Clinicopathological parameters and biological markers predicting non-sentinel node metastasis in sentinel node-positive breast cancer patients

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Abstract. The value of complete axillary lymph node dissection (ALND) has been questioned in invasive breast cancer (IBC) patients with positive sentinel lymph nodes (SLNs) who have no non-sentinel lymph node (NSLN) metastases. Because biological markers have not been systematically studied in this setting, we sought to identify clinicopathological characteristics and biological markers for predicting NSLN metastases in SLN-positive IBC patients. Two hundred and five IBC patients who had at least one positive SLN and received SLN biopsy and ALND were included in our study. We examined the clinicopathological characteristics of their primary tumors, SLNs and NSLNs. We also evaluated the biological markers of the primary tumors by tissue microarray and immunohistochemistry. Of the 205 patients with SLN metastases, 89 patients (43.4%) had additional metastases in NSLNs. The following factors were found to be associated with NSLN metastases: peritumoral lymphovascular invasion (p=0.01), two or more metastatic SLNs (p<0.01), SLN metastasis >2.0 mm (p<0.01) and extranodal extension (p<0.01). Primary tumors >2.0 cm showed more NSLN metastases, but the association was statistically insignificant (p=0.08). In contrast, NSLN metastases were not associated with histologic grade, histologic type, presence of extensive intraductal component, presence of high grade ductal carcinoma *in situ* and number of harvested SLNs. Biological markers such as E-cadherin, CD44, cyclin D1, p21, ER, PR, c-erbB2, p53, Ki-67, luminal (CK7, CK18, CK19) and basal (CK5, p63) markers were not useful predictors of NSLN metastasis in IBC patients with SLN metastases. Multivariate analysis revealed that SLN metastasis >2.0 mm (p=0.01), two or more metastatic SLNs (p=0.03) and extranodal extension (p<0.01) were independent predictors of NSLN metastasis. For the prediction of NSLN metastasis in IBC patients with SLN metastases, light microscopic evaluation of the number, size and extranodal extension of metastatic SLNs by hematoxylin and eosin staining appeared to be critical. However, the biological markers of primary tumor characterized by immunohistochemical staining, such as luminal and basal markers, hormone receptors, E-cadherin, CD44, cyclin D1, p21, c-erbB2, p53 and Ki-67, did not appear to be helpful predictors.

Introduction

The management of breast cancer has changed dramatically over the past two decades. The trend of breast operations has evolved from radical mastectomy to modified radical mastectomy and further to lumpectomy followed by radiation therapy, which is now commonly referred to as breast conservation surgery. A comparable trend has been seen with axillary surgery, where the operation option has evolved from complete axillary node dissection (ALND) to sentinel lymph node (SLN) biopsy (1).

Recent studies have shown that SLN biopsy is highly accurate in predicting the status of non-sentinel axillary lymph nodes (NSLNs) and have also indicated that axillary metastasis in early T stage breast cancer, if present, may be confined only to the SLNs (2). This would allow patients with early breast cancer to avoid the morbidity associated with a full ALND, especially in light of the increasing number of node negative breast cancer patients diagnosed by mammography (3). Because of its lower morbidity compared with ALND, SLN biopsy has become widely used to evaluate lymph node status in patients with breast carcinoma (4). When the

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Abbreviations: IBC, invasive breast cancer; SLN, sentinel lymph node; NSLN, non-sentinel lymph node; ALND, axillary lymph node dissection; CK, cytokeratin; ER, estrogen receptor; PR, progesterone receptor

Key words: sentinel lymph node biopsy, breast neoplasms, biological markers, tissue array analysis

Table I. List of antibodies for immunohistochemistry.

