Original Article

External verification and improvement of the Neo-Bioscore staging system in a Chinese cohort

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Background: Accurately predicting outcomes for patients with breast cancer receiving neoadjuvant chemotherapy (NAC) is critical for clinical decisions. Prognostic models applicable to the Chinese population remain limited. The Neo-Bioscore staging system has been utilized as a predictive model for survival of breast cancer patients after NAC. This study aimed to validate the applicability of Neo-Bioscore in a Chinese population and develop an improved staging system based on it to predict prognosis of Chinese patients more accurately.

Methods: This study retrospectively collected clinicopathological and survival data in patients receiving NAC from February 2005 to August 2018 in PLA General Hospital. Discrimination, calibration and clinical usefulness were used to assess model performance. Univariate and multivariate analyses assessed relationships between clinicopathological factors and disease-specific survival. For model modification, postoperative pathological staging in the Neo-Bioscore was substituted with the posttreatment pathological tumor (ypT) stage and posttreatment pathological lymph node (ypN) stage. Neo-Bioscore and Modified Neo-Bioscore were compared with the American Joint Committee on Cancer (AJCC) staging system.

Results: A total of 436 patients with a median follow-up of 67 months were included. Five-year disease-specific survival (DSS), overall survival, and disease-free survival rates were 88.0%, 87.9%, and 76.8%, respectively. The concordance index (C-index) of the Neo-Bioscore staging system, posttreatment pathological stage (PS), and pretreatment clinical stage (CS) for DSS were 0.78 [95% confidence interval (CI): 0.72–0.83], 0.75 (95% CI: 0.69–0.82), and 0.68 (95% CI: 0.62–0.74), respectively. No significant difference between the Neo-Bioscore and PS was observed in the C-index (P=0.399). ypT and ypN were included in Neo-Bioscore to replace PS and create a modified staging system named MNeo-Bioscore. The C-index for DSS of the MNeo-Bioscore was 0.82 (95% CI: 0.78–0.87), and the calibration curve and decision curve analysis (DCA) curve performed well in internal validation.

Conclusions: The Neo-Bioscore staging system provided precise prognostic stratification for Chinese breast cancer patients receiving NAC; ypN and ypT stage may be substituted for PS to add significant prognostic value for Neo-Bioscore.

Keywords: Breast cancer; neoadjuvant chemotherapy (NAC); Neo-Bioscore; MNeo-Bioscore

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Introduction

Neoadjuvant chemotherapy (NAC) is an application of systematic pre-operative chemotherapy designed to shrink malignant lesions and eliminate potentially micrometastases. NAC allows oncologists to evaluate treatment response and guide subsequent adjustments to treatment regimens based upon tumor burden change. NAC has become the gold-standard treatment for locally advanced breast cancer.

A high pathologic complete response (pCR) rate post-NAC is associated with improved disease-free survival and overall survival (1). To further quantify the relationship between residual tumor and the prognosis of breast cancer patients who do not achieve pCR (non-pCR) after NAC, Jeruss *et al.* (2) developed a staging system that combined pretreatment clinical stage (CS), posttreatment pathological stage (PS), estrogen receptor (ER) status, and nuclear grade (NG) (CPS + EG). Patients with different scores were divided into more refined prognostic subgroups than those of the American Joint Committee on Cancer (AJCC) staging system. Mittendorf *et al.* (3) integrated human epidermal receptor 2 (HER2) status into the previous CPS + EG to develop the Neo-Bioscore staging system, which can be applied in HER2–positive patients treated with trastuzumab. The superior performance of CPS + EG or Neo-Bioscore over PS and CS has been demonstrated in the American and Japanese breast cancer patients receiving NAC (4,5). However, the PS, CPS + EG, and Neo-Bioscore staging systems were proven to be equivalent in predicting Brazilian patients' prognosis (6). Another study based on a Chinese population also did not observe the advantages of CPS + EG and Neo-Bioscore over PS (7). Differences in clinical characteristics of breast cancer patients in different countries may explain these results. The median age of diagnosis of patients in the United States was over 60 years old, while the age of diagnosis of Chinese patients is less than 50 years old. A study evaluating survival differences between Chinese and Caucasian women with breast cancer based on the Surveillance, Epidemiology, and End Results (SEER) database indicated that Chinese breast cancer patients have superior breast cancer-specific survival than Caucasians (8). At present, prognostic models applied to the Chinese population remain limited. It is vital to further validate the performance of Neo-Bioscore in Chinese people to identify a favourable prognostic model for these patients.

Several strategies have been utilized to optimize different prognostic models, such as incorporating other prognostic factors or genetic data, developing interpolation models using continuous, square, and cubic covariates, and optimizing the operation mode. Unfortunately, the high costs of genetic models often make it prohibitive to apply clinically. Xu *et al.* (9) incorporated an additional factor of poor prognosis, HER2-positive status without trastuzumab treatment, to determine an accurate prognosis. We attempted to integrate other prognostic factors into Neo-Bioscore to achieve more precise prognosis predictions.

Researchers have confirmed that pathological nodal status after NAC is significantly correlated to survival and is an independent prognostic factor for local-regional recurrence (10-13). Another staging system, Residual Cancer Burden (RCB), developed based upon the primary tumor and axillary lymph node status after NAC was also reported to be a significant predictor of remote recurrence-free survival. RCB could further categorize patients into different prognostic subgroups when applied to posttreatment AJCC pathologic stage groups (14). Therefore, we would like to explore whether it has an improved predictive ability to replace PS with posttreatment pathological lymph node (ypN) stage and posttreatment pathological tumor (ypT) stage after NAC in Neo-Bioscore.

The objective of the study was to perform an external verification of the Neo-Bioscore in a Chinese cohort from a single institution and then modify the model by replacing PS with two risk factors, ypT and ypN, to improve the prediction of survival outcomes for patients receiving NAC. We present the following article in accordance with the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6738/rc).

Methods

Participants

Clinicopathologic and survival data were collected from a total of 602 patients diagnosed with primary breast cancer

who received NAC and underwent subsequent surgical treatment from February 2005 to August 2018 in the First Medical Center of the PLA General Hospital. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of the Chinese PLA General Hospital (S2021-582-01). Informed consent was waived due to the retrospective nature of the study.

