The relationship between hormone receptor status, tumor size and clinical stage and the efficacy of neoadjuvant chemotherapy for locally advanced breast cancer, predictors of survival, and the establishment of Cox-Based risk prediction model and nomogram

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The relationship between hormone receptor status, tumor size and clinical stage and the efficacy of neoadjuvant chemotherapy for locally advanced breast cancer, predictors of survival, and the establishment of Cox-Based risk prediction model and nomogram

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Abstract:

Background: Neoadjuvant chemotherapy (NAC) is currently mainly used for locally advanced breast cancer (LABC), which can improve the patient's chance of surgery and the probability of breast preservation. However, there is currently no effective way to predict the prognosis of neoadjuvant chemotherapy patients.

Methods: This study collected a total of 158 breast cancer patients who received NAC at the Affiliated Hospital of Zunyi Medical University (Zunyi City, China) from January 1, 2010 to May 31, 2016. All relevant data before and after chemotherapy were collected and evaluate the efficacy of chemotherapy through RECIST and pathology. Survival curves were generated by the Kaplan-Meier method and compared using the log-rank test. A nomogram was developed using a binary logisti regression model with a cross validation.

Results: A total of 158 patients were included in our study. Molecular typing of breast cancer, HER-2 positive & HR positive type 14(8.9%), HER-2 positive & HR negaive type 9(5.7%), Luminal A type 44(24.8%), Luminal B type 43(27.2%), TNBC type 48(30.4%). All included investigators were reinstated according to the AJCC 8th breast cancer staging system, 96(60.8%),40(25.3%) and 22(13.9%) patients were in clinical stages IIB, IIIA and IIIB; 97(61.4%). Depending on tumor size 44(27.8%), and 17(10.8%) patients were in T2, T3 and T4. The overall response rate (ORR) was 64.6% (cCR, 3.8%; cPR, 60.8%). The pCR rates wer 12.0% for breast tumor and axillary nodes. The pCR rates were 17.1% for breast tumor and 30.4% for axillary nodes. Our study found that only differences in HER-2 status could predict different clinical efficacy, and clinical remission (CR+PR) was more likely to be achieved if HER-2 positive than negative (16.7% vs 7.5%, p=0.05). There was no significant difference in the clinical efficacy of neoadjuvant chemotherapy in age, clinical stage, and molecular classification of patients. In a single factor COX proportional hazard model study, it was found that ER positive relative to HR negative(HR 0.479,CI 95% 0.272-0.844, p=0.011), PR positive relative to PR negative (HR 0.423,CI 95% 0.235-0.763, p=0.004), HER-2 positive relative to HER-2 negative (HR 2.011, CI 95% 1.000-4.042, p=0.050), TNBC (HR 2.229, CI 95% 1.189-4.178, p=0.012), and HER-2 (HR 2.808, CI 95% 1.301-6.062, p=0.009) type are more effective than patients with Luminal type breast cancer 0btain a longer survival time, which is considered a protective factor. A nomogram was developed based on the clinical and statistically significant predictors. We could estimate the probability of 3- and 5-year OS. The calibration plot for the probability of OS at 3 or 5 years showed a good correlation between the nomogram prediction and actual observation. ROC curve for 3-year survial with area under the curve = 0.731 (95% confidence interval, 0.663-0.828); ROC curve for 5-year survial with area under the curve = 0.743 (95% confidence interval,

0.652-0.834); ROC curve for 10-year survial with area under the curve = 0.726 (95% confidence interval, 0.639-0.812).
Conclusion: In conclusion, the hormone receptor status before chemotherapy can predict the prognosis of patients in advance, and we successfully constructed nomogram, which can help us predict the OS of patients with NAC. However, there was no significant difference between OS/DFS and pCR/RR in patients with NAC for LABC.
Key words: Locally advanced breast cancer, Neoadjuvant chemotherapy, Pathological response, Nomogram

1. Introduction

Breast cancer is one of the malignant tumors that seriously threaten women's health[1]. According to the latest global cancer statistics[2], the incidence of breast cancer has increased globally, and also in China[3, 4]. Currently, the treatment of breast cancer includes surgery, chemotherapy, radiotherapy, endocrine therapy and targeted therapy, and chemotherapy plays a very important role in the treatment of breast cancer. Chemotherapy includes preoperative neoadjuvant chemotherapy (NAC) and postoperative adjuvant chemotherapy. NAC is a systemic treatment for breast cancer that is prior to definitive surgical treatment. In locally advanced and large operable tumors, NAC may reduce the tumor size and achieve operability, or
reduce the extent of the surgery. Furthermore, it checks then sensitivity of certain drugs to the tumor or determines which drugs will achieve the optimal response[5-8]. One of the main goals of neoadjuvant chemotherapy is to achieve a pathologic complete response (pCR). Patients who achieve pCR after neoadjuvant chemotherapy for breast cancer have a much better prognosis than those who do not.[9, 10]. However, relevant studies have found that 10-35% of breast cancer patients are not sensitive to NAC, and about 5% of breast cancer patients have enlarged tumors after NAC[11, 12]. Instead, they miss the best time for surgery. The well-known clinical trials NSABP b-18 and b-27 combined analysis suggested that age, clinical tumor characteristics before NAC, and pathological nodule status/breast tumor response after NAC predicted the risk of local recurrence in breast cancer patients[13].

However, there is still controversy regarding the evaluation of the efficacy of neoadjuvant chemotherapy for breast cancer and its impact on prognosis. In this study, we explored the factors that influenced the efficacy of NAC, as well as the overall survival (OS) and disease-free survival (DFS) time of patients after NAC. Furthermore, a prognostic model was established to facilitate patients obtaining greater benefits from NAC.

2. Materials and methods

2.1 Patients

This retrospective study was approved by the ethics committee.

A total of 158 breast cancer patients who received NAC at the Affiliated Hospital of Zunyi Medical University (Zunyi, China) between January 01, 2010 and May 31, 2016 were recruited for the present study. All patients were confirmed via biopsy to have breast cancer prior to receiving NAC treatment and they received no other treatment. The inclusion criteria were as follows: 1) Female; 2) Biopsy proven primary invasive breast cancer without distant metastasis; 3) Locally advanced breast cancer, stage IIB-IIIB; 4) No history of other cancers; 5) Complete NAC with no prior treatment; 6) Surgery followed by a pathologic examination performed after completion of NAC. The exclusion criteria were as follows:1) Male; 2) The biopsy was diagnosed as carcinoma in situ and early breast cancer; 3) Bilateral breast cancer; 4) Combined with a history of other cancers; 5) NAC treatment not completed; 6) surgery not performed at our hospital or no postoperative pathologic assessment. The included patients were divided into different subgroups according to the chemotherapy response. A flowchart of the study population is presented in Figure 1.

2.2 Immunohistochemistry and response assessment

Based on nuclear staining of estrogen receptor (ER) and progesterone receptor (PR), we defined < 1% positive tumor cells as ER/PR negative and ≥ 1% positive tumor cells as ER/PR positive[14]. The cutoffs for Ki67 level were < 20% and ≥ 20%. For HER2 status validation, immunohistochemistry (IHC) scored as 3+ was defined as HER2 positive; IHC scored as 0 or 1+ was defined as HER2 negative; and if IHC was scored as 2+ , further confirmation using molecular tests (in situ hybridization [ISH]) was obtained. ISH non-amplified results were defined as HER2 negative, and ISH amplified results were considered HER2 positive. The molecular subtype was classified according to the 13th St Gallen International Breast Cancer Conference (2013) Expert Consensus[15].

The clinical efficacy evaluation were evaluated according to the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1). At least a 30% decrease in the sum of the diameters of the tumor was considered a partial response (PR). Progressive disease (PD) was defined as an increaseof >20% in the sumof the diameters. Stable disease(SD) was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. Evaluation of pathological effect： A pCR was defined as no residual invasive breast cancer in the histopathology specimen of the breast and axillary lymph nodes (ypT0/ypTisN0). A B-pCR was defined as no residual invasive breast cancer in the histopathology specimen of the breast (ypT0/ypTis). A L-pCR was defined as no residual invasive breast cancer in the histopathology specimen of the axillary lymph nodes (ypN0).

2.3 Neoadjuvant chemotherapy regimen

All patients received 2-6 cycles of NAC with the 'TEC/TE' chemotherapy regimen (docetaxel, 75 mg/m 2 ; epirubicin, 75 mg/m 2 ; cyclophosphamide, 500 mg/m 2) before surgery. Patients with positive receptor 2 were given the targeted drug herceptin (8 mg/kg body mass for the first time, followed by 6 mg/kg body mass). Drug treatment for 21 days was considered as 1 cycle and an interval of 20 days occurred following. Image examinations were performed prior to the next NAC cycle. Surgical excision was performed within 20 days after drug treatment. A flow chart illustrating the chemotherapeutic regimen Figure 2.

2.4 Follow-up

All enrolled patients were followed up for a long time by telephone or in an outpatient clinic, focusing on whether the patient had local recurrence, distant metastasis and was still alive. Overall survival (OS) is defined as the patients first diagnosed with breast cancer to the time of death from the disease; Disease-free survival (DFS) was defined as the time after surgery when the patient had no disease metastasis or recurrence.