Antibodies	Clone	Dilution rate	Supplies
E-cadherin	36	1:250	Transduction (San Jose, USA)
CD44	F10-44-2	1:100	Novocastra (Newcastle, UK)
Cyclin D1	P2D11F11	1:50	Novocastra
p21	SX118	1:50	Dako (Glostrup, Denmark)
CK7	OV-TL 12/30	1:100	Dako
CK18	DC10	1:50	Dako
CK19	RCK108	1:50	Dako
CK5	XM26	1:50	Novocastra
p63	4A4	1:50	Dako
ER	6F11	RTU[a]	Ventana (Tucson, AZ, USA)
PR	1A6	RTU[a]	Ventana
c-erbB2	Polyclonal	1:500	Dako
p53	Bp53-11	1:50	Ventana
Ki-67	MIB-1	1:50	Dako

[a]RTU, ready to use.

SLN is positive, a complete ALND is performed. However, it remains uncertain whether a complete ALND results in a survival benefit for patients (5).

Because NSLN involvement is often not detected during an operation, clinicians have hoped to predict the presence of NSLN metastases by the characteristics of the SLNs and the primary tumors, thus avoiding unnecessary ALND. The results of the recently published meta-analysis demonstrated that in the presence of any one of the five characteristics (metastatic size of SLN >2.0 mm, presence of extranodal extension in the SLN, size of primary tumor >2.0 cm, more than one positive SLN, or presence of lymphovascular invasion in the primary tumor), there is a >2-fold increase in the chance of additional metastasis in NSLNs (6). However, whether biological markers could be used to predict NSLN metastasis in breast cancer patients with SLN metastasis has not been studied. In this study, we analyzed the various clinicopathologic features and biological markers in SLN-positive invasive breast carcinomas (IBCs) to determine factors that could help predict the involvement of NSLNs.

Materials and methods

Patient selection. Between January 2002 and December 2004, 1080 breast cancer patients underwent SLN biopsy at the Center for Breast Cancer, National Cancer Center, Gyeonggi-do, South Korea. Patients who received neoadjuvant chemotherapy or previous excisional biopsy, who had SLNs negative for metastasis, who did not undergo a complete ALND, and who had ductal carcinoma *in situ* or microinvasive ductal carcinoma were further excluded from the study. The remaining 205 IBC patients who had at least one positive SLN were included in our study.

Lymphatic mapping and SLN biopsy. The SLN was identified using 1% isosulfan blue dye either alone as described by Giuliano *et al* (7) or in combination with technetium-99m (^{99m}Tc) sulfur colloid as a radioactive tracer as described by Krag *et al* (8). Briefly, after the induction of anesthesia, 3-4 ml of isosulfan blue dye was injected intradermally around the tumor, and the breast was massaged for 5 min. When the radioisotope was used, ^{99m}Tc-antimony trisulfide colloid (Korea Atomic Energy Research Institute) was diluted with saline and injected intradermally 2-4 h before surgery (0.4 mCi) or peritumorally in the early morning on the operation day (4 mCi). Lymphoscintigraphy was performed before surgery, and SLNs were localized with a navigator of the Gamma Guidance System in the operating room. Once localized, the SLNs were removed and sent to the pathology department for examination.

Evaluation of clinicopathologic parameters. All SLNs were measured, sliced into 2.0 mm-thick sections perpendicular to their long axis, and embedded entirely in pre-frozen Tissue-Tek® OCT™ -compound. Frozen sections from two levels were made from these slices and stained with hematoxylin and eosin (H&E). After the frozen section diagnosis, all remaining tissue was fixed in 10% buffered formalin and embedded entirely in paraffin blocks to make permanent sections for histologic examination with routine H&E stain. NSLNs were identified from the fresh fibrofatty ALND specimens. All lymph nodes were bisected along their long axis and embedded in paraffin blocks to make H&E sections. The breast specimens were routinely processed for H&E examination.

