BMJ Open

# Nomogram predicting overall survival for invasive micropapillary carcinoma of the breast: a SEER-based population study.



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2023-072632 |
| Article Type: | Original research |
| Date Submitted by the Author: | 10-Mar-2023 |
| Complete List of Authors: | Liu , Jianpeng; The First Affiliated Hospital of Wenzhou Medical University, Departments of pathology<br>Xi, Wei; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Zhou, Jiahao; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Gao, Wei; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Wu , Qiaolin; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology |
| Keywords: | Breast tumours < ONCOLOGY, Epidemiology < ONCOLOGY, Adult oncology < ONCOLOGY |
| | |

*I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.*

*The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.*

*Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.*

# Nomogram predicting overall survival for invasive micropapillary carcinoma of the breast: a SEER-based population study.

Jianpeng Liu[1], Wei Xi[2], Jiahao Zhou[2], Wei Gao[2], Qiaolin Wu[2]

Departments of [1]pathology, [2]Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

Address correspondence to: Dr. Qiaolin Wu, Department of Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

Tel: +86-0577-55579098; Fax: +86-0577-55579098

E-mail: 634828235@qq.com(QL);

Jianpeng Liu, and Wei Xi have contributed equally to this article.

Abstract

Background: The prognosis of invasive micropapillary carcinoma (IMPC) of the breast is determined by many clinicopathological factors. This study aims to identify prognostic factors and develop reliable nomogram to predict the overall survival (OS) in patients with IMPC.

Methods: The Surveillance, Epidemiology, and End Results (SEER) database was used to screen 754 eligible patients as the study cohort. The whole cohort was randomly divided into a training cohort (n=377) and a validation cohort (n=377). Log-rank test and Cox proportional hazards analysis were used to identify variables and construct a nomogram based on the training cohort. C-index and calibration curves were performed to evaluate the performance of the model in the training cohort and validation cohorts.

Results: Age at diagnosis, hormone receptors, number of positive regional nodes and clinical stage were independent prognostic factors for patients with IMPC. The calibration curves presented excellent consistency between the actual and nomogram-predict survival probabilities in the training and validation cohorts. The C-index values of the nomogram were 0.794 and 0.774 for OS in the training and validation cohorts, respectively.

Conclusion: The novel nomogram provides new insights of the risk of each prognostic factor and can assist doctors in predicting the 1-, 3-, and 5-year OS in patients with IMPC.

Keywords: Invasive micropapillary breast carcinoma; nomogram; prognosis; Surveillance, Epidemiology, and End Results

Strengths and limitations of this study

First, retrospective SEER data lack a pathologic review to identify the diagnosis for each case. Second, we cannot consider the types of systemic therapy administered to patients. Third, the relationship between the degree of micropapillary involvement and clinical outcomes among patients with IMPC remains unclear.

## Background

Breast cancer is the most prevalent malignancy in women with 290,560 newly estimated diagnosed cases and 43,780 estimated deaths in the United States in 2022(1). The subtype invasive micropapillary carcinoma (IMPC) of the breast is characterized by aggressive potential for lymphovascular invasion and lymph node metastasis(2, 3) and accounts for less than 2% of all invasive breast cancers(4). This cancer type has varying classifications and has no available standardized treatment guidelines.

Considering the rarity of this disease, the conduct of clinical trials to evaluate prognostic factors and optimal treatments is difficult. A few studies have discussed the potential pathologic predictors of survival for IMPC(5-8). However, these published analyses of IMPC have been limited by size, thus leading to discrepancies in the reported prevalence of overall survival and significant prognostic indicators.

A nomogram, a simple visual prediction tool based on a prognostic model that includes related clinicopathological factors, allows doctors to access the probabilities of the clinical outcomes of particular individuals(9, 10). Moreover, compared to the American Joint Committee on Cancer (AJCC) TNM stage system, nomograms can provide a more precise estimation of prognosis for some malignancies(11, 12) and help clinicians to make decisions in complex situations in an alternative or novel standard(13, 14).

In this study, we investigated the Surveillance, Epidemiology, and End Results (SEER) database to determine the prognostic effect of clinicopathological factors on overall survival (OS) in patients with IMPC. A novel nomogram was constructed to predict the prognosis for patients with IMPC.

## Methods

### Study cohorts

The data for this study were obtained from 18 registries of the SEER program, and 1,480 patients diagnosed with IMPC of the breast between 1973 and 2013 were included. The inclusion criteria for the data screening were as follows: (1) female

patients who accepted surgery treatment; (2) age older than 18 years; (3) diagnosis confirmed by positive histology; (4) IMPC as the first and primary cancer determined by international rules; (5) survival data with complete and available dates and more than 0 days of survival; and (6) clear clinicopathological information for all the variables of interest including age at diagnosis, race, marital status, primary site, hormone receptors (HRs) [estrogen receptor (ER) and progesterone receptor (PR)], tumor size, grade, laterality, number of positive regional nodes, surgery record, and clinical stage (the 6th edition of AJCC system).

Variables and definitions

The following data were extracted for each patient from the database: age at diagnosis, race (White, and other), marital status at diagnosis, laterality, clinical stage, number of positive regional nodes, tumor size, tumor grade (well-differentiated, moderately differentiated, poorly differentiated, undifferentiated or anaplastic), hormone receptors (HR+ and HR-), surgery record, radiotherapy record, survival months, and vital status. Marital status was classified as married or unmarried. The latter included single, separated, divorced, widowed, and unmarried/domestic partners. OS was defined as the time from diagnosis to death from any cause or to the time of the last follow-up.

Construction and validation of the nomogram

We performed univariate and multivariate Cox regression analyses to determine the prognostic value of the factors. The independent factors were used to build the nomogram for the Wins by using the rms package in R software version 4.1.3. All the significant independent factors in the training cohort were used to build a nomogram to predict the survival rates. The nomogram was validated in the training and the validation cohorts. We used the Harrell concordance index (C-index), the area under the receiver operating characteristic (ROC) curve (AUC) and the calibration curve to assess the discrimination of the nomogram.

Statistical analysis

Our study consolidated the descriptive characteristics of the training and validation cohorts, respectively. Chi-square test or Fisher's exact test was used to

confirm whether significant differences exist in the demographic and clinicopathological features between the training and validation cohorts. The variables were analyzed using Kaplan–Meier survival curves and log-rank tests to evaluate their effects on OS. The prognostic value of each variable was estimated through univariate and multivariate Cox regression analyses. All P values are two sided, and P values under .05 are reported as statistically significant. The SEER data were extracted using SEERStat 8.4.0, and statistical analyses were performed using SPSS version 26.0 (IBM-SPSS Inc., Armonk, NY).

## Results

### Demographics and clinicopathological characteristics

From the SEER database, a total of 754 cases of IMPC were eligible for inclusion criteria. The eligible patients were randomly divided into the training cohort (n=377) and the validation cohort (n=377) by applying 'create Data Partition' function in the package of 'caret' from R version 4.1.3. The demographic and clinicopathological characteristics of the training and validation cohorts are shown in Table 1, and no statistically significant differences were found between the two cohorts. The estimated average OS values were 106.9 months (95% CI: 102.7-111.1 months) in the 377 patients with IMPC in the training cohort, and 108.2 months (95%CI: 104.4-112.1 months) in the validation cohort. The survival curve showed no significant differences between the two cohorts (Figure1A, P= 0.786).

Table 1. Clinicopathologic characteristics of the training and validation cohorts.

| Variables | Training cohort (n=377) ( %) | Validation cohort (n=377) (%) | P value |
|---|---|---|---|
| Age (year) | 58.73±13.19 | 59.90±12.94 | 0.22 |
| Race | | | 0.11 |
| White | 276 (73.2) | 295 (78.2) | |
| Other | 101 (26.8) | 82 (21.8) | |
| Marital status | | | 0.55 |
| Unmarried | 159 (42.2) | 151 (40.1) | |
| Married | 218 (57.8) | 226 (59.9) | |
| Laterality | | | 0.17 |
| Left | 205 (54.4) | 186 (49.3) | |
| Right | 172 (45.6) | 191 (50.7) | |

| | | | |
|---|---|---|---|
| Grade | | | 0.26 |
| I/II | 236 (62.6) | 221 (58.6) | |
| III/IV | 141 (37.4) | 156 (41.4) | |
| HR status | | | 0.55 |
| Positive | 335 (88.9) | 340 (90.2) | |
| Negative | 42 (11.1) | 37 (9.8) | |
| Tumor size (mm) | 24.44±22.78 | 24.71±21.93 | 0.87 |
| <20 | 223 (59.2) | 204 (54.1) | 0.29 |
| 20-50 | 114 (30.2) | 134 (35.5) | |
| >50 | 40 (10.6) | 39 (10.3) | |
| Number of positive regional nodes | | | 0.99 |
| 0 | 179 (47.5) | 175 (46.4) | |
| 1-3 | 118 (31.3) | 121 (32.1) | |
| 4-9 | 45 (11.9) | 47 (12.5) | |
| ≥10 | 35 (9.3) | 34 (9.0) | |
| Stage | | | 0.73 |
| I | 141 (37.4) | 127 (33.7) | |
| II | 148 (39.2) | 160 (42.4) | |
| III | 82 (21.8) | 83 (22.0) | |
| IV | 6 (1.6) | 7 (1.9) | |
| Surgery | | | 0.34 |
| Conserving surgery | 208 (55.2) | 195 (51.7) | |
| Mastectomy | 169 (44.8) | 182 (48.3) | |
| Radiotherapy | | | 0.06 |
| Yes | 238 (63.1) | 213 (56.5) | |
| No | 139 (36.9) | 164 (43.5) | |

Univariate and multivariate Cox proportional hazards analyses

The hazard ratios for OS according to all variables in the univariate or multivariate Cox proportional hazards model are listed in Tables 2 and 3. According to the results of univariate analysis, we found that the race, marital status, laterality, and radiotherapy were not significant factors for OS. After excluding the aforementioned variables, age at diagnosis, grade, HR status, tumor size, number of positive regional nodes, clinical stage, and surgery were determined as prognostic factors in the multivariate Cox proportional hazards model for the OS analysis. As shown in Table 3, age at diagnosis could be a negative prognostic factor for the OS of patients with IMPC. The HR negative subtype exhibited higher risk of death. Compared with patients with IMPC and negative regional node, patients with positive

regional nodes suffered from higher risk of poor prognosis. Interestingly, the subgroups of stages II and III had a significantly lower risk than the stage I group.

Construction and validation of the nomograms.

Table 2. Univariate analysis of OS in the training cohort.

| Variables | HR | 95% CI | P value |
|---|---|---|---|
| Age (year) | 1.035 | 1.005-1.065 | 0.023 |
| Race | | | |
| White | | reference | |
| Other | 1.202 | 0.529-2.732 | 0.660 |
| Marital status | | | |
| Unmarried | | reference | |
| Married | 0.721 | 0.343-1.512 | 0.386 |
| Laterality | | | |
| Left | | reference | |
| Right | 0.915 | 0.435-1.923 | 0.816 |
| Grade | | | |
| I/II | | reference | |
| III/IV | 2.180 | 1.030-4.611 | 0.042 |
| HR status | | | |
| Positive | | reference | |
| Negative | 4.150 | 1.914-8.998 | <0.001 |
| Tumor size (mm) | | | <0.001 |
| <20 | | reference | |
| 20-50 | 1.931 | 0.728-5.119 | 0.186 |
| >50 | 7.960 | 3.339-18.973 | <0.001 |
| Number of positive regional nodes | | | <0.001 |
| 0 | | reference | |
| 1-3 | 1.679 | 0.609-4.632 | 0.317 |
| 4-9 | 3.145 | 0.998-9.914 | 0.050 |
| ≥10 | 8.350 | 3.016-23.115 | <0.001 |
| Stage | | | <0.001 |
| I | | reference | |
| II | 1.040 | 0.365-2.967 | 0.941 |
| III | 3.529 | 1.262-8.419 | 0.015 |
| IV | 19.576 | 4.982-76.921 | <0.001 |
| Surgery | | | |
| Conserving surgery | | reference | |
| Mastectomy | 2.530 | 1.144-5.596 | 0.022 |
| Radiotherapy | | | |
| Yes | | reference | |
| No | 0.780 | 0.368-1.649 | 0.515 |

Table 3. Multivariate analysis of OS in the training cohort.

| Variables | HR | 95% CI | P value |
|---|---|---|---|
| Age (year) | 1.054 | 1.020-1.090 | 0.020 |
| Grade | | | |
| I/II | | reference | |
| III/IV | 1.159 | 0.504-2.666 | 0.728 |
| HR status | | | |
| Positive | | reference | |
| Negative | 5.368 | 2.084-13.830 | 0.001 |
| Tumor size (mm) | | | |
| <20 | | reference | |
| 20-50 | 2.292 | 0.631-8.322 | 0.208 |
| >50 | 4.807 | 0.919-25.153 | 0.063 |
| Number of positive regional nodes | | | |
| 0 | | reference | |
| 1-3 | 18.314 | 1.387-241.811 | 0.027 |
| 4-9 | 10.340 | 1.044-102.388 | 0.046 |
| ≥10 | 26.776 | 3.300-23.115 | 0.002 |
| Stage | | | |
| I | | reference | |
| II | 0.057 | 0.004-0.802 | 0.034 |
| III | 0.096 | 0.100-0.964 | 0.046 |
| IV | 0.211 | 0.170-2.641 | 0.228 |
| Surgery | | | |
| Conserving surgery | | reference | |
| Mastectomy | 1.119 | 0.393-3.190 | 0.833 |

The nomogram for 1-, 3-, and 5-year OS was developed by using the multivariate Cox proportional hazards models as the final prognostic models after factor selection (Figure 1B). The nomogram was internally validated in the training cohort and externally validated in the validation cohort. The AUC values of the ROC curve, which exhibited the discrimination capacity, were 0.830 and 0.764 in the training (Figure 1C) and validation cohorts (Figure 1D), respectively. Moreover, compared with the discriminative ability of the sixth edition AJCC TNM staging classification, the discriminative ability of the nomogram was significantly superior in the training and validation cohorts ($P < 0.001$). The results indicated that the nomogram can efficiently predict OS in patients with IMPC. The calibration plots

also showed great consistency between the actual and nomogram-predicted survival rates in the training (Figure 2A)and testing cohorts (Figure 2B). The C-index values of the nomogram for OS were 0.794 in the training cohort and 0.774 in the validation cohort.

