

Re-Evaluate the Value of Frozen Sections in Diagnoses of Breast Malignancies that Failed to be Diagnosed by Core Needle Biopsy: A Chinese Retrospective Analysis of Clinical Practice

Jialei Xue

Changshu Hospital Affiliated of Nanjing University of Chinese Medicine

Jianwei Li

Fudan University Shanghai Cancer Center

Yue Gong

Fudan University Shanghai Cancer Center

Qiuxia Cui

Changshu Hospital Affiliated of Nanjing University of Chinese Medicine

Li Dai

Changshu Hospital Affiliated of Nanjing University of Chinese Medicine

Tianwei Guo

Changshu Hospital Affiliated of Nanjing University of Chinese Medicine

Zhebin Liu (✉ lysazb@hotmail.com)

Fudan University Shanghai Cancer Center

Guangyu Liu

Fudan University Shanghai Cancer Center

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Abstract

Objective: The value of frozen sections in diagnoses of breast malignancies that failed to be diagnosed by core needle biopsy (CNB) is indeterminate. To re-evaluate and improve the utility of frozen section on this kind of breast malignancy, we conducted a retrospective data analysis and constructed a prediction model.

Method: We reviewed data of breast cancer patients that failed to be diagnosed by CNB (CNB-undiagnosable) in Fudan University Shanghai Cancer Center (FUSCC) from May 1, 2006 to December 31, 2019. Clinical characteristics of patients were collected. the correlation between clinical features and false negative rate (FNR) of frozen sections was explored with logistic regression analysis, after which a nomogram was constructed to predict the probability of false negative.

Result: The diagnostic sensitivity of frozen section on CNB-undiagnosable breast cancer was 67.18%, and the FNR was 32.82%. In multivariate analysis, papillary lesion (OR, 4.251; 95% CI, 2.804-6.492; $P < 0.0001$) and sclerosing adenosis (OR, 3.727; 95% CI, 1.897-7.376; $P = 0.0001$) on CNB were risk factors of false negative, while clustered microcalcifications on mammography (OR, 0.345; 95% CI, 0.216-0.543; $P < 0.0001$) and ultrasonic BI-RADS category 4C-5 (OR, 0.250; 95% CI, 0.081-0.777; $P = 0.0157$) were favorable factors of true positive. The false negative rate of frozen section could be controlled at about 10% by the prediction of nomogram.

Conclusion: Frozen sections are valuable in the diagnosis of CNB-undiagnosable breast cancers. It is recommended to implement the intraoperative frozen sections for high-risk breast lesions with a low probability of false negative indicated by prediction, so as to minimize the occurrence of unnecessary re-operation.

Introduction

Ultrasound-guided core needle biopsy (CNB) is the main diagnostic method for breast cancer [1]. Previous research that reported by our institute showed that the accuracy of CNB could reach 92.4%, but due to the sampling limitations, it still has possibility of false negative (FN) or underestimation of grade, with an underestimation rate of 5.9% and a false negative rate (FNR) of 1.7% [2]. Previous reports have shown that high-risk breast lesions diagnosed by CNB may be upgraded to malignancies in excision biopsy [3, 4], which are bound to undergo radical surgery after definite diagnosis. Therefore, if the diagnosis of the lesion and the radical surgery can be completed in one operation, the efficiency of diagnosis and treatment will be greatly improved. Frozen sections are the most common method for immediate pathological diagnosis intraoperatively, and were widely used in China. However, its sensitivity in the diagnosis of very early breast cancer has been controversial. According to previous reports, the diagnostic accuracy of frozen section for invasive breast cancer is high [5-7], but lower for carcinoma in situ [8]. Early researches also shown that there are still a few patients with malignancies couldn't be diagnosed by frozen sections, and need to wait for the final result of paraffin sections (PS) [9, 10]. So far, as far as we

know, there is no study that investigated the role of frozen sections in the diagnoses of CNB-undiagnosable breast malignancies, therefore, its practical utility in CNB-undiagnosable breast cancer is indeterminate. So, we conducted a retrospective analysis of clinical data, hoping to provide some guidance and help for clinical practice.

