) score**, we utilized a support vector machine (SVM) algorithm to construct a Breast Cancer Pathological Characteristics(PBCS) prediction model. We thoroughly validated the PBCS and compared it to a Pathomics Signatures (PS) prediction model.

Results

In our study, we used Cellprofiler to extract nine pathological features highly correlated with patients' RCB scoring from HE-stained slides of breast cancer NAT. Employing the Support Vector Machine (SVM) algorithm, we developed a pathological prediction label, named PS. Subsequently, through univariate and multivariate analysis, we discovered a significant correlation between HER2 and the patients' RCB scores. Integrating HER2 into PS, we constructed a breast cancer pathological prediction model, named PBCS. PBCS exhibits good performance in predicting the effectiveness of postoperative therapy (RCB 0~I) in both the training sets(AUC0.86[95%CI0.7988-0.9173])and validation sets

(AUC0.83[95%CI0.7219-0.9382]). In the validation set, PBCS significantly outperforms the PS (AUC0.65[95%CI0.5121-0.7886]). Calibration curves and clinical decision curves also strongly support PBCS's ability to effectively predict the efficacy of therapy (RCB 0~I).

Conclusions

PBCS can assist clinical and pathological physicians in accurately predicting patients' post-treatment RCB grading before initiating NAT. This offers a new approach to forecast breast cancer patients' responsiveness to NAT, aiding in devising personalized treatment strategies for patients.

Keywords

Breast cancer, Pathomics, Residual Cancer Burden, Neoadjuvant therapy.

Introduction

Breast cancer, as a malignant tumor, poses a severe threat to women's life and health. During the period from 2014 to 2018, the incidence of breast cancer continued to rise, and has now become the most prevalent malignant tumor among women [1, 2]. Neoadjuvant therapy

(NAT) can assist patients in reducing staging, shrinking tumors, thereby potentially facilitating subsequent surgical treatment[3, 4]. This significantly improves patients' event-free survival(EFS) and overall survival (OS) [5]. thus becoming one of the standard treatment approaches for the aforementioned breast cancer patients [6].

The Residual Cancer Burden (RCB) assessment system was first introduced in 2007, As a continuous numerical value, it comprehensively analyzes the two-dimensional size of the largest residual tumor, the proportion of tumor cells, and the status of lymph node metastasis[7]. This system enables a more comprehensive and effective evaluation of the effectiveness of NAT and patient prognosis in breast cancer[8]. Studies have shown that the pathologic complete response (pCR) after NAT can serve as a marker for patients' disease-free survival (DFS) and overall survival (OS)[9, 10].The team of researchers led by Christina Yau from the University of California, San Francisco, published a multicenter retrospective study in 'The Lancet: Cancer,' revealing that for each unit increase in RCB score, there is an 82% increase in the risk of recurrence, metastasis, or death in breast cancer patients (HR: 1.82),and researchers believe that RCB scoring and grading can independently predict the long-term survival of breast cancer patients in various clinical research centers and within different breast cancer subgroups[10].

However, in clinical practice, not all patients can achieve pathological relief after receiving NAT and some patients do not respond effectively to NAT, while the condition may even worsen in some cases[11]. Therefore, numerous biological indicators have been proposed for predicting the effectiveness of NAT[12, 13]. As we Know, histological type, histological grade, molecular subtypes, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and Ki67 in breast cancer tissues are all related to patients' response to NAT[14]. However, due to the heterogeneity of tumors, the responsiveness to treatment is not always consistent among different patients[15].Further exploration is necessary to identify more precise prognostic markers, providing a theoretical basis for the clinical prognosis assessment of breast cancer and thus enabling correct and effective personalized treatment.

Over the past decade, with the advancement of computer technology, machine learning has made substantial progress in the field of medicine, especially in computer-aided screening, precise diagnostics, and treatment decisions. Machine learning offers a new approach to assist clinicians in classifying medical information and images. Pathologists can extract information about the tumor from pathological slides of tumor tissue. Through visual inspection and evaluation based on diagnostic criteria and their accumulated diagnostic experience, they assess the pathological grading of the tumor. However, due to variations in pathologists' experiences, this diagnostic model still lacks specificity and efficiency.

Recently, researchers from Southwest Medical University developed a multi-scale fusion model based on the Support Vector Machine (SVM) algorithm, combining CT and WSI images of breast cancer patients. This model is designed for predicting the pCR after neoadjuvant chemotherapy (NAC)[16].However, the assessment of pCR in breast cancer patients who received NAC in this study is based on clinical doctors' experience. The evaluation criteria are not standardized in pathological practice, making it challenging to accurately distinguish various degrees of tumor residue.We assume to evaluate the efficacy of breast cancer after NAT using pathological characteristics combined with RCB scoring. We categorize RCB 0-I as the significantly effective therapy group (NAT-sensitive), and RCB II~III as the group showing less significant response to therapy (NAT-resistant)[17].