By reviewing the medical records and archival pathology slides, we analyzed the following clinicopathologic parameters: age, gender, operation type, neoadjuvant chemotherapy, previous excisional biopsy, tumor location, tumor size, tumor border, histologic type, histologic grade, presence of extensive intraductal component, type and grade of ductal carcinoma *in situ* (DCIS), presence of peritumoral lymphovascular invasion, pTNM category, number of SLNs identified, number of metastatic SLNs, largest size of metastases in the SLNs, extranodal extension in metastatic SLNs, and presence or absence of tumor in the NSLNs. If more than one SLN was

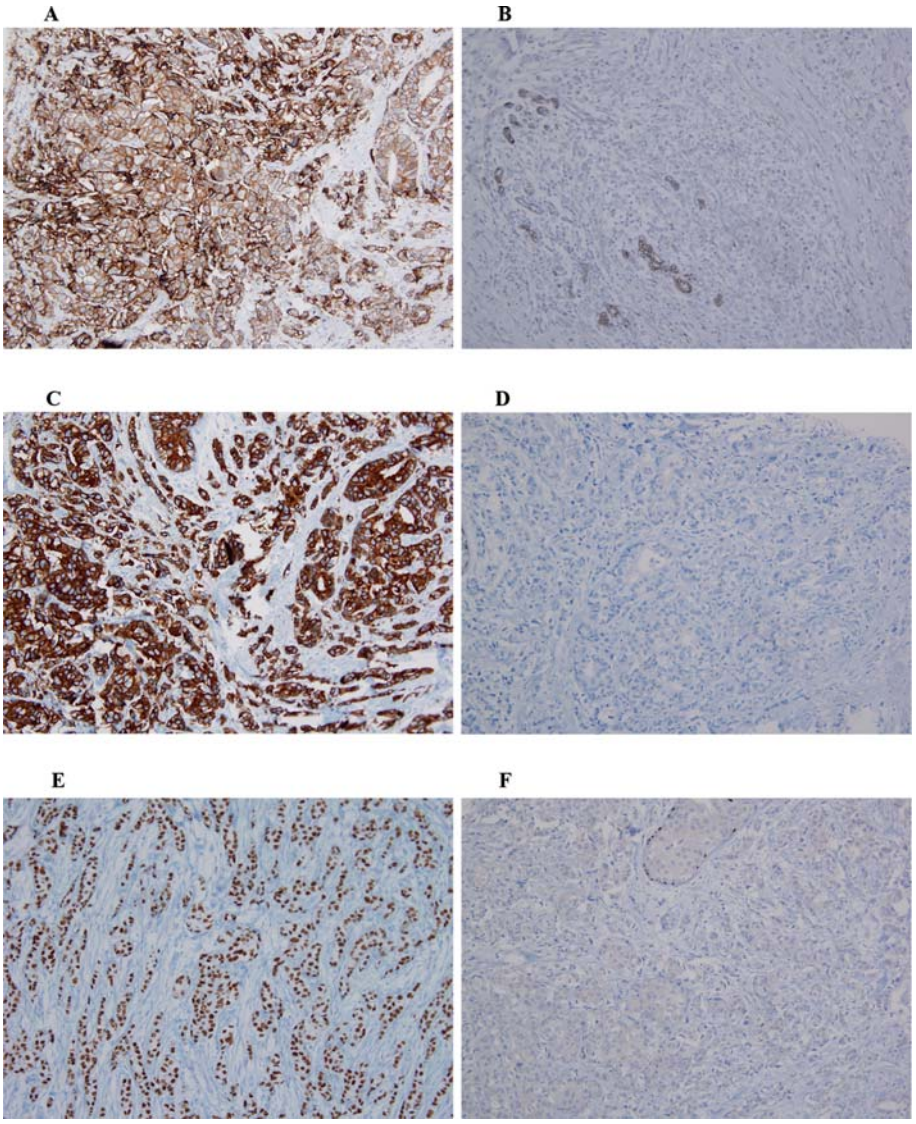

Figure 1. Expression of biological markers in IBCs with SLN metastasis. For membranous staining (E-cadherin, CD44 and c-erbB2), according to HercepTest criteria, scores 0 and 1+ were considered negative and scores 2+ and 3+ were considered positive [(A), positive; (B), negative; x200]. For cytoplasmic staining (CK7, CK18, CK19 and CK5), diffuse cytoplasmic staining with moderate to strong intensity was recorded as positive [(C), positive; (D), negative; x200]. For nuclear staining (ER, PR, p53, Ki-67 and p63), tumors with <10% weakly stained nuclei were considered negative [(E), positive; (F), negative; x200].

submitted, the data for the node with the largest metastatic tumor were entered.