Materials And Methods

Study Population and Data Collection

Our subjects were collected from the database of Fudan University Shanghai Cancer Center (FUSCC) from May 1,2006 to December 31,2019. The eligibility criteria were as follows: 1). The final pathological diagnosis must be breast cancer; 2). CNB was taken preoperatively, but malignancy was not confirmed; 3). Frozen section must be performed for pathological assessment after excisional biopsy immediately.

We collected the baseline characteristics of patients by referring to the electronic medical record system. False negative was defined as that was diagnosed non-malignant by FS but malignant by PS. 11 clinically relevant candidate variables were selected from the database, include age, physical examination symptoms(whether the mass could be palpable, and whether there is nipple discharge), ultrasonographic features(type of ultrasonic image, ultrasonic maximum diameter, whether there is dense punctate strong echo (DPSE) on ultrasonic image, the category of the BI-RADS on ultrasonography (US-BI-RADS)), mammography features(whether there is clustered microcalcifications on mammography, the category of the BI-RADS on mammography (MG-BI-RADS)), pathological features(whether the core needle biopsy contained papillary lesions (PL-CNB) and/or sclerosing adenosis (SA-CNB)).

The need for informed consent was waived because of the retrospective nature of the study, and the study design was approved by the appropriate Ethics Review Board.

Statistical Method

Univariate logistic regression was used to test the associations between FNR of frozen section and clinical characteristics. Multivariable logistic regression with backward selection was performed to identify independent covariates. Factors at the 0.05 level were considered statistically significant. The performance of the nomogram was quantified with respect to discrimination and calibration^[11]. The receiver operating characteristic (ROC) curve was drawn, and the predictive accuracy was assessed by calculating the area under the ROC curve (AUC). The Harrell C-index was used to evaluate discriminatory power^[12]. Calibration was performed using the bootstrapping method and was used to illustrate the relation between the predicted and observed FNR of frozen sections^[13]. Statistical analyses were performed using the rms, Hmisc, pROC, and ggplot2 packages in R version 3.4 (R Foundation for Statistical Computing), including bootstrapping and drawing of the nomogram to visually represent the model.

Result

Baseline Characteristics of Study Population

From May 1,2006 to December 31,2019, a total of 1036 patients with 1039 breast cases (3 of whom had simultaneity bilateral breast malignancies) met the inclusion criteria. 698 (696 patients) were diagnosed by frozen sections and 341 (340 patients) by paraffin sections. Based on the above data, we calculated that the diagnostic sensitivity of frozen section was 67.18%, the FNR was 32.82%.

After removing patients with incomplete image data, 876 patients (876 cases) had complete image data and were selected for logistic regression analysis and nomogram construction, and randomly assigned to the training set and testing set in a ratio of 7:3. The characteristics of the patients are shown in Table1.

Logistic Regression Analysis

In the training set, of 613 patients, 205 (33.44%) were false negative. In the univariate logistic regression analysis, for patients who were sixty years and older (OR, 1.653; 95% CI, 1.029-2.686; P=0.0396), patients who have PL-CNB (OR, 5.037; 95% CI, 3.468-7.366; P < 0.0001), or patients who have SA-CNB (OR, 2.133; 95% CI, 1.161-3.917; P= 0.014), the FNR of frozen section was higher, but lower for those that showed solid image on ultrasonography (OR, 0.286; 95% CI, 0.158-0.505; P < 0.0001), DPSE on ultrasonic image (OR, 0.205; 95% CI, 0.128-0.319; P < 0.0001), US-BI-RADS 4C-5 (OR, 0.273; 95% CI, 0.101-0.737; P = 0.0094), clustered microcalcifications on mammography (OR, 0.203; 95% CI, 0.138-0.294; P < 0.0001), and MG-BI-RADS 4C-5 (OR, 0.203; 95% CI, 0.190-0.750; P = 0.0049).

In multivariate logistic regression analysis with backward stepwise selection, US-BI-RADS 4C-5 (OR, 0.250; 95% CI, 0.081-0.777; P = 0.0157), clustered microcalcifications on mammography (OR, 0.345; 95% CI, 0.216-0.543; P < 0.0001) were associated with lower FNR, but for DPSE on ultrasonic image, the correlation is slightly weaker (OR, 0.595; 95% CI, 0.335-1.044; P = 0.0727). On the contrary, PL-CNB (OR, 4.251; 95% CI, 2.804-6.492; P<0.0001) and SA-CNB (OR, 3.727; 95% CI, 1.897-7.376; P= 0.0001) were associated with higher FNR (show in Table 2).

