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Predictors of internal mammary lymph nodes (IMLN) metastasis and disease-free survival comparison between IMLN-positive and IMLN-negative breast cancer patients

Results from Western China Clinical Cooperation Group (WCCCG) database (CONSORT)

Kang Wang, MD[a], Xiang Zhang, PhD[a], Ke Zheng, PhD, MD[a], Xue-Dong Yin, PhD[a], Lei Xing, MD[a], Ai-Jie Zhang, MD[a], Yang Shi, PhD[b], Ling-Quan Kong, MD[a], Fan Li, PhD[a], Bin-Lin Ma, MD[c], Hui Li, MD[d], Jin-Ping Liu, MD[e], Jun Jiang, PhD[f,*], Guo-Sheng Ren, PhD[a,g,*], Hong-Yuan Li, MD[a,*]

Abstract

Limited studies performed a comprehensive assessment of risk factors for internal mammary lymph nodes (IMLN) metastasis, and disease-free survival (DFS) difference between IMLN-positive and IMLN-negative breast cancer (BC) patients undergoing IMLN dissection and systemic therapies was not clear.

A retrospective study included 1977 BC patients from Western China Clinical Cooperation Group between January 2005 and December 2012. The impact of clinicopathological factors on the occurrence of IMLN metastasis was assessed in univariate and multivariate logistic regression analyses, and a nomogram (model) was constructed to predict the IMLN status. DFS difference was evaluated in univariate and multivariate Cox regression analyses between IMLN-negative and IMLN-positive patients, and univariate analysis was performed to compare DFS between individuals with high and low IMLN metastasis risk defined by proposed nomogram.

Of 1977 enrolled patients, 514 cases underwent IMLN dissection and 1463 cases did not undergo IMLN irradiation or dissection. We found that initial disease symptoms and signs, mammographic calcification, tumor site, number of positive axillary lymph nodes (ALNs), American Joint Committee on Cancer pT stage, and human epidermal growth factor receptor 2 status were associated with IMLN metastasis (all $P < .05$). Those variables were included in nomogram, whose predictive ability was better than that of ALN classification (area under the curve: 0.82 vs 0.76, $P < .001$). Univariate cox proportional hazards model indicated that better DFS was observed in IMLN-negative patients than IMLN-positive group (hazard ratio [HR] = 1.87, 95% confidence interval [CI] = 1.05–3.34; $P = .04$), whereas no significant differences in DFS (HR = 0.99, 95% CI = 0.49–2.00; $P = .97$) were found after adjusting patient-, disease-, and treatment-related factors.

Nipple inversion, mammographic calcification, larger tumor size, medial tumor site, negative HER-2 status, and more positive ALNs are independent risk factors for IMLN metastasis, and the individualized nomogram is a feasible tool to predict the status of IMLN. Equivalent DFS was found between positive and negative IMLN patients who all accepted IMLN dissection and systemic therapies.

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Kang Wang (kang_wang0822@hotmail.com) and Xiang Zhang have contributed equally to this work and should be considered as co-first authors.

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[a] Department of the Endocrine and Breast Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing Medical University, Chongqing, [b] Department of Epidemiology and Biostatistics, West China School of Public Health, Sichuan University, Chengdu, Sichuan, [c] Department of Breast and Neck Surgery, Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, Xinjiang, [d] Department of Breast Surgery, Sichuan Province Tumor Hospital, [e] Department of Breast Surgery, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, Sichuan, [f] Breast Disease Center, Southwest Hospital, Third Military Medical University, [g] Chongqing Key Laboratory of Molecular Oncology and Epigenetics, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China.

[*] Correspondence: Hong-Yuan Li, and Guo-Sheng Ren, Department of the Endocrine and Breast Surgery, The First Affiliated Hospital of Chongqing Medical University, Yuzhong, Youyi Road, Chongqing 400016, China (e-mail: hongy_li@hotmail.com, rengs726@126.com), Jun Jiang, Breast Disease Center, Southwest Hospital, Third Military Medical University, No. 29 Gaotanyan Street, Chongqing 400038, China (e-mail: southwestbreast@163.com).

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Abbreviations: AJCC = American Joint Committee on Cancer system, ALN = axillary lymph node, AUC = area under the curve, BC = breast cancer, C-index = concordance index, CI = confidence interval, DFS = disease-free survival, ER = estrogen receptor, FISH = fluorescence in situ hybridization, HER2 = human epidermal growth factor receptor 2, HR = hazard ratio, IMLN = internal mammary lymph nodes, PR = progesterone receptor.

Keywords: breast cancer, disease-free survival, internal mammary lymph node, nomogram

1. Introduction

Overwhelming evidence from recent years showed obvious survival advantages conferred by additionally internal mammary lymph nodes (IMLN) irradiation especially among axillary lymph nodes (ALNs) positive breast cancer (BC) patients.[1–3] Positron emission tomography/computed tomography, high-resolution ultrasound, magnetic resonance imaging, and IMLN-sentinel lymph nodes biopsy were introduced to directly detect IMLN involvement, still, all these techniques are not enough to guide individualized IMLN irradiation.[4,5]

Although the status of IMLN and its relevant concepts were incorporated into 6th edition of the American Joint Committee on Cancer (AJCC) staging system for more than a decade, the 2016 NCCN Breast Cancer Clinical Practice Guidelines just recommended that IMLN irradiation for patients with at least 4 positive ALNs, and strongly considers IMLN irradiation for patients with 1 to 3 positive ALNs.[6] Nevertheless, prior studies reported that 36.8% to 46.2% patients with more than 4 positive ALNs and 18.8% to 26.7% patients with 1 to 3 positive ALNs were identified as IMLN metastases, and negative IMLN was found in about 70% patients with more than 4 positive ALNs.[7–10] Besides, excess local irradiation therapy can also lead to radiation pneumonitis and myocardial damage.[11] Huang et al included 1679 Chinese BC patients who underwent extended radical mastectomy, and indicated that patients with medial tumor and positive ALNs had a considerable risk of IMLN metastasis.[9] This study did not include tumor biological characteristics in multivariate regression, whereas previous studies indicated that tumor with calcifications, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are predictive of lymph nodes involvement.[12–14] Hence, applying status of ALN to select beneficiaries of IMLN irradiation was not feasible, and more comprehensive models/nomograms integrated with anatomical and biological features needed be proposed to predict the involvement of IMLN individually.