Tissue microarray and immunohistochemical analysis. After review of the archival H&E slides, tissue cores (2.0 mm in diameter) were taken from the representative tumors in paraffin blocks (donor blocks) and arranged in a new recipient paraffin block (tissue-array block) using a Quick-RAY™ (Woo-Ri Medic, Seoul, Korea).

Sections (4-μm-thick) were cut from each tissue array block, deparaffinized and dehydrated. Immunohistochemical staining of these tissue sections was performed with I-View DAB detection kit and Ventana ES autostainer (Ventana, Tucson, USA) with primary antibodies against E-cadherin, CD44, cyclin D1, p21, CK7, CK18, CK19, CK5, p63, ER, PR, c-erbB2, p53 and Ki-67. The details of these primary antibodies are summarized in Table I.

For the evaluation of E-cadherin, CD44 and c-erbB2, membranous staining was scored using the HercepTest (Dako) protocol criteria as follows: no membrane staining or membrane staining in <10% of the tumor cells (score 0); faintly/barely perceptible partial membrane staining in >10% of tumor cells (score 1+); weak to moderate staining of the entire membrane in >10% of tumor cells (score 2+); and strong staining of the entire membrane in >10% of tumor cells (score 3+). Scores 0 and 1+ were considered negative, and scores 2+ and 3+ were considered positive. For CK7, CK18, CK19 and CK5, the diffuse cytoplasmic staining with moderate or strong intensity was recorded as positive. For ER, PR, p53, Ki-67 and p63, the percentage of tumor cells with nuclear staining was scored and a cut-off value of <10% tumor cells with weak nuclear staining intensity was chosen for negative cases (Fig. 1).

Statistical analysis. Correlation between all variables and the presence of metastases in the NSLNs was analyzed using the χ^2 test for categorical values and Student's t-test for continuous variables. Logistic regression was used to assess the effects

Table II. Clinicopathologic features of IBC patients with SLN metastasis.

Characteristics	No. (%) (n=205)
Age (years)	49.5 (29-81)
Gender	
Female	204 (99.5)
Male	1 (0.5)
Operation type	
Lumpectomy	148 (72.2)
Mastectomy	57 (27.8)
Tumor location	
Left	100 (48.8)
Right	105 (51.2)
T stage	
T1	93 (45.3)
T2	108 (52.7)
T3	4 (2.0)
N stage	
N1	155 (75.6)
N2	37 (18.0)
N3	13 (6.4)
Histologic type	
IDC, NOS	193 (94.1)
ILC	3 (1.5)
Others	9 (4.4)
Histologic grade	
1	18 (8.8)
2	108 (52.7)
3	79 (38.5)

of different pathological variables on the presence of NSLN metastases. Two-tailed p-values <0.05 were considered statistically significant. The SPSS 12.0 software package (SPSS Inc., Chicago, IL) was used for all statistical analyses.

Results

Clinical features of included cases. Two hundred and five patients who had SLN metastases and received ALND were included in the study. The mean age of the patients was 49.5 years (range, 29-81 years). All but one patient were women. Lumpectomy was performed in 148 patients and mastectomy in 57 patients. In 100 cases the tumors were located in the left breast; in the other 105 cases they were in the right breast. The mean tumor size was 2.4 cm (range, 0.5-10.0 cm). The clinicopathologic features of the included cases are summarized in Table II.

Characteristics of SLNs and NSLNs. A total of 494 SLNs and 2553 NSLNs were obtained from the 205 patients with SLN metastases. The mean number of identified SLNs was 2.4 (range, 1-8) and the mean number of metastatic SLNs was 1.4 (range, 1-4). The mean size of the largest SLN metastasis was 8.2 mm (range, 0.2-30.0 mm). The mean number of NSLNs harvested with ALND was 12.4 (range, 1-31). Among these 205 patients with SLN metastases, 89 patients (43.4%) had additional metastases in NSLNs. The mean number of NSLNs involved with metastatic tumor was 1.5 (range, 1-22). SLN metastases were micrometastases (≤2.0 mm) in 38 patients (18.5%) and macrometastases (>2.0 mm) in 167 (81.5%) patients. Only 2 (5.3%) of the 38 patients with micrometastatic SLN had further axillary involvement, whereas 87 (52.1%) of the 167 patients with macrometastastic SLNs showed NSLN metastases. This difference was statistically significant (p<0.01).

