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Original Article

Factors predicting non-sentinel lymph node status in breast cancer patients with 1-2 macrometastatic positive sentinel lymph nodes

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Abstract: The aim of this study was to investigate predicting factors and to develop a predictive nomogram for non-sentinel lymph nodes (non-SLNs) metastases in breast cancer patients with 1-2 macrometastatic sentinel lymph nodes (SLNs). Details of clinical, imaging, and pathological features of 374 breast cancer patients with 1-2 SLNs metastases, between January 2010 and June 2015, that underwent sentinel lymph node biopsies (SLNB) and complete axillary lymph node dissections (ALND) were collected. Multivariable logistic regression was used to assess the predicting factors of non-SLNs metastases of these patients. A nomogram was created with these independent predictors. Afterward, the model was applied to 135 breast cancer patients with 1-2 SLNs metastases between July 2015 and December 2016. According to results of multivariate analysis, abnormal sonographic ALNs, perineural invasion, clinical TNM staging (cTNM), lymphovascular invasion (LVI), Ki67, and ratio of metastatic SLNs to total SLNs (pRatio) were identified as independent predictors of non-SLNs metastasis. The nomogram for the modeling population was precise and the area under the receiver operating characteristic (ROC) curve of 0.741 (95% CI: 0.693-0.785). When applied to the validation population, the model predicted non-SLNs status effectively (ROC = 0.763, 95% CI: 0.682-0.832). The nomogram developed may distinguish patients with low risk for positive non-SLNs from high risk patients effectively. It could help surgeons and patients to make decisions on avoidance of ALND surgery for low risk of non-SLNs metastases.

Keywords: Breast cancer, lymph node metastasis, nomogram, sentinel lymph node biopsy

Introduction

Breast cancer patients with lymph node-positives should receive complete axillary lymph node dissection (ALND) traditionally for nodal staging [1]. However, significant short-term and long-term morbidity after ALND, including range of motion, lymphedema, pain, hypesthesia, and paresthesia are significantly higher than in sentinel lymph node biopsies (SLNB) [2]. After the early 1990s, SLNB has been widely adopted as an alternative procedure to axillary lymph node dissection (ALND) for axillary staging progressively [3, 4]. Nevertheless, ALND remains the standard of surgical procedures for breast cancer patients with positive SLNs, according to clinical practice guidelines. However, there is still a controversy. Along with the advancement of SLN biopsy and adjuvant therapies, the addition of ALND has not further improved patient outcomes [5]. ASCO issued updated recommendations for most women with one to two metastatic sentinel lymph nodes planning to receive breast conserving surgery with whole-breast radiotherapy, suggesting that they should not undergo axillary lymph node dissection [6]. However, there is confusion for clinicians in making decisions for patients with one to two metastatic sentinel lymph nodes that undergo mastectomies. Nomograms are mathematical tools that provide probability of a specific outcome or prognostic information for an individual patient by combining related factors. These have been widely studied and applied in breast cancer [7-9]. The primary aim of this study was to determine the clinical-pathological

factors associated with non-SLN metastasis and to develop a nomogram that can predict non-SLN metastasis in patients with 1-2 macrometastatic positive sentinel lymph nodes without considering the operation type.

Materials and methods

Patients

This study retrospectively identified 509 breast cancer patients, meeting the inclusion and exclusion criteria, at Sun Yat-sen Memorial Hospital, between January 2010 and December 2016. Inclusion criteria were as follows: (1) Diagnosed with operable primary invasive breast cancer confirmed by core biopsy or open biopsy; (2) SLN biopsy successfully performed; (3) One or two macrometastases found in SLNs; (4) Additional ALND and radical operations performed; and (5) Informed consent obtained. Exclusion criteria included: (1) Received neoadjuvant therapy (neoadjuvant chemotherapy, neoadjuvant endocrine therapy, or neoadjuvant radiotherapy); (2) Pregnant; (3) Prior surgery at the affected axilla; and (4) Bilateral breast cancer. After surgery, adjuvant chemotherapy, radiotherapy, and endocrine therapy were provided to patients according to National Comprehensive Cancer Network (NCNN) guidelines [10]. According to the date of ALND surgery, patients were divided into two groups: retrospective training group and prospective validation group. The training group included 374 breast cancer patients that received additional ALND due to 1-2 positive macro-metastatic SLNs between January 2010 and June 2015. The validation group included 135 breast cancer patients with 1-2 macro-metastatic positive SLNs that received additional ALND between July 2015 and December 2016. The study was approved by the Ethical Committee of Sun Yat-sen Memorial Hospital. The Institutional Review Board (IRB) approval number was SYSEC-KY-KS-033 and all patients provided written informed consent.