Nomogram Development

On the basis of results from multivariable logistic regression analysis, a nomogram was developed to predict the FNR of frozen section. In the nomogram, the total score is calculated by using clinical and pathologic features, contain BI-RADS category on ultrasonography, DPSE on ultrasonic image, clustered microcalcifications on mammography, PL-CNB, and SA-CNB. This total score can then be used to assign a probability of FN to individual patient using the scale at the bottom of Figure 1.

Nomogram Validation

The resulting nomogram was internally validated using the bootstrap method. We use formula to determine the cutoff value of the validation: $f(x) = \frac{nx(1-FNRx)}{N-nx(1-FNRx)}$, x represents the total score, nx

represents the number of patients with this score and below, FNRx represents the actual false negative rate of patients with this score and below, and N represents the total number of patients. The best cutoff value is obtained at the peak of the formula value curve, that represents the best clinical utility. The cutoff value we set was the total score 135 points (Figure 2), the prediction model had an AUC of 0.794 (95% CI: 0.756-0.831) in the training set, indicating that the multivariate logistic regression model had potentially promising predictive power (Fig. 3A). The model demonstrated an adequate level of accuracy for predicting the FNR of frozen section.

The independent testing set of 263 patients also showed good discriminatory ability, with an AUC of 0.800 (95% CI: 0.736-0.865), indicating that the multivariate logistic regression model in a separate, individual data set of patients had potentially promising predictive power (Fig. 3B).

The calibration was good for the training and testing cohorts and showed no significant difference between the predicted and observed probabilities of failure diagnosis ($P = 1.000$), indicating that the nomogram was well calibrated (Fig. 4).

On the basis of the predicted probability of FN, we calculated the practical FNR of different cutoff points in total patients (876 patients). When predicting the probabilities of patients who were more likely to be FN, the patients with practical FNR accounted for 10% and 10.16% of those who had a predicted probability of FN $\leq 10\%$ and $\leq 15\%$, respectively. Among patients with a predicted probability of FN $\geq 60\%$, $\geq 70\%$, and $\geq 80\%$, the practical FNR accounted for 71.7%, 73.4%, and 87.5%, respectively (shown in Table 3).

These results demonstrated that the individual probability of FN of frozen section could be predicted accurately by combining information from routinely available clinicopathologic variables.

Discussion

In most countries, Frozen sections are often omitted, and paraffin sections are used for post-resection pathological assessment of breast lesions those have risks of upgrading from atypical to malignant. But in a few countries, such as China, frozen sections are still utilized in clinical practice, mainly for making intra-operational decision and avoiding unnecessary re-operations. This may be related to the low acceptance of re-operation in Chinese patients. Of course, minimizing unnecessary re-operations is beneficial for both patients and doctors. Our research showed that, in all patients, the diagnostic sensitivity of frozen section for CNB-undiagnosable breast cancer was 67.18%, and the false-negative rate was 32.82%. This suggests that frozen sections are valuable in the diagnosis of these patients, but further screening is needed to reduce the false negative rate. According to the nomogram, we find that the FNR is more than 70% when the total score exceeds 220, and even reach 87.5% when the total score exceeds 288. For such patients, frozen sections should be omitted. On the contrary, when the total score is lower than 76, the diagnostic sensitivity of frozen section can reach nearly 90%, the incidence of re-operation is significantly reduced.