2.5 Statistical analysis

All statistical calculations were carried out using SPSS 24.0 (SPSS Inc, USA). All P-values were two-sided and the a-value was set at 0.05. To assess differences in categorical and continuous variables, Pearson's Chi square, independent samples t test, and one-way analysis of variance (ANOVA) were performed. A log-rank test and the KaplaneMeier method were used for survival analysis. Prognostic variables were submitted to multivariate analysis using Cox's proportional hazard regression model. The Cox model has an exponential form (see Equation (1)), where t represents the time that the event occurs; h(t) is the hazard function for a subject at time t, determin by a set of m covariates ($X_1,X_2,X_3,\ldots\ldots,X_k$); $\beta_1, \beta_2,\ldots\ldots,\beta_k$ are the regression coefficients that measure the effect size of covariates; exp is the exponential function (exp(X) = ex); $h0(t)$ is the baseline hazard rate, an arbitrary (unknown) function, corresponding to the value of the hazard when all X_i equal zero.

$$h(t) = h_0(t)\exp(\beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k) \quad \ldots\ldots(1)$$

A nomogram based on possible prognostic factors associated with OS/DFS was established using R software, on the basis of the Cox regression model. The performance of the nomogram was assessed with respect to discrimination and calibration. The discriminative abilities of prognostic models were evaluated with Harrell's concordance index (C-index). The C-index estimates the probability of concordance between the observed OS and

OS that are predicted from the model. The value of the C-index statistic ranged from 0.5 to 1, with 1 indicating perfect concordance, 0.5 indicating no better concordance than chance, and higher C-index values indicated a better prognostic model. Calibration was quantified by comparing the predicted OS with that of the observed survival against the nomogram's 3- , 5- and 10-year predicted OS/DFS.

3. Results

3.1 Characteristics of patients

A total of 158 patients were included in our study. By May 31, 2019, the median follow-up was 51.91±23.989 months (range 6-112 months). The median age was 46.91±8.158 years (range 25–68 years). Except for 7 cases of other types of breast cancer, all of the pathological types were invasive ductal carcinoma, of which 80 were right invasive ductal carcinoma and 71 were left invasive ductal carcinoma. All included investigators were reinstated according to the AJCC 8th breast cancer staging system, 96(60.8%),40(25.3%) and 22(13.9%) patients were in clinical stages IIB, IIIA and IIIB stage. Depending on tumor size 44(27.8%), and 17(10.8%) patients were in T2, T3 and T4. The overall response rate (ORR) was 64.6% (cCR, 3.8%; cPR, 60.8%). For both breast and lymph nodes, the pCR rate was 12.0%, while for breast tumors, the pCR rate was 17.1%. Or if only the lymph nodes reached pCR, it was 30.4%.The clinical and pathological data of all patients are shown in table 1.

3.2 Clinical and pathological response to NAC

In 2016, a study found that, in patients accepting NAC, the age, clinical tumor characteristics before NAC and pathological nodal status after NAC/breast tumor response could be used to predict the risk of locoregional recurrence (LRR)[13]. Our study found that only differences in HER-2 status could predict different clinical efficacy, and clinical remission (CR+PR) was more likely to be achieved if HER-2 positive than negative (16.7% vs 7.5%, p=0.05). There was no significant difference in the clinical efficacy of neoadjuvant chemotherapy in age, clinical stage, and molecular classification of patients. However, under the pathological curative effect evaluation system, only breast cancer patients had different HER-2 status before NAC, and different PCRs were achieved, and HER-2 positive status was more able to obtain PCR (26.1% vs 9.6%, P=0.025). The relevant factors affecting the clinical and pathological response of NAC are shown in Table 2.

3.3 Survival outcomes

As shown in Table 3, the Kaplan–Meier method found that the relevant factors affecting the survival of patients accepting NAC included: the overall survival time is longer in patients in early stage, with HER-2 positive, HER-2 positive /HR negative or small size tumor ($p<0.05$); relapse or metastasis was a risk factor that reduced the patient's survival ($p<0.05$) (Table 3 and Figure 3) .The results for DFS seemed to be consistent with OS. Whereas, PCR or CR+PR obtained after NAC did not affect the OS and DFS. This also confirmed that PCR or clinical response was not an alternative endpoint for survival in NAC patients[16]. According to our research, different molecular types had different DFS, and HR+ patients had longer DFS. Tumor size was also an important factor affecting DFS. The smaller the primary tumor diameter, the longer it takes to obtain DFS (Table 4).

3.4 Predictive model

The molecular typing of breast cancer was determined by the status of ER, PR, HER-2, and ki-67, thus we established a multivariate Cox proportional hazards model, and to reduce co-linearity just single factors that had no effect on each other was included, while none of the factors were related to OS. However, in a single factor COX proportional hazard model, it was found that ER positive relative to ER negative (HR 0.479, CI 95% 0.272-0.844, p=0.011), PR positive relative to PR negative (HR 0.423,CI 95% 0.235-0.763, p=0.004), HER-2 positive relative to HER-2 negative (HR 2.011, CI 95% 1.000-4.042, p=0.050), TNBC relative to other subtypes of breast cancer (HR 2.229, CI 95% 1.189-4.178, p=0.012), and HER2 positive & HR positive type relative to other subtypes of breast

cancer (HR 4.596, CI 95% 1.763-11.980, p=0.002) were more obtain a longer survival time, which are considered protective factors (Table 5). Moreover, the prognostic predictive model was made based on factors that were significantly correlated with prognosis, such as state of ER, PR, Her-2, clinical stage and tumor size, analyzed by the Single variable Cox logic hazards model (Table 6). It had good discrimination performance (C-Index 0.7065; 95% CI, 0.633-0.779). The model was verified separately and found that the same 3-, 5- and 10-year are good discrimination performance: ROC curve for 3-year survial with area under the curve is 0.731 (95% confidence interval, 0.633-0.828); ROC curve for 5-year survial with area under the curve is 0.743 (95% confidence interval, 0.652-0.834); ROC curve for 10-year survial with area under the curve is 0.726 (95% confidence interval, 0.639-0.812) (Figure 4). The Prediction Function followed:

$$h(t)=h0(t)exp(-0.261\ ER(+)-0.537\ PR(+)+0.575\ HS(+)-1.187\ CS(IIIA)-18.43\ CS(IIIB)+1.451TS(T3)+19.81TS(T4)) \quad (2)$$

Nomogram is a two-dimensional graph that represents a mathematical function involving multiple predictive variables[17]. A nomogram incorporating all significant independent factors for predicting 3- and 5-year OS was established based on selected variables with hazard ratios (Figure 5). The nomogram incorporates five factors: ER, PR, and HER-2 status, clinical stage, and tumor size. In Figure 3, each predictor has a set of scales, and there is a mapping between each scale and the “Points” scale. The bottoms are the corresponding 3-year and 5-year survival estimates, and the median survival time (years). Each variable was given a score on a points scale. By adding up the total scores projected in the bottom scale, we could estimate the probability of 3- and 5-year OS. The calibration plot for the probability of OS at 3 or 5 years showed a good correlation between the nomogram prediction and actual observation (Figure 6).

The risk model of DFS was formulated by the same method, but the c-index of the model was only 0.656<0.7, 95% CI (0.574,0.738), which was not predictive.

4. Discussion

NAC plays an important role in the treatment of breast cancer. In a well-known clinical trial, the National Surgically Assisted Breast and Bowel Project (NSABP) B-18, which compared the OS and DFS of patients in the preoperative and postoperative chemotherapy groups, showed no statistically significant difference in long-term follow-up. In present study among which NAC reached PCR and some studies indicated that pCR 12% reached after NAC, our result was consistently (11%) with that of Chou et al[18] and the RR (CR+PR) 64.6%. We found that pCR was more available in NAC in patients with HER-2 positive than in those with HER-2 negative, which was consistent with relevant literatures[19, 20]. Studies had reported that those who achieved pCR after NAC predicted better prognosis [12, 21, 22] and longer OS[19]. However, in our study, neoadjuvant breast cancer patients from 2010 to 2016, including up to 10 years, were not found to have achieved clinical remission (CR+PR) or pCR consistent with survival: those who achieved pCR had a longer total survival time. At the 16th St. Gallen International Breast Cancer Conference, it was suggested that alternative endpoints could not replace long-term survival endpoints. Cortazar found that patients with complete pathological response to NAC in breast cancer had significantly improved DFS and OS[23]. However, further analysis from clinical trials, the improvement of the pathological complete remission rate did not show a significant correlation with the patient's OS. In our study, it was found that not all HER-2 positive patients used targeted therapy throughout the course. The reason for this phenomenon may be that Zunyi in China is a relatively remote and poor city, and most of the patients treated were of poor economic conditions. What’s more, the period of time included in this study was from 2010 to 2016, the time span is long, the longest is up to 10 years, and targeted therapy had not been fully invested in clinical treatment. Therefore, when studying the survival prognosis between HER-2+ patients and HER-2- patients, the conclusion was open to question, but combined with the relevant literature,

our results were credible and had certain guiding significance for our clinical work in the future.

Although in our study, we did not find that obtaining pCR had better OS/DFS, the life quality patients who obtained pCR was higher. So what affects whether a patient gets a pCR? In our study, only HER-2 status was correlated with pCR (p = 0,025), and no correlation was found between patients' age and pCR. Chou et al. reported that the age (<50 years) was an important factor (p=0.031) that affected whether the patient obtained pCR: the younger the age, the better the pCR could be achieved in the NAC process[18]. However, Lin et al. retrospectively reported that age was not a factor that affected obtaining pCR, and breast cancer patients in different regions had different factors affecting geting pCR or having a good prognosis in NAC[24, 25]. This may be related to differences in regional, environmental, genetic and ethnic factors. Ki-67 is a nuclear antigen that exists in all stages of the cell cycle, but it is not expressed in the G0 resting phase, so it is an indicator of cell proliferation. Many studies have found that Ki-67 can be used as an important predictor of pCR obtained by chemotherapy.[26], when Ki≤35% (vs Ki-67> 35%), NAC obtains more pCR (p = 0.0001)[27], and this is inconsistent with the conclusions of our study. Our study found that patients with Ki-67 <20% did not get better pCR in NAC (p = 1.4 > 0.05). The inconsistency leading to the conclusion may be related to the cutoff value. The cutoff value taken in this study is 20%, while the cutoff value taken by him is 35. There is no clear conclusion about the size of Ki-67. According to the latest clinical guidelines, the cut-off value of Ki-67 is recommended to be 20%.