Correlations between clinicopathologic parameters and NSLN metastases. Tables III and IV summarize the results of the statistical analyses to determine the relationship between clinicopathologic variables and NSLN metastases. In univariate analysis, the likelihood of additional metastases in NSLNs was significantly higher in patients with peritumoral lymphovascular invasion (p=0.01), two or more involved SLNs (p<0.01), SLN metastasis >2.0 mm (p<0.01) and extranodal extension in SLN metastases (p<0.01). There appeared to be an association between primary tumors >2.0 cm and NSLN metastases, but it was not statistically significant (p=0.08). Histologic grade, histologic type, presence of extensive intraductal component, presence of high grade ductal carcinoma *in situ*, and number of assessed SLNs were not associated with NSLN metastases (Table III).

In multivariate analysis, SLN macrometastasis (>2.0 mm) (OR, 6.94; 95% CI, 1.51-31.96; p=0.01), involvement of more than one SLN (OR, 2.29; 95% CI, 1.16-4.52; p=0.03), and presence of extranodal extension (OR, 3.50; 95% CI, 1.79-6.87; p<0.01) were independent predictors of NSLN metastases (Table IV). However, peritumoral lymphovascular invasion in the primary tumor did not retain a significant association with NSLN metastases (p=0.09). Our results indicated that when all three parameters were present (SLN micrometastasis (≤2.0 mm), only one metastatic SLN and absence of extranodal extension), there was no additional NSLN metastasis in 34 (94.4%) of 36 patients.

Correlation between biological markers and NSLN metastases. As shown in Table V, invasion/metastasis-associated markers (E-cadherin, CD44), cell cycle regulators (cyclin D1, p21), luminal (CK7, CK18, CK19) and basal (CK5, p63) markers, hormone receptors (ER, PR), oncogene (c-erbB2), tumor suppressor gene (p53) and proliferation markers (Ki-67) did not correlate with the frequency of NSLN metastases.

Discussion

During the past ten years, SLN biopsy has been used in breast cancer patients for axillary conservation. There are many reasons for not performing a complete ALND. Through aggressive screening programs and patient self-exam, breast cancer is detected at a much earlier stage than before, resulting in fewer axillary metastases (9). Axillary metastases are

Table III. Clinicopathologic characteristics of IBC patients with positive SLN according to NSLN status.

	NSLN (-) (n=116) (%)	NSLN (+) (n=89) (%)	p-value
Age (mean, years)	48.6±9.3	50.5±10.4	0.17
Tumor size (cm)			0.08
≤2.0	58 (50)	35 (39)	
>2.0	58 (50)	54 (61)	
Mean	2.7±0.9	2.5±1.2	0.09
Histologic grade			0.24
1 and 2	77 (66.4)	54 (60.7)	
3	39 (33.6)	35 (39.3)	
Histologic type			0.34
IDC, NOS	108 (93.1)	85 (95.5)	
Others	8 (6.9)	4 (4.5)	
EIC			0.90
No	56 (48.3)	46 (51.7)	
Yes	60 (51.7)	43 (48.3)	
DCIS			0.14
High grade	54 (46.6)	32 (36.0)	
Non-high grade	62 (53.4)	57 (64.0)	
Peritumoral lympho-vascular invasion			0.01
No	70 (60.3)	38 (42.7)	
Yes	46 (39.7)	51 (57.3)	
No. of harvested SLNs			0.28
≤2	71 (61.2)	50 (56.2)	
>2	45 (38.8)	39 (43.8)	
Mean	2.4±1.2	2.4±1.0	0.95
No. of metastatic SLNs			<0.01
1	93 (80.2)	45 (50.6)	
>1	23 (19.8)	44 (49.4)	
Mean	1.2±.5	1.7±0.9	<0.01
Metastatic size of SLN (mm)			<0.01
≤2.0	36 (31.0)	2 (2.2)	
>2.0	80 (69.0)	87 (97.8)	
Mean	5.2±4.7	12.1±7.2	<0.01
Extranodal extension			<0.01
No	80 (69.0)	23 (25.8)	
Yes	36 (31.0)	66 (74.2)	