Previous research showed that the diagnostic sensitivity of frozen section for ductal carcinoma in situ (DCIS) was only about 50% [8], the main reason was that some DCIS appear as non-mass lesions, which could not be identified by macroscopic examination, that may be leading to sampling errors [8, 9]. In our study, the diagnostic sensitivity of frozen section for pure DCIS was 50.62% (papillary carcinomas are not included), which similar to previous study. In our study, interestingly, malignancies with microcalcifications on mammography were more likely to be diagnosed by frozen sections, that seems to contradict earlier researches. Previous reports had shown that pure microcalcifications on mammography may increase the FNR of frozen section [6, 14]. However, in these reports, invasive cancer and DCIS were not distinguished, and the proportion of DCIS was significantly higher in patients presenting as pure microcalcifications without mass, leading to significant imbalance of tumor stage, which may be the real reason for the difference in FNR. In our study, the staging of patients was very different from previous reports. All the subjects underwent preoperative core needle biopsy, patients diagnosed as malignant were excluded. As a result, the vast majority of invasive cancers had been excluded, resulting in a higher percentage of DCIS in our patients. The proportion of DCIS and DCIS with microinvasive carcinoma (DCIS-M) in our study was nearly 60% (include papillary carcinomas), and the percentage of DCIS+DCIS-M between the microcalcification and non-calcification groups was very similar (57.3% vs 59.7%). Cheng's report also suggested that DCIS with microcalcifications is more likely to be diagnosed in frozen section, probably because the microcalcifications help in localizing the lesion and aids in accurate sampling [8].

We found a higher FNR of frozen section for papillary carcinoma (PC), which is consistent with previous studies [5, 8]. PC is considered to be a rare type of breast cancer with a favorable prognosis, most of which are confined to ducts [15]. In the past decades, PC was considered a variant of intraductal carcinoma. The latest World Health Organization (WHO) Working Group's classification of breast tumors defines PC as a separate subtype of breast carcinoma, which is classified into encapsulated papillary carcinoma and encapsulated papillary carcinoma with invasion [16, 17]. Previous studies have reported that the final diagnosis of PC often requires immunohistochemical examination to differentiate it from benign papilloma [15]. In the preoperative evaluation, ultrasonic images of PC more show solid-cystic lesion, and appearances of mammography more show dense masses without conspicuous microcalcification, due to the limited sampling, preoperative core needle biopsy are usually visible only to a small amount of papillary hyperplasia or atypical hyperplasia lesions, it is coinciding with one of this study results that papillary lesion can increase the FNR of FS.

Our study inevitably has some limitations. First of all, this is a retrospective study, and there are some unavoidable bias factors. Secondly, breast magnetic resonance imaging (MRI) was not included in the preoperative evaluation factors, this is because the large patients base in China and the lack of MRI equipment, most of the patients do not have enough time for preoperative MRI scan. In addition, Patients in this study all received core needle biopsy, instead of vacuum assisted biopsy (VAB), mainly because VAB is not covered by medical insurance in China, and its cost is high. Surgeons usually use VAB to remove small benign lesions, while rarely used in suspected malignant lesions [2, 18].

Conclusion

Frozen sections are valuable in the diagnosis of CNB-undiagnosable breast cancer. Although the overall false negative rate is relatively high, but it can be significantly reduced through prediction. It is recommended to implement the intraoperative frozen sections for high-risk breast lesions with a low probability of false negative indicated by prediction, so as to minimize the occurrence of unnecessary re-operation. Of course, prospective studies are needed to verify this conclusion.

Abbreviations

FS, frozen section; PS, paraffin section; DCIS, ductal carcinoma in situ; PC, papillary carcinoma; US, ultrasonography; DPSE, dense punctate strong echo; US-BI-RADS, the category of the BI-RADS on ultrasonography; MG, mammography; MG-BI-RADS, the category of the BI-RADS on mammography; CNB, core needle biopsy; PL-CNB, core needle biopsy contained papillary lesions; SA-CNB, core needle biopsy contained sclerosing adenosis. FN, false negative; FNA, false negative rate; OR, odds ratio; MRI, magnetic resonance imaging; VAB, vacuum assisted biopsy.

Declarations

Ethics approval and consent to participate

The need for informed consent was waived because of the retrospective nature of the study, and the study design was approved by the appropriate Ethics Review Board.

consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests

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Not applicable

Author Contributions

JX: Conceptualization, methodology, data collection, investigation, writing–original draft, and writing–review and editing. **JL:** Conceptualization, methodology, resources, and data interpretation. **YG:** Statistical analysis and data interpretation. **QC** and **LD:** Data collection and writing–original draft. **TG:** Data collection and data interpretation. **ZL** and **GL:** Supervised the study planning and design; data collection; statistical analysis and data interpretation, full access to all the data in the study and responsibility for the integrity of the data and the accuracy of the data analysis, and article review, revision, and reporting. All authors read and approved the final manuscript.