Compared with patients who selected surgery, the breast cancer patients who had not undergone surgical treatment after NAC achieved cCR and their 5-year OS was not significantly different from those who underwent surgery (p = 0.15)[28]. Does this remind us that some patients could give up surgery when they reach cCR or pCR after NAC, and choose long-term testing or degrading treatment? In our research, this point was not involved, but was worth pondering. Minna et al found that among 202 breast cancer patients who received neoadjuvant chemotherapy, TNBC patients were more able to obtain pCR than other subtype breast cancer patients[29]. However, this conclusion was not reached in our study, probably because Our cohort is not large enough to accurately verify whether different pairs of molecular typing are related to pCR.

In addition to the response after neoadjuvant chemotherapy is related to OS/DFS, is the patient's initial clinical characteristics also related to OS/DFS? In order to find more prognostic factors, we found that the hormone receptor status of the patient before surgery and the size of the patient's tumor were related to the survival prognosis of the patient: the smaller the tumor, the longer the OS/DFS (p <0.001, p = 0.004); compared with HR negative, HR positive can obtain longer OS and DFS (p = 0.003, p = 0,011). Jonathan et al's analysis of 103 neoadjuvant chemotherapy studies for locally advanced breast cancer found that the tumor size of patients before chemotherapy was related to LRR (p = 0.003)[30], and our results are consistent with this. Chou et al confirmed that the younger patients (<50years) is the only independent factor favorable for LRR-freesurvival in patients with breast cancer after NAC with concurrent epirubicin and docetaxel[18]. However, other studies have concluded that age is not related to the prognosis of neoadjuvant chemotherapy[27]. In our study, it was found that the patient's age was not significantly related to OS/DFS. Does this remind us that the age of the patient at the time of chemotherapy is not necessarily a significant factor affecting the prognosis of the patient, and the reference value of the age when we select the patient for neoadjuvant chemotherapy is worthy of further discussion.

Nomograms were considered to be an alternative or new standard for predicting survival and prognosis of malignancies compared to conventional AJCC TNM staging classification[31, 32]. Additionally, nomograms facilitated decision-making under complicated clinical conditions without needing standard guidelines[33, 34]. In order to establish and confirm a nomogram of prognostic factors for breast cancer patients undergoing neoadjuvant chemotherapy, we collected relevant clinical data of 158 neoadjuvant breast cancer patients. The constructed nomogram successfully

predicted the 3-year, 5-year, and 10-year OS of NAC patients, demonstrating good resolution and correction rate. Collecting relevant clinical data of the included patients through statistical analysis and up to 10 years of survival follow-up data, we found that the patient's ER, PR and HER-2 hormone receptor status, clinical stage and tumor size were significantly related to OS, this also confirmed by other researchers[18, 22, 23]. In the constructed nomogram, the C-index were 0.731 for the 3-year forecast, 0.743 for the 5-year forecast, and 0.726 for the 10-year forecast.

This study had several limitations. First, the survival rate predicted using the nomogram had a good correlation with the actual operating system, but it lacked a nomogram that successfully builded a predictable DFS. Secondly, this study was a retrospective study. Of the breast cancer patients recruited from 2010 to 2016, only 158 patients completed the complete course of neoadjuvant chemotherapy, and during this period, as the diagnosis and treatment guidelines, the inclusion of patients was uneven, which has a certain impacted on the results of regional control. Therefore, the provided nomograms needed to be copied and then prospectively verified before they were used in clinical practice.

5. Conclusions

In summary, there is no significant difference in whether OS/DFS of patients with locally advanced breast cancer neoadjuvant chemotherapy achieves pCR/RR. During the clinical process, physicians should not blindly pursue pC/RR. However, the ER/PR/HER-2 expression, clinical stage, and tumor size of patients before chemotherapy are OS that can help us predict in advance.

Supplementary Materials:

The clinical and pathological data of all patients are shown in table 1. The relevant factors affecting the clinical and pathological response of NAC are shown in Table 2. As shown in Table 3, the Kaplan–Meier method found that the relevant factors affecting the survival of NAC. The factors affecting the DFS are shown in Table 4. A nomogram incorporating all significant independent factors for predicting 3-year OS and 5-year OS was established based on selected variables with hazard ratios (Figure 5). The calibration plot for the probability of OS at 3 or 5 years showed a good correlation between the nomogram prediction and actual observation (Figure 6).

Abbreviations:

ER: estrogen receptor; PR: progesterone receptor; HR: Hormone receptor; HER-2: human epidermal growth factor receptor 2; pCR: pathological complete response; cPR: clinical partial response; cSD: clinical stable disease; cPD: clinical progressive diease; R-IDC: Invasive ductal carcinoma of the right breast; L-IDC: Invasive ductal carcinoma of the left breast; B-pCR: breast-pathological complete response; L-pCR:Lymph node-pathological complete response; cPR: clinical partial response; cSD: clinical stable disease; cPD: clinical progressive diease.

Author Contributions:

Conceptualization, G.F.J, L.Q, and S.S.H; methodology, G.F.J, L.Q. and S.S.H; validation, L.Q, X.M.X, X.L, and J.N; formal analysis, G.F.J, S.S.H, L.Q, and X.M.X; investigation, G.F.J, X.M.X, W.L, Z.M.Y, and L.Q.Y; resources, G.F.J, X.M.X, L.Y, and W.Q.Y; data curation, G.F.J and S.S.H; writing-original draft preparation, G.F.J; writing-review and editing, G.F.J, L.Q, and S.S.H; visualization, G.F.J, L.Q, S.S.S, X.M.X, X.L, L.Y, W.Q.Y, and J.N; supervision, L.Q and S.S.H; project administration, L.Q and S.S.H; funding acquisition, L.Q and S.S.H. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials:

The datasets generated and/or analyzed during the current study are not publicly available due to privacy restrictions but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate:

The research protocol has been approved by the Ethics Committee of Zunyi Medical University.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

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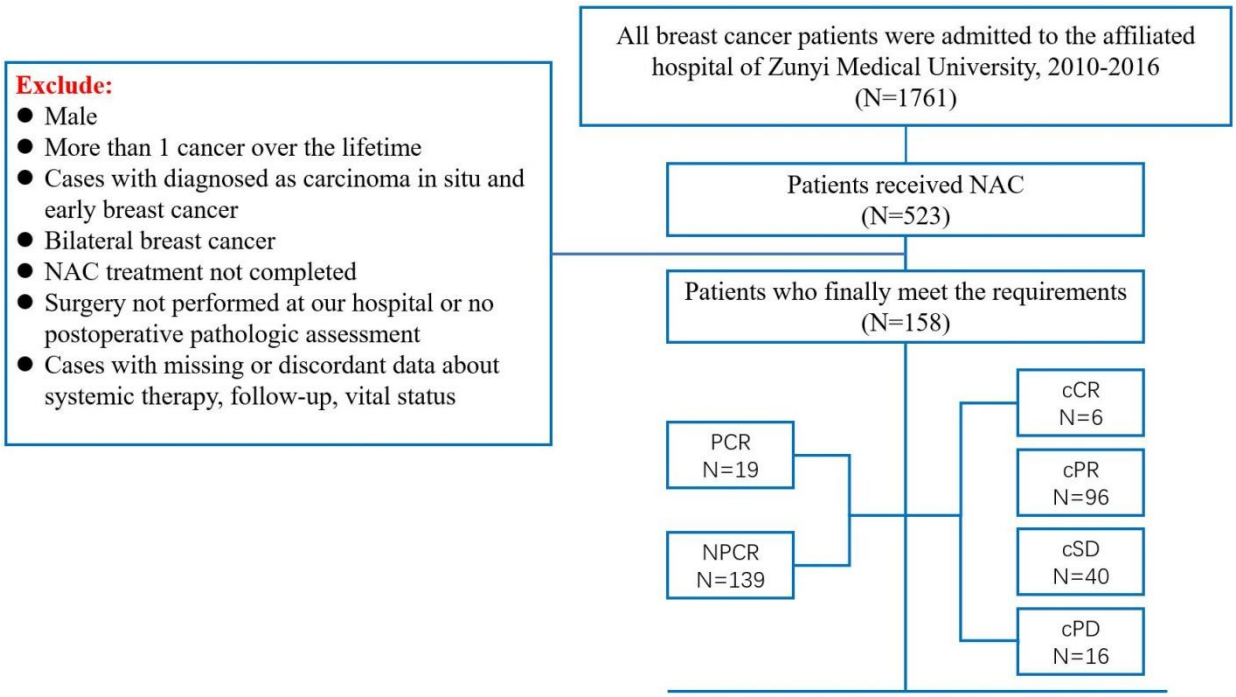

Fig.1 Flow chart of the study population with exclusion criteria.