Previous studies documented that patients with IMLN metastasis had worse survival outcomes than those with IMLN-negative regardless of involvement of ALN,[15–18] but randomized controlled trails consistently indicated that additional IMLN dissection did not show survival benefits compared with radical or modified radical mastectomy.[19–22] Although Veronesi et al[22] suggested that IMLN-positive patients who underwent IMLN dissection had higher annual death rate than corresponding nodal-negative patients, all the subjects enrolled in this study did not received postoperative radiotherapy. Moreover, any anticancer therapy or hormonal manipulation were not considered as prognostic covariables due to absence of documented evidence of primary treatment in this study, and similar results were found by Donegan.[23] Thus, few studies examined the effect of IMLN dissection on disease-free survival (DFS) among patients with different IMLN statuses when system adjuvant therapies were guaranteed for postoperative treatments.

The aim of this study was to investigate the impact of tumor heterogeneity, status of ALN, and calcifications of primary tumor on IMLN status, and to develop a nomogram for clinicians in predicting the IMLN status based on variables available after surgery to decide the best regional nodal irradiation option. In addition, we assessed the DSF difference between IMLN-positive and IMLN-negative patients who all received IMLN dissection and system adjuvant treatments.

2. Methods

2.1. Study design

The data for this study were obtained from Western China Clinical Cooperation Group (WCCCG), which included 23 BC centers in 9 provinces of Western China (i.e., Chongqing, Sichuan, Yunnan, Guizhou, Shanxi, Gansu, Guangxi, Ningxia, and Xinjiang). The whole database included a total of 18,600 patients with BC, which was histologically confirmed. Details about WCCCG had been described previously.[24] Patients with primary BC had undergone breast surgery between January 2005 and December 2012 were potentially enrolled. We excluded patients with neoadjuvant chemotherapy, bilateral tumors, and insufficient data. Only patients with completed data on mammography, ER, PR, HER2 status based on immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) were included. ER and PR positivity were determined IHC when the staining of ≥1% of tumor cells appeared. Tumors were identified as HER2-negative if they received an IHC score of 1+ and as HER2-positive only if they received an IHC score of 3+ or exhibited a HER2 gene expression level that was at least 2-fold higher than normal, as determined FISH. Considering the patients in the previous period did not routinely receive IHC for some prognostic biomarkers like P53 and Ki67, we cannot extract these variables from medical records. Despite this, the tumors were still categorized into 4 BC subtypes according to 2013 St Gallen International Expert Consensus[25]: hormone receptor (HR)-positive/HER2-negative, HR-positive/HER2- positive, HR-negative/HER2-positive, and triple-negative BC. Patient medical records and WCCCG were reviewed for data regarding to age at diagnosis, mammography data, and histopathological information. The surgical field of IMLN dissection was from the first to the fourth intercostal space, involving involved lymph nodes or pleura. The tumor sites were defined according to the quadrant or angles with nipple of primary tumor, and the data were provided by mammography. If the left (right) tumor located in upper outer quadrant, lower outer quadrant or 3 o'clock (9 o'clock), we defined it as lateral site. If the tumor located in nipple–areola, 6 o'clock or 12 o'clock, we defined it as central site. If the left (right) tumor located in upper inner quadrant, lower inner quadrant or 9 o'clock (3 o'clock), we defined it as medial site. The number of metastatic axillary nodes were categorized into 3 groups (node-negative nodes, 1–3 positive nodes, and at least 4 positive nodes), which reflected

the essential cut-offs for status of ALN based on the IMLN irradiation associated recommendations released by the 2016 NCCN Breast Cancer Clinical Practice Guidelines.[6]

We extracted the aforementioned patients with completed survival data including survival status and survival time, who were followed up from 2005 to 2015, and questionnaire results were obtained through phone and outpatient department follow-up ways. Patients in every registry would answered the questions through telephone follow-up or reexamining in outpatient department at least once every 3 months during the first 3 years and then every 6 months thereafter. Clinic doctors would take detailed history or have a completed physical examination at each follow-up visit. Residual breast ultrasound or mammogram, chest radiography, abdominal sonography, whole-body bone scan, or positron emission tomography–computed tomography was routinely performed annually or when tumor relapse was clinically suspected. DFS was defined as the date of the diagnosis to the locoregional or distant recurrence or death from BC, whichever came first, and DFS was considered as censored status if patients were alive until date of last contact.

At last, of 1977 enrolled patients, 514 cases underwent IMLN dissection, 1463 cases did not undergo IMLN irradiation or dissection. This observational study was entirely based on data extracted from patient medical records and was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University.

2.2. Statistical method

We evaluated the distribution of clinicopathological variables between patients with IMLN-positive and IMLN-negative groups using the Pearson chi-squared test or Fisher exact test for categorical variables, and Student *t* for continuous variables. Univariate logistic regression analysis was used to assess the strength of the association between each predictive variable and the status of IMLN, and multivariate logistic regression analysis was applied to identify independent effects of these univariate predictive variables ($P < .05$). To avoid the influence of multi-collinearity between some highly correlated variables, we only included one of them into final model (e.g., N stage and ALN status). In addition of these, an individualized nomogram was constructed based on *rms* package in R software. To validate this model internally through 1000 bootstrap resamples, concordance index (C-index) was calculated for the evaluation of the performance of predicting and discrimination ability by test concordance between predicted probability and actual outcome. Given that the probability of IMLN status was predicted by points based on nomogram or ALN categories recommended by NCCN 2016, we also conducted receiver operating characteristic curve to compare the performance of predicting ability of 2 IMLN status prediction tools, which were measured by area under the curve (AUC) values.

We conducted log-rank tests and cox proportion hazard regressions to examine the difference between patients with IMLN-positive and IMLN-negative in DFS, and calculated hazard ratios (HRs) with 95% confidence interval (CI). We performed univariate analysis to determine potential prognostic variable on DFS, and multivariate analysis was conducted to acquire adjusted HRs. Similar analyses were performed between high and low IMLN metastasis risk groups, which were stratified by cut-off value of score based on developed nomogram.

All *P* values reported are 2-sided, which $<.05$ were considered statistically significant. All analyses were conducted using R software (version 3.4.1).