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References

1. Ma JF, Chen LY, Wu SL, et al. Clinical practice guidelines for ultrasound-guided breast lesions and lymph nodes biopsy: Chinese society of breast surgery (CSBrS) practice guidelines 2021. *Chin Med J (Engl)* 2021 May 19
2. Hao S, Liu ZB, Ling H, Chen JJ, Shen JP, Yang WT, Shao ZM. Changing attitudes toward needle biopsies of breast cancer in shanghai: experience and current status over the past 8 years. *Onco Targets Ther* 2015;8:2865–71.
3. Schiaffino S, Calabrese M, Melani EF, Trimboli RM, Cozzi A, Carbonaro LA, Di Leo G, Sardanelli F. Upgrade Rate of Percutaneously Diagnosed Pure Atypical Ductal Hyperplasia: Systematic Review and Meta-Analysis of 6458 Lesions. *Radiology* 2020 Jan;294(1):76–86.
4. Pawloski KR, Christian N, Knezevic A, Wen HY, Van Zee KJ, Morrow M, Tadros AB. Atypical ductal hyperplasia bordering on DCIS on core biopsy is associated with higher risk of upgrade than conventional atypical ductal hyperplasia. *Breast Cancer Res Treat* 2020 Dec;184(3):873–880.
5. Niu Y, Fu XL, Yu Y, Wang PP, Cao XC. Intra-operative frozen section diagnosis of breast lesions: a retrospective analysis of 13,243 Chinese patients. *Chin Med J (Engl)* 2007 Apr 20;120(8):630-5.
6. Bianchi S, Palli D, Ciatto S, Galli M, Giorgi D, Vezzosi V, Del Turco MR, Cataliotti L, Cardona G, Zampi G. Accuracy and reliability of frozen section diagnosis in a series of 672 nonpalpable breast lesions. *Am J Clin Pathol* 1995 Feb;103(2):199–205.
7. Stolnicu S, Rădulescu D, Pleșea IE, Dobru D, Podoleanu C, Pintilei DR. The value of intraoperative diagnosis in breast lesions. *Rom J Morphol Embryol* 2006;47(2):119–23.
8. Cheng L, Al-Kaisi NK, Liu AY, Gordon NH. The results of intraoperative consultations in 181 ductal carcinomas in situ of the breast. *Cancer* 1997 Jul 1;80(1):75-9.
9. Cserni G. Pitfalls in frozen section interpretation: a retrospective study of palpable breast tumors. *Tumori* 1999 Jan-Feb;85(1):15–8.
10. Fechner RE. Frozen section examination of breast biopsies. Practice parameter. *Am J Clin Pathol* 1995 Jan;103(1):6–7.

11. Coutant C, Olivier C, Lambaudie E, et al. Comparison of models to predict nonsentinel lymph node status in breast cancer patients with metastatic sentinel lymph nodes: a prospective multicenter study. *J Clin Oncol*. 2009;27:2800–2808.
12. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med*.1996;15:361–387.
13. Steyerberg EW, Harrell FE Jr, Borsboom GJ, Eijkemans MJ, Vergouwe Y, Habbema JD. Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. *J Clin Epidemiol*.2001;54:774–781.
14. Tinnemans JG, Wobbles T, Holland R, Hendriks JH, van der Sluis RF, Lubbers EJ, de Boer HH. Mammographic and histopathologic correlation of nonpalpable lesions of the breast and the reliability of frozen section diagnosis. *Surg Gynecol Obstet* 1987 Dec;165(6):523–9.
15. Mogal H, Brown DR, Isom S, Griffith K, Howard-McNatt M. Intracystic papillary carcinoma of the breast: a SEER database analysis of implications for therapy. *Breast*. 2016;27:87–92.
16. Tan PH, Schnitt SJ, van de Vijver MJ, Ellis IO, Lakhani SR. Papillary and neuroendocrine breast lesions: the WHO stance. *Histopathology*.2015;66(6):761–770.
17. board Wcote. *Breast Tumours*. 5th ed. Lyon: IARC Press; 2019.
18. Li SJ, Hao XP, Hua B, et al. Clinical practice guidelines for ultrasound-guided vacuum-assisted breast biopsy: Chinese Society of Breast Surgery (CSBrS) practice guidelines 2021. *Chin Med J (Engl)* 2021 Jun 2.