Breast ultrasonography and biopsy procedures

Bilateral breast ultrasonic scanning was performed to detect possible lesions on all patients. Once a breast lesion was detected, the following data were recorded: location, maximum diameter, and characteristics (including shape, margin, inner echo, posterior echo, and color Doppler characteristics). Additionally, the characteristics of axillary lymph nodes were recorded. Abnormal sonographic ALNs were defined as axillary lymph nodes with abnormal features, including large size, cortical thickening (diffuse or eccentric), loss of fatty hilum, loss of oval shape, or abnormal cortical blood flow. Suspicious breast masses were detected by core needle biopsies. Abnormal sonographic ALNs were detected by lymph node fine needle aspiration or core needle biopsies. All biopsy procedures were performed by dedicated senior breast surgeons with ultrasonic guidance.

SLN biopsy and surgery procedure

SLNs were identified with blue dye and/or radiocolloid. SLN was defined as any blue-stained node, any node with a blue-stained lymphatic channel leading directly to it, any node with a radioactive count of 10 % or more of the most radioactive node, or any pathologically palpable node. ALND was performed when SLNs were shown to be positive by pathological evaluation. Breast-conserving surgeries (BCS) or mastectomies were performed successfully for all patients.

Histopathologic evaluation

Pathologic examinations of the primary tumor and axillary lymph nodes were performed by two experienced pathologists. Pathologic evaluations of primary tumors were performed with hematoxilin and eosin (H&E) and immunohistochemical (IHC) staining, postoperatively. A standardized pathology reporting form was used. The pathologic size of the primary tumor, nuclear and histologic grade, lympho-vascular invasion (LVI), numbers of excised LNs, SLNs, and metastatic SLNs were recorded. Results of IHC staining of primary tumors, including estrogen and progesterone receptor (ER and PR), Her2/neu, Ki67, P53, Topoisomerase 2 alpha (TOPO2α), and CK5/6 status, were also detected. Her2/neu immunoreactivity was scored as 0, 1+, 2+, and 3+, according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. Her-2/neu IHC scores of 0 and 1+ were classified as HER2 negative and 3 as positive by a pathologist. Her-2/neu status was further investigated by FISH if the pathologist scored it as 2. Breast cancer patients were classified

Table 1. Comparison of clinical and pathological characteristics of the modeling group and validation group

Characteristics		Modeling group (n = 374)	Validation group (n = 135)	*p-value*	t/X^2/Z
Age (years)		48.9±11.6	47.2±9.6	0.097	1.667
Reproductive history	Yes	351 (93.9%)	130 (96.3%)	0.285	1.142
	No	23 (6.1%)	5 (3.7%)		
Family history of breast cancer	Yes	15 (4.0%)	4 (3.0%)	0.582	0.303
	No	359 (96.0%)	131 (97.0%)		
History of tumors	Yes	20 (5.3%)	3 (2.2%)	0.134	2.246
	No	354 (94.7%)	132 (97.8%)		
Menstruation status	Yes	142 (38.0%)	47 (34.8%)	0.516	0.422
	No	232 (62.0%)	88 (65.2%)		
Menopausal age (years)		50.0±3.7	50.0±3.4	0.916	0.106
Side	Left	205 (54.8%)	75 (55.6%)	0.882	0.022
	Right	169 (45.2%)	60 (44.4%)		
Multifocal	Unifocal	335 (89.6%)	128 (94.8%)	0.069	3.317
	Multifocal	39 (10.4%)	7 (5.2%)		
Tumor location	UOQ	190 (50.8%)	59 (43.7%)	0.139	8.133
	UIQ	55 (14.7%)	29 (21.5%)		
	LIQ	37 (9.9%)	16 (11.9%)		
	LOQ	47 (12.6%)	14 (10.4%)		
	Central	37 (9.9%)	10 (7.4%)		
	Others	8 (2.1%)	7 (5.1%)		
Clinical tumor size	T1	184 (49.2%)	71 (52.6%)	0.522	1.298
	T2	181 (48.4%)	59 (43.7%)		
	T3	9 (2.4%)	5 (3.7%)		
Abnormal sonographic ALNs	Yes	100 (26.7%)	45 (33.3%)	0.146	2.118
	No	274 (73.3%)	90 (66.7%)		
Perineural invasion	Yes	14 (3.7%)	3 (2.2%)	0.399	0.711
	No	360 (96.3%)	132 (97.8%)		
cTNM staging	I	69 (18.4%)	30 (22.2%)	0.151	3.786
	II	288 (77.0%)	94 (69.6%)		
	III	17 (4.6%)	11 (8.2%)		
Operative type	Mastectomy	142 (38.0%)	62 (45.9%)	0.106	2.616
	BCS	232 (62.2%)	73 (54.1%)		
Tumor type	IDC	317 (84.8%)	122 (90.4%)	0.144	3.879
	ILC	16 (4.3%)	6 (4.4%)		
	Others	41 (10.9%)	7 (5.2%)		
Histological grade	I	24 (6.4%)	4 (3.0%)	0.117	5.882
	II	148 (39.6%)	67 (49.6%)		
	III	145 (38.8%)	49 (36.3%)		
	N/A	57 (15.5%)	15 (11.1%)		
LVI	Yes	163 (43.6%)	70 (51.9%)	0.098	2.733
	No	211 (56.4%)	65 (48.1%)		
ER	Positive	293 (78.3%)	115 (85.2%)	0.087	2.920
	Negative	81 (21.7%)	20 (14.8%)		
PR	Positive	229 (61.2%)	89 (65.9%)	0.334	0.933
	Negative	145 (38.8%)	46 (34.1%)		
HER2	Positive	76 (20.3%)	36 (26.7%)	0.214	3.086