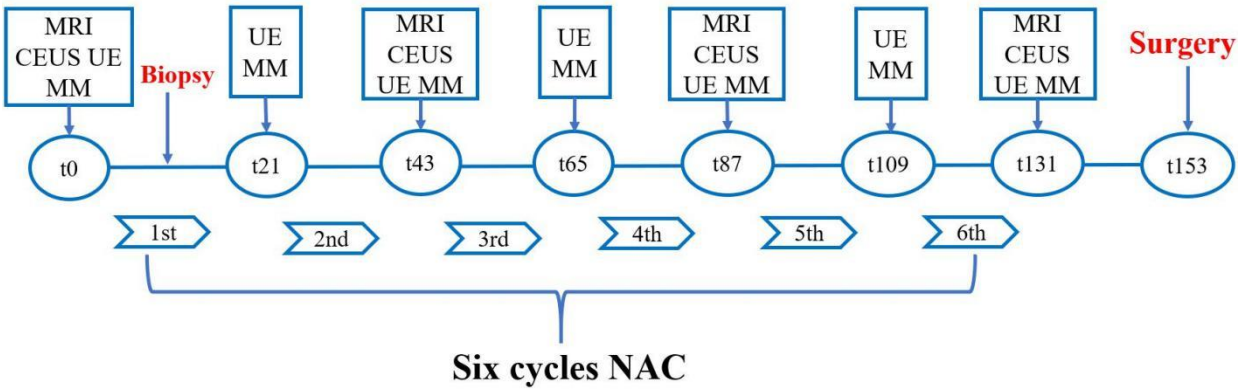

Fig.2 Flow chart of NAC. Each cycle lasted for 21 days and surgical excision was performed within 20 days after 6 cycles. Thorough examinations are performed every two sessions. (CEUS, contrast-enhanced ultrasonography; UE, ultrasound elastography; MM, mammary gland molybdenum target; MRI, magnetic resonance imaging)

TABLE 1. Clinical, and pathological characteristics of breast cancer patients who received neoadjuvant chemotherapy

characteristics	N(%)	characteristics	N(%)
Age(years)		**HER2 status**	
Mean(SD)	46.91(8.158)	**HER2 negative**	135（85.4）
Median(Range)	47(25-68)	**HER2 positive**	23(14.6)
Follow-up time		**HR & HER2**	
Median(Range)	51（6-112）	**HR negative/HER2 negative**	48(30.4)
Tumor pathology(biopsy)		**HR negative/HER2 positive**	14(8.9)
R-IDC	80(50.6)	**HR positive/HER2 negative**	87(55.2)
L-IDC	71(45.0)	**HR positive/HER2 positive**	9(5.5)
Others	7（4.4）	**Molecular classification**	
Estrogen receptor(ER)		**HER2 positive & HR positive type**	14(8.9)
ER negative	67(42.4)	**HER2 positive & HR negative type**	9(5.7)
ER positive	91(57.6)	**Luminal A type**	44(24.8)
Progesterone receptor(PR)		**Luminal B type**	43（27.2）
PR negative	78(49.4)	**TNBC type**	48（30.4）
PR positive	80(50.6)	**Clinical stage**	
Hormone receptors(HR)		**IIB**	96（60.8）
HR negative	62(39.2)	**IIIA**	40（25.3）
(ER negative and PR negative)			
HR positive	96(60.8)	**IIIB**	22（13.9）
(ER positive and/or PR negative)			
Tumor size		**Clinical response**	
T2	97（61.4）	**cCR**	6(3.8)
T3	44（27.8）	**cPR**	96(60.8)
T4	17（10.8）	**cSD**	40(25.3)
Grade		**cPD**	16(10.1)
G1	5(3.2)	**RR(cCR+cPR)**	102(64.6)
G2	108(68.4)	**non-RR(cSD+cPD)**	56(35.4)
G3	45(28.5)	**Pathological response**	
Postoperative lymph node metastasis		**pCR**	19（12.0）
NO(0)	47(29.7)	**Non-pCR**	139(88.0)
N1(1-3)	56(35.4)	**Pathological response of Breast**	
N2(4-9)	38(24.1)	**B-pCR**	27(17.1)
N3(≥10)	17(10.8)	**non-B-PCR**	131(82.9)
Chemotherapy drugs		**Pathological response of Lymph node**	
Taxol & Anthracycline	78(49.4)	**L-pCR**	48(30.4)
Taxol & Anthracycline & CTX	80(50.6)	**Non-L-pCR**	110(69.6)
Total patients	158(100)	**Total patients**	158(100)

Abbreviations: R-IDC: Invasive ductal carcinoma of the right breast; L-IDC: Invasive ductal carcinoma of the left breast; ER: estrogen receptor; PR: progesterone receptor; HR: Hormone receptor; HER-2: human epidermal growth factor receptor 2; pCR: pathological complete response; B-pCR: breast-pathological complete response; L-pCR:Lymph node-pathological complete response; cPR: clinical partial response; cSD: clinical stable disease; cPD: clinical progressive diease.

TABLE 2 . Factors influencing the efficacy of neoadjuvant chemotherapy in breast cancer patients

characteristics	CR+PR	SD+PD	P	PCR	NPCR	p
	N(%)	N(%)		N(%)	N(%)	
Age at entry (years)						
≤50	75(66.4)	38(33.6)	0.45	15(13.3)	98(86.7)	0.444 [a]
>50	27(60.0)	1840.0)		4(8.9)	41(91.1)	
Clinical stage			0.241			0.901 [a]
IIB	66(68.8)	30(31.3)		12(12.5)	84(87.5)	
IIIA	25(62.5)	15(37.5)		5(12.5)	35(87.5)	
IIIB	11(50.0)	11(50.0)		2(9.1)	20(90.9)	
Estrogen receptor(ER)						
ER negative	43(64.2)	24(35.8)	0.932	10(14.9)	57(85.1)	0.336
ER positive	59(64.8)	32(35.2)		9(9.9)	82(90.9)	
Progesterone receptor(PR)						
PR negative	53(67.9)	25(32.1)	0.379	13(16.7)	65(83.3)	0.077
PR positive	49(61.3)	31(38.8)		6(7.5)	74(92.5)	
Hormone receptors(HR)						
HR negative	41(66.1)	21(33.9)	0.74	10(16.1)	52(83.9)	0.202
(ER negative and PR negative)						
HR positive	61(63.5)	35(36.5)		9(9.4)	87(90.6)	
(ER positive and/or PR negative)						
HER2 status						
HER2 negative	83(61.5)	52(38.5)	0.050 [a]	13(9.6)	122(90.4)	0.025 [a]
HER2 positive	19(82.6)	4(17.4)		6(26.1)	17(73.9)	
HR & HER2						
HR negative/HER2 negative	28(58.3)	20(35.7)	0.122 [a]	7(14.6)	41(85.4)	0.058 [a]
HR negative/HER2 positive	13(92.9)	1(1.8)		3(21.4)	11(78.6)	
HR positive/HER2 negative	55(63.2)	32(36.8)		6(6.9)	81(93.1)	
HR positive/HER2 positive	6(66.7)	3(33.3)		3(33.3)	6(66.7)	
Molecular classification			0.172 [a]			
HER2 positive & HR positive type	13(92.9)	1(7.1)		3(21.4)	11(78.6)	0.093 [a]
HER2 positive & HR negative type	6(66.7)	3(33.3)		3(33.3)	3(66.7)	

Luminal A type	28(63.6)	16(36.4)		2(4.5)	42(95.5)	
Luminal B type	28(65.1)	15(34.9)		4(9.3)	39(90.7)	
TNBC	27(56.3)	21(43.8)		7(14.6)	41(85.4)	
Grade						0.161[a]
G1+G2	74(65.5)	39(34.5)	0.699	11(9.7)	102(90.3)	
G3	28(62.2)	17(37.8)		8(17.8)	37(82.2)	
Tumor size			0.246			0.766 [a]
T2	66(68.0)	31(32.0)		13(13.4)	84(86.6)	
T3	28(63.6)	16(36.4)		4(9.1)	40(90.9)	
T4	8(47.1)	9(52.9)		2(11.8)	15(88.2)	
Ki67			0.855			0.104
<20	44(63.8)	25(36.2)		5(7.2)	6492.8)	
⩾20	58(65.2)	31(34.8)		14(15.7)	75(84.3)	
Chemotherapy drugs			0.063			0.181
Taxol & Anthracycline	38(56.7)	29(43.3)		5(7.5)	62(92.5)	
Taxol & Anthracycline & CTX	49(72.1)	19(27.9)		10(14.7)	58(85.3)	

Abbreviations: ER: estrogen receptor; PR: progesterone receptor; HR: Hormone receptor; HER-2: human epidermal growth factor receptor 2; pCR: pathological complete response; cPR: clinical partial response; cSD: clinical stable disease; cPD: clinical progressive diease.

TABLE 3. Factors related to OS

Factors	OS(months)		χ2	P	Rate		
	Mean	95%CI			1-OS(%)	3-OS(%)	5-OS(%)
Clinical response			**0.279**	**0.597**			
cCR+cPR	70.325	62.772-77.928			96.3	76.8	63.2
cSD+cPD	82.538	70.734-94.341			93.9	75.1	73.1
Pathological response			1.321	0.25			
pCR	71.938	61.528-82.347			93.8	81.3	81.3
NpCR	71.166	71.166-87.085			94.8	75.5	66.3
Postoperative lymph node metastasis			0.427	0.935			
NO（0）	72.906	61.596-84.217			94.6	70.3	65.6
N1（1-3）	73.161	63.516-82.806			95.7	78.4	65.5
N2（4-9）	82.734	69.422-96.046			96.9	84.4	75.5
N3（≥10）	67.2	48.273-86.127			93.3	66.7	55.6
Molecular classification			11.63	0.02			
HER2 positive & HR positive type	45.818	26.144-65.492			81.8	45.5	45.5
HER2 positive & HR negative type	90.311	67.785-88.644			88.9	88.9	71.1
Luminal A type	74.388	66.574-82.201			94.4	86.1	75.8
Luminal B type	78.214	67.785-88.644			97	84.8	76.1
TNBC	57.86	49.199-66.522			92.9	66.1	59.9
Hormone receptors(HR)			9.024	0.003			
HR negative **(ER negative and PR negative)**	54.999	46.912-63.086			90.6	61.8	56.4
HR positive (ER positive and/or PR negative)	89.818	81.172-98.465			98.7	85.9	75.8
HER2 status			1.429	0.232			
HER2 negative	73.718	67.492-79.943			96.4	77.7	70
HER2 positive	71.583	49.925-93.242			90	65	54.2
Grade			0.611	0.435			
G1-2	82.875	74.207-91.544			95.6	77.6	68.9
3	59.8	51.828-67.772			95.1	73.2	66.4
Tumor size			15.77	<0.001			
T2	77.733	70.653-84.813			95.2	81.8	76.8
T3	79.156	65.195-93.117			97.1	73.5	66.3
T4	39.262	27.464-51.060			92.9	50	22.2
Age at entry (years)			0.095	0.758			
≤50	81.272	72.695-89.850			95.8	77.1	68.6
>50	71.06	59.150-82.971			94.3	73.9	66.1