## Discussion

Invasive micropapillary carcinoma of breast, which was first described by Siriaunkgul and Tavassoli in 1993(4), is a rare variant of invasive breast carcinoma (IDC). Histologically, it is a subtype characterized by small papillary structures that lack true central fibrovascular cores and lie within empty stromal spaces(15, 16). Historically, standard IDC treatment was used to treat patients with IMPC. However, notable differences in histological characters and prognosis exist between IMPC and IDC(17); as such, treating IMPC as IDC would be inappropriate. Accurate predictions of prognosis of patients with IMPC patients could effectively help clinicians to take proper treatment modalities. This study aims to build a nomogram capable of predicting the prognosis of IMPC based on a larger population database of the Surveillance, Epidemiology, and End Results (SEER) program.

In this study, we equally divided 754 patients with IMPC from the SEER database into two cohorts. We developed an effective nomogram that contains four independent prognostic factors including age at diagnosis, HR, number of positive regional nodes, and clinical stage. The nomogram, derived from the Cox regression model to predict the 1-, 3-, and 5-year OS of patients with IMPC, was verified to have good discrimination capacity. Moreover, the nomogram showed better prediction ability for OS than that of the sixth edition AJCC TNM staging classification (AUCs in the ROC curve: 0.830 and 0.651 in the training cohort and 0.764 and 0.633 in the validation cohort, respectively).

Hormone receptors play important role in prognosis of breast cancer(18, 19). A previous study showed that the 5-year OS was 59% in 100 patients with IMPC with a mean age of 50 years and 46% HR positivity(7). In another study, 72 patients with IMPC with a mean age of 46 years and 75% HR positivity had 86% 5-year OS(5). In

comparison, our study population was older (mean age of 59.3 years) and had a higher percentage of HR positivity (89.5%). The higher HR positivity in the present study may contribute to the better 5-year OS (91.1%)(20). The Cox-regression analysis result also proved that HR negativity could lead to significantly poor OS in patients with IMPC (HR 5.368; 95%CI 2.084–13.830; P=0.001).

Lymph node metastasis is widely considered as an unfavorable prognostic factor in clinical practice(21, 22). Axillary lymph node metastasis is commonly seen in patients with IMPC at first diagnosis. The rate of lymphatic and lymph nodal spread ranged from 33% to 95%(4, 15, 23, 24). The value and necessity of sentinel lymph node biopsy (SLNB) or axillary dissection in patients with IMPC remains controversial. Walsh et al. found that regional lymph nodes can be involved even at early stage of IMPC lesions. The team highly recommended a thorough regional lymph node examination to patients with IMPC (16). However, Paterakos et al. were skeptical to the utility of SLNB for patients with IMPC due to the high frequency of multiple positive regional lymph nodes(25). In the present study, we found that patients with IMPC with even one positive regional lymph node would suffer higher risk than patients with negative lymph node. Patients with IMPC and 10 or more positive lymph nodes are at the highest risk (OR 26.776; 95%CI 3.300-23.115; P=0.002). Thus, axillary dissection, or SLNB at minimum, should be performed to correctly access the risk and adopt suitable treatment regimens for patients with IMPC.

This study has some limitations. First, retrospective SEER data lack a pathologic review to identify the diagnosis for each case. Second, we cannot consider the types of systemic therapy administered to patients. Hormonal blockade therapy and chemotherapy could significantly affect the outcome of patients. Third, the relationship between the degree of micropapillary involvement and clinical outcomes among patients with IMPC remains unclear. Although some previous small case series studies have revealed that an increasing percentage of micropapillary component was not associated with more lymph node metastasis and worse survival(23, 26), it need to be further validated in large-scale studies.

## Conclusions

In conclusion, age at diagnosis, HR status, number of positive regional nodes, and clinical stage were independent prognostic factors for patients with IMPC. We constructed a nomogram to predict OS in patients with IMPC based on a large-scale population from the SEER database. This accessible nomogram will help doctors to adopt proper treatment regimens in clinical practice.

## List of abbreviations

invasive micropapillary carcinoma (IMPC)
overall survival (OS)
Surveillance, Epidemiology, and End Results (SEER)
American Joint Committee on Cancer (AJCC)
hormone receptors (HRs)
estrogen receptor (ER)
progesterone receptor (PR)
receiver operating characteristic (ROC)
area under the curve (AUC)
invasive breast carcinoma (IDC)

## Acknowledgments

This work was supported by a grant from the Incubation project [NO.FHY2019064].

Conflict of interest: The authors declare that they have no conflict of interest.

## Reference

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33.

2. Luna-More S, Gonzalez B, Acedo C, Rodrigo I, Luna C. Invasive micropapillary

carcinoma of the breast. A new special type of invasive mammary carcinoma. Pathol Res Pract. 1994;190(7):668-74.

3. Fu L, Ikuo M, Fu XY, Liu TH, Shinichi T. [Relationship between biologic behavior and morphologic features of invasive micropapillary carcinoma of the breast]. Zhonghua Bing Li Xue Za Zhi. 2004;33(1):21-5.

4. Siriaunkgul S, Tavassoli FA. Invasive micropapillary carcinoma of the breast. Mod Pathol. 1993;6(6):660-2.

5. Yu JI, Choi DH, Park W, Huh SJ, Cho EY, Lim YH, et al. Differences in prognostic factors and patterns of failure between invasive micropapillary carcinoma and invasive ductal carcinoma of the breast: matched case-control study. Breast. 2010;19(3):231-7.

6. Yu JI, Choi DH, Huh SJ, Cho EY, Kim K, Chie EK, et al. Differences in Prognostic Factors and Failure Patterns Between Invasive Micropapillary Carcinoma and Carcinoma With Micropapillary Component Versus Invasive Ductal Carcinoma of the Breast: Retrospective Multicenter Case-Control Study (KROG 13-06). Clin Breast Cancer. 2015;15(5):353-61 e1-2.

7. Chen L, Fan Y, Lang RG, Guo XJ, Sun YL, Cui LF, et al. Breast carcinoma with micropapillary features: clinicopathologic study and long-term follow-up of 100 cases. Int J Surg Pathol. 2008;16(2):155-63.

8. Chen AC, Paulino AC, Schwartz MR, Rodriguez AA, Bass BL, Chang JC, et al. Prognostic markers for invasive micropapillary carcinoma of the breast: a population-based analysis. Clin Breast Cancer. 2013;13(2):133-9.

9. Iasonos A, Schrag D, Raj GV, Panageas KS. How to build and interpret a nomogram for

cancer prognosis. J Clin Oncol. 2008;26(8):1364-70.

10. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP. Nomograms in oncology: more than meets the eye. Lancet Oncol. 2015;16(4):e173-80.

11. Diao JD, Ma LX, Sun MY, Wu CJ, Wang LJ, Liu YL, et al. Construction and validation of a nomogram to predict overall survival in patients with inflammatory breast cancer. Cancer Med. 2019;8(12):5600-8.

12. Yang J, Pan Z, Zhao F, Feng X, Liu Q, Li Y, et al. A nomogram for predicting survival in patients with nodular melanoma: A population-based study. Medicine (Baltimore). 2019;98(24):e16059.

13. Liu H, Zheng SQ, Li XY, Zeng ZH, Zhong JS, Chen JQ, et al. Derivation and Validation of a Nomogram to Predict In-Hospital Complications in Children with Tetralogy of Fallot Repaired at an Older Age. J Am Heart Assoc. 2019;8(21):e013388.

14. Song J, Sun Y, Cao H, Liu Z, Xi L, Dong C, et al. A novel pyroptosis-related lncRNA signature for prognostic prediction in patients with lung adenocarcinoma. Bioengineered. 2021;12(1):5932-49.

15. Pettinato G, Manivel CJ, Panico L, Sparano L, Petrella G. Invasive micropapillary carcinoma of the breast: clinicopathologic study of 62 cases of a poorly recognized variant with highly aggressive behavior. Am J Clin Pathol. 2004;121(6):857-66.

16. Walsh MM, Bleiweiss IJ. Invasive micropapillary carcinoma of the breast: eighty cases of an underrecognized entity. Hum Pathol. 2001;32(6):583-9.

17. Nassar H, Wallis T, Andea A, Dey J, Adsay V, Visscher D. Clinicopathologic analysis of invasive micropapillary differentiation in breast carcinoma. Mod Pathol. 2001;14(9):836-41.

18. Clark GM, Osborne CK, McGuire WL. Correlations between estrogen receptor, progesterone receptor, and patient characteristics in human breast cancer. J Clin Oncol. 1984;2(10):1102-9.

19. Osborne CK, Fisher E, Redmond C, Knight WA, Yochmowitz MG, McGuire WL. Estrogen receptor, a marker for human breast cancer differentiation and patient prognosis. Adv Exp Med Biol. 1981;138:377-85.

20. Luna-More S, Casquero S, Perez-Mellado A, Rius F, Weill B, Gornemann I. Importance of estrogen receptors for the behavior of invasive micropapillary carcinoma of the breast. Review of 68 cases with follow-up of 54. Pathol Res Pract. 2000;196(1):35-9.

21. Kuru B, Camlibel M, Dinc S, Gulcelik MA, Alagol H. Prognostic significance of axillary node and infraclavicular lymph node status after mastectomy. Eur J Surg Oncol. 2003;29(10):839-44.

22. Hur MH, Ko S. Metastatic axillary node ratio predicts recurrence and poor long-term prognosis in patients with advanced stage IIIC (pN3) breast cancer. Ann Surg Treat Res. 2017;92(5):340-7.

23. Zekioglu O, Erhan Y, Ciris M, Bayramoglu H, Ozdemir N. Invasive micropapillary carcinoma of the breast: high incidence of lymph node metastasis with extranodal extension and its immunohistochemical profile compared with invasive ductal carcinoma. Histopathology. 2004;44(1):18-23.

24. Adrada B, Arribas E, Gilcrease M, Yang WT. Invasive micropapillary carcinoma of the breast: mammographic, sonographic, and MRI features. AJR Am J Roentgenol. 2009;193(1):W58-63.

25. Paterakos M, Watkin WG, Edgerton SM, Moore DH, 2nd, Thor AD. Invasive micropapillary carcinoma of the breast: a prognostic study. Hum Pathol. 1999;30(12):1459-63.

26. Kim MJ, Gong G, Joo HJ, Ahn SH, Ro JY. Immunohistochemical and clinicopathologic characteristics of invasive ductal carcinoma of breast with micropapillary carcinoma component. Arch Pathol Lab Med. 2005;129(10):1277-82.