3. Results

3.1. Patient characteristics

A total of 1977 eligible patients were enrolled in this study according to inclusion criteria, 514 individuals of them underwent IMLN dissection, 1463 patients of them with survival data did not undergo IMLN dissection or radiation. The flow

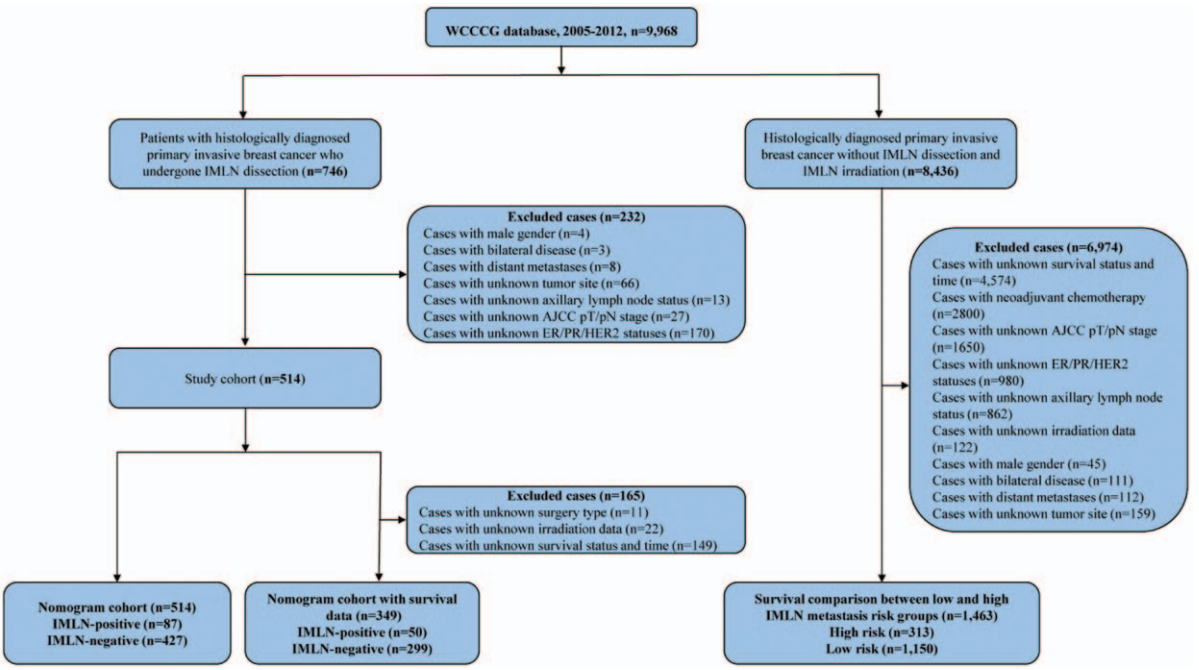

Figure 1. Flow chart for the data screening.

Table 1

Demographics for eligible patients according to IMLN status (n = 514).

Characteristics	IMLN-negative, N (427)	IMLN-positive, N (87)	P*
Age at diagnosis, y†	46.8 ± 9.9	44.9 ± 10.0	.10[a]
Marital status			
Married	417 (97.7)	82 (94.3)	.15[b]
Never married/widowed/divorced	10 (2.3)	5 (5.7)	
Menopausal status			
Premenopausal	260 (60.9)	58 (66.7)	.51
Postmenopausal	165 (38.6)	29 (33.3)	
No. of parity			
0	30 (7.0)	10 (11.5)	.29
1–2	339 (79.4)	68 (78.2)	
>2	58 (13.6)	9 (10.3)	
Initial disease symptoms and signs			
Breast lump	384 (89.9)	73 (83.9)	.06[b]
Breast pain	24 (5.6)	5 (5.7)	
Nipple discharge	7 (1.6)	1 (1.1)	
Nipple inversion	12 (2.8)	8 (9.2)	
Mammographic calcification			
No	396 (92.7)	64 (73.6)	**<.001**
Yes	31 (7.3)	23 (26.4)	
Laterality			
Left	230 (53.9)	55 (63.2)	.11
Right	197 (46.1)	32 (36.8)	
Tumor site			
Lateral	235 (55.0)	44 (50.6)	.17[b]
Central	60 (14.1)	8 (9.2)	
Medial	96 (22.5)	22 (25.3)	
Lateral and central	18 (4.2)	4 (4.6)	
Central and medial	6 (1.4)	2 (2.3)	
Occupying the whole breast	12 (2.8)	7 (8)	
Histologic type			
Ductal	350 (82.0)	70 (80.5)	.47
Lobular	38 (8.9)	11 (12.6)	
Other	39 (9.1)	6 (6.9)	
No. of positive ALN			
0	201 (47.1)	9 (10.3)	**<.001**
1–3	118 (27.6)	16 (18.4)	
≥4	108 (25.3)	62 (71.3)	
AJCC pT stage			
pT1	87 (20.4)	12 (13.8)	**.004**
pT2	274 (64.2)	50 (57.5)	
pT3	61 (14.3)	20 (23.0)	
pT4	5 (1.2)	5 (5.7)	
AJCC pN stage			
pN0	200 (46.8)	0 (0.0)	**<.001**
pN1	118 (27.6)	3 (3.4)	
pN2	61 (14.3)	8 (9.2)	
pN3	48 (11.2)	76 (87.4)	
AJCC stage			
I	50 (11.7)	0 (0.0)	**<.001**
IIA	154 (36.1)	0 (0.0)	
IIB	93 (21.8)	3 (3.4)	
IIIA	125 (29.3)	79 (90.8)	
IIIB	5 (1.2)	5 (5.7)	
ER			
Negative	181 (42.4)	43 (49.4)	.23
Positive	246 (57.6)	44 (50.6)	
PR			
Negative	181 (42.4)	41 (47.1)	.42
Positive	246 (57.6)	46 (52.9)	
HER2			
Negative	385 (90.2)	86 (98.9)	**.008**
Positive	42 (9.8)	1 (1.1)	
Tumor subtypes			
HR-positive/HER2-negative	269 (63.0)	55 (63.2)	**.03**
HR-positive/HER2-positive	30 (7.0)	0 (0.0)	
HR-negative/HER2-positive	12 (2.8)	1 (1.1)	
Triple negative	116 (27.2)	31 (35.6)	

AJCC = American Joint Committee on Cancer system, ALN = axillary lymph node, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, IMLN = internal mammary lymph node, PR = progesterone receptor.

* Pearson chi-squared test, except [a]Student *t* test and [b]Fisher exact test for R*C contingency tables as computed by "fisher.test" function in R.

† Values are mean (standard deviation).

Bold emphasized values were considered statistically significant.

Table 2

Demographics for patients with survival data according to IMLN status (n = 349).