	Negative	268 (71.7%)	92 (68.1%)		
	NA	30 (8.0%)	7 (5.2%)		
Ki67	<14%	82 (21.9%)	29 (21.5%)	0.915	0.011
	≥14%	292 (78.1%)	106 (78.5%)		
BMI	<18.5	27 (7.2%)	9 (6.7%)	0.917	0.173
	18.5-25	253 (67.6%)	89 (65.9%)		
	≥25	94 (25.2%)	36 (27.4%)		
TOPO2α	Positive	191 (51.1%)	67 (49.6%)	0.937	0.131
	Negative	168 (44.9%)	63 (46.7%)		
	NA	15 (4.0%)	5 (3.7%)		
CK5/6	Positive	24 (6.4%)	7 (5.2%)	0.135	4.006
	Negative	201 (53.7%)	86 (63.7%)		
	NA	149 (39.9%)	42 (31.1%)		
P53	Positive	181 (48.4%)	79 (58.5%)	0.130	4.078
	Negative	180 (48.1%)	52 (38.5%)		
	NA	13 (3.5%)	4 (3.0%)		
CEA (ng/ml)		1.5 (0.9-2.3)	1.6 (1.0-2.6)	0.181	-1.338
CA153 (U/ml)		11.7 (8.4-17.3)	12.9 (10.0-17.6)	0.097	-1.659
CA125 (U/ml)		11.5 (7.4-17.7)	12.0 (9.3-17.1)	0.120	-1.555
CYFRA21-1 (ng/ml)		2.2 (1.7-2.9)	2.3 (1.8-3.1)	0.478	-0.709
pRatio*	≤ 0.25	120 (32.1%)	56 (41.5%)	0.112	4.376
	>0.25-≤0.5	162 (43.3%)	54 (40.0%)		
	>0.5	92 (24.6%)	25 (18.5%)		
Intrinsic subtype	Luminal A	73 (19.5%)	20 (14.8%)	0.091	9.481
	Luminal B (HER2-)	169 (45.2%)	61 (45.2%)		
	Luminal B (HER2+)	38 (10.2%)	26 (19.3%)		
	HER2 overexpressing	29 (7.8%)	6 (4.4%)		
	TNBC	38 (10.2%)	12 (8.9%)		
	N/A	27 (7.1%)	10 (7.4%)		
Period† (months)	<3	251 (67.1%)	77 (57.0%)	0.088	4.864
	3-6	53 (14.2%)	28 (20.7%)		
	>6	70 (18.7%)	30 (23.3%)		
Number of positive SLNs	1	274 (73.3%)	89 (65.9%)	0.106	2.610
	2	100 (26.7%)	46 (34.1%)		