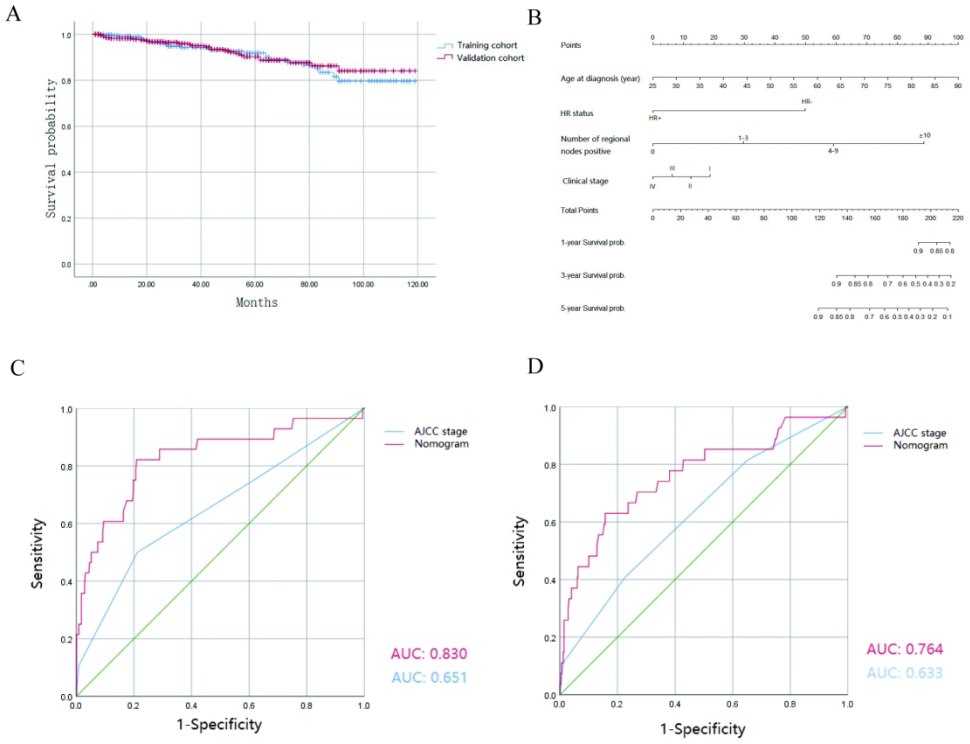


Figure 1A. Kaplan-Meier survival curves of the patients with IMPC in the training and validation cohorts.
Notes: The survival curves showed no significant differences between the 2 cohorts (P= 0.786).

Figure 1B. Nomogram for predicting 1-, 3-, 5-year OS for patients with the prognosis factors.
Notes: The total points are calculated by summing up the points for each factor. The predicted probability of OS can be obtained by projecting the location of the total points to the bottom scales. Abbreviations: OS= overall survival.

Figure 1C and Figure 1D. ROC curves for discrimination in the training and validation cohorts.
Notes: (Figure 1C) In the training cohort, the AUC of the ROC curve of the nomogram and the sixth edition AJCC TNM staging classification was 0.830 and 0.651, respectively (P＜0.001). (Figure 1D) In the validation cohort, the AUC of the ROC curve of the nomogram and the sixth edition AJCC TNM staging classification was 0.764 and 0.633, respectively (P＜0.001). Abbreviations: AJCC= American Joint Committee on Cancer; AUC= area under the curve; ROC= receiver operating characteristic.

185x141mm (300 x 300 DPI)

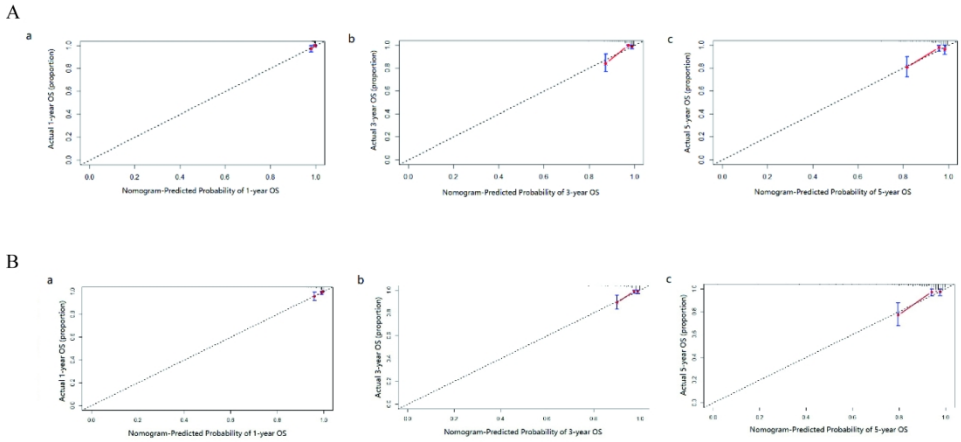


Figure 2. Calibration curves for predictions for the 1-year (a), 3-year (b), 5-year (c) OS in the training cohort (Figure 2A) and in the testing cohort (Figure 2B).
Notes: (Figure 2A) The nomogram-predicted probability of OS is plotted on the X-axis, and the actual OS is plotted on the Y-axis. (Figure 2B) The nomogram-predicted probability of OS is plotted on the X-axis, and the actual OS is plotted on the Y-axis.

213x94mm (300 x 300 DPI)

BMJ Open

# Nomogram predicting overall prognosis for invasive micropapillary carcinoma of the breast: a SEER-based population study.



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2023-072632.R1 |
| Article Type: | Original research |
| Date Submitted by the Author: | 24-May-2023 |
| Complete List of Authors: | Liu , Jianpeng; The First Affiliated Hospital of Wenzhou Medical University, Departments of pathology<br>Xi, Wei; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Zhou, Jiahao; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Gao, Wei; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Wu , Qiaolin; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology |
| Primary Subject Heading: | Oncology |
| Secondary Subject Heading: | Epidemiology |
| Keywords: | Breast tumours < ONCOLOGY, Epidemiology < ONCOLOGY, Adult oncology < ONCOLOGY |
| | |

*I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.*

*The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.*

*Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.*

# Nomogram predicting overall prognosis for invasive micropapillary carcinoma of the breast: a SEER-based population study.

Jianpeng Liu[1], Wei Xi[2], Jiahao Zhou[2], Wei Gao[2], Qiaolin Wu[2]

Departments of [1]pathology, [2]Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

Address correspondence to: Dr. Qiaolin Wu, Department of Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

Tel: +86-0577-55579098; Fax: +86-0577-55579098

E-mail: 634828235@qq.com(QL);

Jianpeng Liu, and Wei Xi have contributed equally to this article.

Abstract

**Objectives** The prognosis of invasive micropapillary carcinoma (IMPC) of the breast is determined by many clinicopathological factors. This study aims to identify prognostic factors and develop reliable nomogram to predict the overall survival (OS) in patients with IMPC.

**Design** Log-rank test and Cox proportional hazards analysis were used to identify variables and construct a nomogram based on the training cohort. C-index and calibration curves were performed to evaluate the performance of the model in the training cohort and validation cohorts.

**Setting** We collected the patient data from the Surveillance, Epidemiology, and End Results (SEER) database. This database holds data related to the cancer incidence from 18 population-based cancer registries in the United States.

**Participants** The SEER database was used to screen 754 eligible patients as the study cohort. The whole cohort was randomly divided into a training cohort (n=377) and a validation cohort (n=377).

**Results** Age at diagnosis, hormone receptors, number of positive regional lymph-nodes and clinical stage were independent prognostic factors for patients with IMPC. The calibration curves presented excellent consistency between the actual and nomogram-predict survival probabilities in the training and validation cohorts. The C-index values of the nomogram were 0.794 and 0.774 for OS in the training and validation cohorts, respectively.

**Conclusions** The novel nomogram provides new insights of the risk of each prognostic factor and can assist doctors in predicting the 1-, 3-, and 5-year OS in patients with IMPC.

Keywords: Invasive micropapillary breast carcinoma; nomogram; prognosis; Surveillance, Epidemiology, and End Results

Strengths and limitations of this study

The data was downloaded from the SEER database, which provides a representative population-based cohort.

Prognostic factors were determined by univariate and multivariate Cox proportional hazards regression analyses and used to develop nomograms to predict 1-, 3-, and 5-year overall survival of patients with invasive micropapillary carcinoma (IMPC).

To compare the accuracy of the nomograms with that of American Joint Committee on Cancer 6 staging, we used the Harrell concordance index (C-index), the area under the receiver operating characteristic (ROC) curve (AUC) and the calibration curve to assess the discrimination of the nomograms.

This research was a retrospectively large-sample study, the casual basis of this research was difficult to conclude.

The diagnosis of IMPC in each case cannot be validated by pathologic assessments, and the relationship between the degree of micropapillary involvement and clinical outcomes among patients with IMPC yet to be determined.

## Introduction

Breast cancer is the most prevalent malignancy in women with 290,560 newly estimated diagnosed cases and 43,780 estimated deaths in the United States in 2022[1]. The special type invasive micropapillary carcinoma (IMPC) of the breast is characterized by aggressive potential for lymphovascular invasion and lymph node metastasis and accounts for less than 2% of all invasive breast cancers[2-4]. Hormonal and HER-2 positivity in invasive micropapillary carcinoma (IMPC) of the breast is also commoner when compared to other Non-Specific Type (NST) carcinomas. IMPC occurs either as a pure form or more often as a component of mixed NST carcinoma[5-7]. This cancer type has varying classifications and has no available standardized treatment guidelines.

Considering the rarity of this disease, the conduct of clinical trials to evaluate prognostic factors and optimal treatments is difficult. A few studies have discussed the potential pathologic predictors of survival for IMPC[8-15]. However, these published analyses of IMPC have been limited by size, thus leading to discrepancies in the reported prevalence of overall survival and significant prognostic indicators.

A nomogram, a simple visual prediction tool based on a prognostic model that includes related clinicopathological factors, allows doctors to access the probabilities of the clinical outcomes of particular individuals[16 17]. Moreover, compared to the American Joint Committee on Cancer (AJCC) TNM stage system, nomograms can provide a more precise estimation of prognosis for some malignancies[18 19] and help clinicians to make decisions in complex situations in an alternative or novel standard[20 21].

In this study, we investigated the Surveillance, Epidemiology, and End Results (SEER) database to determine the prognostic effect of clinicopathological factors on overall survival (OS) in patients with IMPC. A novel nomogram was constructed to predict the prognosis for patients with IMPC.

## Methods

### Study cohorts

The data for this study were obtained from 18 registries of the SEER program, and 1,480 patients diagnosed with IMPC of the breast between 1973 and 2013 were included. The inclusion criteria for the data screening were as follows: (1) female patients who accepted surgery treatment; (2) age older than 18 years; (3) diagnosis confirmed by histopathological report; (4) IMPC as the first and primary cancer determined by international rules; (5) survival data with complete and available dates and more than 0 days of survival; and (6) clear clinicopathological information for all the variables of interest including age at diagnosis, race, marital status, primary site, hormone receptors (HRs) [estrogen receptor (ER) and progesterone receptor (PR)], tumor size, grade, laterality, number of positive regional lymph-nodes, surgery record, and clinical stage (the 6th edition of AJCC system).

Variables and definitions

The following data were extracted for each patient from the database: age at diagnosis, race (white and others), marital status at diagnosis, laterality, clinical stage, number of positive regional lymph-nodes, tumor size, tumor grade (well-differentiated, moderately differentiated, poorly differentiated, undifferentiated or anaplastic), hormone receptors (HR+ and HR-), surgery record, radiotherapy record, survival months, and vital status. Marital status was classified as married or unmarried. The latter included single, separated, divorced, widowed, and unmarried/domestic partners. OS was defined as the time from diagnosis to death from any cause or to the time of the last follow-up.

Construction and validation of the nomogram

We performed univariate and multivariate Cox regression analyses to determine the prognostic value of the factors. The independent factors were used to build the nomogram for the Wins by using the rms package in R software version 4.1.3. All the significant independent factors in the training cohort were used to build a nomogram to predict the survival rates. The nomogram was validated in the training and the validation cohorts. We used the Harrell concordance index (C-index), the area under the receiver operating characteristic (ROC) curve (AUC) and the calibration curve to assess the discrimination of the nomogram.

## Statistical analysis

Our study consolidated the descriptive characteristics of the training and validation cohorts, respectively. Chi-square test or Fisher's exact test was used to confirm whether significant differences exist in the demographic and clinicopathological features between the training and validation cohorts. The variables were analyzed using Kaplan–Meier survival curves and log-rank tests to evaluate their effects on OS. The prognostic value of each variable was estimated through univariate and multivariate Cox regression analyses. All P values are two sided, and P values under .05 are reported as statistically significant. The SEER data were extracted using SEERStat 8.4.0, and statistical analyses were performed using SPSS version 26.0 (IBM-SPSS Inc., Armonk, NY).

## Results

### Demographics and clinicopathological characteristics

From the SEER database, a total of 754 cases of IMPC were eligible for inclusion criteria. The eligible patients were randomly divided into the training cohort (n=377) and the validation cohort (n=377) by applying 'create Data Partition' function in the package of 'caret' from R version 4.1.3. The demographic and clinicopathological characteristics of the training and validation cohorts are shown in Table 1, and no statistically significant differences were found between the two cohorts. The estimated average OS values were 106.9 months (95% CI: 102.7-111.1 months) in the 377 patients with IMPC in the training cohort, and 108.2 months (95%CI: 104.4-112.1 months) in the validation cohort. The survival curve showed no significant differences between the two cohorts (Figure1, P= 0.786).