Only patients who underwent core needle biopsy and were diagnosed with invasive breast cancer were included. All patients were treated with an anthracycline- and/or taxane-based NAC regimen. The number of chemotherapy cycles ranged from 2 to 8. After NAC patients received surgical treatment, including breast-conserving surgery, mastectomy, and axillary assessment. Postoperative adjuvant therapy regimens and radiotherapy were based on the patient's preoperative treatment and postoperative pathological results. During the screening, 166 patients were excluded: 32 had metastatic breast cancer, 12 with bilateral breast cancer, 13 with inflammatory breast cancer, and 4 with other malignant tumors; 105 had incomplete medical records. The final analysis included 436 eligible patients.

Clinicopathologic data

Data collected included basic demographic data (age, gender), menopausal stage, CS, PS, ypT, ypN, ER status, HER2 status, progesterone receptor (PR) status, pretreatment Ki67 level, posttreatment Ki67 level, and tumor grade. CS was based on physical examination, mammography, ultrasonography, and magnetic resonance imaging of the breast and regional nodal basins before NAC. CS and PS were determined according to the 8th edition AJCC guidelines. ER, PR, HER2 status, pretreatment Ki67 and histological grading were obtained from the core needle biopsy at diagnosis. We utilized the Nottingham histological grading (rather than nuclear grade) due to its widespread use in clinical practice, which was consistent with the Mayo Clinic validation cohort (15). Posttreatment Ki67 was determined from surgical pathology reports.

ER/PR positive status was defined as 1% or greater of the tumor cells stained for the protein by immunohistochemical. HER2 status was defined as positive when 3 + on immunohistochemistry or fluorescence *in situ* hybridization demonstrated gene amplification. Nottingham histological grade was determined by evaluating tubule formation, nuclear pleomorphism, and mitotic count under the microscope, with a score of 1 to 3 assigned for each parameter. In this system, a combined score of 3–5 points indicated grade 1 (G1), 6–7 points for grade 2 (G2), and 8–9 points for grade 3 (G3). Ki67 level was evaluated by immunohistochemical analysis, and the cutoff value was defined as 14%. pCR was defined as no invasive lesions in the primary breast and axillary.

Outcomes

Disease-specific survival (DSS) (16) was calculated as the time from diagnosis to death caused by breast cancer; overall survival (OS) was defined as the time from diagnosis to death from any cause; disease-free survival (DFS) was defined as the time from diagnosis to the first disease progression (locoregional, distant relapse, ipsilateral or contralateral invasive breast cancer, ductal carcinoma *in situ*, or an invasive second primary cancer) or death from any cause. Data for patients who had no event at the cutoff date for the final analysis were censored at the time of the last disease assessment for DFS and at the last follow-up date for OS and DSS.

All patients were followed every six months by telephone or clinic. The follow-up was completed until July 29, 2021. The follow-up personnel blinded the patients' identity information and clinicopathologic information.

Statistical analysis

Based on the Neo-Bioscore staging system published by Mittendorf *et al.* (3), the point assignment of each component, including pretreatment clinical stage, posttreatment pathologic stage, ER status, tumor grade 3, and the HER2 status, is shown in *Table 1*. Scores of each patient were calculated by summarizing the scores of each component, and patients with the same score were classified into identical subgroups. Continuous variables were converted to categorical variables with a cutoff value of 40 for age and 14% for Ki67. Cox proportional hazards model was used to determine risk factors for DSS. PS was replaced with the ypT and ypN stage in the Neo-Bioscore to create the MNeo-Bioscore (modified Neo-Bioscore) staging system. Here ypT and ypN were each assigned 2 points, and the score distribution of the MNeo-Bioscore is depicted in *Table 1*.

DSS, OS, and DFS rates were calculated for each subgroup of the four models (Neo-Bioscore, MNeo-Bioscore, PS, and CS) utilizing the Kaplan-Meier method

Table 1 Point assignments for Neo-Bioscore and MNeo-Bioscore staging systems

Cancer stage	Score
Neo-Bioscore (7 points)	
CS	
I	0
IIA	0
IIB	1
IIIA	1
IIIB	2
IIIC	2
PS	
0	0
I	0
IIA	1
IIB	1
IIIA	1
IIIB	1
IIIC	2
Tumor marker	
ER negative	1
Grade 3	1
HER2 negative	1
MNeo-Bioscore (9 points)	
CS	
I	0
IIA	0
IIB	1
IIIA	1
IIIB	2
IIIC	2
ypT stage	
T0	0
T1	1
T2	1
T3	2
T4	2
ypN stage	
N0	0
N1	1
N2	1
N3	2
Tumor marker	
ER negative	1
Grade 3	1
HER2 negative	1

CS, pretreatment clinical stage; PS, posttreatment pathologic stage; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; ypT stage, posttreatment pathological tumor stage; ypN stage, posttreatment pathological lymph node stage.

and compared by log-rank test, and Kaplan-Meier curves were plotted. The concordance indexes (C-index) of the four models were calculated; the receiver operating characteristic (ROC) curves were plotted for the four models, and the area under the curve (AUC) values were calculated to assess discrimination. The calibration curves for 3- and 5-year DSS of Neo-Bioscore and MNeo-Bioscore were drawn to evaluate the calibration. For clinical utility, decision curve analysis (DCA) with the threshold probability and net benefit delineated as the horizontal and vertical coordinates to assess the net benefits of the model was performed (17). For the internal validation of the MNeo-Bioscore, C-index and AUC of MNeo-Bioscore were calculated and calibration curves were plotted using the bootstrap resampling method. To compare the four staging systems, the C-index was compared by the "compareC" package. We further drew a time-dependent receiver operating characteristic curve (tdROC) based on the AUC at each time point for the categorized models.

A two-sided αof 0.05 was used for all tests. Statistical analyses were conducted with R version 3.3.1 (The R Foundation of Statistical Computing, Vienna, Austria).