Therefore, based on whole-slide images (WSI) of breast cancer H&E stained slides and pathological analysis methods, we establish a model to classify the chemotherapy outcomes after breast cancer NAT. This aims to identify patients who can achieve a good chemotherapy response, providing guidance for clinicians in devising personalized treatment strategies.

Patients and Methods

H&E slicer

Research Design

A total of 289 patients records who underwent NAT and surgical treatment at Renmin Hospital of Wuhan University between January 2021 and June 2023 were collected. Based on the inclusion criteria, 201 patients were ultimately included. Among them, 20 cases were excluded due to insufficient cancer tissue in the biopsy samples to extract enough histological features, 16 cases were excluded due to concomitant presence of other malignancies or diagnosed distant metastases at the time of diagnosis, 11 cases were excluded due to incomplete clinical or pathological information, 4 cases were excluded due to the destruction of tumor masses in the surgical samples, making the RCB assessment inaccurate, and 37 cases were excluded due to unclear image quality in the scanned WSI images, making it impossible to accurately extract Primary Score (PS). Finally, a total of 201 eligible patients were obtained (Figure2). Clinical baseline information of the patients was collected preoperatively, including age, histological grading, histological type, ER, PR, androgen receptor (AR), HER2, Ki67, and cytological lymph node involvement. This retrospective study has obtained approval from the Ethics Review Committee of Renmin Hospital of Wuhan University (Ethics No.WDRY2024-K04) and has been exempted from the requirement for informed consent.

Pathological Response Assessment

Currently, there are three commonly used methods for prognostic assessment of NAT in breast cancer, namely the Miller-Payne system, the American Joint Committee on Cancer (AJCC) ypTNM staging, and the RCB system[18, 19]. Among these, the RCB assessment system can consider the dimensions of the primary tumor, the number of tumor bed cells, and the axillary lymph node burden. Therefore, the RCB score demonstrates very good accuracy, objectivity, and feasibility in predicting the prognosis of NAT for breast cancer[10, 20]. This study also utilized the RCB score to evaluate the therapeutic effects of neoadjuvant treatment for breast cancer, with the RCB index calculated using an online-based calculator (calculation website: http://www.mdanderson.org/breast_cancer_RCB). According to the calculation results of the RCB index, RCB classification is divided into four levels: RCB 0 (complete pathological response,pCR), RCB I ($0 < \text{RCB} \leq 1.36$, minimal residual lesions), RCB II ($1.36 < \text{RCB} \leq 3.28$, moderate residual lesions), and RCB III ($\text{RCB} > 3.28$, extensive residual lesions)[21]. (Figure3)

Patients were treated according to the "Guidelines and Norms for the Diagnosis and Treatment of Breast Cancer" developed by the Breast Cancer Committee of the Chinese Anti-Cancer Association in 2019[22]. All patients underwent 3 to 9 cycles of NAT, with chemotherapy regimens based on taxanes, carboplatin, and anthracycline drugs. HER2-positive patients also received treatment with trastuzumab. After completion of NAT, surgical resection was performed. Postoperatively, sampling and assessment were conducted following the methods recommended in the "Expert Consensus on Pathological Diagnosis of Neoadjuvant Therapy for Breast Cancer"[23]. Based on the postoperative RCB grading of

patients, RCB grades 0 to I were classified as the significantly effective therapy group, while RCB grades II to III were classified as the insignificantly effective therapy group.

Pathomics

Development and Validation of Breast Cancer Pathomics Prediction Model

The predictive values of the Pathomics model were combined with independent clinical predictive factors using the SVM to construct Pathomics Breast Cancer Signatures (PBCS), for the prediction of RCB scores in patients undergoing NAT for breast cancer. We conducted a comprehensive evaluation of the model using the following methods. Receiver Operating Characteristic (ROC) curve analysis, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were employed to assess the discriminative performance[24]. We assessed the model's calibration using calibration curves. Decision Curve Analysis (DCA) was performed on all models to quantify the net benefit for patients at different probability thresholds, thereby evaluating the clinical value of our predictive model in this study .

Sample Preparation and Selection of Regions of Interest

All included breast cancer tissue H&E stained slides were prepared from formalin-fixed paraffin-embedded samples. Subsequently, the pathology department director of Renmin Hospital of Wuhan University selected the slides that best represented the tumor cell heterogeneity. KFBIO (KF-PRO-020) was used to scan the selected slides at 20X magnification to acquire Whole Slide Images (WSI) of the patients, and digitize the images into SVS format files. Managed using (K-Viewer, version: 1.7.0.21), under the quality control of the director of the Pathology Department at Renmin Hospital of Wuhan University, two pathologists selected 10 non-overlapping screenshots per case containing the most tumor cells, each with a field of view of 1800×900 pixels. We saved the selected screenshots as formatted files(JPEG). Using Photoshop (Version: 24.0.0), we segmented all screenshots into non-overlapping small frame images (500×500 pixels) for subsequent analysis.