Table 1. Clinicopathologic characteristics of the training and validation cohorts.

| Variables | | Training cohort (n=377) ( %) | Validation cohort (n=377) (%) | P value |
|---|---|---|---|---|
| Age (year) | | 58.73±13.19 | 59.90±12.94 | 0.22 |
| Race | | | | 0.11 |
| | White | 276 (73.2) | 295 (78.2) | |
| | Other | 101 (26.8) | 82 (21.8) | |
| Marital status | | | | 0.55 |

| | | | |
|---|---|---|---|
| Unmarried | 159 (42.2) | 151 (40.1) | |
| Married | 218 (57.8) | 226 (59.9) | |
| Laterality | | | 0.17 |
| Left | 205 (54.4) | 186 (49.3) | |
| Right | 172 (45.6) | 191 (50.7) | |
| Grade | | | 0.26 |
| I/II | 236 (62.6) | 221 (58.6) | |
| III/IV | 141 (37.4) | 156 (41.4) | |
| HR status | | | 0.55 |
| Positive | 335 (88.9) | 340 (90.2) | |
| Negative | 42 (11.1) | 37 (9.8) | |
| Tumor size (mm) | 24.44±22.78 | 24.71±21.93 | 0.87 |
| <20 | 223 (59.2) | 204 (54.1) | 0.29 |
| 20-50 | 114 (30.2) | 134 (35.5) | |
| >50 | 40 (10.6) | 39 (10.3) | |
| Number of positive regional nodes | | | 0.99 |
| 0 | 179 (47.5) | 175 (46.4) | |
| 1-3 | 118 (31.3) | 121 (32.1) | |
| 4-9 | 45 (11.9) | 47 (12.5) | |
| ≥10 | 35 (9.3) | 34 (9.0) | |
| Stage | | | 0.73 |
| I | 141 (37.4) | 127 (33.7) | |
| II | 148 (39.2) | 160 (42.4) | |
| III | 82 (21.8) | 83 (22.0) | |
| IV | 6 (1.6) | 7 (1.9) | |
| Surgery | | | 0.34 |
| Conserving surgery | 208 (55.2) | 195 (51.7) | |
| Mastectomy | 169 (44.8) | 182 (48.3) | |
| Radiotherapy | | | 0.06 |
| Yes | 238 (63.1) | 213 (56.5) | |
| No | 139 (36.9) | 164 (43.5) | |

Univariate and multivariate Cox proportional hazards analyses

The hazard ratios for OS according to all variables in the univariate or multivariate Cox proportional hazards model are listed in Tables 2 and 3. According to the results of univariate analysis, we found that the race, marital status, laterality, and radiotherapy were not significant factors for OS. After excluding the aforementioned variables, age at diagnosis, grade, HR status, tumor size, number of positive regional lymph-nodes, clinical stage, and surgery were determined as

prognostic factors in the multivariate Cox proportional hazards model for the OS analysis. As shown in Table 3, age at diagnosis could be a negative prognostic factor for the OS of patients with IMPC. The HR negative special type exhibited higher risk of death. Compared with patients with IMPC and negative regional node, patients with positive regional lymph-nodes suffered from higher risk of poor prognosis. Interestingly, the subgroups of stages II and III had a significantly lower risk than the stage I group.

Table 2. Univariate analysis of OS in the training cohort.

| Variables | HR | 95% CI | P value |
|---|---|---|---|
| Age (year) | 1.035 | 1.005-1.065 | 0.023 |
| Race | | | |
| White | | reference | |
| Other | 1.202 | 0.529-2.732 | 0.660 |
| Marital status | | | |
| Unmarried | | reference | |
| Married | 0.721 | 0.343-1.512 | 0.386 |
| Laterality | | | |
| Left | | reference | |
| Right | 0.915 | 0.435-1.923 | 0.816 |
| Grade | | | |
| I/II | | reference | |
| III/IV | 2.180 | 1.030-4.611 | 0.042 |
| HR status | | | |
| Positive | | reference | |
| Negative | 4.150 | 1.914-8.998 | <0.001 |
| Tumor size (mm) | | | <0.001 |
| <20 | | reference | |
| 20-50 | 1.931 | 0.728-5.119 | 0.186 |
| >50 | 7.960 | 3.339-18.973 | <0.001 |
| Number of positive regional nodes | | | <0.001 |
| 0 | | reference | |
| 1-3 | 1.679 | 0.609-4.632 | 0.317 |
| 4-9 | 3.145 | 0.998-9.914 | 0.050 |
| ≥10 | 8.350 | 3.016-23.115 | <0.001 |
| Stage | | | <0.001 |
| I | | reference | |
| II | 1.040 | 0.365-2.967 | 0.941 |
| III | 3.529 | 1.262-8.419 | 0.015 |
| IV | 19.576 | 4.982-76.921 | <0.001 |
| Surgery | | | |

| | | | |
|---|---|---|---|
| Conserving surgery | | reference | |
| Mastectomy | 2.530 | 1.144-5.596 | 0.022 |
| Radiotherapy | | | |
| Yes | | reference | |
| No | 0.780 | 0.368-1.649 | 0.515 |

Table 3. Multivariate analysis of OS in the training cohort.

| Variables | HR | 95% CI | P value |
|---|---|---|---|
| Age (year) | 1.054 | 1.020-1.090 | 0.020 |
| Grade | | | |
| I/II | | reference | |
| III/IV | 1.159 | 0.504-2.666 | 0.728 |
| HR status | | | |
| Positive | | reference | |
| Negative | 5.368 | 2.084-13.830 | 0.001 |
| Tumor size (mm) | | | |
| <20 | | reference | |
| 20-50 | 2.292 | 0.631-8.322 | 0.208 |
| >50 | 4.807 | 0.919-25.153 | 0.063 |
| Number of positive regional nodes | | | |
| 0 | | reference | |
| 1-3 | 18.314 | 1.387-241.811 | 0.027 |
| 4-9 | 10.340 | 1.044-102.388 | 0.046 |
| ≥10 | 26.776 | 3.300-23.115 | 0.002 |
| Stage | | | |
| I | | reference | |
| II | 0.057 | 0.004-0.802 | 0.034 |
| III | 0.096 | 0.100-0.964 | 0.046 |
| IV | 0.211 | 0.170-2.641 | 0.228 |
| Surgery | | | |
| Conserving surgery | | reference | |
| Mastectomy | 1.119 | 0.393-3.190 | 0.833 |

Construction and validation of the nomograms.

The nomogram for 1-, 3-, and 5-year OS was developed by using the multivariate Cox proportional hazards models as the final prognostic models after factor selection (Figure 2). The nomogram was internally validated in the training cohort and externally validated in the validation cohort. The AUC values of the ROC curve, which exhibited the discrimination capacity, were 0.830 and 0.764 in the

training and validation cohorts, respectively (Figure 3). Moreover, compared with the discriminative ability of the sixth edition AJCC TNM staging classification, the discriminative ability of the nomogram was significantly superior in the training and validation cohorts ($P < 0.001$). The results indicated that the nomogram can efficiently predict OS in patients with IMPC. The calibration plots also showed great consistency between the actual and nomogram-predicted survival rates in the training and testing cohorts (Figure 4, Figure 5). The C-index values of the nomogram for OS were 0.794 in the training cohort and 0.774 in the validation cohort.

## Patient and public involvement

No patient involved.

## Discussion

Invasive micropapillary carcinoma of breast, which was first described by Siriaunkgul and Tavassoli in 1993[4], is a rare variant of invasive breast carcinoma (IBC). Histologically, it is a special type characterized by small papillary structures that lack true central fibrovascular cores and lie within empty stromal spaces[22 23]. Historically, standard IBC treatment was used to treat patients with IMPC. However, notable differences in histological characters and prognosis exist between IMPC and IBC[24]; as such, treating IMPC as IBC would be inappropriate. Accurate predictions of prognosis of patients with IMPC patients could effectively help clinicians to take proper treatment modalities. This study aims to build a nomogram capable of predicting the prognosis of IMPC based on a larger population database of the Surveillance, Epidemiology, and End Results (SEER) program.

In this study, we equally divided 754 patients with IMPC from the SEER database into two cohorts. We developed an effective nomogram that contains four independent prognostic factors including age at diagnosis, HR, number of positive regional lymph-nodes, and clinical stage. The nomogram, derived from the Cox regression model to predict the 1-, 3-, and 5-year OS of patients with IMPC, was verified to have good discrimination capacity. Moreover, the nomogram showed

better prediction ability for OS than that of the sixth edition AJCC TNM staging classification (AUCs in the ROC curve: 0.830 and 0.651 in the training cohort and 0.764 and 0.633 in the validation cohort, respectively).

Hormone receptors play important role in prognosis of breast cancer[25 26]. A previous study showed that the 5-year OS was 59% in 100 patients with IMPC with a mean age of 50 years and 46% HR positivity[10]. In another study, 72 patients with IMPC with a mean age of 46 years and 75% HR positivity had 86% 5-year OS[8]. In comparison, our study population was older (mean age of 59.3 years) and had a higher percentage of HR positivity (89.5%). The higher HR positivity in the present study may contribute to the better 5-year OS (91.1%)[27]. The Cox-regression analysis result also proved that HR negativity could lead to significantly poor OS in patients with IMPC (HR 5.368; 95%CI 2.084–13.830; P=0.001).

Lymph node metastasis is widely considered as an unfavorable prognostic factor in clinical practice[28 29]. Axillary lymph node metastasis is commonly seen in patients with IMPC at first diagnosis. The rate of lymphatic and lymph nodal spread ranged from 33% to 95%[4 22 30 31]. The value and necessity of sentinel lymph node biopsy (SLNB) or axillary dissection in patients with IMPC remains controversial. Walsh et al. found that regional lymph nodes can be involved even at early stage of IMPC lesions. The team highly recommended a thorough regional lymph node examination to patients with IMPC[23]. However, Paterakos et al. were skeptical to the utility of SLNB for patients with IMPC due to the high frequency of multiple positive regional lymph nodes[32]. In the present study, we found that patients with IMPC with even one positive regional lymph node would suffer higher risk than patients with negative lymph node. Patients with IMPC and 10 or more positive lymph nodes are at the highest risk (OR 26.776; 95%CI 3.300-23.115; P=0.002). Thus, axillary dissection, or SLNB at minimum, should be performed to correctly access the risk and adopt suitable treatment regimens for patients with IMPC.

This study has some limitations. First, retrospective SEER data lack a pathologic review to identify the diagnosis for each case. Second, we cannot consider the types of systemic therapy administered to patients. Hormonal blockade therapy and

chemotherapy could significantly affect the outcome of patients. Third, the relationship between the degree of micropapillary involvement and clinical outcomes among patients with IMPC remains unclear. Although some previous small case series studies have revealed that an increasing percentage of micropapillary component was not associated with more lymph node metastasis and worse survival[30 33], it need to be further validated in large-scale studies.

## Conclusions

In conclusion, age at diagnosis, HR status, number of positive regional lymph-nodes, and clinical stage were independent prognostic factors for patients with IMPC. We constructed a nomogram to predict OS in patients with IMPC based on a large-scale population from the SEER database. This accessible nomogram will help doctors to adopt proper treatment regimens in clinical practice.

## List of abbreviations

invasive micropapillary carcinoma (IMPC)
overall survival (OS)
Surveillance, Epidemiology, and End Results (SEER)
American Joint Committee on Cancer (AJCC)
hormone receptors (HRs)
estrogen receptor (ER)
progesterone receptor (PR)
receiver operating characteristic (ROC)
area under the curve (AUC)
invasive breast carcinoma (IBC)

Jianpeng Liu and Wei Xi contributed equally.

Contributors: Jianpeng Liu and Wei Xi designed and conducted the study. Jiahao Zhou provide suggestions in revision. Wei Gao analysed the data. Qiaolin Wu drafted the manuscript. Jianpeng Liu are responsible for the critical revision. All authors have approved the final version of the manuscript. Qiaolin Wu responsible for the overall content as the guarantor.

Funding: This work was supported by a grant from the Incubation project (NO.FHY2019064).

Disclaimer: The funder had no role in study design, data collection and analysis, or writing of the manuscript.

Conflict of interest: The authors declare that they have no conflict of interest.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not Applicable.

Ethics statements: Not Applicable.

Reference

[dataset]1. Siegel R L, Miller K D, Fuchs H E*, et al.* Cancer statistics, 2022. CA: a cancer journal for clinicians 2022;72(1):7-33.https://doi.org/10.3322/caac.21708

[dataset]2. Luna-Moré S, Gonzalez B, Acedo C*, et al.* Invasive micropapillary carcinoma of the breast. A new special type of invasive mammary carcinoma. Pathology, research and practice 1994;190(7):668-74.https://doi.org/10.1016/s0344-0338(11)80745-4

[dataset]3. Fu L, Ikuo M, Fu X Y*, et al.* Relationship between biologic behavior and morphologic features of invasive micropapillary carcinoma of the breast. Chinese Journal of Pathology 2004

[dataset]4. Siriaunkgul S, Tavassoli F A. Invasive micropapillary carcinoma of the breast. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 1993;6(6):660-2.