Results

Patient characteristics

Of the 436 patients included, 66.3% had clinical stage II disease (IIA, 31.0%; IIB, 35.3%), and 25.5% had clinical

Table 2 Clinicopathologic DSS data using univariate Cox proportional hazards regression analysis

Variables	Total, N (%)	DSS	
		HR (95% CI)	P value
Age (years)			
≤40	105 (24.1)	Reference	
>40	331 (75.9)	0.51 (0.291–0.882)	0.016
Menopausal status (female)			
Postmenopausal	125 (28.7)	Reference	
Premenopausal	311 (71.3)	0.76 (0.44–1.33)	0.342
CS			
I	36 (8.3)		
IIA	135 (31.0)	0.84 (0.164–4.361)	0.84
IIB	154 (35.3)	5.64 (1.34–23.711)	0.018
IIIA	58 (13.3)	6.95 (1.528–31.573)	0.012
IIIB	24 (5.5)	4.64 (0.845–25.475)	0.077
IIIC	29 (6.7)	6.56 (1.264–34.057)	0.025
ER status			
Positive	277 (63.5)	Reference	
Negative	159 (36.5)	1.2 (0.703–2.055)	0.502
PR status			
Positive	301 (69.0)	Reference	
Negative	135 (31.0)	1.18 (0.671–2.067)	0.569
HER2 status			
Positive	156 (35.8)	Reference	
Negative	280 (64.2)	1.59 (0.881–2.876)	0.123
Pretreatment Ki67			
<14	41 (9.4)	Reference	
≥14	335 (76.8)	1.81 (0.649–5.032)	0.258
Unknown	60 (13.8)		
Posttreatment Ki67			
<14	125 (28.7)	Reference	
≥14	219 (50.2)	2.67 (1.336–5.335)	0.005
Unknown	92 (21.1)		
Tumor grade			
I	14 (3.2)	Reference	
II	275 (63.1)	Reference	
III	147 (33.7)	2.02 (1.192–3.412)	0.009
ypT stage			
T0	77 (17.7)	Reference	
T1	173 (39.7)	2.01 (0.579–7.014)	0.271
T2	145 (33.3)	5.25 (1.597–17.287)	0.006
T3	41 (9.4)	10.06 (2.804–36.125)	<0.0001
ypN stage			
N0	189 (43.3)	Reference	
N1	126 (28.9)	5.61 (2.21–14.268)	<0.0001
N2	70 (16.1)	9.48 (3.671–24.466)	<0.0001
N3	51 (11.7)	18.92 (7.46–47.967)	<0.0001

DSS, disease-specific survival; HR, hazard ratio; CI, confidence interval; CS, pretreatment clinical stage; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; ypT stage, posttreatment pathological tumor stage; ypN stage, posttreatment pathological lymph node stage.

stage III disease (IIIA, 13.3%; IIIB, 5.5%; IIIC, 6.7%). pCR rate was 12.6% in our cohort, with the remaining patients having pathologic stage I (17.0%), IIA (28.9%), IIB (11.7%), IIIA (18.1%), IIIB (0%), or IIIC (11.7%) disease. The pathological T stages of the patients were T0 (17.7%), T1 (39.7%), T2 (33.3%), T3 (9.4%), and T4 stage (0%); the pathological N stages were N0 (43.3%), N1 (28.9%), N2 (16.1%), and N3 (11.7%). Additionally, 76.8% of patients had Ki67≥14% pretreatment and 50.2% posttreatment. Clinical characteristics are displayed in *Table 2*.

The median follow-up time was 67 months (2-192 months). During the entire follow-up period, 59 patients (13.5%) died, (56 died from breast cancer), and 101 patients (23.1%) recurred or metastasized.

External validation of the Neo-Bioscore

The Kaplan-Meier curves of CS, PS, and Neo-Bioscore are shown in *Figure 1A-1C*, respectively, depicting that the three staging systems could significantly distinguish the DSS among different groups (P<0.0001). The ROC curves for 3-year and 5-year DSS are in *Figure 2*. The C-index for the Neo-Bioscore was 0.78 [95% confidence interval (CI): 0.72–0.83], and the AUC for 3-year and 5-year DSS was 0.76 (95% CI: 0.68–0.85) and 0.81 (95% CI: 0.76–0.87),