IDC, invasive ductal carcinoma; NOS, not otherwise specified; EIC, extensive intraductal component; DCIS, ductal carcinoma *in situ*.

Table IV. Significant predictive factors associated with NSLN metastasis on univariate and multivariate analyses.

Factors	Odds ratio (95% CI)	p-value
Univariate analysis		
Peritumoral lymphovascular invasion	2.04 (1.17-3.58)	0.01
No. of metastatic SLNs	3.95 (2.13-7.32)	<0.01
Metastatic size of SLN	19.58 (4.57-83.94)	<0.01
Extranodal extension	6.38 (3.44-11.81)	<0.01
Multivariate analysis		
No. of metastatic SLNs	2.29 (1.16-4.52)	0.03
Metastatic size of SLN	6.94 (1.51-31.96)	0.01
Extranodal extension	3.50 (1.79-6.87)	<0.01

CI, confidence interval.

found in 4-37% of cancers considered stage I and II according to the American Joint Committee on Cancer staging system (10). Thus, 63-96% of patients would undergo ALND with no therapeutic benefit. Another argument against ALND is that currently further chemotherapy or hormonal therapy is recommended to patients with tumor size greater than 1 cm regardless of lymph node status (11). In addition, data from the National Surgical Adjuvant Breast and Bowel Project (B-04) conducted by Fisher *et al* (12) did not show any survival advantage of patients who had immediate ALND compared with patients who waited.

While there is a general consensus regarding the omission of axillary clearance in SLN negative patients, there still remains a substantial proportion of SLN-positive patients who have metastases limited to the SLNs (13). Accordingly, up to 50-60% of the patients with positive SLNs seem to undergo ALND with negative NSLNs. Our current understanding of breast cancer suggests that these patients do not benefit from complete ALND and are exposed to its potential morbidity (14). Several studies (1,2,5,14-41) have attempted to identify factors that may be associated with NSLN metastases and predict the incidence using data from patients with positive SLNs. The studies reported in English literature are summarized in Table VI. Based on these studies (1,2,5,14-41), the metastatic size in the SLNs appears to be the most important predictive factor of positive NSLN in multivariate analysis.

Our study showed that in univariate analysis, NSLN metastases were significantly associated with SLN macrometastases (>2.0 mm) (p<0.01), more than one involved SLNs (p<0.01), extranodal extension in metastatic SLNs (p<0.01), and the presence of peritumoral lymphovascular invasion in the primary tumor (p=0.01). Our multivariate analysis revealed that SLN macrometastasis (>2.0 mm) (p=0.01), involvement of more than one SLN (p=0.03), and the presence of extranodal extension (p<0.01) were independent predictors of NSLN metastases, but peritumoral lymphovascular invasion in the primary tumor did not retain a significant association (p=0.09). Efforts have been made to identify whether the patients' SLN status could accurately predict the need for a complete ALND versus no further surgery (1,2,5,14-41). The results of these

Table V. Biological markers of IBCs with SLN metastasis according to NSLN status.