Extraction of Pathomics Features

We used CellProfiler (version 4.0.7)[25](an open-source image analysis software developed by the Broad Institute in Cambridge, Massachusetts) to extract quantitative pathological features from the selected pathology screenshots. We used the 'UnmixColors' module to separate images of H&E staining into grayscale images for Hematoxylin and Eosin staining. Additionally, we utilized the 'ColorToGray' module to convert the H&E images into grayscale images for further analysis. We conducted two measurements on the images. In the first comprehensive measurement, we accumulated a total of 285 primary features. We evaluated the image quality features of the grayscale images of H&E, Hematoxylin, and Eosin using the 'MeasureImageQuality' and 'MeasureImageIntensity' modules. Subsequently, by utilizing the 'MeasureLocalization' module, we computed, on a per-pixel basis throughout the entire image, the co-localization and correlation of intensities between each Hematoxylin and Eosin staining image. Assessing the granularity features of each image was done using the 'MeasureGranularity' module. These features summarize three types of images. In the second measurement, we extracted detailed features in the Hematoxylin staining. First, we identified the primary and secondary objects and then measured them separately, obtaining 831 pathomics features. The extracted pathomics features were aggregated through the mean values of the features from 10 screenshots of each WSI. We cumulatively extracted 1116

pathomics features, which were randomly divided into training and validation sets in a 7:3 ratio for subsequent model construction. For a detailed summary of the extracted pathomics features, please refer to the supplementary materials (FigureS1-S3).

Construction of Pathomics Models

Constructing pathomics features can unveil tumor information from the tumor microenvironment. However, these features are high-dimensional data, which adversely affect the prediction of RCB grading. Therefore, we should obtain the features most closely associated with RCB 0-I levels in the training set. Initially, normalizing all variables and conducting a U-Test on each feature to eliminate redundant features. To thoroughly extract discriminative features in this process, the threshold for the p-value was set at 0.05. Subsequently, considering the correlation between features, we conducted a correlation analysis. If the correlation coefficient between two features exceeded 0.8, one of the features was excluded. Next, the Least Absolute Shrinkage and Selection Operator (LASSO) algorithm was used to select the extracted features, and tenfold cross-validation was employed to choose the value of Lambda to determine the optimal features. Based on the optimal features from the aforementioned pathomics characteristics, we constructed a Pathomics Prediction Model using the Support Vector Machine (SVM) algorithm. Subsequently, Pathomics Predictive Values were utilized to build a signature, named Pathomics Signatures (PS).

Development and Validation of Breast Cancer Pathomics Prediction Model

The predictive values of the Pathomics model were combined with independent clinical predictive factors using the SVM to construct Pathomics Breast Cancer Signatures (PBCS), for the prediction of RCB scores in patients undergoing NAT for breast cancer. We conducted a comprehensive evaluation of the model using the following methods. Receiver Operating Characteristic (ROC) curve analysis, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were employed to assess the discriminative performance [26, 27]. We assessed the model's calibration using calibration curves. Decision Curve Analysis (DCA) was performed on all models to quantify the net benefit for patients at different probability thresholds, thereby evaluating the clinical value of our predictive model in this study.

Statistical Analysis

All statistical analyses were performed using R Studio (Version 4.1.1; R Studio, <http://www.R-project.org>) and Jupyter Notebook (Version 6.4.11). The differences in categorical variables were calculated using the chi-square test. The differences in continuous variables were analyzed using the Mann-Whitney U test. All tests were two-tailed, and a two-tailed p-value < 0.05 was considered statistically significant.

Results

The correlation between clinicopathological features and RCB grading.

This study included a total of 201 female breast cancer patients who underwent NAT, with an average age of 49.4 (ranging from 22 to 76) years. Postoperative specimens were assessed using the RCB grading system, with 77 patients (38.3%) classified as RCB 0, 29 patients (14.4%) as RCB I, 58 patients (28.8%) as RCB II, and 37 patients (18.4%) as RCB III. According to the clinical efficacy of therapy, RCB 0-I (106 cases) was defined as effective therapy, and RCB II-III (95 cases) was defined as ineffective. The chi-square test showed that

histological grade, histological type, ER, PR, HER2, and Ki67 proliferation index were all associated with the efficacy of NAT (Table S1). Further univariate logistic regression analysis indicated a correlation between histological grade, histological type, ER, PR, HER2, and Ki67 proliferation index with the efficacy of NAT. Patients with higher histological grades, non-special type invasive breast cancer, negative expression of ER and PR, positive expression of HER2, and a high Ki67 proliferation index ($\geq 30\%$) were more likely to achieve RCB 0-I, and this association was statistically significant ($p < 0.05$). Multivariate logistic regression analysis revealed that HER2 is an independent predictive factor influencing the efficacy of NAT ($p < 0.05$) (Table 1). The data of 201 cases were randomly divided into training and validation sets at a ratio of 7:3, resulting in 140 cases in the training set and 61 cases in the validation set. Chi-square tests were performed on both sets, demonstrating differences in ER, PR, and Ki67 expression between the groups. Additionally, there was no significant statistical difference observed between the two groups of patients (Table S2).