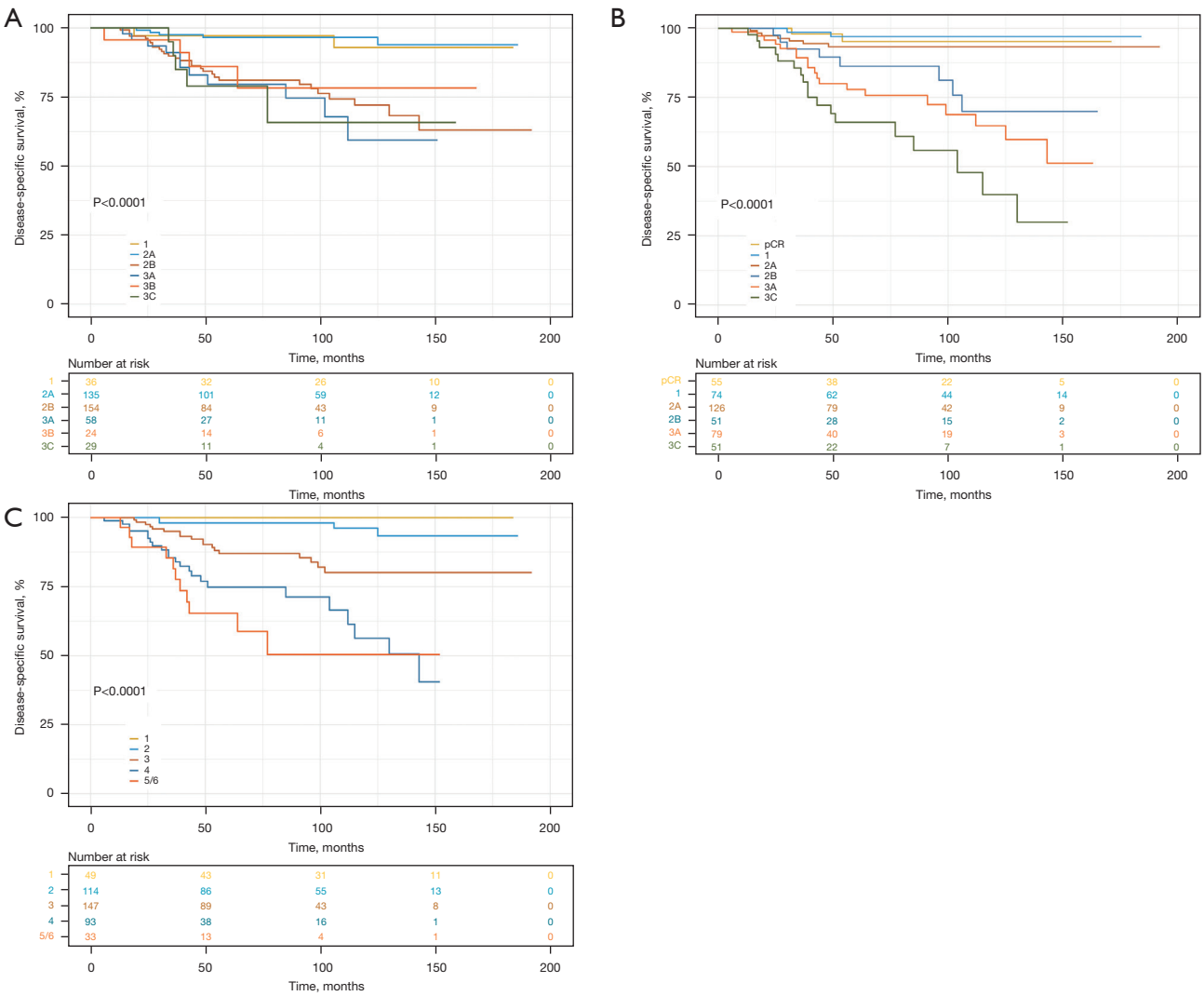

Figure 1 Kaplan-Meier survival curves for disease-specific survival (16) in patients with breast cancer receiving NAC. (A) DSS determined based on the CS. (B) DSS determined based on the PS. (C) DSS determined based on the Neo-Bioscore. pCR, pathologic complete response; NAC, neoadjuvant chemotherapy; DSS, disease-specific survival; CS, pretreatment clinical stage; PS, posttreatment pathologic stage.

respectively. The C-index of PS and CS was 0.75 (95% CI: 0.69–0.82) and 0.68 (95% CI: 0.62–0.74), and the C-index of the Neo-Bioscore was significantly superior to CS (P<0.001) though not significantly different from PS (P=0.399). The calibration curves of the Neo-Bioscore at 3- and 5-year DSS are shown in *Figure 3A*,*3B*. The Neo-Bioscore fit well based on our patient cohort.

Development and internal validation of the MNeo-Bioscore

Univariate analysis disclosed that age, CS, ypT stage, ypN stage, tumor grade 3, and posttreatment Ki67 were significantly correlated with DSS (*Table 2*). In further multivariate analysis, age, ypN, and tumor grade 3 were independent prognostic factors related to DSS (*Table 3*).

Combining the biological effects of aggressive tumor lesions after NAC and the risk ratio of patients in each substage, the single PS variable in Neo-Bioscore was replaced with the ypT stage and ypN stage, each of which was assigned 2 points to create the modified prognostic model (MNeo-Bioscore), and the distribution of scores are presented in *Table 1*. The total score for each patient was the sum of the scores of each component. The DSS curves

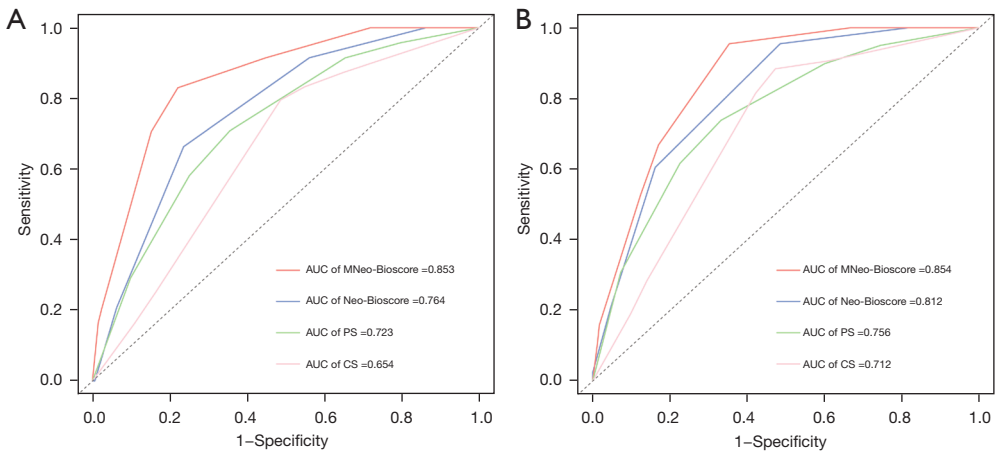

Figure 2 Receiver operating characteristic curves in predicting the 3-year disease-specific survival and 5-year disease-specific survival for the Neo-Bioscore, MNeo-Bioscore CS, PS. (A) predicting the 3-year disease-specific survival for four staging systems. (B) Predicting the 5-year disease-specific survival for four staging systems. AUC, area under the receiver operating characteristic curve; CS, pretreatment clinical stage; PS, posttreatment pathologic stage.

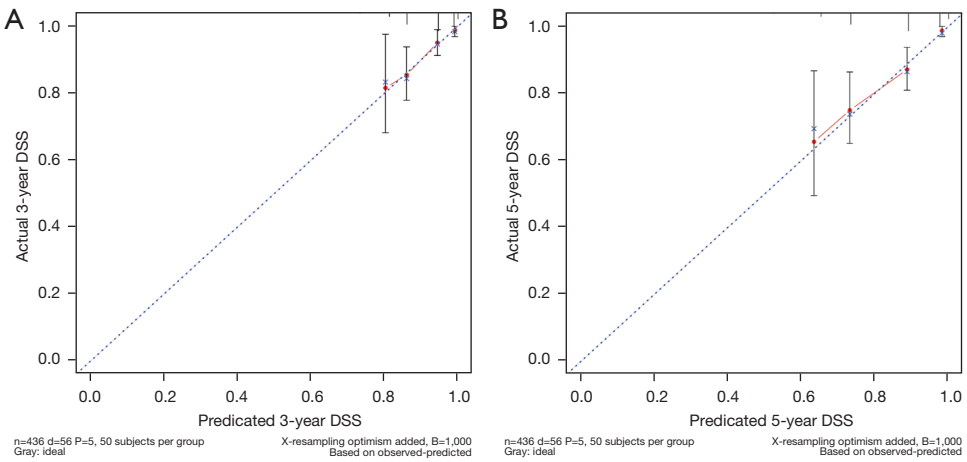

Figure 3 Calibration curve for all patients based on the 3-year disease-specific survival and 5-year disease-specific survival in the Neo-Bioscore. (A) 3-year disease-specific survival in the Neo-Bioscore. (B) 5-year disease-specific survival in the Neo-Bioscore. DSS, disease-specific survival.

based on ypT and ypN are shown in *Figure 4*. The MNeo-Bioscore extended the scoring system to a total of 10 distinct 5-year DSS, OS, and DFS subgroups ranging from 0 to 9 points. Note that there were no patients in the study cohort with a score of 9. The DSS curves of distinct groups are shown in *Figure 5*, with significant differences between the groups (P<0.0001). The DSS of patients in the 7/8 group was significantly worse than that of the other groups.