Data are presented as mean ± SD, median (interquartile range) or number (%). UIQ = upper inner quadrant; UOQ = upper outer quadrant; LIQ = lower inner quadrant; LOQ = lower outer quadrant; cTNM = clinical TNM staging; BCS = breast-conserving surgery; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; N/A = not available; LVI = lymphovascular invasion; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor-2; BMI = body mass index; TOPO2α = Topoisomerase 2 alpha; P53 = tumor protein 53; CEA = carcino-embryonic antigen; CA15-3 = carbohydrate antigen 15-3; CA125 = carbohydrate antigen 125; CYFRA21-1 = cytokeratin fragment 19; SLNs = sentinel lymph nodes; pRatio*, ratio of metastatic SLNs to total SLNs; Period†, time of seeing a doctor.

into five intrinsic subtypes based on different possible IHC combinations of ER, PR, HER2, and Ki67 status: luminal A [ER-positive and/or PR-positive, HER2-negative, and low Ki67 labeling index (<14%)], luminal B (HER2-negative) [ER- and/or PR-positive, HER2-negative, and high Ki67 (≥14%)], luminal B (HER2-positive) (ER and/or PR-positive, HER2-positive, and any Ki67), HER2 overexpressing (ER- and PR-negative and HER2-positive), and TNBC (ER- and PR-negative and Her2-negative).

Bisected SLNs were quickly frozen in liquid nitrogen and a single 5-lm-thick section stained with H&E was examined intraoperatively. If the section was positive for metastasis, ALND was performed immediately. After frozen-section analysis, the remaining frozen tissue was fixed

Table 2. Univariate analysis of non-SLN metastasis in the training group

Characteristics		Non-SLNs metastasis (n = 154)	No non-SLNs metastasis (n = 220)	*p-value*	t/X^2/Z
Age (years)		49.33±11.66	48.58±11.50	0.535	-0.621
Reproductive history	Yes	141 (91.6%)	210 (95.5%)	0.123	2.383
	No	13 (8.4%)	10 (4.5%)		
Family history of breast cancer	Yes	5 (3.2%)	10 (4.5%)	0.529	0.397
	No	149 (96.8%)	210 (95.5%)		
History of tumors	Yes	7 (4.5%)	13 (5.9%)	0.564	0.333
	No	147 (95.5%)	207 (94.1%)		
Menstruation status	Yes	56 (36.4%)	86 (39.1%)	0.286	0.593
	No	98 (63.6%)	134 (60.9%)		
Menopausal age (years)		50.36±3.05	49.65±3.09	0.183	1.339
Side	Left	84 (54.5%)	121 (55.0%)	0.931	0.008
	Right	70 (45.5%)	99 (45.0%)		
Multifocal	Unifocal	139 (90.3%)	196 (89.1%)	0.716	0.132
	Multifocal	15 (9.7%)	24 (10.9%)		
Tumor location	UOQ	87 (56.5%)	103 (46.8%)	0.298	6.088
	UIQ	21 (13.6%)	34 (15.5%)		
	LIQ	12 (7.8%)	25 (11.4%)		
	LOQ	16 (10.4%)	31 (14.1%)		
	Central	13 (8.4%)	24 (10.9%)		
	Others	5 (3.3%)	3 (1.3%)		
Clinical tumor size	T1	68 (44.2%)	116 (52.7%)	0.265	2.658
	T2	82 (53.2%)	99 (45.0%)		
	T3	4 (2.6%)	5 (2.3%)		
Abnormal sonographic ALNs	Yes	54 (35.1%)	46 (20.9%)	0.002	9.267
	No	100 (64.9%)	174 (79.1%)		
Perineural invasion	Yes	12 (7.8%)	2 (0.9%)	0.001	11.911
	No	142 (92.2%)	218 (99.1%)		
cTNM staging	I	16 (10.4%)	53 (24.1%)	0.001	14.080
	II	127 (82.5%)	161 (73.2%)		
	III	11 (7.1%)	6 (2.7%)		
Operative type	Mastectomy	65 (42.2%)	77 (35.0%)	0.157	1.998
	BCS	89 (57.8%)	143 (65.0%)		
Tumor type	IDC	132 (85.7%)	185 (84.1%)	0.390	1.883
	ILC	4 (2.6%)	12 (5.5%)		
	Others	18 (11.7%)	23 (10.4%)		
Histological grade	I	6 (3.9%)	18 (8.2%)	0.125	4.158
	II	59 (38.3%)	89 (40.5%)		
	III	67 (43.5%)	78 (35.5%)		
	N/A	22 (14.3%)	35 (15.8%)		
LVI	Yes	82 (53.2%)	81 (36.8%)	0.002	9.944
	No	72 (46.8%)	139 (63.2%)		
ER	Positive	123 (79.9%)	170 (77.3%)	0.548	0.360
	Negative	31 (20.1%)	50 (22.7%)		
PR	Positive	100 (64.9%)	129 (58.6%)		
	Negative	54 (35.1%)	91 (41.4%)	0.219	1.514
HER2	Positive	36 (23.4%)	40 (18.2%)	0.427	1.702