Selection of Pathomics Features and Construction of the PS Model

Utilizing U-Test and Spearman correlation analysis, redundant features were eliminated, resulting in 12 pathomics features from 201 patients (Figure 4). Subsequently, Lasso was used for further feature selection. We performed tenfold cross-validation to find the optimal lambda value. We chose the features with a lambda value equivalent to one standard error. Ultimately, 9 pathomics features were obtained. Please refer to the supplementary file for details. We extracted 9 highly correlated pathomics features with patient RCB scores from H&E stained slices of breast cancer NAT patients using CellProfiler. Subsequently, we employed the SVM algorithm to construct a PS. The ROC curve evaluated the model's predictive performance, showing an AUC of 0.75 in the training set and 0.65 in the validation set, indicating acceptable accuracy of the model (Figure 5).

Development and Validation of PBCS

The results of the multifactor regression analysis showed that HER2 is an independent predictive factor influencing the efficacy of NAT. Thus, integrating HER2 with PS into the training set, a comprehensive predictive model PBCS was developed using the SVM algorithm. The model's discriminative performance was evaluated using the ROC curve. PBCS accurately predicted effective therapy (RCB0-I) in the training set (AUC 0.86 [95% CI 0.7988-0.9173]) and the validation set (AUC 0.83 [95% CI 0.7219-0.9382]) (Table 2).

Discussion

Due to the high incidence and increasing trend of breast cancer among patients, NAT combined with surgical treatment as a standardized approach brings hope to an increasing number of patients. However, due to individual variations, different patients also respond differently to therapy [28]. Hence, numerous biological indicators predicting treatment response are gradually becoming a hot area in clinical research. Currently, decisions regarding whether patients should undergo NAT are largely based on clinical pathological features. Numerous studies have shown that higher histological grading, a high Ki67 proliferation index, negative expression of ER and PR, as well as positive expression of HER2, are all associated with a favorable treatment response [29]. Among these, the molecular subtypes determined by ER, PR, HER2, and Ki67 serve as one of the most important indicators for

predicting NAT. This suggests that patients with HER2-enriched and triple-negative breast cancer are recommended candidates for NAT, while those with ER and PR positive expression (Luminal subtype) may benefit more from hormonal therapy or surgical treatment. Klein et al.'s research also indicates a more pronounced sensitivity of HER2-positive and triple-negative breast cancer patients to neoadjuvant treatments[30]. Therefore, in the complex clinical practice, relying solely on clinical pathological features does not yield satisfactory results, necessitating the urgent development of more accurate biological markers to predict patients' treatment responses.

In the field of digital pathology, artificial intelligence techniques such as support vector machine algorithms and convolutional neural networks have been used to assist in the diagnosis of oncologic pathology[31-35]. Previous studies have confirmed the effectiveness of using ultrasound images of tumors to predict the outcome of pathological complete response to neoadjuvant chemotherapy in breast cancer patients[36]. Recently, some investigators have developed a CT-based radiological model that is effective in predicting the validity of axillary pathologic complete response after neoadjuvant chemotherapy[37]. In this study, based on clinical pathological features and pathological histological characteristics, we developed a predictive model for the efficacy of NAT in breast cancer using an SVM algorithm. This model aims to forecast the RCB score in breast cancer patients after neoadjuvant treatment. The predictive model based on pathological characteristics showed complete accuracy in predicting the RCB score, demonstrating good performance in discrimination, calibration, and at the level of clinical decision-making. Internally validated within our center, the model exhibited a good AUC, high sensitivity, specificity, and NPV. In clinical practice, our research provides an effective and replicable tool to predict the RCB score in breast cancer patients undergoing NAT. This tool can assist in timely adjustment of treatment strategies for patients with ineffective treatment (RCB II-III), enabling the development of personalized treatment plans for them.

For breast cancer patients, accurately predicting their RCB classification before initiating NAT can significantly maximize their treatment benefits and aid in the early development of personalized treatment strategies. In the traditional treatment process, pathologists often use optical microscopes to determine the tumor's malignancy, differentiation degree, and growth pattern. Clinical physicians then combine the patients' molecular characteristics and their own experience to decide on whether to use NAT. However, due to limitations in optical microscope magnification, variations in pathologists' experiences, and the heterogeneity of tumors among different patients, numerous biological markers do not always accurately predict a patient's response to NAT. This leads to cases where patients with ineffective treatment (RCB II-III) are subjected to NAT strategies. This not only fails to cause a significant regression in tumor cells but also brings numerous side effects to patients and might delay the appropriate timing for treatment. With the application of cell analysis software and the rapid advancement of computer storage technology, the digitization of pathological slides has become achievable. Within histopathological sections lie a multitude of microscopic structural details that elude the naked eye, reflecting the characteristics of tumor cells and microenvironment features[38]. This information can directly indicate the malignancy level of the tumor and its response to treatment.