[dataset]5. Verras G I, Tchabashvili L, Mulita F*, et al.* Micropapillary Breast Carcinoma: From Molecular Pathogenesis to Prognosis. Breast cancer (Dove Medical Press) 2022;14:41-61.https://doi.org/10.2147/bctt.s346301

[dataset]6. Akrida I, Mulita F. The clinical significance of HER2 expression in DCIS. Medical oncology 2022;40(1):16.https://doi.org/10.1007/s12032-022-01876-9

[dataset]7. Verras G I, Mulita F, Tchabashvili L*, et al.* A rare case of invasive micropapillary carcinoma of the breast. Przeglad menopauzalny = Menopause review 2022;21(1):73-80.https://doi.org/10.5114/pm.2022.113834

[dataset]8. Yu J I, Choi D H, Park W*, et al.* Differences in prognostic factors and patterns of failure between invasive micropapillary carcinoma and invasive ductal carcinoma of

the breast: matched case-control study. Breast (Edinburgh, Scotland) 2010;19(3):231-7.https://doi.org/10.1016/j.breast.2010.01.020

[dataset]9. Yu J I, Choi D H, Huh S J, *et al.* Differences in Prognostic Factors and Failure Patterns Between Invasive Micropapillary Carcinoma and Carcinoma With Micropapillary Component Versus Invasive Ductal Carcinoma of the Breast: Retrospective Multicenter Case-Control Study (KROG 13-06). Clinical breast cancer 2015;15(5):353-61.e1-2.https://doi.org/10.1016/j.clbc.2015.01.008

[dataset]10. Chen L, Fan Y, Lang R G, *et al.* Breast carcinoma with micropapillary features: clinicopathologic study and long-term follow-up of 100 cases. International journal of surgical pathology 2008;16(2):155-63.https://doi.org/10.1177/1066896907307047

[dataset]11. Chen A C, Paulino A C, Schwartz M R, *et al.* Prognostic markers for invasive micropapillary carcinoma of the breast: a population-based analysis. Clinical breast cancer 2013;13(2):133-9.https://doi.org/10.1016/j.clbc.2012.10.001

[dataset]12. Li D, Zhong C, Cheng Y, *et al.* A Competing Nomogram to Predict Survival Outcomes in Invasive Micropapillary Breast Cancer. Journal of Cancer 2019;10(27):6801-12.https://doi.org/10.7150/jca.27955

[dataset]13. Ye F G, Xia C, Ma D, *et al.* Nomogram for predicting preoperative lymph node involvement in patients with invasive micropapillary carcinoma of breast: a SEER population-based study. BMC cancer 2018;18(1):1085.https://doi.org/10.1186/s12885-018-4982-5

[dataset]14. Chen Y, Yu C, Chen D, *et al.* A prognostic nomogram based on risk assessment for invasive micropapillary carcinoma of the breast after surgery. Cancer

medicine 2023;12(7):8050-62.https://doi.org/10.1002/cam4.5595

[dataset]15. Zhao Y, Li S, Yan L*, et al.* Nomogram for predicting overall survival in patients with invasive micropapillary carcinoma after breast-conserving surgery: A population-based analysis. Frontiers in surgery 2022;9:1009149.https://doi.org/10.3389/fsurg.2022.1009149

[dataset]16. Iasonos A, Schrag D, Raj G V*, et al.* How to build and interpret a nomogram for cancer prognosis. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2008;26(8):1364-70.https://doi.org/10.1200/jco.2007.12.9791

[dataset]17. Balachandran V P, Gonen M, Smith J J*, et al.* Nomograms in oncology: more than meets the eye. The Lancet Oncology 2015;16(4):e173-80.https://doi.org/10.1016/s1470-2045(14)71116-7

[dataset]18. Diao J D, Ma L X, Sun M Y*, et al.* Construction and validation of a nomogram to predict overall survival in patients with inflammatory breast cancer. Cancer medicine 2019;8(12):5600-08.https://doi.org/10.1002/cam4.2470

[dataset]19. Yang J, Pan Z, Zhao F*, et al.* A nomogram for predicting survival in patients with nodular melanoma: A population-based study. Medicine 2019;98(24):e16059.https://doi.org/10.1097/md.0000000000016059

[dataset]20. Liu H, Zheng S Q, Li X Y*, et al.* Derivation and Validation of a Nomogram to Predict In-Hospital Complications in Children with Tetralogy of Fallot Repaired at an Older Age. Journal of the American Heart Association 2019;8(21):e013388.https://doi.org/10.1161/jaha.119.013388

[dataset]21. Song J, Sun Y, Cao H*, et al.* A novel pyroptosis-related lncRNA signature for

prognostic prediction in patients with lung adenocarcinoma. Bioengineered 2021;12(1):5932-49.https://doi.org/10.1080/21655979.2021.1972078

[dataset]22. Pettinato G, Manivel C J, Panico L*, et al.* Invasive micropapillary carcinoma of the breast: clinicopathologic study of 62 cases of a poorly recognized variant with highly aggressive behavior. American journal of clinical pathology 2004;121(6):857-66.https://doi.org/10.1309/xtj7-vhb4-9ud7-8x60

[dataset]23. Walsh M M, Bleiweiss I J. Invasive micropapillary carcinoma of the breast: eighty cases of an underrecognized entity. Human pathology 2001;32(6):583-9.https://doi.org/10.1053/hupa.2001.24988

[dataset]24. Nassar H, Wallis T, Andea A*, et al.* Clinicopathologic analysis of invasive micropapillary differentiation in breast carcinoma. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 2001;14(9):836-41.https://doi.org/10.1038/modpathol.3880399

[dataset]25. Clark G M, Osborne C K, McGuire W L. Correlations between estrogen receptor, progesterone receptor, and patient characteristics in human breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1984;2(10):1102-9.https://doi.org/10.1200/jco.1984.2.10.1102

[dataset]26. Osborne C K, Fisher E, Redmond C*, et al.* Estrogen receptor, a marker for human breast cancer differentiation and patient prognosis. Advances in experimental medicine and biology 1981;138:377-85.https://doi.org/10.1007/978-1-4615-7192-6_23

[dataset]27. Luna-Moré S, Casquero S, Pérez-Mellado A*, et al.* Importance of estrogen receptors for the behavior of invasive micropapillary carcinoma of the breast. Review

of 68 cases with follow-up of 54. Pathology, research and practice 2000;196(1):35-9.https://doi.org/10.1016/s0344-0338(00)80019-9

[dataset]28. Kuru B, Camlibel M, Dinc S*, et al.* Prognostic significance of axillary node and infraclavicular lymph node status after mastectomy. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology 2003;29(10):839-44.https://doi.org/10.1016/j.ejso.2003.08.003

[dataset]29. Hur M H, Ko S. Metastatic axillary node ratio predicts recurrence and poor long-term prognosis in patients with advanced stage IIIC (pN3) breast cancer. Annals of surgical treatment and research 2017;92(5):340-47.https://doi.org/10.4174/astr.2017.92.5.340

[dataset]30. Zekioglu O, Erhan Y, Ciris M*, et al.* Invasive micropapillary carcinoma of the breast: high incidence of lymph node metastasis with extranodal extension and its immunohistochemical profile compared with invasive ductal carcinoma. Histopathology 2004;44(1):18-23.https://doi.org/10.1111/j.1365-2559.2004.01757.x

[dataset]31. Adrada B, Arribas E, Gilcrease M*, et al.* Invasive micropapillary carcinoma of the breast: mammographic, sonographic, and MRI features. AJR American journal of roentgenology 2009;193(1):W58-63.https://doi.org/10.2214/ajr.08.1537

[dataset]32. Paterakos M, Watkin W G, Edgerton S M*, et al.* Invasive micropapillary carcinoma of the breast: a prognostic study. Human pathology 1999;30(12):1459-63.https://doi.org/10.1016/s0046-8177(99)90168-5

[dataset]33. Kim M J, Gong G, Joo H J*, et al.* Immunohistochemical and clinicopathologic

characteristics of invasive ductal carcinoma of breast with micropapillary carcinoma component. Archives of pathology & laboratory medicine 2005;129(10):1277-82.https://doi.org/10.5858/2005-129-1277-iaccoi

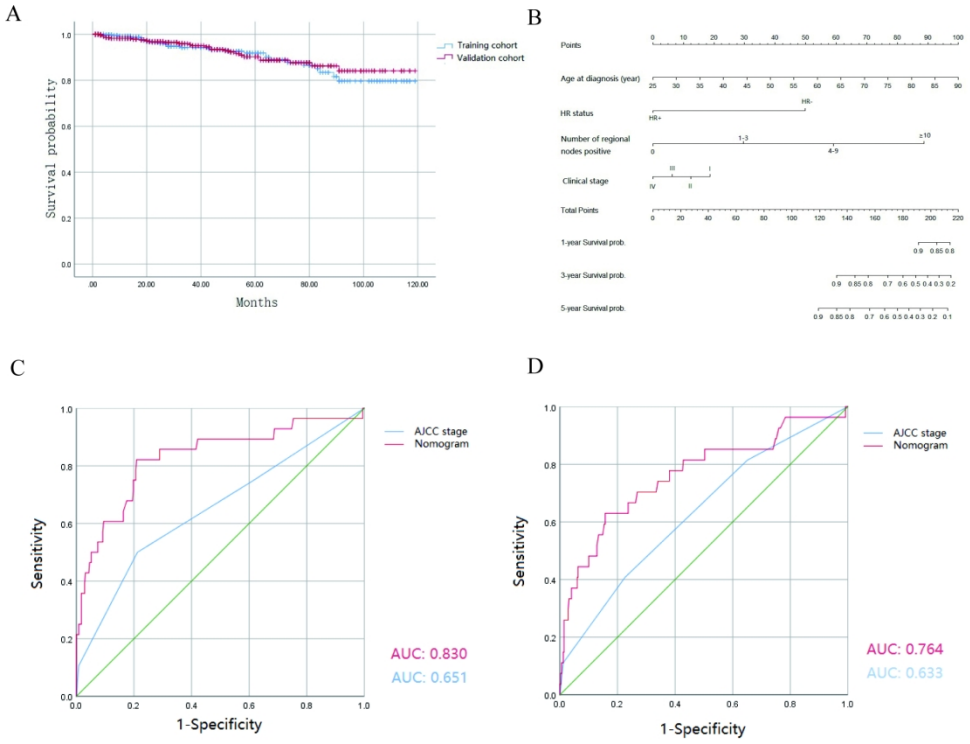


Figure 1A. Kaplan-Meier survival curves of the patients with IMPC in the training and validation cohorts.
Notes: The survival curves showed no significant differences between the 2 cohorts (P= 0.786).

Figure 1B. Nomogram for predicting 1-, 3-, 5-year OS for patients with the prognosis factors.
Notes: The total points are calculated by summing up the points for each factor. The predicted probability of OS can be obtained by projecting the location of the total points to the bottom scales. Abbreviations: OS= overall survival.

Figure 1C and Figure 1D. ROC curves for discrimination in the training and validation cohorts.
Notes: (Figure 1C) In the training cohort, the AUC of the ROC curve of the nomogram and the sixth edition AJCC TNM staging classification was 0.830 and 0.651, respectively (P＜0.001). (Figure 1D) In the validation cohort, the AUC of the ROC curve of the nomogram and the sixth edition AJCC TNM staging classification was 0.764 and 0.633, respectively (P＜0.001). Abbreviations: AJCC= American Joint Committee on Cancer; AUC= area under the curve; ROC= receiver operating characteristic.

185x141mm (300 x 300 DPI)

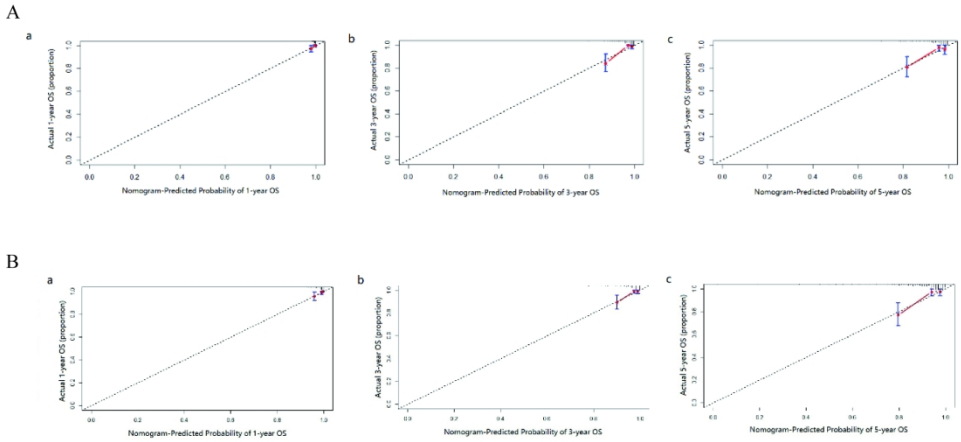


Figure 2. Calibration curves for predictions for the 1-year (a), 3-year (b), 5-year (c) OS in the training cohort (Figure 2A) and in the testing cohort (Figure 2B).
Notes: (Figure 2A) The nomogram-predicted probability of OS is plotted on the X-axis, and the actual OS is plotted on the Y-axis. (Figure 2B) The nomogram-predicted probability of OS is plotted on the X-axis, and the actual OS is plotted on the Y-axis.