	Negative	105 (68.2%)	163 (74.1%)		
	NA	13 (8.4%)	17 (7.7%)		
Ki67	<14	25 (16.2%)	57 (25.9%)	0.026	4.954
	≥14	129 (83.8%)	163 (74.1%)		
BMI	<18.5	11 (7.2%)	16 (7.3%)	0.308	2.358
	18.5-25	98 (63.6%)	155 (70.5%)		
	≥25	45 (29.2%)	49 (22.2%)		
TOPO2α	Positive	91 (59.1%)	100 (45.5%)	0.031	6.951
	Negative	57 (37.0%)	111 (50.5%)		
	NA	6 (3.9%)	9 (4.0%)		
CK5/6	Positive	7 (4.5%)	17 (7.7%)	0.291	2.470
	Negative	80 (52.0%)	121 (55.0%)		
	NA	67 (43.5%)	82 (37.3%)		
P53	Positive	78 (50.6%)	103 (46.8%)	0.764	0.537
	Negative	71 (46.1%)	109 (49.5%)		
	NA	5 (3.3%)	8 (3.7%)		
CEA (ng/ml)		1.70 (1.0-2.53)	1.30 (0.9-2.1)	0.048	-1.980
CA153 (U/ml)		11.6 (8.6-16.4)	11.7 (8.3-17.6)	0.787	-0.270
CA125 (U/ml)		11.6 (8.0-18.2)	11.6 (8.2-17.4)	0.652	-0.451
CYFRA21-1 (ng/ml)		2.3 (1.7-3.3)	2.2 (1.7-2.9)	0.476	-0.713
pRatio*	≤ 0.25	36 (23.4%)	84 (38.2%)	<0.001	19.635
	>0.25-≤0.5	63 (40.9%)	99 (45.0%)		
	>0.5	55 (35.7%)	37 (16.8%)		
Intrinsic subtype	Luminal A	19 (12.3%)	54 (24.5%)	0.036	11.912
	Luminal B (HER2-)	77 (50.0%)	92 (41.8%)		
	Luminal B (HER2+)	21 (13.6%)	17 (7.7%)		
	HER2 overexpressing	11 (7.1%)	18 (8.2%)		
	TNBC	14 (9.1%)	24 (10.9%)		
	N/A	12 (7.9%)	15 (6.9%)		
Period† (months)	3	103 (66.9%)	148 (67.3%)	0.150	0.928
	3-6	23 (14.9%)	30 (13.6%)		
	>6	28 (18.2%)	42 (19.1%)		
Number of positive SLNs	1	105 (68.2%)	169 (76.8%)	0.063	3.449
	2	49 (31.8%)	51 (23.2%)		

Data are presented as mean ± SD, median (interquartile range) or number (%). SLNs = sentinel lymph nodes; UIQ = upper inner quadrant; UOQ = upper outer quadrant; LIQ = lower inner quadrant; LOQ = lower outer quadrant; cTNM = clinical TNM staging; BCS = breast-conserving surgery; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; N/A = not available; LVI = lymphovascular invasion; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor-2; BMI = body mass index; TOPO2α = Topoisomerase 2 alpha; P53 = tumor protein 53; CEA = carcino-embryonic antigen; CA15-3 = carbohydrate antigen 15-3; CA125 = carbohydrate antigen 125; CYFRA21-1 = cytokeratin fragment 19; pRatio*, ratio of metastatic SLNs to total SLNs; Period†, time of seeing a doctor.

in formalin and embedded in paraffin for routine pathologic examinations, as previously described.