In our study, the PS model, solely based on pathology image extraction in the validation

set, showed an AUC of 0.65 for predicting NAT in breast cancer, yielding reasonably reliable results regarding predicting patient responses to neoadjuvant treatment. Additionally, considering that patients' clinical characteristics, particularly HER2 status, are closely associated with NAT efficacy, the PCBS model constructed by combining PS with the patients' clinical feature HER2 exhibited a significant enhancement in predictive value (AUC=0.83). The PBCS achieves a predictive value for NAT in breast cancer with an AUC (>0.8), similar to the results in Zhang et al.'s study[16]. They used radiomics, pathomics, and deep learning pathomics to predict pCR in breast cancer NAC. However, their assessment of patients' pathological response relies on the clinical pathologists' experience, which holds a degree of subjectivity. In contrast to their study, our research adopts a more advanced and objective method, utilizing RCB classification to evaluate the effectiveness of NAT in patients, hence providing a more accurate assessment of treatment outcomes. During the construction of our model, we conducted two extractions of our pathological images using CellProfile. The PBCS model demonstrated high sensitivity and NPV in an independent validation set, indicating its reliability in identifying individuals with no significant response to neoadjuvant treatment.

Although our research pioneered the introduction of RCB classification as an evaluation tool for pathological remission of NAT for breast cancer patients, providing a stronger objectivity in distinguishing treatment effects, the study still has certain limitations. Firstly, due to random assignment of cases in this study, the proportion of triple-negative breast cancer patients was significantly higher in the training set compared to the validation set, causing statistically significant differences in ER, PR, and Ki-67 between the training and validation set patients. Further single-factor and multi-factor regression analyses conducted separately on the training and validation sets showed that high histological grading, negative expression of ER and PR, positive expression of HER2, and a high Ki67 ($\geq 30\%$) proliferation index were all influencing factors on patients' chemotherapy response, leading to poor uniformity in clinical pathological factors between the training and validation sets. Secondly, our study is a single-center retrospective study where all breast cancer patients originated from the same medical institution. The sample size is limited. In the future, we aim to acquire sample data from more medical institutions and conduct prospective studies to validate the accuracy and applicability of PBCS.

Conclusion

We have developed the PBCS, a novel artificial intelligence model that predicts the RCB score after NAT for breast cancer by integrating pathology biopsy WSI and clinical pathological features. This model aids clinical physicians in preparing for NAT by predicting the therapy response, enabling timely adjustment of personalized treatment strategies for patients.

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Table 1 Univariate and multivariate regression analysis of clinicopathological features and NAT

Factors	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P	OR (95%CI)	P
Age (%)				
>50 vs ≤50	0.81 (0.463~1.405)	0.449		
Histological grade (%)				
3 vs 1~2	0.33 (0.177~0.597)	<0.001	0.569 (0.199~1.628)	0.293
Histological type (%)				
Other invasive histology vs Invasive carcinoma of no special type	6.44 (1.801~23.004)	0.004	4.783 (0.820~27.889)	0.082
ER status (%)				
Positive vs Negative	4.33 (2.370~7.927)	<0.001	1.748 (0.630~4.850)	0.283
PR status (%)				
Positive vs Negative	5.76 (3.130~10.600)	<0.001	2.160 (0.791~5.897)	0.133
AR status (%) *				
Positive vs Negative	1.249 (0.608~2.566)	0.544		
HER2 (%)				
Positive vs Negative	0.03 (0.009~0.098)	<0.001	0.029 (0.008~0.106)	<0.001
Ki67 expression status (cut-of 30%) (%)				
High (≥30%) vs Low (<30%)	0.29 (0.142~0.578)	<0.001	0.521 (0.152~1.779)	0.298

Table 2 Discrimination performance of predict models for predicting RCB0-I in breast cancer patients

Training set	AUC (95%CI)	SEN(%)	SPE(%)	ACC(%)	PPV(%)	NPV(%)
PS	0.754513 (0.6663 – 0.8427)	0.911392	0.573770	0.764286	0.734694	0.833333
PSBC	0.858062 (0.7988 – 0.9173)	0.772152	0.770492	0.771429	0.813333	0.723077
Test set	AUC (95%CI)	SEN(%)	SPE(%)	ACC(%)	PPV(%)	NPV(%)

PS	0.650327 (0.5121 – 0.7886)	0.962963	0.323529	0.606557	0.530612	0.916667
PSBC	0.830065 (0.7219 – 0.9382)	0.666667	0.941176	0.819672	0.900000	0.780488