	NSLN (-) (n=116) (%)	NSLN (+) (n=89) (%)	p-value
E-cadherin			0.95
Negative	9 (7.8)	6 (6.7)	
Positive	101 (87.0)	79 (88.8)	
NA	6 (5.2)	4 (4.5)	
CD44			0.29
Negative	70 (60.3)	50 (56.2)	
Positive	41 (35.3)	30 (33.7)	
NA	5 (4.3)	9 (10.1)	
Cyclin D1			0.18
Negative	59 (50.9)	52 (58.4)	
Positive	40 (34.9)	23 (25.8)	
NA	17 (14.6)	14 (15.7)	
p21			0.78
Negative	99 (85.3)	73 (82.0)	
Positive	12 (10.4)	10 (11.2)	
NA	5 (4.3)	6 (6.8)	
CK7			0.77
Negative	10 (8.6)	10 (11.2)	
Positive	102 (87.9)	75 (84.3)	
NA	4 (3.5)	4 (4.5)	
CK18			0.36
Negative	17 (14.7)	7 (7.9)	
Positive	94 (81.0)	77 (86.5)	
NA	5 (4.3)	5 (5.6)	
CK19			0.52
Negative	6 (5.2)	3 (3.4)	
Positive	104 (89.7)	78 (87.6)	
NA	6 (5.1)	8 (9.0)	
CK5			0.21
Negative	101 (87.0)	75 (84.3)	
Positive	9 (7.7)	4 (4.5)	
NA	6 (5.3)	10 (11.2)	
p63			0.29
Negative	100 (86.2)	79 (88.8)	
Positive	8 (6.9)	2 (2.2)	
NA	8 (6.9)	8 (9.0)	
Cell type			0.09
Basal	15 (12.9)	5 (5.6)	
Luminal	91 (78.5)	77 (86.5)	
NA	10 (8.6)	7 (7.9)	
ER			0.44
Negative	24 (20.7)	21 (23.6)	
Positive	92 (79.3)	68 (76.4)	
PR			0.13
Negative	42 (36.2)	40 (44.9)	
Positive	74 (63.8)	49 (55.1)	
c-erbB2			0.32
Negative	98 (84.5)	68 (76.4)	
Positive	18 (15.5)	21 (23.6)	
p53			0.78
Negative	93 (80.2)	68 (76.4)	
Positive	23 (19.2)	21 (23.6)	
Ki-67			0.33
<10%	66 (56.9)	47 (52.8)	
≥10%	50 (43.1)	42 (47.2)	

NA, not applicable.

previous studies are consistent with our results in determining that macrometastatic SLNs (>2.0 mm), extranodal extension, peritumoral lymphovascular invasion, and two or more metastatic SLNs were correlated with NSLN metastasis, except that we did not find a statistically significant association between primary tumor size and NSLN metastasis.

Our study indicated that there was no additional NSLN metastasis in 34 (94.4%) of 36 patients with all three parameters, i.e., SLN micrometastasis (≤2.0 mm), only one metastatic SLN, and the absence of extranodal extension. In this case, the possibility of additional NSLN metastases was only 5.6%, which was similar to that of patients with only SLN micrometastasis. Therefore, we considered SLN micrometastasis as the most important negative predictive factor of NSLN involvement in IBC patients with SLN metastasis. Since Van Zee and colleagues reported the use of a nomogram to calculate the likelihood of metastases in NSLNs (42), several other groups have proposed new predictive models (43-46) and compared their predictive probabilities (47,48). These studies have suggested that the nomograms might be good discriminators of NSLN metastases in SLN-positive IBC patients, but because of their limitations and imperfections they should be used with caution in clinical applications (42-48).

Recent studies using gene expression profiles have classified breast carcinomas into luminal, normal breast-like, HER2 overexpressing, and basal-like groups, with the latter two associated with poor outcome (49). Tissue microarray-based study demonstrated that cyclin E expression was increased in cyclin D1 expressing tumors and was associated with poor survival in node-negative breast cancer patients (50). Abraham *et al* (51) suggested that the prevalence of $CD44^+/CD24^-$ cells in breast cancer may not be a prognostic factor but may be associated with distant metastasis. We attempted to demonstrate whether expression of different protein markers could predict NSLN metastasis in IBC patients with SLN metastasis using tissue microarray sections that were immunostained for these biological markers. Our study revealed that invasion/metastasis-associated markers

Table VI. Summary of reported studies on predictive factors for NSLN metastases in English literature.