Statistical analysis

Details of the subjects were categorized to the training dataset for building a nomogram system and the validation dataset for validating. SPSS, version 19, for windows (SPSS Inc., Chicago, USA) was employed for statistical analysis. Chi-square test or Fisher's exact test was employed for testing statistical significance of association between two discrete variables. Descriptive statistics and t-tests were used for between-group or within-group comparisons of independent samples. All variables with p values of less than 0.1 were then added to multivariate logistic regression analysis to determine whether the clinical-pathological

Table 3. Multivariate logistic analysis of non-SLN metastasis in the training group

	B	S.E.	Wald	*p-value*	OR	95% CI	
						Lower	Upper
Abnormal sonographic ALNs	1.110	0.281	15.623	<0.001	3.034	1.750	5.262
Perineural invasion	2.343	0.814	8.275	0.004	10.410	2.110	51.366
cTNM staging							
II vs. I	1.262	0.353	12.796	<0.001	3.533	1.769	7.055
III vs. I	1.479	0.645	5.256	0.022	4.387	1.239	15.530
LVI	0.745	0.237	9.919	0.002	2.106	1.325	3.349
Ki67	0.742	0.299	6.148	0.013	2.100	1.168	3.775
pRatio*							
≤0.25 vs. >0.5	-1.237	0.320	14.979	<0.001	0.290	0.155	0.543
>0.25-≤0.5 vs. >0.5	-0.665	0.294	5.123	0.024	0.514	0.289	0.915
Constant	-2.036	0.488	17.390	<0.001	0.131		

SLNs = Sentinel lymph nodes; LVI = lymphovascular invasion; cTNM staging = clinical TNM staging; pRatio*, ratio of metastatic SLNs to total SLNs.

variables correlated with non-SLNs positivity. In the multivariate logistic model, a forward stepwise approach was performed and factors with a *p* value less than 0.05 are considered statistically significant. Additionally, 95% confidence intervals (CI) were calculated. The nomogram system was developed using R software (version 3.2.3) (http://www.r-project.org) with all independent variables for prediction of non-SLNs metastasis. Area under the receiver operating characteristic (ROC) curve (AUC) was calculated using MedCalc for Windows software version 17.2 (MedCalc Software, Mariakerke, Belgium) to assess the predictive power of the scoring system. Nomogram performance in terms of calibration ability was evaluated using the Hosmer-Lemeshow-type χ^2 statistics.

Results

Clinicopathologic characteristics of the training group and validation group

During the study period, the study population consisted of 509 breast cancer patients. Median patient age was 48.89±11.55 years (range 23-83 years). Moreover, 194 (38.11%) out of 509 patients with 1-2 SLNs macrometastasis had non-SLNs metastasis, while 315 (61.89%) did not. A total of 315 (61.89%) out of 509 patients underwent breast-conserving surgeries, while 194 (38.11%) received mastectomies. There was a total of 374 patients (training group) for the development of the nomogram system and 135 patients (validation group) for validation of the nomogram system. Clinical and pathological characteristics of patients between the training group and validation group did not differ significantly ($p>0.05$) (**Table 1**).

Univariate analysis of non-SLNs metastasis in the training group

Based on the results of univariate analysis, variables that were significantly associated with incidence of non-SLNs metastasis in an 1-2 SLNs positive patient included abnormal sonographic ALNs, perineural invasion, clinical TNM staging (cTNM staging), LVI, Ki67, TOPO2α, CEA, ratio of metastatic SLNs to total SLNs (pRatio), intrinsic subtype, and numbers of positive SLNs ($p<0.1$) (**Table 2**).

Multivariate logistic analysis of non-SLNs metastasis in the training group

According to multivariate analysis, abnormal sonographic ALNs, perineural invasion, clinical TNM staging, LVI, Ki67, and pRatio were identified as independent predictors of non-SLNs metastasis (**Table 3**).

Construction and application of a novel nomogram

A novel nomogram of non-SLNs metastasis was constructed, according to results of multivariate logistic analysis. As shown in **Figure 1**, rows 2 through 7 represent variables. Vertical lines should be made between each variable and the uppermost row (points). In this way, the effects of each variable are determined by a defined number of points, which should be