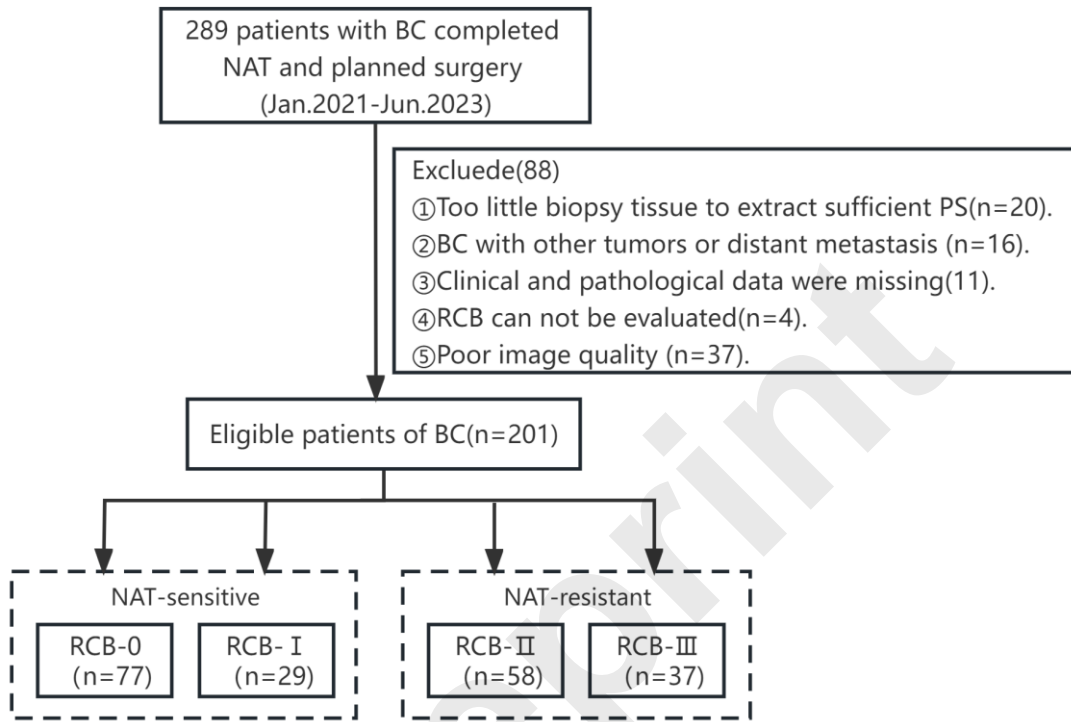


Figure1 Flow diagram of patient cohort selection

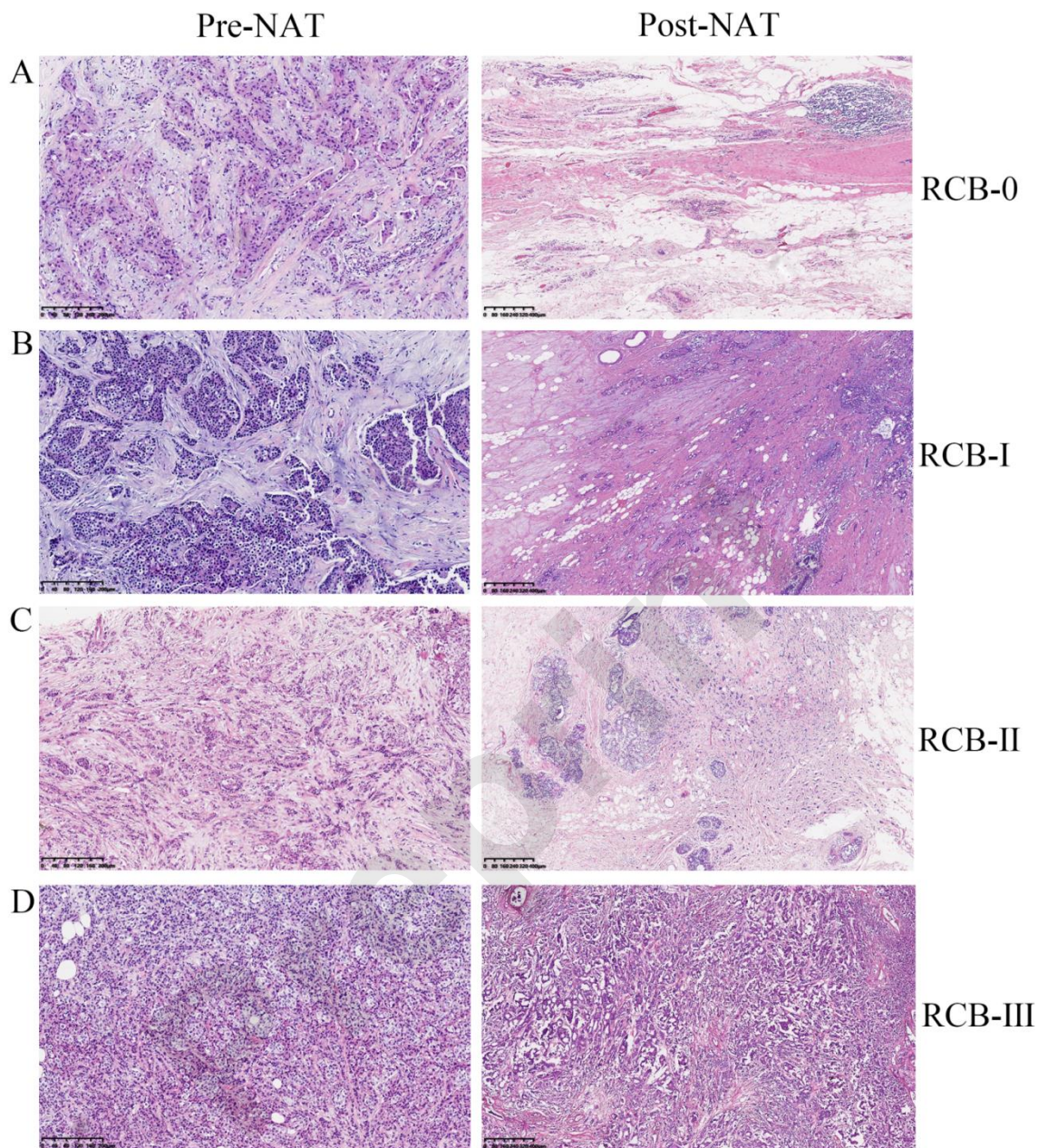


Figure2 Histological images of breast cancer patients before and after NAT. RCB-0: Tumor cells disappeared completely after NAT.(A); RCB-I:Only a small amount of tumor remained after NAT(B); RCB- II: There was moderate