Characteristics	IMLN-negative, N (299)	IMLN-positive, N (50)	P*
Age at diagnosis, y†	46.4 ± 9.4	43.3 ± 9.3	.03[a]
Disease-free survival, mo‡	33 (3–98)	58.5 (5–89)	<.001[a]
Disease relapse			
No	251 (83.9)	35 (70.0)	.018
Yes	48 (16.1)	15 (30.0)	
Mammographic calcification			
No	284 (95.0)	39 (78.0)	<.001[b]
Yes	15 (5.0)	11 (22.0)	
Tumor site			
Lateral	175 (58.5)	28 (56.0)	.04[b]
Central	57 (19.1)	5 (10.0)	
Medial	66 (22.1)	15 (30.0)	
Lateral and central	1 (0.3)	2 (4.0)	
Histologic type			
Ductal	245 (81.9)	38 (76.0)	.36
Lobular	28 (9.4)	8 (16.0)	
Other	26 (8.7)	4 (8.0)	
No. of positive ALN			
0	127 (42.5)	6 (12.0)	<.001
1–3	96 (32.1)	11 (22.0)	
≥4	76 (25.4)	33 (66.0)	
AJCC pT stage			
pT1	68 (22.7)	7 (14.0)	.007
pT2	191 (63.9)	29 (58.0)	
pT3	39 (13.0)	12 (24.0)	
pT4	1 (0.3)	2 (4.0)	
AJCC pN stage			
pN0	127 (42.5)	0 (0.0)	<.001
pN1	96 (32.1)	0 (0.0)	
pN2	48 (16.1)	6 (12.0)	
pN3	28 (9.4)	44 (88.0)	
AJCC stage			
I	36 (12.0)	0 (0.0)	<.001
IIA	103 (34.4)	0 (0.0)	
IIB	70 (23.4)	0 (0.0)	
IIIA	89 (29.8)	48 (96.0)	
IIIB	1 (0.3)	2 (4.0)	
ER			
Negative	122 (40.8)	23 (46.0)	.49
Positive	177 (59.2)	27 (54.0)	
PR			
Negative	121 (40.5)	22 (44.0)	.64
Positive	178 (59.5)	28 (56.0)	
HER2			
Negative	273 (91.3)	50 (100.0)	.04[b]
Positive	26 (8.7)	0 (0.0)	
Tumor subtypes			
HR-positive/HER2-negative	198 (66.2)	33 (66.0)	.14[b]
HR-positive/HER2-positive	20 (6.7)	0 (0.0)	
HR-negative/HER2-positive	6 (2.0)	0 (0.0)	
Triple negative	75 (25.1)	17 (34.0)	
Surgery type			
Breast conserving surgery	9 (3.0)	0 (0.0)	<.001[b]
Modified radical mastectomy	277 (92.6)	39 (78.0)	
Extensive radical mastectomy	13 (4.3)	11 (22.0)	
Chemotherapy			
TE	236 (78.9)	35 (70.0)	.34
CEF	43 (14.4)	11 (22.0)	
Others	20 (6.7)	4 (8.0)	
Endocrine therapy			
No	92 (30.8)	21 (42.0)	.12
Yes	207 (69.2)	29 (58.0)	
Radiotherapy			
No	218 (72.9)	9 (18.0)	<.001
Yes	81 (27.1)	41 (82.0)	

AJCC = American Joint Committee on Cancer system, ALN = axillary lymph node, CEF = cyclophosphamide, epirubicin, and fluorouracil, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, IMLN = internal mammary lymph node, PR = progesterone receptor, TE = docetaxel plus epirubicin.

* Pearson chi-squared test, except [a]Student *t* test and [b]Fisher exact test for R*C contingency tables as computed by "fisher.test" function in R.

† Values are mean (standard deviation).

‡ Values are median (range).

Table 3

Demographics for patients with survival data according to IMLN risk stratified by nomogram (n = 1463).

Characteristics	Low risk of IMLN metastasis, N (1150)	High risk of IMLN metastasis, N (313)	P*
Age at diagnosis, y†	48.9 ± 10.4	44.6 ± 10.5	<.001[a]
Disease-free survival, mo‡	17 (3–111)	14 (3–89)	.05[a]
Disease relapse			
No	1045 (90.9)	259 (82.7)	<.001
Yes	105 (9.1)	54 (17.3)	
Initial disease symptoms and signs			
Breast lump	1046 (91.0)	263 (84.0)	<.001
Breast pain	76 (6.6)	28 (8.9)	
Nipple discharge	17 (1.5)	7 (2.2)	
Nipple inversion	11 (1.0)	15 (4.8)	
Mammographic calcification			
No	1065 (92.6)	238 (76.0)	<.001
Yes	85 (7.4)	75 (24.0)	
Tumor site			
Lateral	699 (60.8)	178 (56.9)	.008
Central	175 (15.2)	54 (17.3)	
Medial	254 (22.1)	66 (21.1)	
Lateral and central	17 (1.5)	7 (2.2)	
Central and medial	3 (0.3)	3 (1.0)	
Occupying the whole breast	2 (0.2)	5 (1.6)	
No. of positive ALN			
0	820 (71.3)	11 (3.5)	<.001
1–3	292 (25.4)	69 (22.0)	
≥4	38 (3.3)	233 (74.4)	
AJCC pT stage			
pT1	470 (40.9)	76 (24.3)	<.001
pT2	599 (52.1)	193 (61.7)	
pT3	49 (4.3)	22 (7.0)	
pT4	32 (2.8)	22 (7.0)	
ER			
Negative	384 (33.4)	94 (30.0)	.26
Positive	766 (66.6)	219 (70.0)	
PR			
Negative	434 (37.7)	123 (39.3)	.62
Positive	716 (62.3)	190 (60.7)	
HER2			
Negative	969 (84.3)	308 (98.4)	<.001
Positive	181 (15.7)	5 (1.6)	

AJCC = American Joint Committee on Cancer system, ALN = axillary lymph node, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, IMLN = internal mammary lymph node, PR = progesterone receptor.

* Pearson chi-squared test, except [a]Student *t* test and [b]Fisher exact test for R*C contingency tables as computed by "fisher.test" function in R.

† Values are mean (standard deviation).

‡ Values are median (range).

chart was shown in Fig. 1, and Tables 1 to 3 illustrated clinicopathological characteristics of corresponding patients included. Of those patients who underwent IMLN dissection, 427 BC patients had IMLN-negative statuses and 87 patients had IMLN metastases. The mean age of the IMLN-negative group was similar with that of the patients who were IMLN involvement (46.8 ± 9.9 vs 44.9 ± 10.0, $P = .10$). Among patients with IMLN metastasis, 71.3% and 18.4% patients of them had at least 4 positive and 1 to 3 positive ALNs, respectively, and IMLN negative was found in 52.9% patients with ALN metastasis. In addition, mammographic calcification, AJCC pathological stage, HER2 status, and tumor subtype defined by IHC varied significantly across the 2 IMLN status groups ($P < .05$).

3.2. Predictors of IMLN status

Associations of clinicopathological factors with metastatic IMLN incidence were studied by univariate and multivariate logistic regression analyses (Table 4). Univariate logistic regression analyses indicated that initial disease symptoms and signs, mammographic calcification, tumor site, number of positive ALN, AJCC pT stage, and HER2 status. After adjusting other predictive variables, exception for AJCC pT stage, residual variables remained to be independent risk factors ($P < .05$). Patients with nipple inversion breast, mass with calcification, foci located in medial site, 1 to 3, or at least 4 metastatic ALNs were all strongly associated with IMLN metastasis (Table 3).