Clinical stage			8.443	0.015			
IIB	76.707	69.498-83.916			95.1	81.6	75.1
IIIA	71.804	60.309-83.299			97	72.7	68.7
IIIB	57.553	36.748-78.359			93.8	56.3	33.8
Ki67			0.337	0.561			
<20	80.783	69.955-91.610			95.0	73.3	62.7
⩾20	74.013	66.330-81.697			95.8	78.7	72.6
HR & HER2			11.963	0.008			
HR negative/HER2 negative	57.686	49.032-66.341			92.9	66.1	59.7
HR negative/HER2 positive	45.818	26.144-65.492			81.8	45.5	45.5
HR positive/HER2 negative	79.562	72.402-86.722			98.6	85.5	76.1
HR positive/HER2 positive	90.311	64.465-116.157			88.9	88.9	71.1
Relapse & Metastatic status							
Yes	-	-	89.954	<0.001	-	-	-
No	**-**	**-**			**-**	**-**	**-**

Abbreviations: ER: estrogen receptor; PR: progesterone receptor; HR: Hormone receptor; HER-2: human epidermal growth factor receptor 2; pCR: pathological complete response; cPR: clinical partial response; cSD: clinical stable disease; cPD: clinical progressive diease.

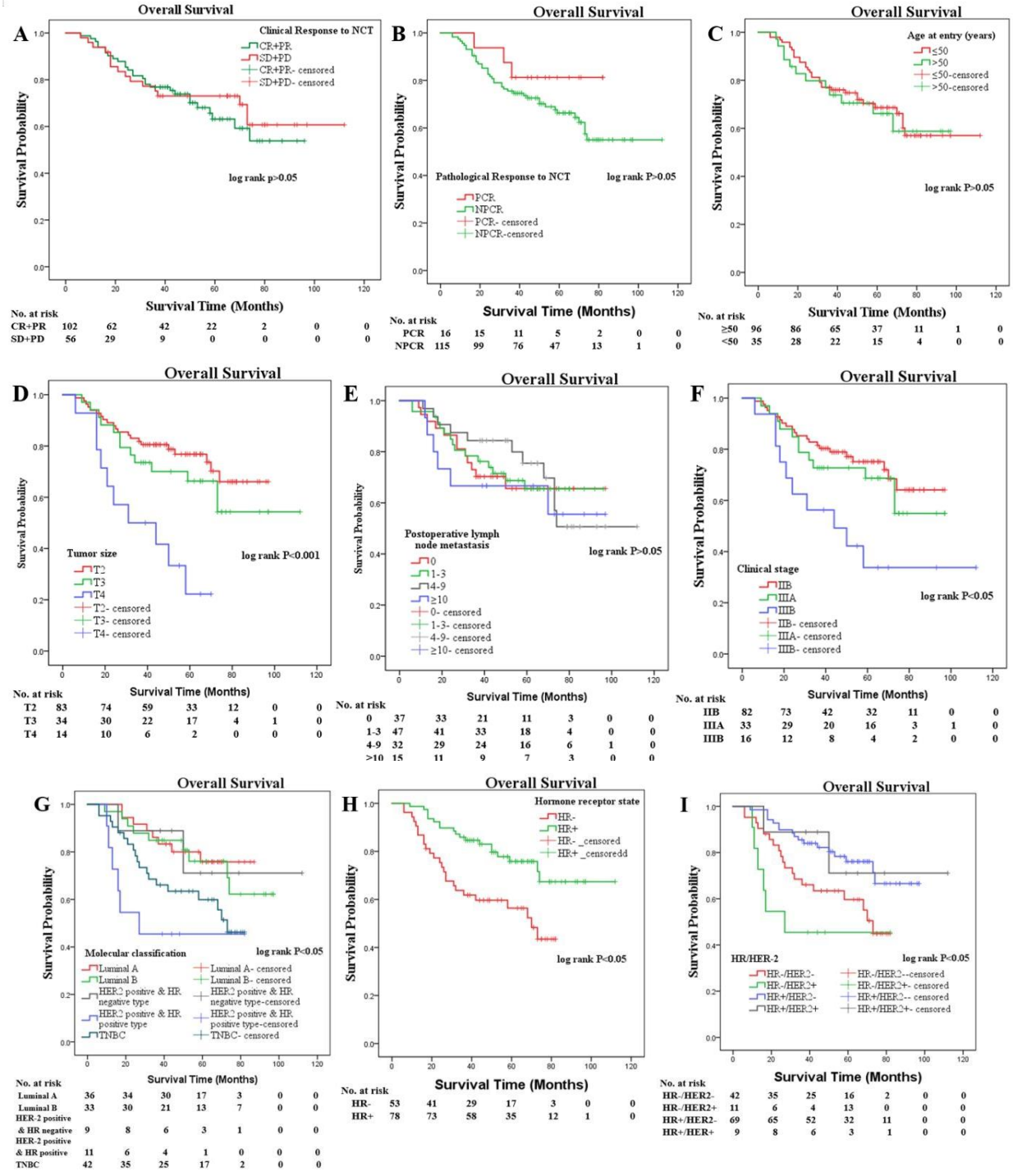

Figure 3. Kaplan–Meier survival plots for patients: (A) for all subtypes of clinical response, (B) for all subtypes of pathological response, (C) age, (D) tumor size (E) postoperative lymph node metastasis, (F) Clinical stage, (G) molecular classification, (H) hormone receptor status, (I)HR/HER-2 status.

TABLE 4. Factors related to DFS

Factors	DFS(months)		χ2	P	Rate	
	Mean	95%CI			3-DFS(%)	5-DFS(%)
Clinical response			**0.292**	**0.589**		
CR+PR	66.055	57.584-74.525			72	60.3
SD+PD	66.171	54.138-78.203			67.8	58.1
Pathological response			0.68	0.41		
PCR	63.467	50.551-76.382			80	60
NPCR	67.813	59.833-75.793			70.4	57.1
Postoperative lymph node metastasis			2.198	0.532		
NO（0）	68.52	54.674-82.365			74.2	68
N1（1-3）	67.811	57.218-78.405			72.4	65.8
N2（4-9）	67.197	52.843-81.552			72.4	52.4
N3（≥10）	49.744	29.955-69.532			61.5	41
Molecular classification						
HER2 positive & HR positive type	32	12.469-51.531	13.428	0.009	30	30
HER2 positive & HR negative type	88.875	68.478-109.272			87.5	87.5
Luminal A type	66.404	57.380-75.428			82.4	70.3
Luminal B type	67.991	54.698-81.285			74.2	58.4
TNBC type	51.777	41.588-61.966			67	53.6
Hormone receptors(HR)			6.437	0.011		
HR negative (ER negative and PR negative)	47.518	37.909-57.127			58.4	42.1
HR positive (ER positive and/or PR negative)	76.378	67.816-84.939			79.4	67
HER2 status			1.327	0.249		
HER2 negative	68.357	60.949-75.766			74.6	60.9
HER2 positive	60.778	40.384-81.171			55.6	55.6
Grade			0.161	0.688		
1-2	67.879	59.058-76.701			70	58
3	57.591	48.498-66.683			75.4	63.8
Age at entry (years)			0.148	0.701		
≤50	69.707	61.175-78.238			73.2	60.2
＞50	64.234	50.154-78.314			67	57.6
Clinical stage			5,256	0.072		
IIB	68.388	60.334-76.441			78.5	62.3
IIIA	66.29	52.222-80.359			68.8	59.9
IIIB	46.231	22.691-69.770			38.5	38.5
Tumor size			11.195	0.004		
T2	69.012	61.112-76.913			79,1	63.2

T3	69.053	54.358-83.748			69	60.6
T4	26.636	12.112-41.161			27.3	27.3
HR & HER2			13.152	0.004		
HR negative/HER2 negative	51.495	41.308-61.682			67	52.7
HR negative/HER2 positive	32	12.469-51.531			30	30
HR positive/HER2 negative	72.436	63.762-81.110			78.4	64.9
HR positive/HER2 positive	88.875	68.550-109.272			87.5	87.5

Abbreviations: ER: estrogen receptor; PR: progesterone receptor; HR: Hormone receptor; HER-2: human epidermal growth factor receptor 2; pCR: pathological complete response; cPR: clinical partial response; cSD: clinical stable disease; cPD: clinical progressive diease.