Study	Year published	No. of patients	IHC on NSLN	Tumor size	Metastasis size	Extranodal extension	LVI	Total no. of SLNs	No. of positive SLNs	Histologic grade	Histologic type	Age	ER	PR	HER2/neu	Tumor palpable
Reynolds *et al* (2)	1999	60	Yes	M	M		N			N	N	N	U	N		
Chu *et al* (5)	1999	157	No	M	M		N		U	N	N	N	N	N	N	
Turner *et al* (15)	2000	194	Yes	U	U	U	U		U	U		N	N	N	N	N
Abdessalam*et al* (1)	2001	100	No	N	U	U	U		N	N	N	N	N	N	N	N
Weiser *et al* (16)	2001	206	No	M	M		U			N	N	N				
Rahusen *et al* (17)	2001	93	Yes	N	U		N		U	N		N	N			N
Wong *et al* (18)	2001	389	No	M				N	M	N		N				
Cserni *et al* (19)	2001	69	No	M	M	M				N						
Kamath *et al* (20)	2001	101	No	U	U											
Canavese *et al* (21)	2001	72	No	N	N											
Sachdev *et al* (22)	2002	55	No	M	M		M				N	N				
Mignotte *et al* (23)	2002	120	No	N	N											
Hwang *et al* (24)	2003	131	No	M	M	N	M	M	N	N	N	N	N	N		
Iterson *et al* (25)	2003	135	No	M	M				M	N						
Nos *et al* (26)	2003	263	No	M	M	U	U		U	N		N	N	N		
Travagli *et al* (27)	2003	50	No	N	N		M		N	N		N	N			
Stitzenberg *et al* (28)	2003	70	No	U	U	M										
Cserni *et al* (14)	2004	150	No	M	M	M	N	M	M	N		N				
Joseph *et al* (29)	2004	70	No	U	U	M	N	N					N	N		
Farshid *et al* (30)	2004	82	No	N	U	U	N	M	N	N	N	N	N	N		
Goyal *et al* (31)	2004	201	No	N	M	U	N	N	U	N	N	N				
Fleming *et al* (32)	2004	54	No	N	U	U	N			N	N	N				
Viale *et al* (33)	2005	1228	No	U	M		M		M	U	N	N	N	N	N	
Hung *et al* (34)	2005	55	No	U	U	U	N		U	N			N			
Callejo *et al* (35)	2005	23	Unknown		U	U	U									
Yu *et al* (36)	2005	286	No	M	M		U		M	U	N	N	U	U	U	
Ozmen *et al* (37)	2006	148	No	M	M	M	N		N	N	N	N	N	N		
Bolster *et al* (38)	2007	541	No	M	M		U			N		N	N	N		
Kapur *et al* (39)	2007	58	No	U	U	U	N		N	N	N		N	N	N	
Jinno *et al* (40)	2008	131	No	N	U		M	N	M	N		N	N	N		
Fougo *et al* (41)	2009	143	Unknown	M			M									
Present study	2010	205	No	N	M	M	U	N	M	N	N	N	N	N	N	

IHC, immunohistochemistry; NSLN, non-sentinel lymph nodes; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor; SLNs, sentinel lymph nodes; U, factors found to be statistically significant by univariate analysis; M, factors found to be statistically significant by multivariate analysis; N, factors not found to be statistically significant.

(E-cadherin, CD44), cell cycle regulators (cyclin D1, p21), luminal (CK7, CK18, CK19) and basal (CK5, p63) markers, hormone receptors (ER, PR), oncogene (c-erbB2), tumor suppressor gene (p53), and proliferation markers (Ki-67) did not predict the presence of NSLN metastasis.

For the prediction of NSLN metastasis in IBC patients with SLN metastasis, it is more important to evaluate the number, size, and extranodal extension of metastatic SLNs by H&E staining than to examine the primary tumors by immunohistochemical staining for luminal and basal markers, hormone receptors, E-cadherin, CD44, cell cycle regulators, c-erbB2 and p53, and Ki-67. According to the results of ACOSOG trials Z0010 and Z0011 (52), until the nomograms for predicting NSLN metastases are more accurately determined, delayed complete ALND remains an alternative management option for patients with SLN metastases.

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