213x94mm (300 x 300 DPI)

BMJ Open

# Nomogram predicting overall prognosis for invasive micropapillary carcinoma of the breast: a SEER-based population study.



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2023-072632.R2 |
| Article Type: | Original research |
| Date Submitted by the Author: | 14-Jun-2023 |
| Complete List of Authors: | Liu , Jianpeng; The First Affiliated Hospital of Wenzhou Medical University, Departments of pathology<br>Xi, Wei; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Zhou, Jiahao; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Gao, Wei; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology<br>Wu , Qiaolin; The First Affiliated Hospital of Wenzhou Medical University, Anesthesiology |
| Primary Subject Heading: | Oncology |
| Secondary Subject Heading: | Epidemiology |
| Keywords: | Breast tumours < ONCOLOGY, Epidemiology < ONCOLOGY, Adult oncology < ONCOLOGY |
| | |

*I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.*

*The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.*

*Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.*

# Nomogram predicting overall prognosis for invasive micropapillary carcinoma of the breast: a SEER-based population study.

Jianpeng Liu[1], Wei Xi[2], Jiahao Zhou[2], Wei Gao[2], Qiaolin Wu[2]

Departments of [1]pathology, [2]Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

Address correspondence to: Dr. Qiaolin Wu, Department of Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

Tel: +86-0577-55579098; Fax: +86-0577-55579098

E-mail: 634928235@qq.com(QL);

Jianpeng Liu, and Wei Xi have contributed equally to this article.

Abstract

**Objectives** The prognosis of invasive micropapillary carcinoma (IMPC) of the breast is determined by many clinicopathological factors. This study aims to identify prognostic factors and develop reliable nomogram to predict the overall survival (OS) in patients with IMPC.

**Design** Log-rank test and Cox proportional hazards analysis were used to identify variables and construct a nomogram based on the training cohort. C-index and calibration curves were performed to evaluate the performance of the model in the training cohort and validation cohorts.

**Setting** We collected the patient data from the Surveillance, Epidemiology, and End Results (SEER) database. This database holds data related to the cancer incidence from 18 population-based cancer registries in the United States.

**Participants** The SEER database was used to screen 754 eligible patients as the study cohort. The whole cohort was randomly divided into a training cohort (n=377) and a validation cohort (n=377).

**Results** Age at diagnosis, hormone receptors, number of positive regional lymph-nodes and clinical stage were independent prognostic factors for patients with IMPC. The calibration curves presented excellent consistency between the actual and nomogram-predict survival probabilities in the training and validation cohorts. The C-index values of the nomogram were 0.794 and 0.774 for OS in the training and validation cohorts, respectively.

**Conclusions** The novel nomogram provides new insights of the risk of each prognostic factor and can assist doctors in predicting the 1-, 3-, and 5-year OS in patients with IMPC.

Keywords: Invasive micropapillary breast carcinoma; nomogram; prognosis; Surveillance, Epidemiology, and End Results

Strengths and limitations of this study

- The data was downloaded from the SEER database, which provides a representative population-based cohort.

- Prognostic factors were determined by univariate and multivariate Cox proportional hazards regression analyses and used to develop nomograms to predict 1-, 3-, and 5-year overall survival of patients with invasive micropapillary carcinoma (IMPC).
- We used the Harrell concordance index (C-index), the area under the receiver operating characteristic (ROC) curve (AUC) and the calibration curve to assess the discrimination of the nomograms.
- This research was a retrospectively large-sample study, the casual basis of this research was difficult to conclude.

## Introduction

Breast cancer is the most prevalent cancer in women and one of the most rapidly increasing human malignancies worldwide. In the USA, the number of newly estimated diagnosed cases and deaths were 290,560 and 43,780, respectively, in 2022[1]. The invasive micropapillary carcinoma (IMPC) of the breast, which characterized by aggressive lymphovascular invasion and metastasis, accounting for less than 2% of all invasive breast cancers[2-5]. Hormonal and HER-2 positivity in invasive micropapillary carcinoma (IMPC) of the breast is also commoner when compared to other Non-Specific Type (NST) carcinomas. IMPC occurs either as a pure form or more often as a component of mixed NST carcinoma[6-8]. This cancer type has varying classifications and has no available standardized treatment guidelines.

Considering the rarity of this disease, the conduct of clinical trials to evaluate prognostic factors and optimal treatments is difficult. A few studies have discussed the potential pathologic predictors of survival for IMPC[5 9-16]. However, the discrepancies caused by the limited IMPC cases in the reported prevalence of overall survival and significant clinicopathological factors were difficult to exclude.

A nomogram, a simple visual prediction tool based on a prognostic model that includes related clinicopathological factors, allows doctors to access the probabilities of the clinical outcomes of particular individuals[17 18]. Moreover, compared to the American Joint Committee on Cancer (AJCC) TNM stage system, nomograms can provide a more precise estimation of prognosis for some malignancies[19 20] and help clinicians to make decisions in complex situations in an alternative or novel standard[21-23].

In this study, we investigated the Surveillance, Epidemiology, and End Results (SEER) database to evaluate the prognostic clinicopathological indicators on overall survival (OS) in patients with IMPC. A novel nomogram was constructed to predict the prognosis for patients with IMPC.

## Methods

### Study cohorts

The data for this study were obtained from 18 registries of the SEER program, and 1,480 patients diagnosed with IMPC of the breast between 1973 and 2013 were included. The personal information from the SEER database is untracked and unavailable. The inclusion criteria for the data screening were as follows: (1) female patients who accepted surgery treatment; (2) age older than 18 years; (3) diagnosis confirmed by histopathological report; (4) IMPC as the first and primary cancer determined by international rules; (5) survival data with complete and available dates and more than 0 days of survival; and (6) clear clinicopathological information for all the variables of interest including age at diagnosis, race, marital status, primary site, hormone receptors (HRs) [estrogen receptor (ER) and progesterone receptor (PR)], tumor size, grade, laterality, number of positive regional lymph-nodes, surgery record, and clinical stage (the 6th edition of AJCC system).

Variables and definitions

The following data were extracted for each patient from the database: age at diagnosis, race (white and others), marital status at diagnosis, laterality, clinical stage, number of positive regional lymph-nodes, tumor size, tumor grade (well-differentiated, moderately differentiated, poorly differentiated, undifferentiated or anaplastic), hormone receptors (HR+ and HR-), surgery record, radiotherapy record, survival months, and vital status. Marital status was classified as married or unmarried. The latter included single, separated, divorced, widowed, and unmarried/domestic partners. OS was defined as the time from diagnosis to death from any cause or to the time of the last follow-up.

Construction and validation of the nomogram

The univariate and multivariate Cox regression analyses were performed to determine the potential prognostic factors. The independent factors were used to build the nomogram for the Wins by using the rms package in R software version 4.1.3. And the annual survival rates were analysed by using the survival and rms packages in R software. All the significant independent factors in the training cohort were used to build a nomogram to predict the survival rates. The nomogram was validated in the training and the validation cohorts. We used the Harrell

concordance index (C-index), the area under the receiver operating characteristic (ROC) curve (AUC) and the calibration curve to assess the discrimination of the nomogram.

Statistical analysis

Our study consolidated the descriptive characteristics of the training and validation cohorts, respectively. Chi-square test or Fisher's exact test was used to confirm whether significant differences exist in the demographic and clinicopathological features between the training and validation cohorts. The variables were analyzed using Kaplan–Meier survival curves and log-rank tests to evaluate their effects on OS. The ROC-AUC calculation was performed by the function of "ROC curve" in SPSS version 26.0. All P values are two sided, and P values under .05 are considered as statistically significant. The SEER data were extracted using SEERStat 8.4.0, and statistical analyses were performed using SPSS version 26.0 (IBM-SPSS Inc., Armonk, NY).

Patient and public involvement

No patient involved.

Results

Demographics and clinicopathological characteristics

From the SEER database, a total of 754 cases of IMPC were eligible for inclusion criteria. The eligible patients were randomly divided into the training cohort (n=377) and the validation cohort (n=377) by applying 'create Data Partition' function in the package of 'caret' from R version 4.1.3. The demographic and clinicopathological characteristics of the training and validation cohorts are shown in Table 1, and no statistically significant differences were found between the two cohorts. The estimated average OS values were 106.9 months (95% CI: 102.7-111.1 months) in the 377 patients with IMPC in the training cohort, and 108.2 months (95%CI: 104.4-112.1 months) in the validation cohort. The survival curve showed no significant differences between the two cohorts (Figure 1A, P= 0.786).

Table 1. Clinicopathologic characteristics of the training and validation cohorts.

| Variables | Training cohort (n=377) ( %) | Validation cohort (n=377) (%) | P value |
|---|---|---|---|
| Age (year) | 58.73±13.19 | 59.90±12.94 | 0.22 |
| Race | | | 0.11 |
| White | 276 (73.2) | 295 (78.2) | |
| Other | 101 (26.8) | 82 (21.8) | |
| Marital status | | | 0.55 |
| Unmarried | 159 (42.2) | 151 (40.1) | |
| Married | 218 (57.8) | 226 (59.9) | |
| Laterality | | | 0.17 |
| Left | 205 (54.4) | 186 (49.3) | |
| Right | 172 (45.6) | 191 (50.7) | |
| Grade | | | 0.26 |
| I/II | 236 (62.6) | 221 (58.6) | |
| III/IV | 141 (37.4) | 156 (41.4) | |
| HR status | | | 0.55 |
| Positive | 335 (88.9) | 340 (90.2) | |
| Negative | 42 (11.1) | 37 (9.8) | |
| Tumor size (mm) | 24.44±22.78 | 24.71±21.93 | 0.87 |
| <20 | 223 (59.2) | 204 (54.1) | 0.29 |
| 20-50 | 114 (30.2) | 134 (35.5) | |
| >50 | 40 (10.6) | 39 (10.3) | |
| Number of positive regional nodes | | | 0.99 |
| 0 | 179 (47.5) | 175 (46.4) | |
| 1-3 | 118 (31.3) | 121 (32.1) | |
| 4-9 | 45 (11.9) | 47 (12.5) | |
| ≥10 | 35 (9.3) | 34 (9.0) | |
| Stage | | | 0.73 |
| I | 141 (37.4) | 127 (33.7) | |
| II | 148 (39.2) | 160 (42.4) | |
| III | 82 (21.8) | 83 (22.0) | |
| IV | 6 (1.6) | 7 (1.9) | |
| Surgery | | | 0.34 |
| Conserving surgery | 208 (55.2) | 195 (51.7) | |
| Mastectomy | 169 (44.8) | 182 (48.3) | |
| Radiotherapy | | | 0.06 |
| Yes | 238 (63.1) | 213 (56.5) | |
| No | 139 (36.9) | 164 (43.5) | |

## Univariate and multivariate Cox proportional hazards analyses

The hazard ratios for OS according to all variables in the univariate or multivariate Cox proportional hazards model are listed in Tables 2 and 3. According to the results of univariate analysis, we found that the race, marital status, laterality, and radiotherapy were not significant factors for OS. After excluding the aforementioned variables, age at diagnosis, grade, HR status, tumor size, number of positive regional lymph-nodes, clinical stage, and surgery were determined as prognostic factors in the multivariate Cox proportional hazards model for the OS analysis. As shown in Table 3, age at diagnosis could be a negative prognostic factor for the OS of patients with IMPC. The HR negative special type exhibited higher risk of death. Compared with patients with IMPC and negative regional node, patients with positive regional lymph-nodes suffered from higher risk of poor prognosis. Interestingly, the subgroups of stages II and III had a significantly lower risk than the stage I group.