Table5. Multivariate and single-variate Cox proportional hazards model for breast cancer patients

Variable	Multivariate			Single-variate		
	HR	**95%CI**	**P-value**	**HR**	**95%CI**	**P-value**
Age at entry (years)						
≤50	1	-		1	-	
>50	0.936	0.480-1.826	0.846	1.14	0.621-2.094	0.672
Clinical stage						
IIB	1	-		1	-	
IIIA	0.294	0.036-2.422	0.255	1.288	0.656-2.532	0.462
IIIB	NA	NA	0.996	2.45	1.225-4.901	0.011
Estrogen receptor(ER)						
ER negative	1	-		1	-	
ER positive	0.736	0.324-1.670	0.463	0.479	0.272-0.844	0.011
Progesterone receptor(PR)						
PR negative	1	-		1	-	
PR positive	0.571	0.239-1.362	0.206	0.423	0.235-0.763	0.004
HER2 status						
HER2 negative	1	-		1	-	
HER2 positive	1.835	0.837-4.019	0.129	2.011	1.000-4.042	0.05
HR & HER2						
HR negative/HER2 negative	-	-		1	--	
HR negative/HER2 positive	-	-		1.931	0.847-4.404	0.118
HR positive/HER2 negative	-	-		0.439	0.234-0.823	0.01
HR positive/HER2 positive	-	-		0.516	0.121-2.207	0.372
Molecular classification						
Luminal A type	**-**	-		1	-	
Luminal B type	**-**	-		1.107	0.449-2.729	0.825
HER2 positive & HR negative type	**-**	-		1.228	0.265-5.688	0.793
HER2 positive & HR positive type	**-**	-		4.596	1.763-11.980	0.002
TNBC	**-**	-		3.251	1.070-5.166	0.033
Grade						
G1+G2	1	-		1	-	
G3	1.129	0.605-2.071	0.72	1.4	0.769-2.551	0.271
Tumor size						
T2	1	-		1	-	
T3	4.316	0.550-33.864	0.164	1.275	0.656-2.480	0.474
T4	NA	NA	0.996	4.171	2.064-8.428	<0.001
Ki-67						
<20%	1	-		1	-	
⩾20%	0.852	0.459-1.583	0.613	0.89	0.506-1.564	0.685

Clinical response						
CR+PR	1	-		1	-	
CD+SD	0.92	0.492-1.720	0.793	0.899	0.498-1.623	0.724
Pathological response						
NPCR	1	-		1	-	
PCR	1.574	0.615-4.030	0.345	1.185	0.469-2.993	0.719

Abbreviations: ER: estrogen receptor; PR: progesterone receptor; HR: Hormone receptor; HER-2: human epidermal growth factor receptor 2; pCR: pathological complete response; cPR: clinical partial response; **cSD:** clinical stable disease; **cPD:** clinical progressive diease.

TABLE 6. Prediction model for breast cancer patients

	HR	95%CI	P-value
ER(VS. negative)			
positive	0.770	(1.345,1.720)	0.524
PR(VS. negative)			
positive	0.585	(0.250,1.365)	0.215
HER-2 status (VS. negative)			
positive	1.778	(0.852,3.706)	0.125
Clinical stage (VS. IIB)			
IIIA	0.305	(0.040,2.304)	0.250
IIIB	NA	NA	0.996
Tumor size (VS. T2)			
T3	4.266	(0.588,30.947)	0.151
T4	NA		0.996

Abbreviations: ER: estrogen receptor; PR: progesterone receptor; HR: Hormone receptor; HER-2: human epidermal growth factor receptor 2.

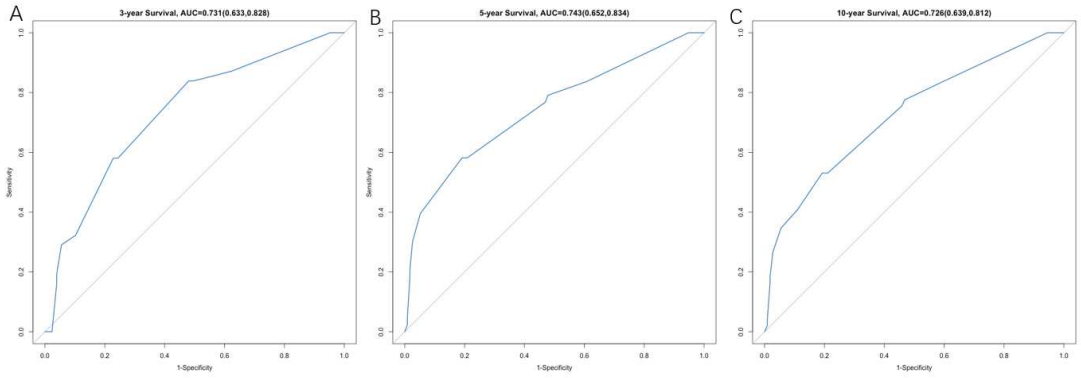

Figure 4. The receiver-operating characteristics (ROC) curve in the prediction function: (A) ROC curve for 3-year survial with area under the curve = 0.731 (95% confidence interval, 0.633-0.828); (B) ROC curve for 5-year survial with area under the curve = 0.743 (95% confidence interval, 0.652-0.834); (C) ROC curve for 10-year survial with area under the curve = 0.726 (95% confidence interval, 0.639-0.812).

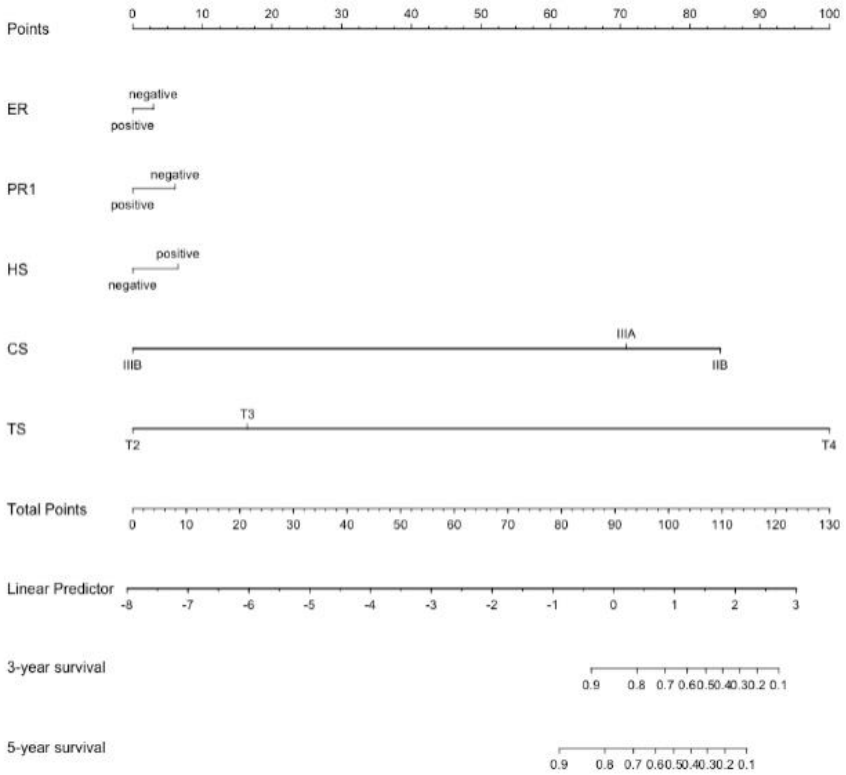

Figure 5. Nomogram to predict the probability of 3-year OS and 5-year OS.

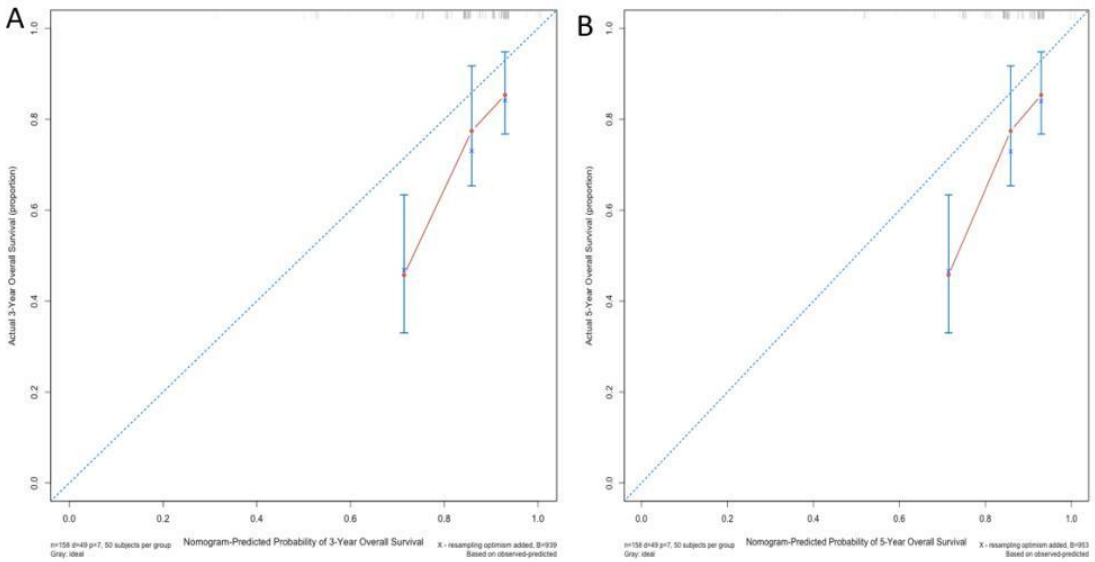

Figure 6. Calibration plot showing nomogram-predicted 3-year OS probabilities with the actual 5-year OS (A) and the nomogram-predicted 2-year OS with the actual 2-year OS (B).