The MNeo-Bioscore was internally verified through the bootstrap resampling method with a C-index of 0.82 (95% CI: 0.78–0.87); the ROC curves for 3- and 5-year DSS were shown in *Figure 2*, and the AUC was 0.85 (95% CI: 0.78–0.92) and 0.85 (95% CI: 0.81–0.90), respectively. The calibration curves of MNeo-Bioscore for 3- and 5-year DSS are shown in *Figure 6A,6B*, with the MNeo-Bioscore showing a good fit.

Comparison of CS, PS, Neo-Bioscore, and MNeo-Bioscore

The 5-year DSS, OS, and DFS of the study population

Table 3 Multivariable Cox proportional hazards regression analysis for DSS

Variables	Total, N (%)	DSS	
		HR (95% CI)	P value
Age (years)			
≤40	105 (24.1)	Reference	
>40	331 (75.9)	0.379 (0.206–0.697)	0.002
CS			
I	36 (8.3)	Reference	
IIA	135 (31.0)	0.398 (0.071–2.219)	0.293
IIB	154 (35.3)	1.509 (0.325–6.994)	0.599
IIIA	58 (13.3)	1.832 (0.342–9.806)	0.479
IIIB	24 (5.5)	1.307 (0.208–8.231)	0.776
IIIC	29 (6.7)	2.837 (0.465–17.298)	0.258
Posttreatment Ki67			
<14	125 (28.7)	Reference	
≥14	219 (50.2)	1.728 (0.837–3.564)	0.139
Unknown	92 (21.1)		
Tumor grade			
I/II	289 (66.3)	Reference	
III	147 (33.7)	1.858 (1.051–3.287)	0.033
ypT stage			
T0	77 (17.7)	Reference	
T1	173 (39.7)	1.381 (0.301–6.343)	0.678
T2	145 (33.3)	2.547 (0.561–11.552)	0.226
T3	41 (9.4)	2.149 (0.489–9.452)	0.311
ypN stage			
N0	189 (43.3)	Reference	
N1	126 (28.9)	4.193 (1.61–10.925)	0.003
N2	70 (16.1)	5.648 (2.038–15.649)	0.001
N3	51 (11.7)	11.506 (4.254–31.117)	<0.001

DSS, disease-specific survival; HR, hazard ratio; CI, confidence interval; CS, pretreatment clinical stage; ypT stage, posttreatment pathological tumor stage; ypN stage, posttreatment pathological lymph node stage.

were 88.0% (95% CI: 84.6–91.5%), 87.9% (95% CI: 84.5–91.4%), and 76.8% (95% CI: 72.5–81.4%). The 5-year DSS, OS, and DFS of the different subgroups based upon CS, PS, Neo-Bioscore, and MNeo-Bioscore are depicted in *Table 4*. Due to the small number of patients, those with scores 5 and 6 in Neo-Bioscore were combined into the score 5/6 group; scores 7 and 8 in the MNeo-Bioscore were combined into the score 7/8 group, and score 0 and 1 in the MNeo-Bioscore were combined into the score 0/1 group. No patients had 9 points in the MNeo-Bioscore and none with 7 in the Neo-Bioscore. Compared with the other three staging systems, the MNeo-Bioscore staging system could identify a subgroup of very high-risk patients with a lower 5-year DSS (47.8% 95% CI: 27.0–84.4%).

The C-index of the MNeo-Bioscore based on 5-year DSS was 0.82 (95% CI: 0.78–0.87), and the differences between MNeo-Bioscore and the other three staging systems were significant (P<0.05). The ROC curves of PS and CS for 3- and 5-year are displayed in *Figure 2*. The AUC value of MNeo-Bioscore was significantly improved compared to Neo-Bioscore and PS in predicting 3-year DSS (P<0.05), while in predicting 5-year DSS, MNeo-Bioscore was superior to PS (P<0.05) but was not statistically different to Neo-Bioscore (P=0.056). The tdROC based on the four staging systems is shown in *Figure* 7. The MNeo-Bioscore performed the best, followed by the Neo-Bioscore, PS, and CS models in order.

The DCA curves based on CS, PS, Neo-Bioscore, and MNeo-Bioscore are shown in *Figure 8A,8B*. MNeo-Bioscore showed the best clinical usefulness, followed by the Neo-Bioscore and PS, while the CS demonstrated the worst performance.

Discussion

It is critical for clinicians to formulate precise individualized treatment plans for patients with breast cancer who fail to achieve pCR after NAC. Developing an effective prognostic model and dividing patients into appropriate prognostic subgroups according to the risk of disease progression is imperative for guiding individualized follow-up treatment. In this study, based upon a cohort composed of Chinese patients treated in one institution, the Neo-Bioscore exhibited superior discrimination and accuracy in the classification of DSS prediction. However, no noticeable improvement was observed over PS. This finding was consistent with the results of Xu *et al.*'s (9) study based on another Chinese population. Since trastuzumab for the treatment of HER2-positive tumors has been proven to prolong the survival of patients, the Neo-Bioscore defined HER2 negative as an adverse prognostic factor and assigned a score of 1 point (3). HER2-positive patients in China