Table 2. Univariate analysis of OS in the training cohort.

| Variables | HR | 95% CI | P value |
|---|---|---|---|
| Age (year) | 1.035 | 1.005-1.065 | 0.023 |
| Race | | | |
| White | | reference | |
| Other | 1.202 | 0.529-2.732 | 0.660 |
| Marital status | | | |
| Unmarried | | reference | |
| Married | 0.721 | 0.343-1.512 | 0.386 |
| Laterality | | | |
| Left | | reference | |
| Right | 0.915 | 0.435-1.923 | 0.816 |
| Grade | | | |
| I/II | | reference | |
| III/IV | 2.180 | 1.030-4.611 | 0.042 |
| HR status | | | |
| Positive | | reference | |
| Negative | 4.150 | 1.914-8.998 | <0.001 |
| Tumor size (mm) | | | <0.001 |
| <20 | | reference | |
| 20-50 | 1.931 | 0.728-5.119 | 0.186 |
| >50 | 7.960 | 3.339-18.973 | <0.001 |
| Number of positive regional nodes | | | <0.001 |
| 0 | | reference | |

| | | | |
|---|---|---|---|
| 1-3 | 1.679 | 0.609-4.632 | 0.317 |
| 4-9 | 3.145 | 0.998-9.914 | 0.050 |
| ≥10 | 8.350 | 3.016-23.115 | <0.001 |
| Stage | | | <0.001 |
| I | | reference | |
| II | 1.040 | 0.365-2.967 | 0.941 |
| III | 3.529 | 1.262-8.419 | 0.015 |
| IV | 19.576 | 4.982-76.921 | <0.001 |
| Surgery | | | |
| Conserving surgery | | reference | |
| Mastectomy | 2.530 | 1.144-5.596 | 0.022 |
| Radiotherapy | | | |
| Yes | | reference | |
| No | 0.780 | 0.368-1.649 | 0.515 |

Table 3. Multivariate analysis of OS in the training cohort.

| Variables | HR | 95% CI | P value |
|---|---|---|---|
| Age (year) | 1.054 | 1.020-1.090 | 0.020 |
| Grade | | | |
| I/II | | reference | |
| III/IV | 1.159 | 0.504-2.666 | 0.728 |
| HR status | | | |
| Positive | | reference | |
| Negative | 5.368 | 2.084-13.830 | 0.001 |
| Tumor size (mm) | | | |
| <20 | | reference | |
| 20-50 | 2.292 | 0.631-8.322 | 0.208 |
| >50 | 4.807 | 0.919-25.153 | 0.063 |
| Number of positive regional nodes | | | |
| 0 | | reference | |
| 1-3 | 18.314 | 1.387-241.811 | 0.027 |
| 4-9 | 10.340 | 1.044-102.388 | 0.046 |
| ≥10 | 26.776 | 3.300-23.115 | 0.002 |
| Stage | | | |
| I | | reference | |
| II | 0.057 | 0.004-0.802 | 0.034 |
| III | 0.096 | 0.100-0.964 | 0.046 |
| IV | 0.211 | 0.170-2.641 | 0.228 |
| Surgery | | | |
| Conserving surgery | | reference | |
| Mastectomy | 1.119 | 0.393-3.190 | 0.833 |

Construction and validation of the nomograms.

The nomogram for 1-, 3-, and 5-year OS was developed by using the multivariate Cox proportional hazards models as the final prognostic models after factor selection (Figure 1B). The nomogram was internally validated in the training cohort and externally validated in the validation cohort. The AUC values of the ROC curve, which exhibited the discrimination capacity, were 0.830 and 0.764 in the training and validation cohorts, respectively (Figure 1C and Figure 1D). Moreover, compared with the discriminative ability of the sixth edition AJCC TNM staging classification, the discriminative ability of the nomogram was significantly superior in the training and validation cohorts ($P < 0.001$). The results indicated that the nomogram can efficiently predict OS in patients with IMPC. The calibration plots also showed great consistency between the actual and nomogram-predicted survival rates in the training and testing cohorts (Figure 2A and Figure 2B). The C-index values of the nomogram for OS were 0.794 in the training cohort and 0.774 in the validation cohort.

## Discussion

Invasive micropapillary carcinoma of breast is a rare variant of invasive breast carcinoma (IBC)[4]. Histologically, it is a special type characterized by small papillary structures that lack true central fibrovascular cores and lie within empty stromal spaces[24 25]. Historically, patients with IMPC were usually treated with standard IBC treatment. However, notable differences in histological characters and prognosis exist between IMPC and IBC[26]; as such, treating IMPC as IBC would be inappropriate. Accurate predictions of prognosis of patients with IMPC patients could effectively help clinicians to take proper treatment modalities. This study aims to build a nomogram capable of predicting the prognosis of IMPC based on a larger population database of the Surveillance, Epidemiology, and End Results (SEER) program.

In this study, we equally divided 754 patients with IMPC from the SEER database into two cohorts. We developed an effective nomogram that contains four independent prognostic factors including age at diagnosis, HR, number of positive

regional lymph-nodes, and clinical stage. The nomogram, derived from the Cox regression model to predict the 1-, 3-, and 5-year OS of patients with IMPC, was verified to have good discrimination capacity. Moreover, the nomogram showed better prediction ability for OS than that of the sixth edition AJCC TNM staging classification (AUCs in the ROC curve: 0.830 and 0.651 in the training cohort and 0.764 and 0.633 in the validation cohort, respectively).

Hormone receptors play important role in prognosis of breast cancer[27 28]. A previous study showed that the 5-year OS was 59% in 100 patients with IMPC with a mean age of 50 years and 46% HR positivity[12]. In another study, 72 patients with IMPC with a mean age of 46 years and 75% HR positivity had 86% 5-year OS[10]. In comparison, our study population was older (mean age of 59.3 years) and had a higher percentage of HR positivity (89.5%). The higher HR positivity in the present study may contribute to the better 5-year OS (91.1%)[29]. The Cox-regression analysis result also proved that HR negativity could lead to significantly poor OS in patients with IMPC (HR 5.368; 95%CI 2.084–13.830; P=0.001).

Lymph node metastasis is widely considered as an unfavorable prognostic factor in clinical practice[30 31]. Axillary lymph node metastasis is commonly seen in patients with IMPC at first diagnosis. The rate of lymphatic and lymph nodal spread ranged from 33% to 95%[4 24 32 33]. The value and necessity of sentinel lymph node biopsy (SLNB) or axillary dissection in patients with IMPC remains controversial. Walsh et al. found that regional lymph nodes can be involved even at early stage of IMPC lesions. The team highly recommended a thorough regional lymph node examination to patients with IMPC[25]. However, Paterakos et al. were skeptical to the utility of SLNB for patients with IMPC due to the high frequency of multiple positive regional lymph nodes[34]. In the present study, we found that patients with IMPC with even one positive regional lymph node would suffer higher risk than patients with negative lymph node. Patients with IMPC and 10 or more positive lymph nodes are at the highest risk (OR 26.776; 95%CI 3.300-23.115; P=0.002). Thus, axillary dissection, or SLNB at minimum, should be performed to correctly access the risk and adopt suitable treatment regimens for patients with IMPC.

This study has some limitations. First, retrospective SEER data lack a pathologic review to identify the diagnosis for each case. Second, we cannot consider the types of systemic therapy administered to patients. Hormonal blockade therapy and chemotherapy could significantly affect the outcome of patients. Third, the relationship between the degree of micropapillary involvement and clinical outcomes among patients with IMPC remains unclear. Although some previous small case series studies have revealed that an increasing percentage of micropapillary component was not associated with more lymph node metastasis and worse survival[32 35], it need to be further validated in large-scale studies.

## Conclusions

In conclusion, age at diagnosis, HR status, number of positive regional lymph-nodes, and clinical stage were independent prognostic factors for patients with IMPC. We constructed a nomogram to predict OS in patients with IMPC based on a large-scale population from the SEER database. This accessible nomogram will help doctors to adopt proper treatment regimens in clinical practice.

## List of abbreviations

invasive micropapillary carcinoma (IMPC)
overall survival (OS)
Surveillance, Epidemiology, and End Results (SEER)
American Joint Committee on Cancer (AJCC)
hormone receptors (HRs)
estrogen receptor (ER)
progesterone receptor (PR)
receiver operating characteristic (ROC)
area under the curve (AUC)
invasive breast carcinoma (IBC)

Jianpeng Liu and Wei Xi contributed equally.

Contributorship statement: Jianpeng Liu and Wei Xi designed and conducted the study. Jiahao Zhou provide suggestions in revision. Wei Gao analysed and

interpreted the data. Wei Xi drafted, revised, finalised and submitted the manuscript. Jianpeng Liu are responsible for the critical revision. Qiaolin Wu approved the submission of the manuscript. Qiaolin Wu has full responsbility for the overall content as the guarantor, had access to the data and controlled the decision to publish.

Competing interests: The authors declare that they have no conflict of interest.

Funding: This work was supported by a grant from the hospital incubation funding project of the First Affiliated Hospital of Wenzhou Medical University (NO.FHY2019064).

Disclaimer: The funder had no role in study design, data collection and analysis, or writing of the manuscript.

Patient consent for publication: Not Applicable.

Ethics statements: This study was reviewed by the Ethics Committee in Clinical Research of the First Affiliated Hospital of Wenzhou Medical University. The data are anonymous, and the requirement for informed consent was therefore waived.

Data sharing statement: Data are available in a public, open access repository. Data are fully accessible at https://seer.cancer.gov/data-software/. The datasets used in this study are available from the corresponding author on reasonable request. The corresponding author has full access to all the data used in this study and had final responsibility for the decision to submit the study for publication.

## Reference


1. Siegel R L, Miller K D, Fuchs H E, *et al.* Cancer statistics, 2022. CA Cancer J Clin 2022;72(1):7-33.https://doi.org/10.3322/caac.21708

2. Luna-More S, Gonzalez B, Acedo C, *et al.* Invasive micropapillary carcinoma of the breast. A new special type of invasive mammary carcinoma. Pathol Res Pract 1994;190(7):668-74.https://doi.org/10.1016/S0344-0338(11)80745-4

3. Fu L, Ikuo M, Fu X Y, *et al.* [Relationship between biologic behavior and morphologic features of invasive micropapillary carcinoma of the breast]. Zhonghua Bing Li Xue Za Zhi 2004;33(1):21-5.

4. Siriaunkgul S, Tavassoli F A. Invasive micropapillary carcinoma of the breast. Mod Pathol 1993;6(6):660-2.

5. Chen A C, Paulino A C, Schwartz M R, *et al.* Prognostic markers for invasive micropapillary carcinoma of the breast: a population-based analysis. Clin Breast Cancer 2013;13(2):133-9.https://doi.org/10.1016/j.clbc.2012.10.001

6. Verras G I, Tchabashvili L, Mulita F, *et al.* Micropapillary Breast Carcinoma: From Molecular Pathogenesis to Prognosis. Breast Cancer (Dove Med Press) 2022;14:41-61.https://doi.org/10.2147/BCTT.S346301

7. Akrida I, Mulita F. The clinical significance of HER2 expression in DCIS. Med Oncol 2022;40(1):16.https://doi.org/10.1007/s12032-022-01876-9

8. Verras G I, Mulita F, Tchabashvili L, *et al.* A rare case of invasive micropapillary carcinoma of the breast. Prz Menopauzalny 2022;21(1):73-80.https://doi.org/10.5114/pm.2022.113834

9. Chen E, Du Y, Chen Y, *et al.* A web-based novel model for predicting prognostic value in patients with invasive micropapillary carcinoma in breast cancer: a real-world data retrospective cohort study. Updates Surg 2023.https://doi.org/10.1007/s13304-023-01530-7

10. Yu J I, Choi D H, Park W, *et al.* Differences in prognostic factors and patterns of failure between invasive micropapillary carcinoma and invasive ductal carcinoma of the breast: matched case-control study. Breast 2010;19(3):231-7.https://doi.org/10.1016/j.breast.2010.01.020

11. Yu J I, Choi D H, Huh S J, *et al.* Differences in Prognostic Factors and Failure Patterns Between Invasive Micropapillary Carcinoma and Carcinoma With Micropapillary Component Versus Invasive Ductal Carcinoma of the Breast: Retrospective Multicenter Case-Control Study (KROG 13-06). Clin Breast Cancer 2015;15(5):353-61 e1-2.https://doi.org/10.1016/j.clbc.2015.01.008

12. Chen L, Fan Y, Lang R G, *et al.* Breast carcinoma with micropapillary features: clinicopathologic study and long-term follow-up of 100 cases. Int J Surg Pathol 2008;16(2):155-63.https://doi.org/10.1177/1066896907307047

13. Li D, Zhong C, Cheng Y, *et al.* A Competing Nomogram to Predict Survival Outcomes in Invasive Micropapillary Breast Cancer. J Cancer 2019;10(27):6801-12.https://doi.org/10.7150/jca.27955

14. Ye F G, Xia C, Ma D, *et al.* Nomogram for predicting preoperative lymph node involvement in patients with invasive micropapillary carcinoma of breast: a SEER population-based study. BMC Cancer

2018;18(1):1085.https://doi.org/10.1186/s12885-018-4982-5

15. Chen Y, Yu C, Chen D, *et al.* A prognostic nomogram based on risk assessment for invasive micropapillary carcinoma of the breast after surgery. Cancer Med 2023;12(7):8050-62.https://doi.org/10.1002/cam4.5595