Figures

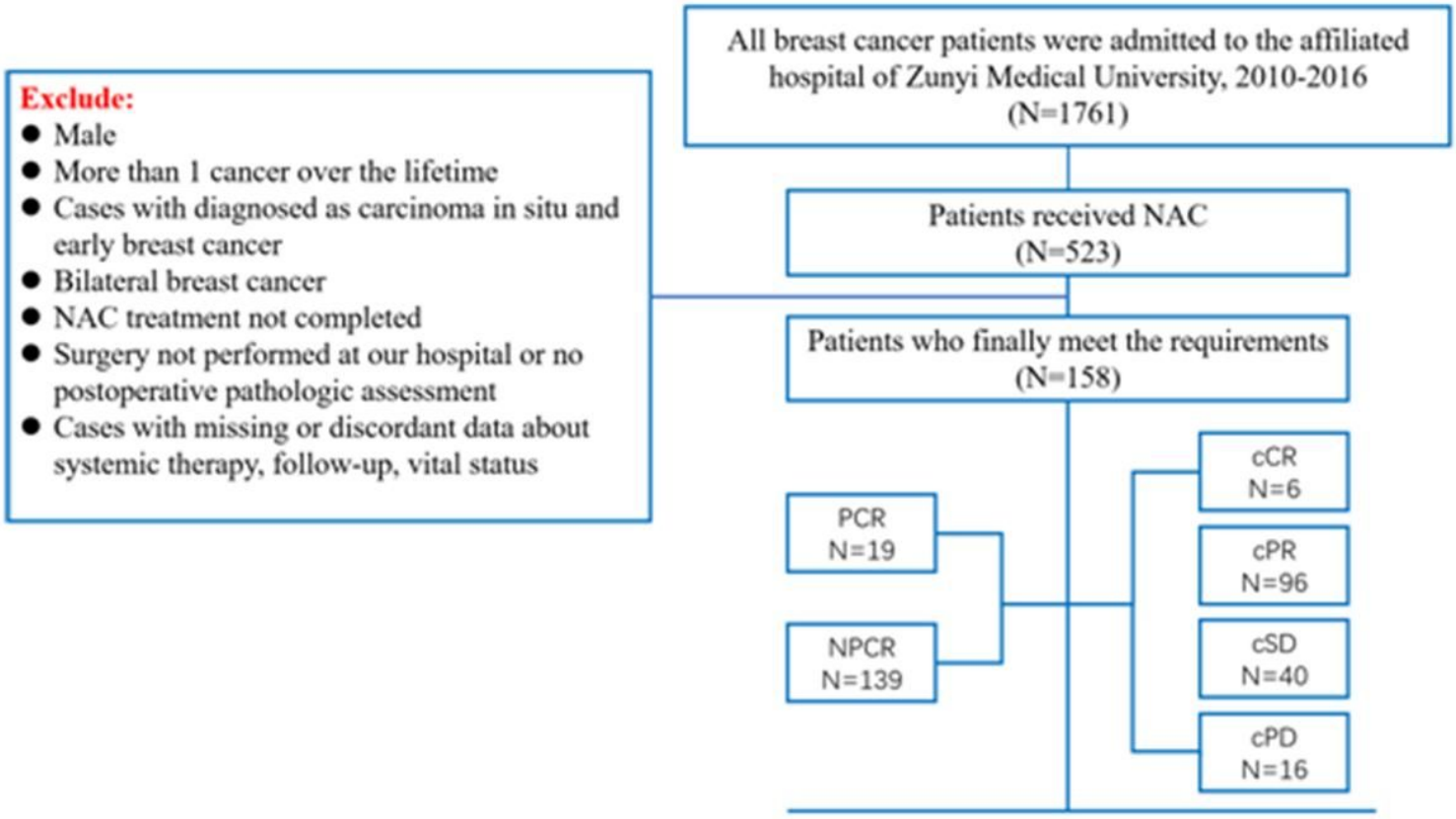

Figure 1

Flow chart of the study population with exclusion criteria.

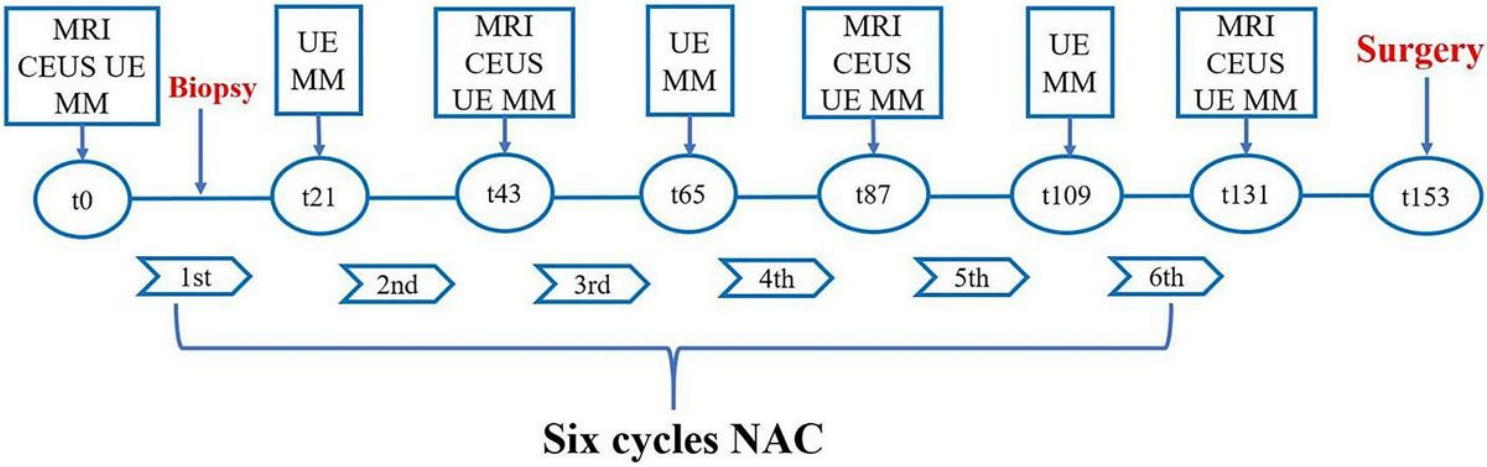

Figure 2

Flow chart of NAC. Each cycle lasted for 21 days and surgical excision was performed within 20 days after 6 cycles. Thorough examinations are performed every two sessions. (CEUS, contrast-enhanced ultrasonography; UE, ultrasound elastography; MM, mammary gland molybdenum target; MRI, magnetic resonance imaging)

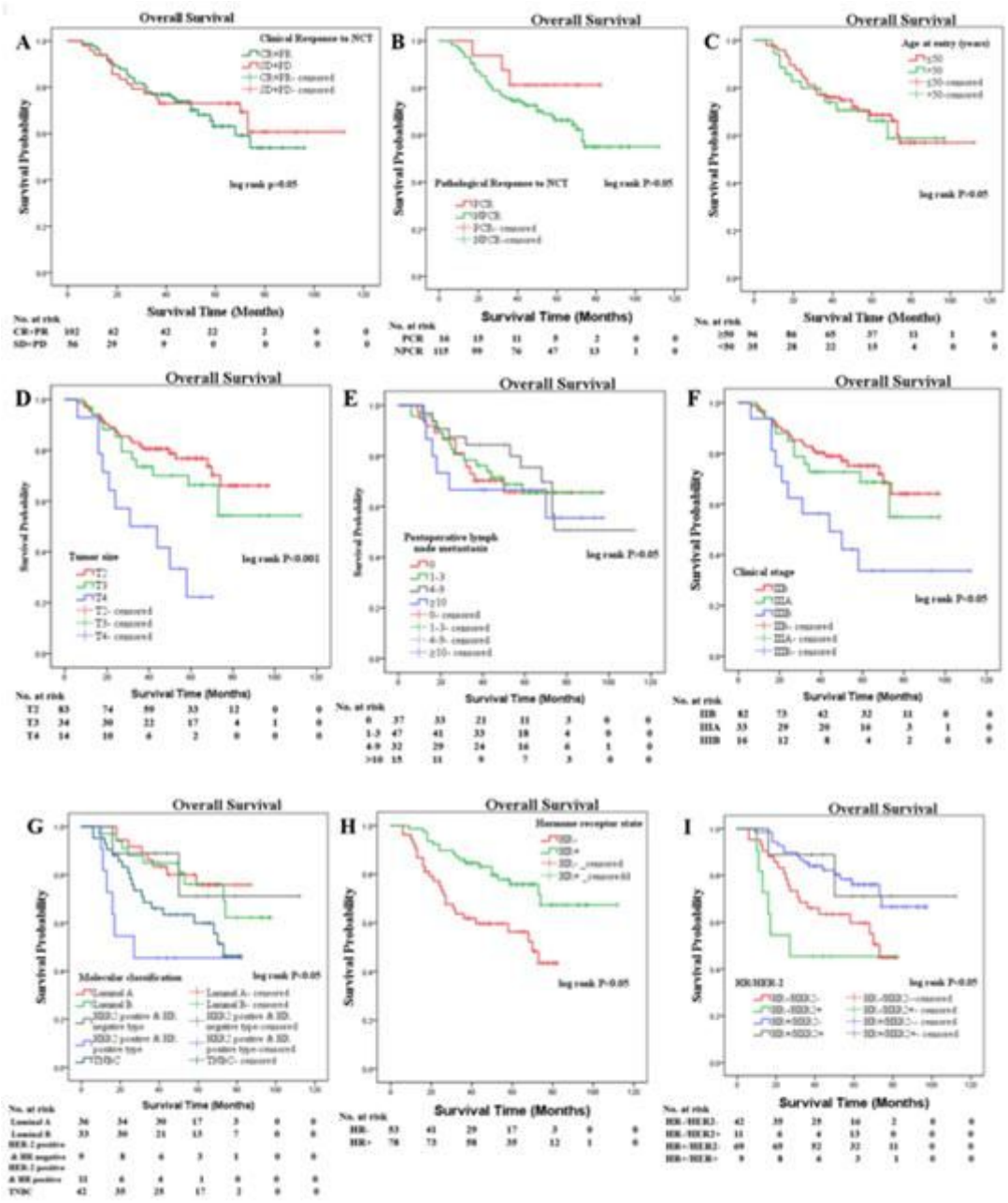

Figure 3

Kaplan–Meier survival plots for patients: (A) for all subtypes of clinical response, (B) for all subtypes of pathological response, (C) age, (D) tumor size (E) postoperative lymph node metastasis, (F) Clinical stage, (G) molecular classification, (H) hormone receptor status, (I)HR/HER-2 status.