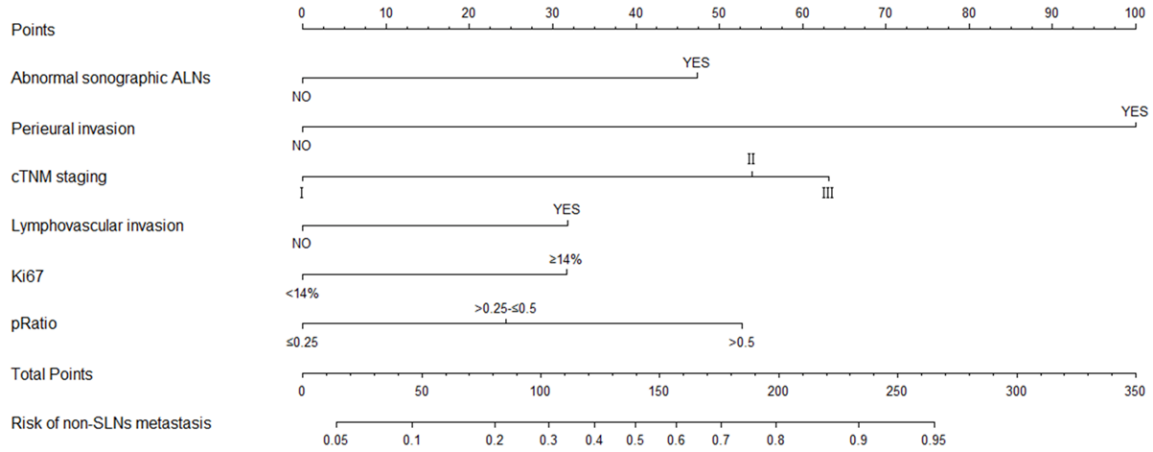

Figure 1. Nomogram for predicting non-sentinel lymph nodes metastasis.

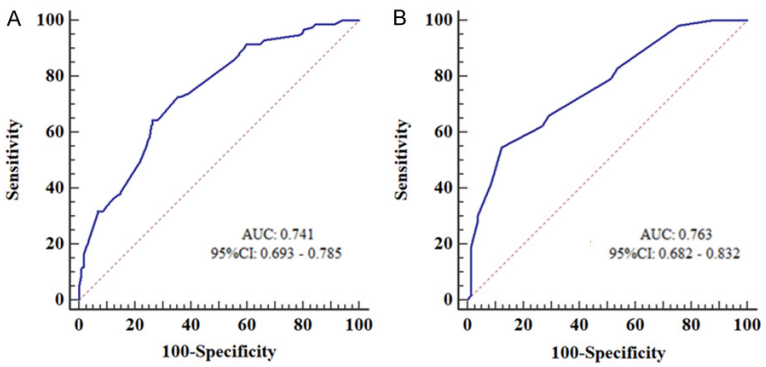

Figure 2. Receiver operating characteristic (ROC) curve calculation for the nomogram applied to the training group (left A) and validation group (right B).

summed and located in row 8 (total points). Vertical lines should be made between row 8 and 9 (predicted value) to get the predicted probability of non-SLNs metastasis (**Figure 1**). Next, ROC analysis was performed to investigate the predictive efficiency of the nomogram. The area under the ROC (AUC) curve for the nomogram on training group was 0.741 (95% CI: 0.693-0.785), indicating potentially promising predictive power of the multivariate logistic regression model. The AUC for the nomogram of the validation group was 0.763 (95% CI: 0.682-0.832) (**Figure 2**). The nomogram was well calibrated (**Figure 3**).

Discussion

According to previous studies, the 5-year-local recurrence rates were not statistically significantly different between SLNB and ALND groups [11, 12]. However, in the SEER Database-study, 184 of 22,986 women experienced local recurrence, with significantly more in the SLND group compared to the ALND group [13]. Studies have not adequately confirmed, however, whether ALND can be omitted in breast cancer patients with positive sentinel lymph nodes.

Non-SLNs were negative in 61.89% of patients with 1-2 positive SLNs in the present study. According to clinical practice guidelines, ALND should be performed on these patients. However, they would not benefit for staging and outcomes from the ALND. The aim of this study was to distinguish low-risk patients from all patients with 1-2 macro-metastatic positive SLNs. ALND may be avoided in these low-risk patients and incidence of complications can be reduced effectively.

Several previous studies have been conducted to determine risk factors associated with axillary lymph node metastasis. Variables significantly associated with incidence of non-SLN metastasis have varied from different studies and centers [7, 14, 15]. In this study, perineural invasion, LVI, Ki67, pRatio, abnormal sonographic ALN, and cTNM were independent predictive factors for non-SLN metastasis in breast cancer patients with 1-2 metastatic SLNs.