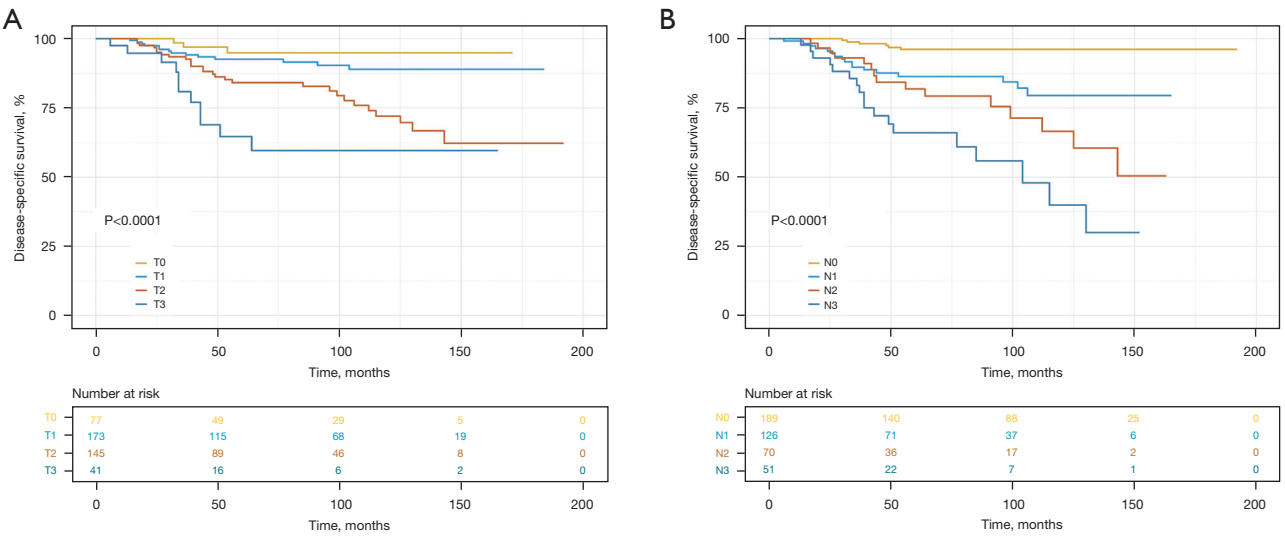

Figure 4 Kaplan-Meier survival curves for disease-specific survival (16) in patients with breast cancer receiving NAC. (A) DSS determined based on the ypT stage. (B) DSS determined based on the ypN stage. NAC, neoadjuvant chemotherapy; ypT stage, posttreatment pathological tumor stage; ypN stage, posttreatment pathological lymph node stage; DSS, disease-specific survival.

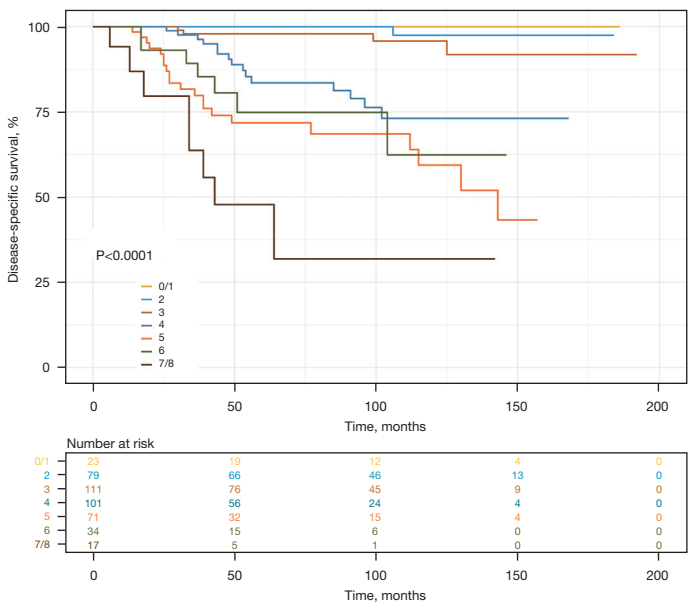

Figure 5 Kaplan-Meier survival curves for disease-specific survival (16) in patients with breast cancer receiving NAC based on the MNeo-Bioscore. NAC, neoadjuvant chemotherapy.

frequently are not treated with trastuzumab due to its high cost. Therefore, the Neo-Bioscore has limited accuracy in predicting the prognosis of HER2-positive patients in that population. Xu *et al.* (18) incorporated HER2-positive status without trastuzumab treatment into Neo-Bioscore, regarding it as more of a risk factor than a HER2-negative status and assigning it a score of 2 points. Interestingly, the improved Neo-Bioscore showed a better 5-year DSS stratification ability than PS (AUC 0.79 *vs.* 0.65; P=0.03) (9).

Considering the independent prognostic role of axillary

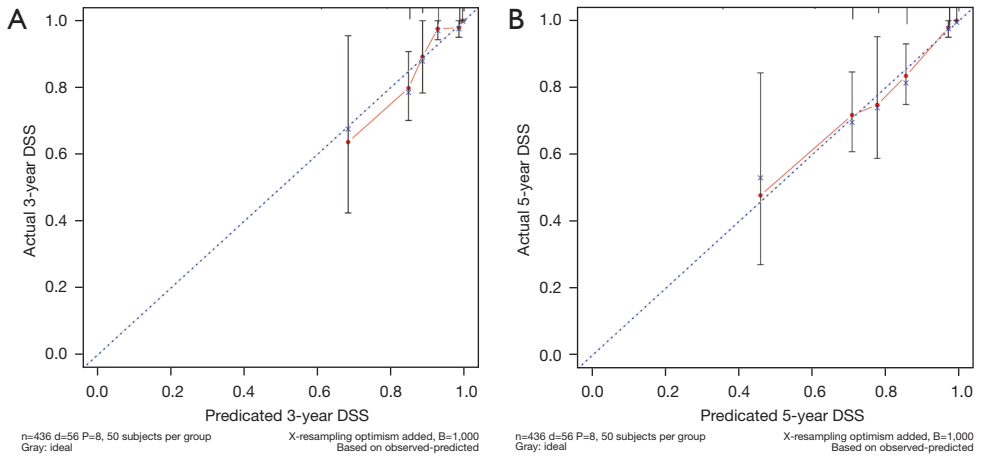

Figure 6 Calibration curve for the bootstrap resampling patients based on the 3-year disease-specific survival and 5-year disease-specific survival in the MNeo-Bioscore. (A) 3-year disease-specific survival in the MNeo-Bioscore. (B) 5-year disease-specific survival in the MNeo-Bioscore. DSS, disease-specific survival.