3.3. Nomograms predicting IMLN status

The results from the multivariate regression analyses were used to construct the nomogram that predicted involvement of internal mammary nodes (Fig. 2). In internal validation, C-index for the nomograms to predict IMLN status was 0.82 (95% CI: 0.81–0.83). The AUC of nomogram (0.82, 95% CI:

Table 4

Univariate and multivariate logistic regressions for prediction of IMLN status (n = 514).

Variables	IMLN status (node-positive vs node-negative)			
	Univariate analysis*		Multivariate analysis†	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age at diagnosis, y	0.98 (0.96, 1.00)	.10		
Marital status				
Married	Reference			
Not married	2.54 (0.85, 7.63)	.10		
Menopausal status				
Premenopausal	Reference			
Postmenopausal	0.78 (0.48, 1.27)	.31		
No. of parity				
0	Reference			
1–2	0.60 (0.28, 1.29)	.19		
>2	0.47 (0.17, 1.27)	.14		
Initial disease symptoms and signs				
Breast lump	Reference		Reference	
Breast pain	1.10 (0.41, 2.97)	.86	0.94 (0.29, 3.08)	.92
Nipple discharge	0.75 (0.09, 6.20)	.79	1.63 (0.17, 15.48)	.67
Nipple inversion	3.51 (1.39,8.88)	**.01**	3.25 (0.99, 10.71)	**.05**
Mammographic calcification				
No	Reference		Reference	
Yes	4.59 (2.52, 8.37)	**<.001**	3.85 (1.82, 8.12)	**<.001**
Laterality				
Left	Reference			
Right	0.68 (0.42, 1.09)	.11		
Tumor site				
Lateral	Reference		Reference	
Central	0.71 (0.32, 1.59)	.41	0.95 (0.38, 2.40)	.92
Medial	1.22 (0.70, 2.15)	.48	2.22 (1.12, 4.39)	**.02**
Lateral and central	1.19 (0.38, 3.68)	.77	1.18 (0.32, 4.36)	.80
Central and medial	1.78 (0.35, 9.11)	.49	0.57 (0.07, 4.76)	.61
Occupying the whole breast	3.12 (1.16, 8.35)	**.02**	1.59 (0.41, 6.20)	.51
Histologic type				
Ductal	Reference			
Lobular	1.45 (0.71, 2.97)	.31		
Other	0.77 (0.31, 1.89)	.57		
No. of positive ALN				
0	Reference		Reference	
1–3	3.03 (1.30, 7.07)	**.01**	3.63 (1.50, 8.75)	**.004**
≥4	12.82 (6.13, 26.80)	**<.001**	15.63 (6.96, 35.06)	**<.001**
AJCC pT stage				
pT1	Reference		Reference	
pT2	1.32 (0.67, 2.60)	.42	0.92 (0.43, 1.95)	.82
pT3	2.38 (1.08, 5.22)	**.03**	0.93 (0.36, 2.42)	.88
pT4	7.25 (1.83, 28.78)	**.01**	2.79 (0.43, 18.09)	.28
ER				
Negative	Reference			
Positive	0.75 (0.47, 1.20)	.23		
PR				
Negative	Reference			
Positive	0.83 (0.52, 1.31)	.42		
HER2				
Negative	Reference		Reference	
Positive	0.11 (0.01, 0.79)	**.03**	0.11 (0.01, 0.82)	**.03**

AJCC = American Joint Committee on Cancer system, ALN = axillary lymph node, CI = confidence interval, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, IMLN = internal mammary lymph node, OR = odds ratio, PR = progesterone receptor.

* Univariate logistic regression analysis.

† Multivariate analysis adjusted by initial disease symptoms and signs, mammography, tumor site, and no. of positive.

Bold emphasized values were considered statistically significant.

0.71–0.81) is higher than that of ALN classification (0.76, 95% CI: 0.78–0.87) (Fig. 3), and the cut-off value of score based on nomogram is 192. These results consistently indicated that the predicting ability and discrimination of the models were generally good.

3.4. Survival analysis

The median DFS for 349 enrolled patients who underwent IMLN dissection was 33 months (range: 3–98) in IMLN-negative group and 58.5 months (range: 5–89) in IMLN-positive group,