residual lesion in the tumor bed after NAT (C) ; RCB- III: There was extensive residual lesion in the tumor bed after NAT (D)

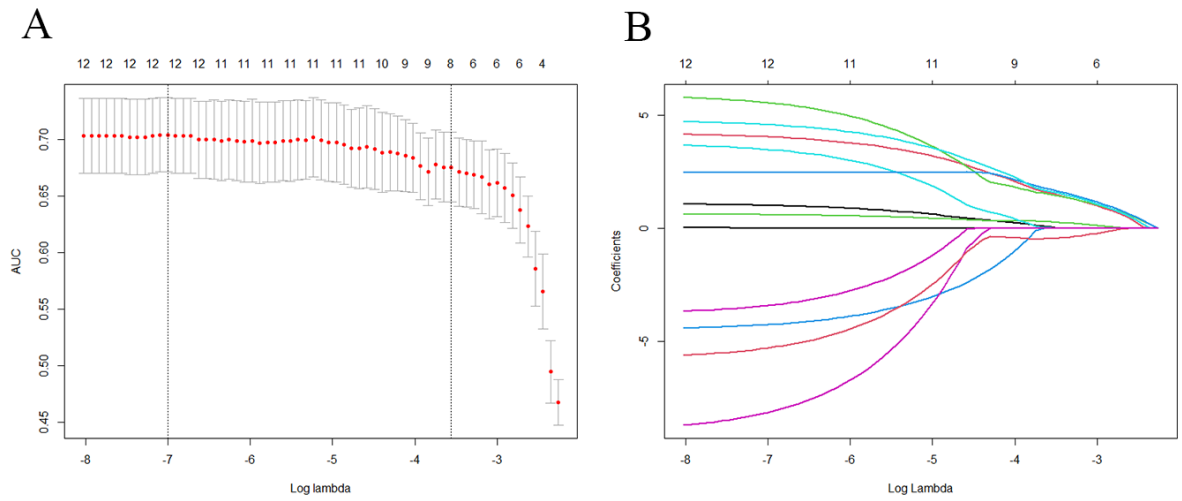


Figure3. Feature selection process. pathomics features (A, B) were selected by the LASSO model with tuning parameter (λ) using fivefold cross-validation via minimum and 1se criteria