lymph node burden on patient survival after NAC, we incorporated ypT and ypN as independent factors into the Neo-Bioscore, replacing the pathological stage, to refine the Neo-Bioscore scoring system. Compared with the Neo-Bioscore, the MNeo-Bioscore model included the proportion of residual tumor lesions by superimposing the independent prognostic effects of residual tumor and axillary lymph node burden after NAC. As a result, the MNeo-Bioscore identified a subgroup of extremely high-risk patients with a 5-year disease-specific survival of 47.8 (95% CI: 27.0–84.4). Kantor *et al.* (19) found that RCB, which consists of residual tumor burden and axillary lymph node burden as 2 separate independent factors which had better discrimination of estimated 7-year DFS and OS after NAC compared with Neo-Bioscore. Moreover, Laas *et al.* (20) further demonstrated that RCB also had superior discriminatory performance to the Neo-Bioscore in breast cancer subtypes based on ER, PR, and HER2 status. These results are consistent with our findings and suggest that the direct additive effect of residual tumor burden and axillary lymph node burden alone is superior to the AJCC-TNM anatomic staging.

The advantages of MNeo-Bioscore were reflected in the prognostic stratification of stage III tumors. For patients with PS stage 0, I, IIA, and IIB, the scores remained unchanged or only adjusted by 1 point before and after model revision in the pathological staging module, and the scores remained the same within subgroups, except for patients with stage IIA whose scores were adjusted from 1 point to 1–2 points. Meanwhile, the score of IIIA patients was changed from 1 to 1–3 points, the score for patients with stage IIIB disease was adjusted from 1 point to 2–3 points, and the stage IIIC patient score was modified from 2 to 2–4 points. Thus, there was a broader range of patient scores in the MNeo-Bioscore. The staging system identified extremely high-risk patients with stage $T_{3-4}N_3$ disease with a score of 4 points which are more likely to benefit from postoperative adjuvant chemotherapy. Some disadvantages to the MNeo-Bioscore were also evident. Since tumor size and nodal status were treated entirely independently, the synergistic effect of tumor aggressiveness was ignored. As a result, a subset of patients with stage IIIA scored 1 point, lower than stage IIB or even equal to stage I. This phenomenon does not correspond with the current perceptions regarding tumor staging. Therefore, the improvement of MNeo-Bioscore was mainly reflected in identifying extremely high-risk patients, with relatively attenuated risk stratification for intermediate risk patients. Subsequent work should likely include developing a clustered MNeo-Bioscore, in which patients will be classified into low, intermediate, and high risk based on the total score obtained by the MNeo-Bioscore, after which the predictive efficacy of the modified model should be verified.

The role of Ki67 as a predictor of response to neoadjuvant hormonal therapy is well-established (21,22). However, its value in predicting response to NAC remains controversial (23). Several studies have shown that high Ki67 expression before NAC is significantly associated with the pCR rate after NAC (24,25), while others have found that the Ki67 index after NAC, but not before NAC, to be

Table 4 Five-year disease-specific survival, overall survival, and disease-free survival based on clinical stage, pathological stage, Neo-Bioscore staging system, and MNeo-Bioscore staging system

Staging system	Score/ stage	Patient No.	DSS		OS		DFS	
			5-year rate (%)	95% CI	5-year rate (%)	95% CI	5-year rate (%)	95% CI
CS								
	I	36	97.1	91.8–100	97.1	91.8–100	97.2	92.0–100
	IIA	135	96.6	93.4–100	96.6	93.4–99.9	90.0	84.6–95.9
	IIB	154	81.1	74.1–88.6	80.9	74.0–88.3	67.2	59.4–76.1
	IIIA	58	79.6	67.8–93.6	79.6	67.8–93.6	74.3	61.3–90.0
	IIIB	24	86.0	72.5–100	86.0	72.5–100	55.3	36.9–82.7
	IIIC	29	78.9	62.4–99.8	78.9	62.4–99.8	54.3	37.1–79.6
PS								
	0	55	95.2	88.9–100	100		91.6	84.0–99.9
	I	74	97.0	93.0–100	97.0	93.0–100	92.7	86.7–99.1
	IIA	126	93.3	88.6–98.3	93.3	88.6–98.3	80.7	73.2–89.1
	IIB	50	86.3	75.7–98.4	86.3	75.7–98.4	78.4	66.7–92.1
	IIIA	79	77.9	67.8–89.5	75.4	65.3–87.3	60.3	49.0–74.2
	IIIC	52	66.0	52.4–83.2	64.4	50.8–81.5	46.2	32.5–65.6
Neo-Bioscore								
	1	49	100		100		100	
	2	114	98.1	95.5–100	98.1	95.5–100	90.6	84.9–96.7
	3	147	87.0	80.8–93.7	88.2	82.3–94.5	71.8	63.9–80.6
	4	93	74.8	64.9–86.3	72.7	62.7–84.3	59.9	48.4–74.0
	5/6	33	65.4	49.3–86.7	65.4	43.9–86.7	46.2	30.3–70.6
MNeo-Bioscore								
	0/1	23	100		100		95.5	87.1–100
	2	79	97.5	92.8–100	100		95.7	91.1–100
	3	111	97.9	95.1–100	98.9	96.9–100	84.3	77.0–92.3
	4	101	83.5	74.9–93.1	85.2	77.1–94.3	73.8	64.5–84.4
	5	71	71.8	60.8–84.7	69.1	58.0–82.2	56.3	44.4–71.4
	6	34	74.8	58.8–95.2	72.1	56.0–92.7	62.2	45.5–85.0
	7/8	17	47.8	27.0–84.4	47.8	27.0–84.4	25.6	9.01–72.7

DSS, disease-specific survival; OS, overall survival; DFS, disease-free survival; CI, confidence interval; CS, pretreatment clinical stage; PS, posttreatment pathologic stage.