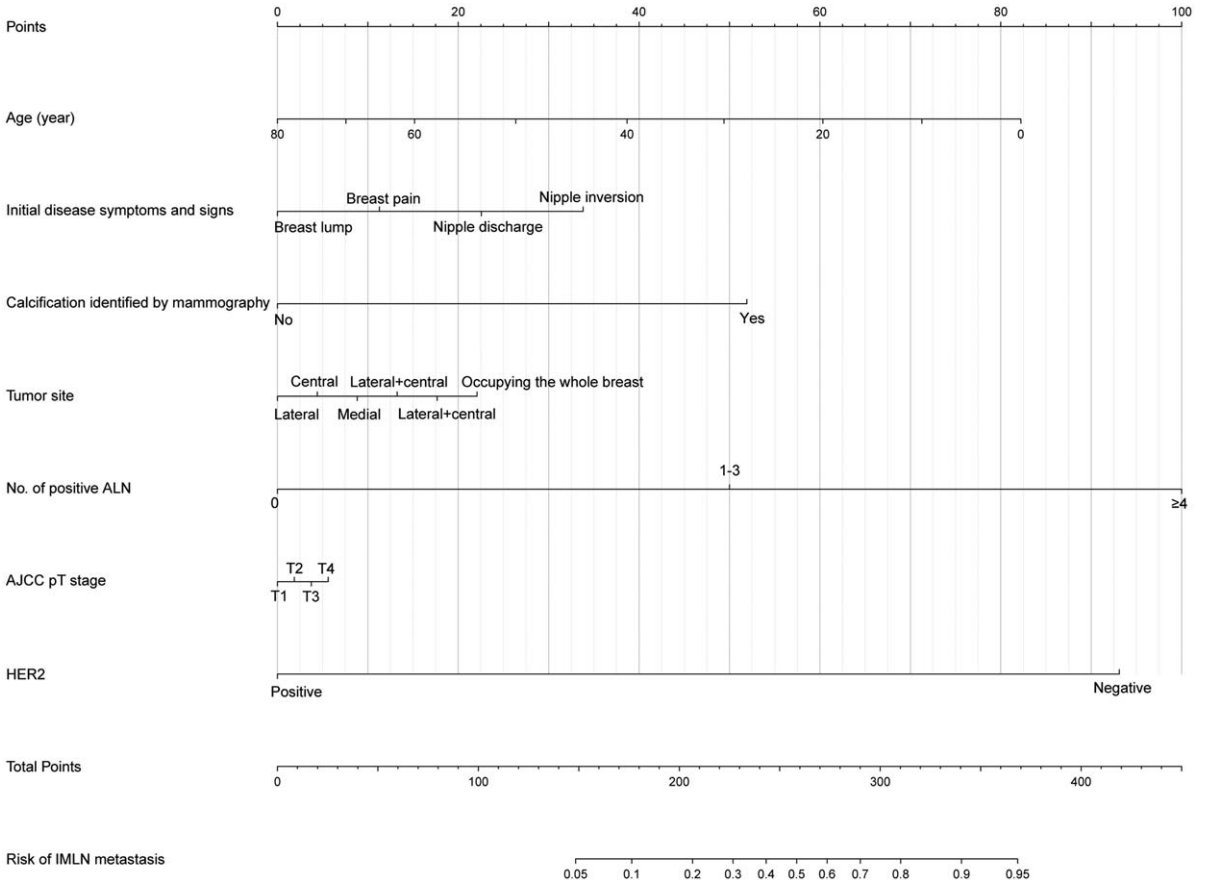

Figure 2. Nomogram predicting the status of IMLN. The total score for each patient is assigned by drawing a vertical line from the appropriate point for each predictor down to the score scale, and summing these scores. To obtain the predicted probability of IMLN metastasis, a vertical line is drawn from the total score scale up to the predicted probability scale in the lower part of the nomogram. AJCC = American Joint Committee on Cancer system, ALN = axillary lymph node, BCS = breast conserving surgery, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, IMLN = internal mammary lymph nodes, PR = progesterone receptor.

respectively. The 3- and 5-DFS rates in the IMLN-negative group are 89.3% and 86.6%, respectively, which were significantly higher than those in the IMLN-positive group (78% and 72%, respectively, log-rank P value = 0.04; HR = 1.87, 95% CI: 1.05–3.34; Fig. 4A). To adjust potential modifier-effects, multivariate cox proportional hazards model including significant variables in univariate analysis was conducted, whereas no significant differences in DFS (HR = 0.99, 95% CI: 0.49–2.00; $P = .97$, Table 5) between IMLN-positive group and IMLN-negative group.

IMLN metastasis risk stratification was carried out by stratifying patients into low-risk and high-risk groups based on cut-off value of scores calculated by our nomogram. As results, among 349 with known IMLN status, 220 patients were placed in the low-risk group with a score of <192, and 129 patients were placed in the high-risk group with a score of >192. Among 1463 patients without receiving IMLN dissection or irradiation, 313 of them were categorized into low-risk group, remaining 1150 patients in low-risk group. The DFS was significantly different between the low- and high-risk groups, regardless of whether comparisons were conducting among patients who underwent IMLN local-therapy or not (all $P < .05$) (Fig. 4B and C).

4. Discussion

This study provides contemporary information from a multi-central population-based data set on the association of tumor anatomical and biological characteristics with IMLN metastasis risk. This updates earlier reports[4,7,9,10,22] and indicates that ALN status, tumor with calcification, initial symptoms and signs, tumor site, and the status of HER2 are independent risk factors for IMLN metastasis, which is comparable with previous studies.[6,7,9,12,14] The developed nomogram is a valid tool that can help doctors in IMLN irradiation strategy making. Meanwhile, equivalent DFS is found between IMLN-positive and IMLN-negative groups after IMLN dissection, which may owe more to the influence of system adjuvant therapies.

In the final nomogram, ALN status remained a key factor to predict the IMLN metastasis. Of those positive IMLN patients, nearly 80% subjects were identified with ALN metastasis. Nevertheless, we found that 304 patients with ALN metastasis occurred IMLN metastasis (25%), which was similar with that reported by Huang et al (27%). Obviously, over-treatment like irradiation for internal mammary district would conducted according to the current NCCN Breast Cancer Clinical Practice Guidelines. A large amount of studies[9,18,19,23,26] also found

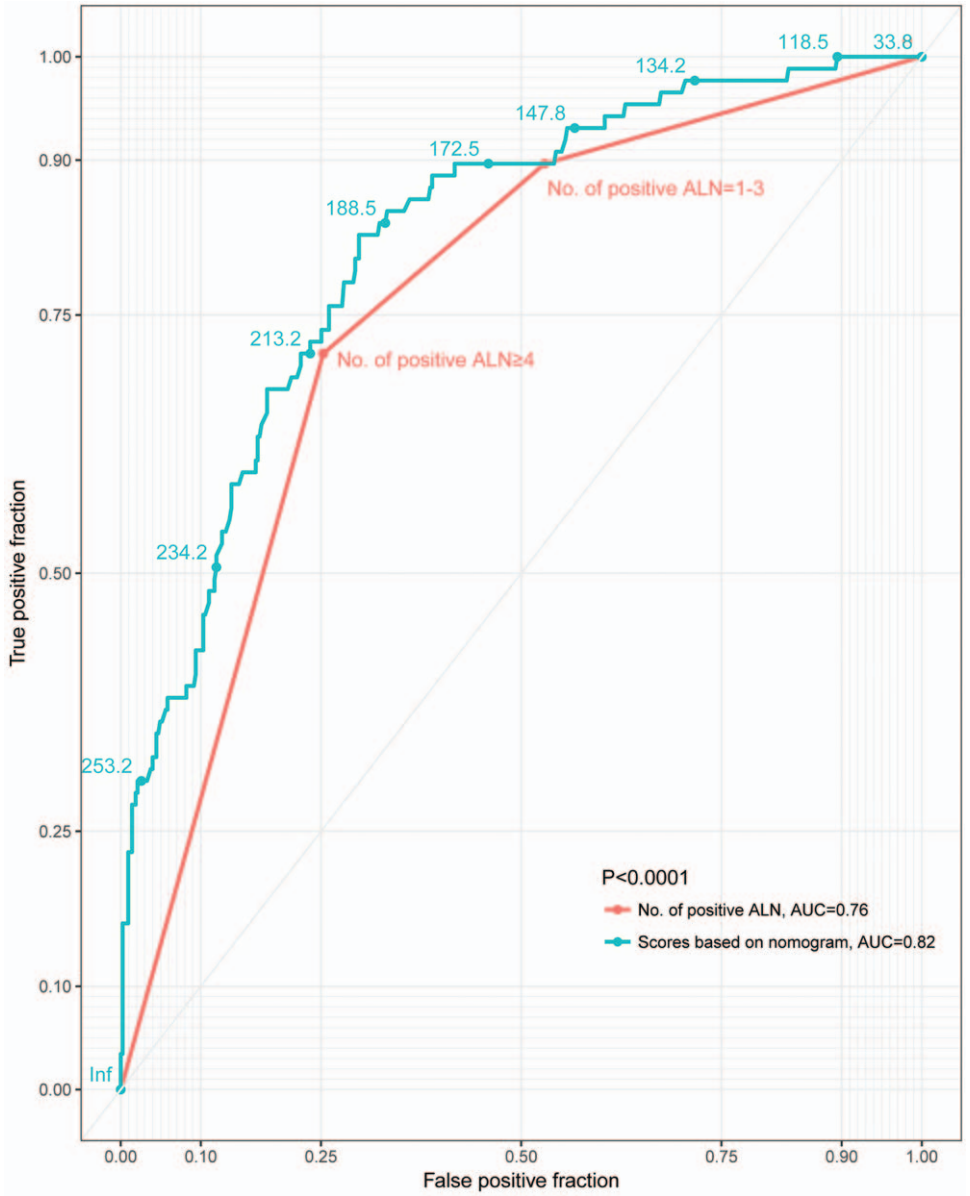

Figure 3. Receiver operating characteristic curves representing the discriminatory ability of the nomograms and ALN categories in predicting axillary nodal status. ALN = axillary lymph node.