Perineural invasion is a marker of poor outcomes, signifying more advanced disease in

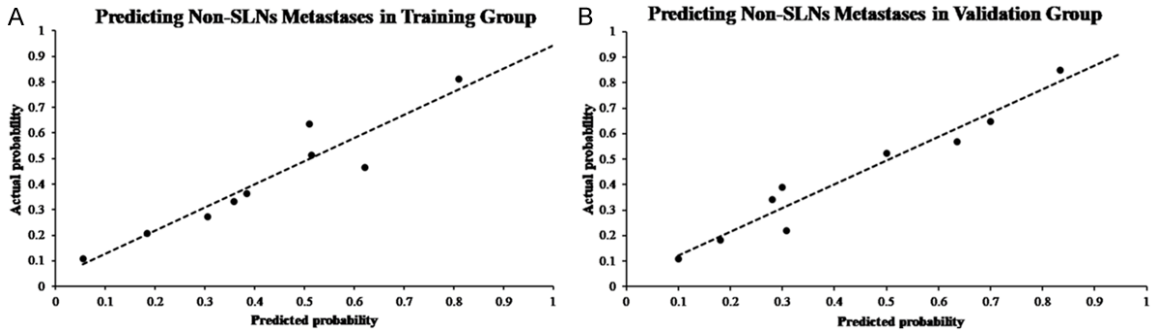

Figure 3. Calibration of the nomogram for predicting non-sentinel lymph nodes metastasis in training group (left A) and validation group (right B). The x-axis shows the predicted probability of non-sentinel lymph nodes metastasis and the y-axis shows the observed probability of non-sentinel lymph nodes metastasis.

many malignancies, including breast cancer [16, 17]. Reported rates of perineural invasion in breast cancer range from 3% to 38% [18-20]. The rate of perineural invasion was 4.07% (14/374) in the training group in the present study. Moreover, there was a significant radical difference between breast cancer patients with or without non-sentinel lymph node metastasis (7.79% vs. 0.91%). Chen et al. also reported that neural invasion was significantly associated with incidence of non-SLN metastasis in SLN-positive patients. based on results of univariate analysis [14]. It may be an important route of metastatic spread and a risk factor for lymph node metastasis mechanisms of breast cancer. Lympho-vascular invasion has been thought to play as an active role in poor prognosis in breast cancer [21, 22]. Furthermore, it has been confirmed that LVI is independent predictor of breast lymph node metastasis [7, 14, 23]. Lymphatic vessels not only provide an entrance for tumor cells to penetrate, but also make several key contributions to tumor metastasis, such as provision of a niche for cancer stem cells and modulation of antitumor immune responses [24, 25].

Ki67, a tumor proliferation marker, was demonstrated to be a predictive factor of non-SLN metastasis of breast cancer in the present study. However, it was not the same as the results of several published nomograms before [15, 26-28]. Studies have confirmed that a high Ki67 index significantly correlates with positive lymph node status [29, 30]. Low ratio of metastatic SLNs to total SLNs predicted a low risk for positive non-SLN, according to Kuru's study [31], as well as in the present study. It may have the same meaning of predictive factors, like number of positive SLNs and number of negative SLNs, in others studies [9, 14].

The nomogram in the present study was considered to have fine predictive effects. It was well calibrated. For low-risk patients with 1-2 macro-metastatic positive SLNs, ALND may be avoided. However, omitting ALND in patients with macro-metastases may be associated with higher regional recurrence rates. According to previous studies, for low-risk breast-conserving surgery patients, radiation therapy should be performed regularly. However, for low-risk mastectomy patients, post-mastectomy radiation therapy may be required. More high-quality randomized controlled trials are needed to carry out research, providing a more reliable basis for clinical practice.

The present study had several limitations. No information was collected concerning mammograms and breast MRIs, which may have improved prediction accuracy. This study was a single center experience and the samples may not have been large enough to construct a perfect nomogram. Multicenter studies from different countries and regions should be researched. Due to discrepant surgical, imaging, and pathologic techniques from different centers, there was a great variation in predictive factors for non-SLN metastasis among breast cancer patients with 1-2 positive SLNs. The present nomogram may not applicable to other centers. All nomograms and scoring systems may not have a utility for all patient populations. Thus, a unique model may be created and validated for each clinic.

In conclusion, for patients with invasive breast cancer and 1-2 positive SLNs, neural invasion,

LVI, Ki67, pRatio, abnormal sonographic ALN, and cTNM were independent predictive factors of non-sentinel lymph node status involvement. A novel nomogram was constructed and validated effectively. This may assist surgeons and patients in making decisions concerning avoidance of ALND surgery for low risk of non-SLN metastases for individual patients. Validation studies will be performed in the future, including investigations from other centers.

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Disclosure of conflict of interest

None.

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