the only prognostic marker of long-term outcomes (26,27). Furthermore, Matsubara *et al.* (28) and Cabrera-Galeana *et al.* (29) found that the reduction rate of Ki67 after NAC was significantly associated with recurrence-free survival (RFS), and the change of Ki67 before and after NAC was also a significant predictor of patient outcome. Sheri *et al.* (30) combined Ki67 with RCB after NAC as the residual proliferative cancer burden (RPCB), which

provided significantly more prognostic information than used alone. This effect might be associated with resistant and highly proliferative disease in patients with high levels of Ki67 in resected tumors that cannot be eradicated by chemotherapy. In this study, 14% was used as the cutoff value of Ki67 before/after NAC, which is also the cutoff value to distinguish between luminal A and luminal B breast cancer subtypes. The results suggested that posttreatment, instead of pretreatment, the Ki67 level was significantly associated with DSS, and neither were independent prognostic factors. Two main reasons were considered to explain this result. Firstly, the cutoff value of the Ki67 level in this study was small. Tanei *et al.* (26) found that using 50% as the cutoff value of Ki67 before NAC had the highest combined sensitivity for predicting prognosis. Secondly, pretreatment Ki67 and posttreatment KI67 were analyzed as two independent factors in this study. However, considering that Ki67 in different patients responds differently to NAC, the change of Ki67 before and after NAC may provide more significant prognostic implications. Future work will require the introduction of Ki67 into the MNeo-Bioscore and exploring the optimal source and cutoff value of Ki67, which could provide additional prognostic information.

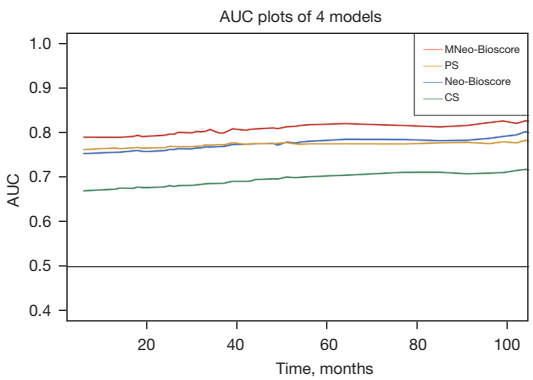

Figure 7 Time-dependent receiver operating characteristic curve based on clinical stage, pathological stage, Neo-Bioscore, and MNeo-Bioscore staging systems. AUC, area under the curve; PS, posttreatment pathological stage; CS, pretreatment clinical stage.

Mittendorf *et al.* (3) confirmed that the CPS + EG staging systems fit nearly identically when ER status was defined at 2 different cutoff values: 1% and 10%. In our study, 1% was selected as the cutoff value. Although ER and HER2 status were not independent prognostic factors for 5-year DSS in this study, ER-negative status was still an essential factor for a poor prognosis considering the molecular subtypes of breast cancer. Additionally, our study applied the Nottingham (also known as Elston-Ellis) histological grade to define tumor grade rather than the nuclear grade. The Nottingham histological grade modified the Scarff-Bloom-Richardson grading system, which has lower inter-observer and intra-observer variability than the nuclear grading system (31), and which evaluates three parameters: tubule formation ratio, nuclear pleomorphism, and mitotic activity counting. This grading system has become the most widely used histological grading system globally for breast cancer and is accepted by a significant number of authoritative bodies, including the World Health Organization (WHO) (32), Union for International Cancer Control (UICC), and the

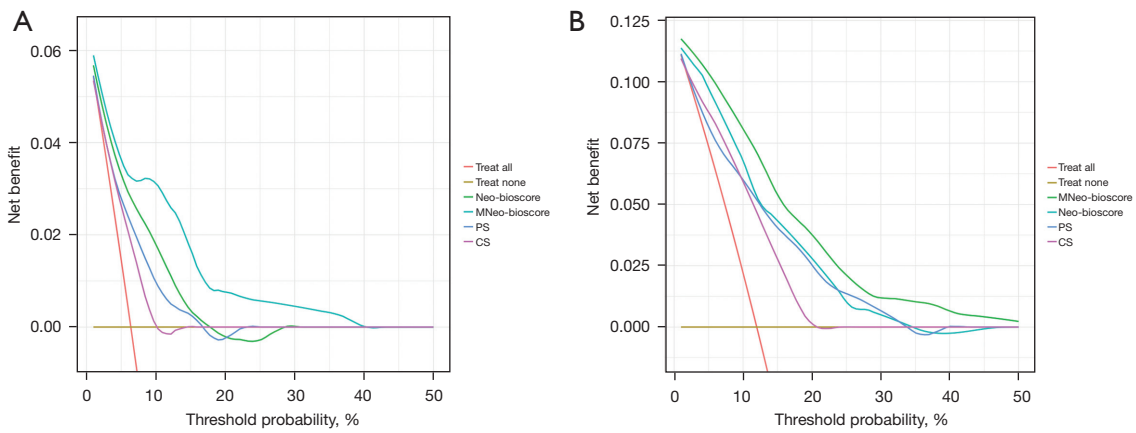

Figure 8 Decision curve of the PS, CS, Neo-Bioscore, and MNeo-Bioscore staging systems at 3 and 5 years. (A) Neo-Bioscore, and the MNeo-Bioscore staging systems at 3 years. (B) Neo-Bioscore, and MNeo-Bioscore staging system at 5 years. PS, posttreatment pathology stage; CS, pretreatment clinical stage.

AJCC (33). Abdelsattar *et al.* (15) validated the stratified prognostic ability of the CPS + EG staging system using the Nottingham grade instead of nuclear grading in an external cohort. In our study, Nottingham histological grade exhibited an independent association with 5-year DSS in multivariate analysis, and the Neo-Bioscore scoring system still showed an excellent ability to stratify prognosis.

Despite promising findings, some limitations to our study should be addressed. First, the data examined were limited, and retrospective in nature and thus, our study was prone to selection and recall bias. Therefore, these results should be confirmed by prospective studies. Second, the number of cases in the higher and lower score groups was small, and adjacent score groups had to be combined for survival analysis. Future studies should incorporate a more significant number of cases for internal validation and employ external validation of the MNeo-Bioscore in combination with patient data from divergent medical centers.

Conclusions

The Neo-Bioscore staging system could precisely stratify the prognosis of patients with breast cancer after NAC in a single-institution Chinese population. Incorporating the ypN and ypT stages in the Neo-Bioscore staging system may optimize the prognostic stratification of patients receiving NAC to improve patient outcomes.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6738/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of the Chinese PLA General Hospital (S2021-582-01). Informed consent was waived due to the retrospective nature of the study.

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