Tables

Due to technical limitations, tables 1 to 3 are only available as a download in the Supplemental Files section.

Figures

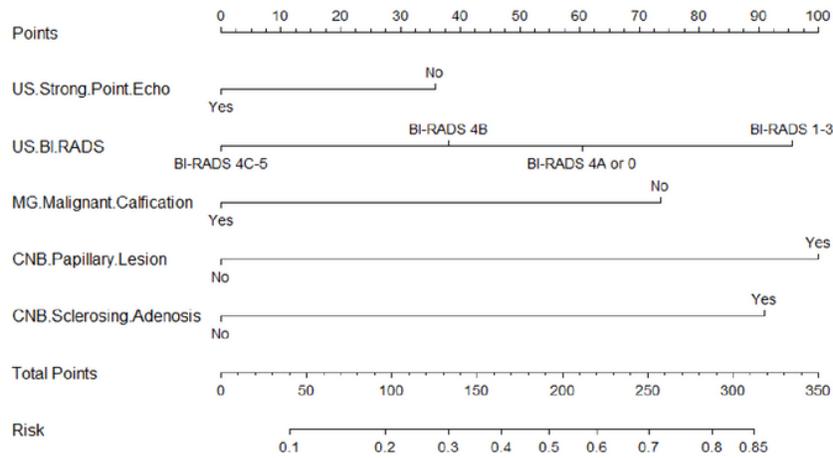


Figure 1

This is the nomogram for predicting the probability of false negative of frozen section in patients with high risk breast lesion. To calculate the probability, identify the predictor points on the uppermost point scale that correspond to each patient variable and sum them. The total points projected in the bottom scale indicate the probability of false negative.

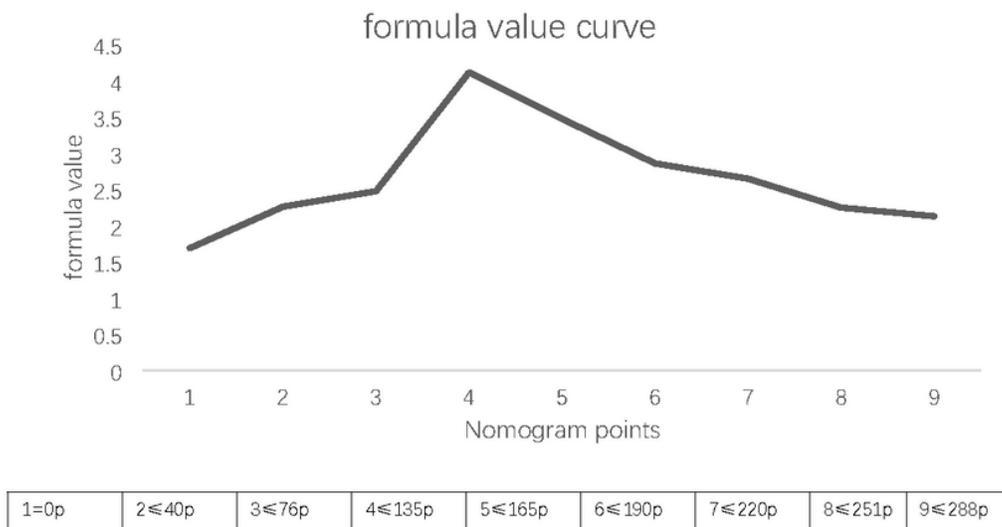


Figure 2

formula value curve shows the best cutoff value is 135.