16. Zhao Y, Li S, Yan L, *et al.* Nomogram for predicting overall survival in patients with invasive micropapillary carcinoma after breast-conserving surgery: A population-based analysis. Front Surg 2022;9:1009149.https://doi.org/10.3389/fsurg.2022.1009149

17. Iasonos A, Schrag D, Raj G V, *et al.* How to build and interpret a nomogram for cancer prognosis. J Clin Oncol 2008;26(8):1364-70.https://doi.org/10.1200/JCO.2007.12.9791

18. Balachandran V P, Gonen M, Smith J J, *et al.* Nomograms in oncology: more than meets the eye. Lancet Oncol 2015;16(4):e173-80.https://doi.org/10.1016/S1470-2045(14)71116-7

19. Diao J D, Ma L X, Sun M Y, *et al.* Construction and validation of a nomogram to predict overall survival in patients with inflammatory breast cancer. Cancer Med 2019;8(12):5600-08.https://doi.org/10.1002/cam4.2470

20. Yang J, Pan Z, Zhao F, *et al.* A nomogram for predicting survival in patients with nodular melanoma: A population-based study. Medicine (Baltimore) 2019;98(24):e16059.https://doi.org/10.1097/MD.0000000000016059

21. Liu H, Zheng S Q, Li X Y, *et al.* Derivation and Validation of a Nomogram to Predict In-Hospital Complications in Children with Tetralogy of Fallot Repaired at an Older

Age. J Am Heart Assoc 2019;8(21):e013388.https://doi.org/10.1161/JAHA.119.013388

22. Song J, Sun Y, Cao H, *et al.* A novel pyroptosis-related lncRNA signature for prognostic prediction in patients with lung adenocarcinoma. Bioengineered 2021;12(1):5932-49.https://doi.org/10.1080/21655979.2021.1972078

23. Wang J, Chen L, Nie Y, *et al.* Nomogram for Predicting the Overall Survival of Patients With Breast Cancer With Pathologic Nodal Status N3. Clin Breast Cancer 2020;20(6):e778-e85.https://doi.org/10.1016/j.clbc.2020.06.002

24. Pettinato G, Manivel C J, Panico L, *et al.* Invasive micropapillary carcinoma of the breast: clinicopathologic study of 62 cases of a poorly recognized variant with highly aggressive behavior. Am J Clin Pathol 2004;121(6):857-66.https://doi.org/10.1309/XTJ7-VHB4-9UD7-8X60

25. Walsh M M, Bleiweiss I J. Invasive micropapillary carcinoma of the breast: eighty cases of an underrecognized entity. Hum Pathol 2001;32(6):583-9.https://doi.org/10.1053/hupa.2001.24988

26. Nassar H, Wallis T, Andea A, *et al.* Clinicopathologic analysis of invasive micropapillary differentiation in breast carcinoma. Mod Pathol 2001;14(9):836-41.https://doi.org/10.1038/modpathol.3880399

27. Clark G M, Osborne C K, McGuire W L. Correlations between estrogen receptor, progesterone receptor, and patient characteristics in human breast cancer. J Clin Oncol 1984;2(10):1102-9.https://doi.org/10.1200/JCO.1984.2.10.1102

28. Osborne C K, Fisher E, Redmond C, *et al.* Estrogen receptor, a marker for human breast

cancer differentiation and patient prognosis. Advances in experimental medicine and biology 1981;138:377-85.https://doi.org/10.1007/978-1-4615-7192-6_23

29. Luna-More S, Casquero S, Perez-Mellado A, *et al.* Importance of estrogen receptors for the behavior of invasive micropapillary carcinoma of the breast. Review of 68 cases with follow-up of 54. Pathol Res Pract 2000;196(1):35-9.https://doi.org/10.1016/S0344-0338(00)80019-9

30. Kuru B, Camlibel M, Dinc S, *et al.* Prognostic significance of axillary node and infraclavicular lymph node status after mastectomy. Eur J Surg Oncol 2003;29(10):839-44.https://doi.org/10.1016/j.ejso.2003.08.003

31. Hur M H, Ko S. Metastatic axillary node ratio predicts recurrence and poor long-term prognosis in patients with advanced stage IIIC (pN3) breast cancer. Ann Surg Treat Res 2017;92(5):340-47.https://doi.org/10.4174/astr.2017.92.5.340

32. Zekioglu O, Erhan Y, Ciris M, *et al.* Invasive micropapillary carcinoma of the breast: high incidence of lymph node metastasis with extranodal extension and its immunohistochemical profile compared with invasive ductal carcinoma. Histopathology 2004;44(1):18-23.https://doi.org/10.1111/j.1365-2559.2004.01757.x

33. Adrada B, Arribas E, Gilcrease M, *et al.* Invasive micropapillary carcinoma of the breast: mammographic, sonographic, and MRI features. AJR Am J Roentgenol 2009;193(1):W58-63.https://doi.org/10.2214/AJR.08.1537

34. Paterakos M, Watkin W G, Edgerton S M, *et al.* Invasive micropapillary carcinoma of the breast: a prognostic study. Hum Pathol 1999;30(12):1459-63.https://doi.org/10.1016/s0046-8177(99)90168-5

35. Kim M J, Gong G, Joo H J, *et al.* Immunohistochemical and clinicopathologic characteristics of invasive ductal carcinoma of breast with micropapillary carcinoma component. Arch Pathol Lab Med 2005;129(10):1277-82.https://doi.org/10.5858/2005-129-1277-IACCOI

Figure legend

Figure 1A. Kaplan-Meier survival curves of the patients with IMPC in the training and validation cohorts.

Notes: The survival curves showed no significant differences between the 2 cohorts (P= 0.786).

Figure 1B. Nomogram for predicting 1-, 3-, 5-year OS for patients with the prognosis factors.

Notes: The total points are calculated by summing up the points for each factor. The predicted probability of OS can be obtained by projecting the location of the total points to the bottom scales. Abbreviations: OS= overall survival.

Figure 1C and Figure 1D. ROC curves for discrimination in the training and validation cohorts.

Notes: (Figure 1C) In the training cohort, the AUC of the ROC curve of the nomogram and the sixth edition AJCC TNM staging classification was 0.830 and 0.651, respectively ($P < 0.001$). (Figure 1D) In the validation cohort, the AUC of the ROC curve of the nomogram and the sixth edition AJCC TNM staging classification was 0.764 and 0.633, respectively ($P < 0.001$). Abbreviations: AJCC= American Joint Committee on Cancer; AUC= area under the curve; ROC= receiver operating characteristic.

Figure 2. Calibration curves for predictions for the 1-year (a), 3-year (b), 5-year (c) OS in the training cohort (Figure 2A) and in the testing cohort (Figure 2B).

Notes: (Figure 2A) The nomogram-predicted probability of OS is plotted on the X-axis, and the actual OS is plotted on the Y-axis. (Figure 2B) The nomogram-predicted probability of OS is plotted on the X-axis, and the actual OS is plotted on the Y-axis.

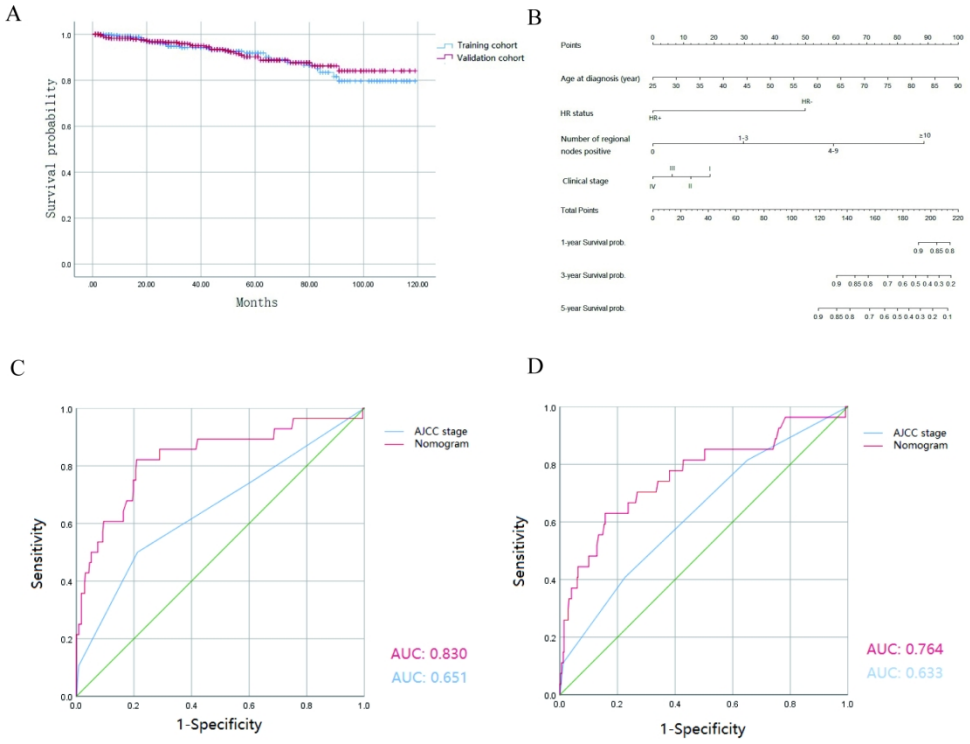


Figure 1A. Kaplan-Meier survival curves of the patients with IMPC in the training and validation cohorts.
Notes: The survival curves showed no significant differences between the 2 cohorts (P= 0.786).

Figure 1B. Nomogram for predicting 1-, 3-, 5-year OS for patients with the prognosis factors.
Notes: The total points are calculated by summing up the points for each factor. The predicted probability of OS can be obtained by projecting the location of the total points to the bottom scales. Abbreviations: OS= overall survival.

Figure 1C and Figure 1D. ROC curves for discrimination in the training and validation cohorts.
Notes: (Figure 1C) In the training cohort, the AUC of the ROC curve of the nomogram and the sixth edition AJCC TNM staging classification was 0.830 and 0.651, respectively (P＜0.001). (Figure 1D) In the validation cohort, the AUC of the ROC curve of the nomogram and the sixth edition AJCC TNM staging classification was 0.764 and 0.633, respectively (P＜0.001). Abbreviations: AJCC= American Joint Committee on Cancer; AUC= area under the curve; ROC= receiver operating characteristic.

185x141mm (300 x 300 DPI)

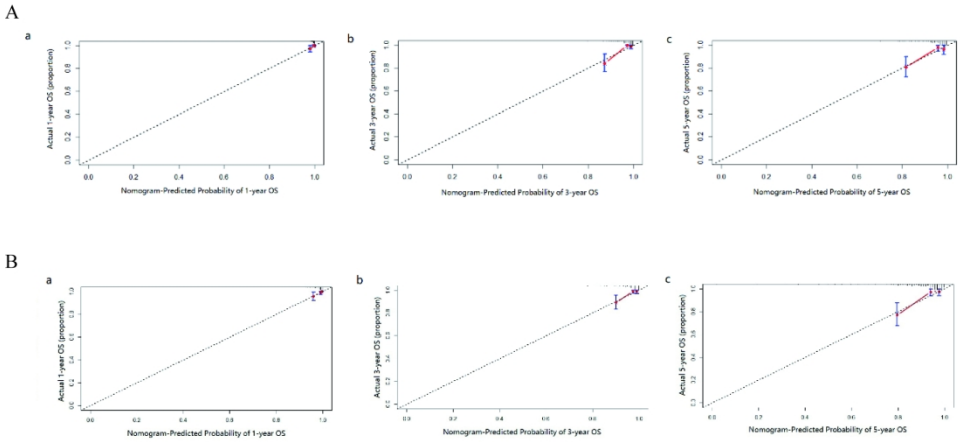


Figure 2. Calibration curves for predictions for the 1-year (a), 3-year (b), 5-year (c) OS in the training cohort (Figure 2A) and in the testing cohort (Figure 2B).
Notes: (Figure 2A) The nomogram-predicted probability of OS is plotted on the X-axis, and the actual OS is plotted on the Y-axis. (Figure 2B) The nomogram-predicted probability of OS is plotted on the X-axis, and the actual OS is plotted on the Y-axis.

213x94mm (300 x 300 DPI)

**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies***

| Section/Topic | Item # | Recommendation | Reported on page # |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 5 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | 5 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | *5* |
| Bias | 9 | Describe any efforts to address potential sources of bias | 5 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 5 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 5 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 5 |
| | | (*c*) Explain how missing data were addressed | 5 |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | 5 |
| | | (*e*) Describe any sensitivity analyses | 5 |
| **Results** | | | |

| | | | |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 6 |
| | | (b) Give reasons for non-participation at each stage | 6 |
| | | (c) Consider use of a flow diagram | 6 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 6 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 6 |
| | | (c) Summarise follow-up time (eg, average and total amount) | 6 |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 8 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 10 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 10 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 10 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 10 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 10 |
| **Limitations** | | | |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 12 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 12 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 13 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.