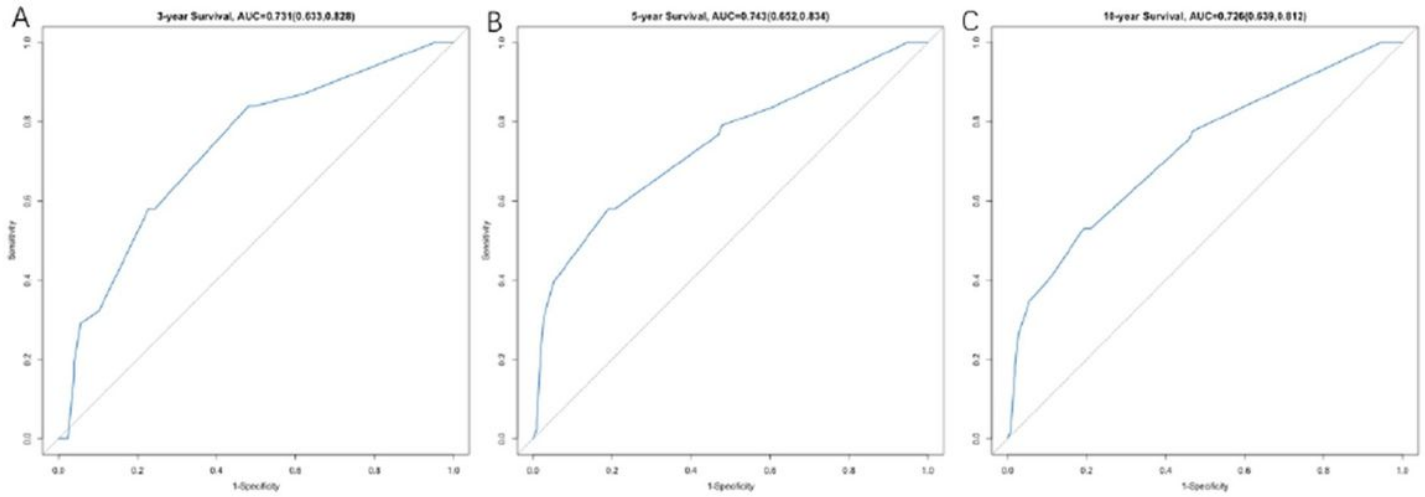

Figure 4

The receiver-operating characteristics (ROC) curve in the prediction function: (A) ROC curve for 3-year survial with area under the curve = 0.731 (95% confidence interval, 0.633-0.828); (B) ROC curve for 5-year survial with area under the curve = 0.743 (95% confidence interval, 0.652-0.834); (C) ROC curve for 10-year survial with area under the curve = 0.726 (95% confidence interval, 0.639-0.812).

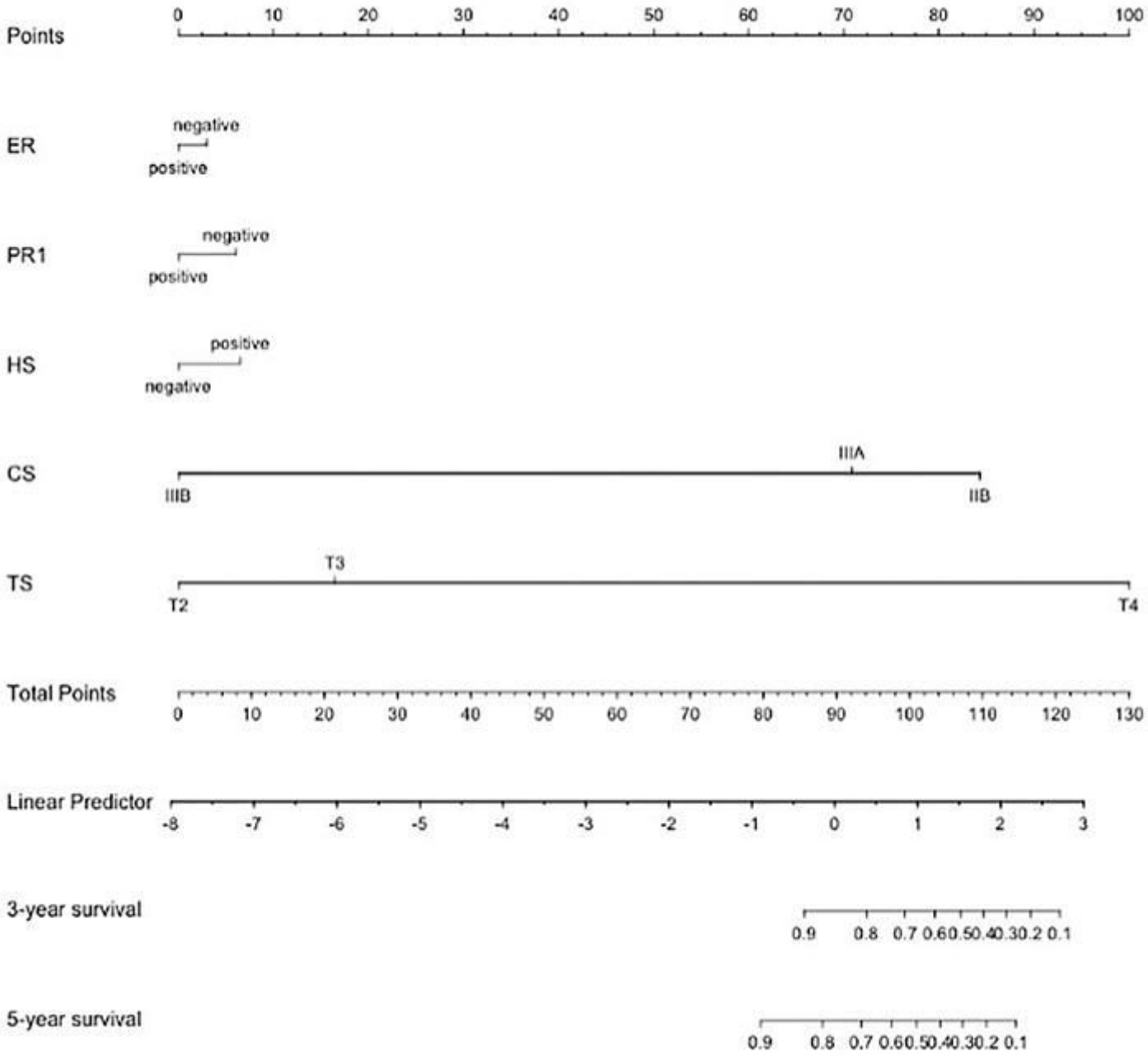

Figure 5

Nomogram to predict the probability of 3-year OS and 5-year OS.

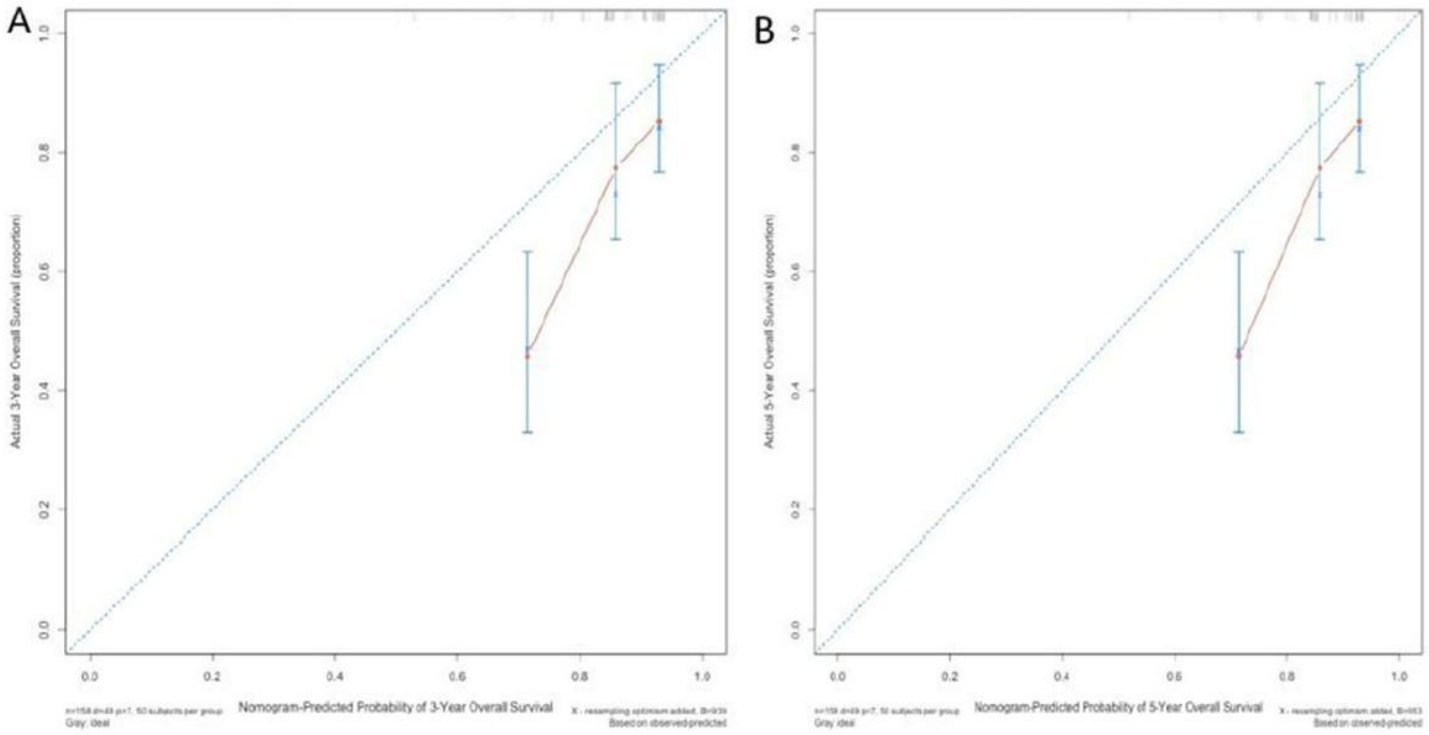

Figure 6

Calibration plot showing nomogram-predicted 3-year OS probabilities with the actual 5-year OS (A) and the nomogram-predicted 2-year OS with the actual 2-year OS (B).