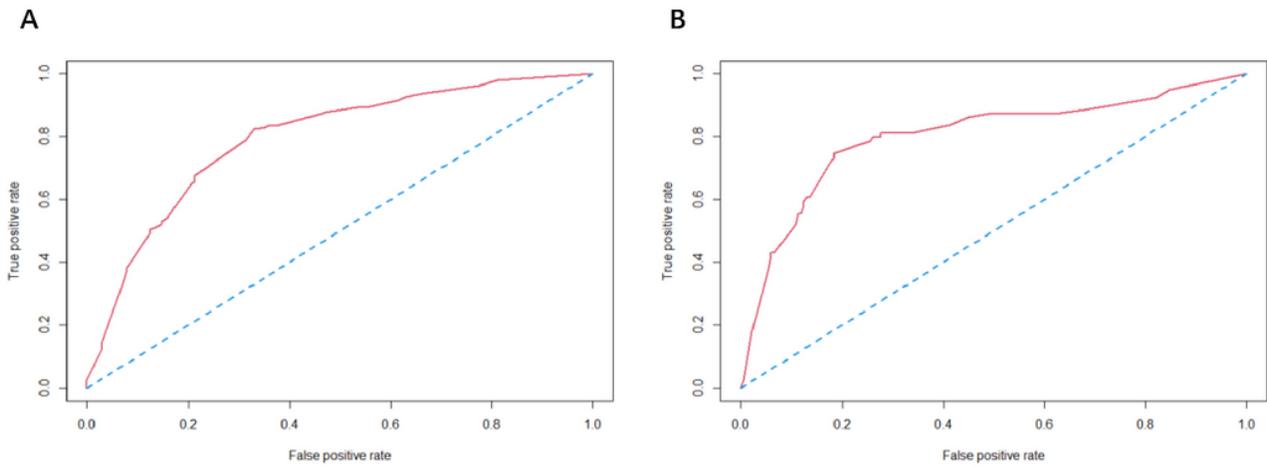


Figure 3

Receiver operating characteristic curves (ROCs) are shown for the prediction model in the training and validation cohorts. (A) The ROC curve in the training set indicates an area under the curve (AUC) of 0.794 (95% CI, 0.756-0.831). (B) For discrimination in the validation set, the ROC indicates an AUC of 0.800 (95% CI, 0.736-0.865).

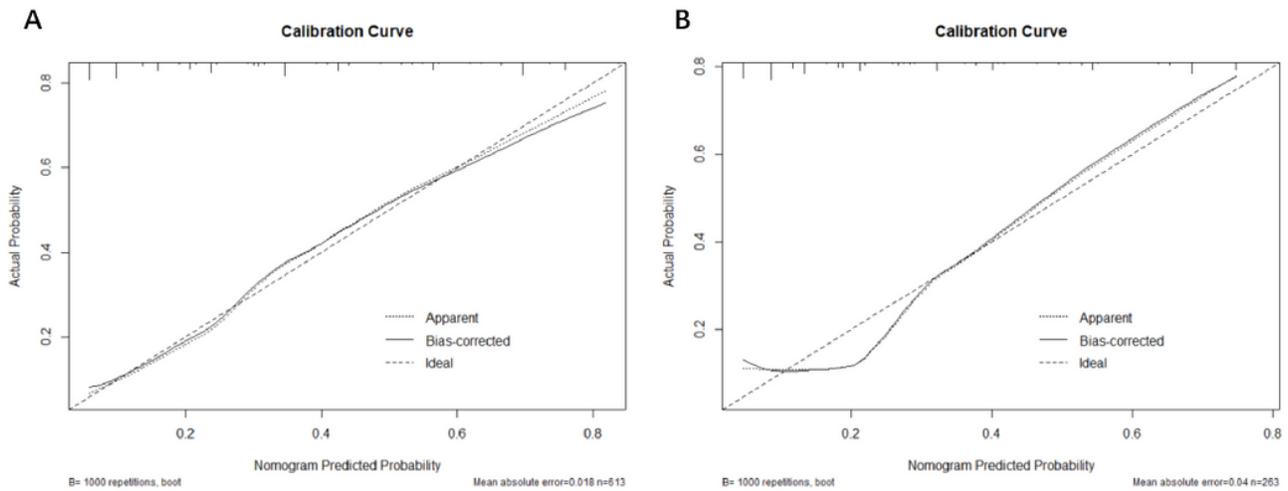


Figure 4

Calibration curves illustrate the observed and predicted false negative rates for patients with high risk breast lesion. (A) the training cohort and (B) the validation cohort. The horizontal axis indicates the predicted probabilities measured by the nomogram, and the vertical axis indicates the actual probabilities. For the calibration plot, $P = 1.000$.

Supplementary Files

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