medial tumor to be strongly associated with a higher rate of IMLN involvement. We defined the tumor site as more comprehensive model that considered those cross-sectional tumors and multiple lesions; unexpectedly, medial tumor was still an independent risk factor to impact IMLN metastasis. Subsequently, Chen et al[16] raised that ALN status combined with tumor site could be an effective way for IMLN prediction, and up to 65% patients with medial tumors and positive ALN had involvement of IMLN. Nevertheless, the effect of tumor site on prediction of IMLN status in the nomogram was limited. Accordingly, Wang et al[27] hypothesized that IMLN sentinel lymph nodes received the lymphatic drainage from not only the primary tumor area but also the entire breast parenchyma, which was also validated that different tracers injected into the different sites of the intraparenchymal reached the same IMLN sentinel lymph node. Furthermore, a prospective cohort study[1] based on Danish Breast Cancer Cooperative Group documented that IMLN irradiation increased overall survival in patients with early-stage node-positive BC, but no survival difference between irradiation and nonirradiation groups was found in medial/central subgroup regardless of number of positive ALNs. In addition, we seemed to ignore that tumor site included not only horizontal position but also vertical position, after all, the dermal and subdermal lymphatic flow is rarely directed to IMLN.[28–31]

A recent study[12] revealed that HER2-positive BC was associated with ALN metastasis, which was independent with HR status. Controversially, Crabb et al[13] suggested that HER2-positive tumors do not differ in risk of ALN involvement compared with the luminal subtype, and they yielded that basal BC molecular subtype predicted a lower incidence of axillary nodal metastasis. In our study, we are first to report that significantly a lower proportion of HER2-positive tumors were

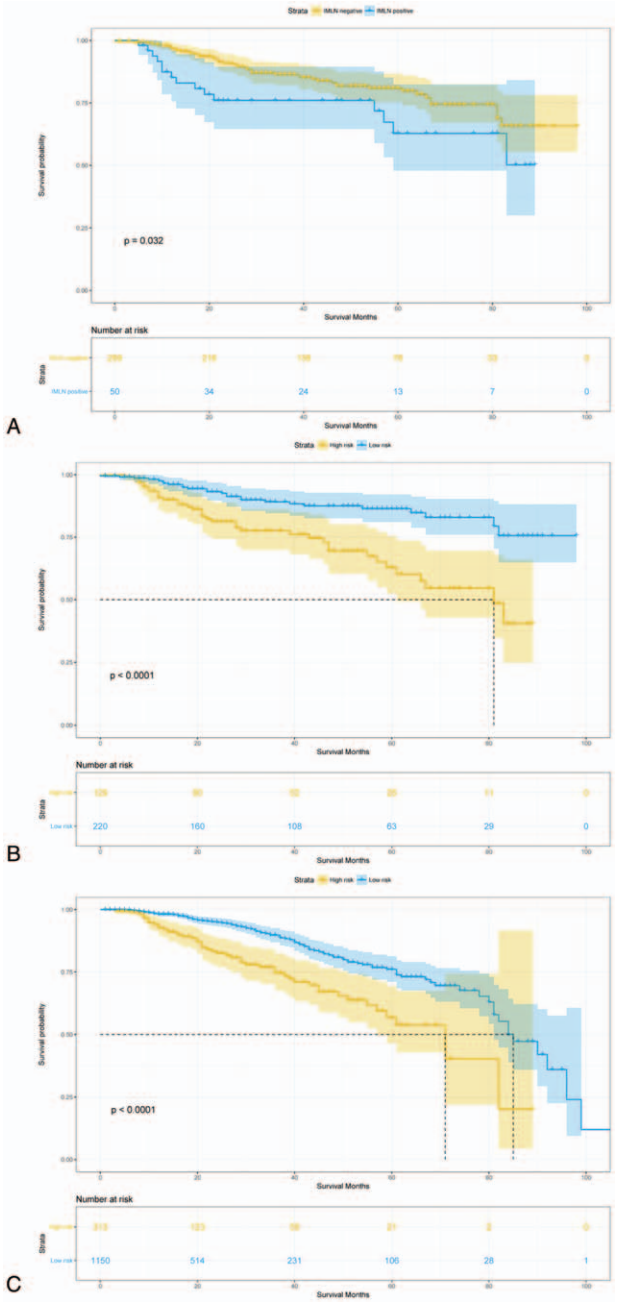

Figure 4. Disease-free survival comparison between (A) IMLN-positive and IMLN-negative patients who underwent IMLN dissection (N = 349). (B)* High and low risk of IMLN metastasis patients who underwent IMLN dissection (N = 349). (C)* High and low risk of IMLN metastasis patients who did not underwent IMLN dissection or IMLN irradiation (N = 1463). IMLN = internal mammary lymph nodes. *High- and low-risk IMLN metastasis group was stratified according to the IMLN nomogram.

found in IMLN-positive group compared with those in IMLN-negative group. We had tried to use molecular subtypes as a covariable (HR-positive/HER2-negative, HR-positive/HER2-positive, HR-negative/HER2-positive, and triple-negative), and no difference in IMLN metastasis risk was observed between groups. Interestingly, Gingras et al[32] failed to demonstrate a DFS benefit of regional nodal irradiation in HER2-positive, node-positive patients treated with adjuvant HER2-targeted therapy, and 131 enrolled patients treated with IMLN irradiation. It was inferred that HER2-positive tumor tends to be nodal negative based on this indirect outcome. More evidence for associations of HR/HER2 status with IMLN metastasis risk should be provided to guide clinicians' decision making like axillary surgery and locoregional radiation.