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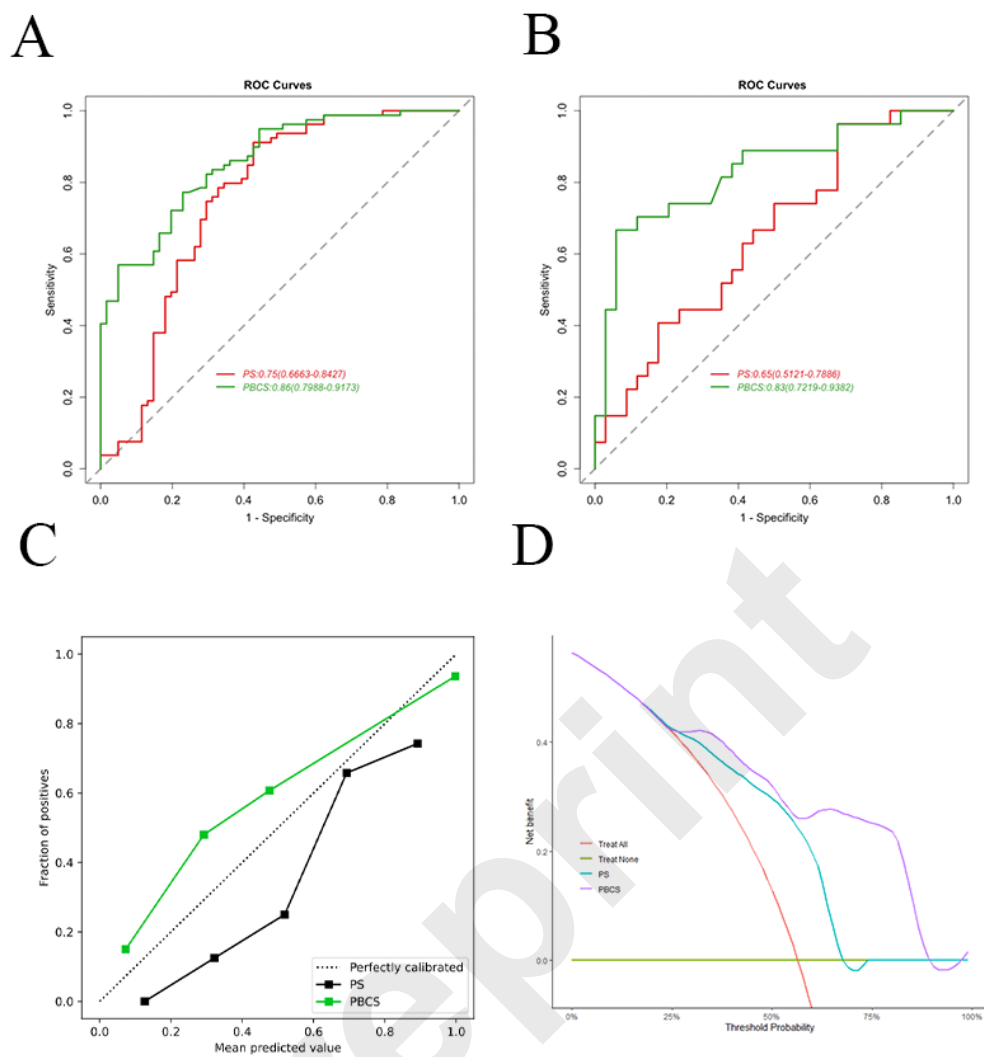
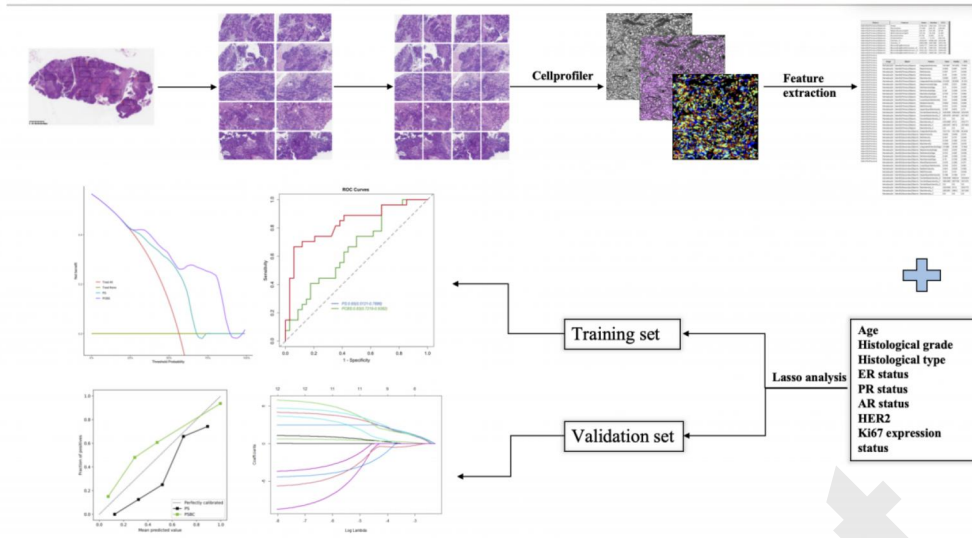


Figure 4. ROC analysis of predict models for predicting Significant therapy response group in the training set (A) and validation set (B), respectively. C Calibration curves of models in training set on discriminating Group with significant effect of therapy versus Group with insignificant effect of therapy. D Decision curve analysis in training set using PS, PBCS .



Workflow diagram of the research: manually segmenting and cropping pathological images. Employing CellProfiler for feature extraction. Building histopathological features (PS), integrating patient clinical characteristics for constructing breast cancer histopathological features (PBCS), and assessing the model's performance.

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