The associations of tumors with calcifications and nipple inversion with increased IMLN metastasis risk were first revealed in this study. Likewise, our previous study[14] had pointed out that patients with mammographic calcifications were also characterized by large tumor sizes, ALN positivity, and other unfavorable pathological features. Interestingly, although incidence of an underlying breast carcinoma in subjects with nipple inversion varied from 5% to >50%,[33] the relationships between BC with nipple inversion and clinicopathological features or prognostic outcomes had been little addressed.

A prior early BC trialists' collaborative group meta-analysis[34] showed the favorable effect of regional radiotherapy including IMLN irradiation after mastectomy on survival outcomes especially in patients with positive nodes, which was comparable with results reported by recent large-scale studies.[1–3] Inversely, 2 cooperative randomized trials[19,22] revealed no benefit from surgical dissection of the IMLN. Unexpectedly, this study demonstrated that patients with positive IMLN had better median DFS compared with negative nodal patients (58.5 vs 33 months). On the one hand, almost all of patients with positive IMLN were 100% HER2-negative, which contributed to satisfying DFS. On the other hand, almost all of patients (82%) with positive IMLN received radiotherapy that was also a crucial factor for DFS. Naturally, no difference in DFS was further found after adjusting these prognostic variables. These findings simultaneously supported that the effect of surgical dissection of IMLN was limited from another angle, and randomized, well-designed clinical trials are urgently needed to compare the effectiveness of IMLN dissection and IMLN irradiation.

To our knowledge, this is the first clinical study including a large number of surgical series in western China to explore the associations of contemporarily clinicopathological characteristics with IMLN metastasis risk among BC patients, and to develop a nomogram to predict IMLN status individually. Some limitations of this study should be acknowledged, and our results ought to be interpreted with cautions. Some important confounding factors such as status of Ki67 and P53, nuclear grade, and anti-HER2 therapy were missing in most of enrolled patients, which may have influenced our results. IMLN irradiation had favorable effects on survival among early BC patients,[1–3] and question remains whether survival advantages would be changed when IMLN was involved or not. In addition, although the sample size in this study was only next to that in Huang et al's study, limited numbers of patients maybe lead to decline of statistical power especially in survival analyses (n = 349). Lastly, we cannot entirely control the quality of primary data, and pathological diagnosis from multiple hospital will lead to inevitable bias.

5. Conclusion

The independent risk factors of IMLN metastasis are nipple inversion, mammographic calcification, large tumor size, medial tumor site, negative HER2 status, and more positive ALNs. The developed nomogram is a valid predictive tool to

Table 5

Univariate and multivariate cox regressions for breast cancer patients with known IMLN status (n = 349).

	Univariate analysis*		Multivariate analysis†	
Variables	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
IMLN metastasis				
No	Reference		Reference	
Yes	1.87 (1.05, 3.34)	**.04**	0.99 (0.49, 2.00)	.97
Age at diagnosis, y	0.98 (0.96, 1.01)	.20		
Mammographic calcification				
No	Reference		Reference	
Yes	2.65 (1.44, 4.91)	**.002**	1.74 (0.87, 3.52)	.12
Histologic type				
Ductal	Reference	.95		
Lobular	0.90 (0.42, 1.93)	.79		
Other	0.92 (0.39, 2.16)	.85		
No. of positive ALN				
0	Reference	**<.001**	Reference	
1–3	2.40 (1.14, 5.05)	**.02**	2.24 (1.03, 4.83)	**.04**
≥4	5.01 (2.58, 9.73)	**<.001**	5.26 (1.94, 14.26)	**.001**
AJCC pT stage				
pT1	Reference	**<.001**	Reference	
pT2	1.15 (0.57, 2.32)	.71	0.71 (0.34, 1.50)	.37
pT3	3.51 (1.59, 7.76)	**.002**	1.55 (0.64, 3.77)	.34
pT4	47.34 (11.77, 190.35)	**<.001**	11.44 (2.36, 55.58)	**.003**
ER				
Negative	Reference		Reference	
Positive	0.45 (0.27, 0.75)	**.002**	0.72 (0.32, 1.62)	.42
PR				
Negative	Reference		Reference	
Positive	0.43 (0.26, 0.72)	**.001**	0.84 (0.36, 1.93)	.68
HER2				
Negative	Reference		Reference	
Positive	2.82 (1.34, 5.97)	**.01**	3.33 (1.51, 7.38)	**.003**
Surgery type				
BCS	Reference	.10		
MRM	1.07 (0.15, 7.80)	.95		
ERM	2.72 (0.33, 22.62)	.35		
Chemotherapy				
TE	Reference	.79		
CEF	1.24 (0.66, 2.35)	.51		
Others	1.12 (0.44, 2.81)	.82		
Endocrine therapy				
No	Reference		Reference	
Yes	0.40 (0.24, 0.66)	**<.001**	0.70 (0.25, 1.98)	.50
Radiotherapy				
No	Reference		Reference	
Yes	2.56 (1.56, 4.21)	**<.001**	0.84 (0.34, 2.10)	.71

AJCC = American Joint Committee on Cancer system, ALN = axillary lymph node, BCS = breast conserving surgery, CEF = cyclophosphamide, epirubicin, and fluorouracil, CI = confidence interval, ER = estrogen receptor, ERM = extensive radical mastectomy, HER2 = human epidermal growth factor receptor 2, HR = hazard ratio, IMLN = internal mammary lymph node, MRM = modified radical mastectomy, PR = progesterone receptor, TE = docetaxel plus epirubicin.

* Univariate cox regression analysis.

† Multivariate analysis adjusted by IMLN status; mammograph results; no. of positive ALN; AJCC pT stage; status of ER, PR, and HER2; endocrine therapy; and radiotherapy.

Bold emphasized values were considered statistically significant.

facilitate postoperative decision making for additional irradiation therapy of internal mammary district. Among those patients who underwent IMLN dissection, DFS in positive IMLN patients was no different with negative nodal patients.

Author contributions

Hong-Yuan Li and Kang Wang conceived the study idea. Kang Wang and Xiang Zhang performed data mining and statistical analyses, interpreted results of statistical analyses. Kang Wang drafted the initial manuscript. Hong-Yuan Li made critical comment and revision for the initial manuscript, and had primary responsibility for the final content. All authors reviewed and approved the final manuscript.

Conceptualization: Kang Wang, Lei Xing, Fan Li.
Data curation: Xiang Zhang, Ai-Jie Zhang, Hui Li.
Formal analysis: Jin-Ping Liu.
Methodology: Xue-Dong Yin, Yang Shi, Bin-Lin Ma.
Project administration: Ling-Quan Kong, Jun Jiang, Guo-Sheng Ren.
Resources: Ke Zheng, Lei Xing, Fan Li.
Software: Kang Wang.
Supervision: Hong-Yuan Li.

Writing – original draft: Kang Wang.
Writing – review and editing: Hong-Yuan Li.

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