

Histologic evaluation of sentinel and non-sentinel axillary lymph nodes in breast cancer by multilevel sectioning and predictors of non-sentinel metastasis

Piyarat Jeeravongpanich¹, Tuenjai Chuangsuwanich², Chulaluk Komoltri³, Adune Ratanawichitrasin⁴

¹Pathology Unit, Songkhla Hospital, Songkhla 90000, Thailand; ²Department of Pathology, ³Office for Research and Development, ⁴Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

Correspondence to: Tuenjai Chuangsuwanich, M.D. Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Email: tuenjai_ch@yahoo.com.

Abstract: Sentinel lymph node (SLN) provides accurate nodal staging for breast cancer. This technique has been introduced in Siriraj Hospital since 1998. The goal of this study is to assess its accuracy in predicting the state of the axilla, and compare the results of standard examination and multilevel sectioning. A retrospective analysis of 195 breast cancer patients who underwent both SLN biopsy (using dye alone as the lymphatic mapping) and axillary node dissection during 1998-2002 were analyzed. All slides including SLNs and the non-SLNs (NSLNs) were reviewed and multilevel study was performed on all SLNs and NSLNs [four levels of hematoxylin-eosin (HE) at 200 µm interval and keratin stains on the first and fourth levels]. Of 195 patients, 30% of cases were SLN-positive (32 NSLN-positive and 27 NSLN-negative). Additional study could detect positive axillary nodes 10.8% (4 SLN-positive and 5 NSLN-positive) more than standard HE stain. The false negative rate increased from 20.3% to 24.1%. The concordance between SLN and NSLN statuses was 89.7%. The sensitivity was 75.9%. By multivariate analysis, the significant predictors for axillary node metastasis were tumor size of more than 2.2 cm, histologic type of invasive ductal carcinoma (IDC), not otherwise specified (NOS) and lymphovascular invasion (LVI). By univariable analysis, the significant predictors of NSLN metastasis after positive-SLN were outer location of the tumor, LVI and perinodal extension. In conclusion, use of multilevel and immunohistochemistry increased detection of positive-SLNs. Caution should be kept in accepting SLN biopsy using peritumoral dye technique alone as the procedure for staging due to a high false-negative rate. The concordance rate of 89.7% confirmed the reliability of SLN. Outer location of tumor, LVI and perinodal extension is significant predictors of positive-NSLN after positive-SLN.

Keywords: Breast cancer; immunohistochemistry study; multilevel study; non-sentinel lymph node (NSLN); sentinel lymph node (SLN)



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Introduction

Axillary node status is one of the most important prognostic indicators in breast cancer and is of particular value in considering the choice of adjuvant therapy. Axillary node dissection has long been the standard procedure for determining the nodal stage in breast cancer but its complications can be disabling. During the past three decades, the surgical management of breast cancer has

changed significantly. Several large multicenter trials have established breast conservation procedure with the advent of sentinel lymph node (SLN) biopsy (1-5). The SLN biopsy is rapidly gaining popularity as a staging procedure for breast cancer. It enables selective targeting of the first tumor draining lymph nodes, where the initial metastasis will form. Conceptually, a negative SLN predicts the absence of tumor metastasis in the other regional lymph

nodes with a high degree of accuracy. Multiple studies showed that SLN examination had a sensitivity of 83.4-100.0% for the detection of axillary lymph node disease and the accuracy in determining axillary lymph node status in comparison with standard axillary lymph node dissection was 92-96.4% (3,5-9).

At present, the sentinel node biopsy is widely used as a definite staging procedure of breast cancer. It has been shown through numerous studies that routine histological examination of dissected nodes may be inadequate depending on the thoroughness of examination. In a number of large studies using different histopathologic techniques, the SLN false-negative rate has varied between 0-11% (2,3,6,8,9). Many investigators have reported finding micrometastases that were not detected by routine sectioning of lymph nodes, but were identified by multiple sectioning and additional immunohistochemistry staining (1,4,6,10-16). However, the optimal pathologic examination of the SLN has not yet been determined. The method used is individualized in each hospital. The study of SLN using isosulfan blue as the dye in Siriraj Hospital was first established by Ratanawichitrasin *et al.* in 1998 (8). The SLN and NSLN simultaneous removals were designed to assess the accuracy in prediction of the state of the axilla. The intensive histologic study of these samples was planned to assess the validity of SLN biopsy, compare the results using standard examination and multilevel sectioning, identify the accurate metastatic size, and determine the predictive factors of metastasis in NSLN when the SLN is positive.

Materials and methods

This study has been approved by the institutional ethic committee (IEC), approval number Si 197/2005. It was a retrospective study using subjects from the study of "Lymphatic mapping and SLN biopsy in breast cancer patients" by Ratanawichitrasin *et al.*, Department of Surgery, Siriraj Hospital, in 1998-2002. There were 215 patients in an early stage (T0-2, tumor size mostly <3 cm, all cell types and clinical N0) and without pre-operative adjuvant therapy who underwent a primary tumor operation, SLN and NSLN dissections. The SLN mappings were performed via peritumoral injection with isosulfan blue dye. Twenty patients were excluded from this study due to failure to identify the SLNs (15 patients) and partial loss of data (5 patients), resulting in a total of 195 patients. All cases were reviewed for clinical information, e.g., age, size of tumor, presenting symptoms and location. Slides were

reviewed by two pathologists for histological data including histologic type of primary tumor, grade (Nottingham combined histologic grade using Elston-Ellis modification of Scarff-Bloom Richardson grading system), lymphovascular invasion (LVI), estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu status, number of lymph nodes with metastasis and size of metastatic deposits and perinodal invasion (by standard histological examination). Subjects were classified into four groups, i.e., SLN-negative, SLN-positive, NSLN-negative and NSLN-positive. All tissue blocks with SLN or NSLN-negative groups were sectioned for 3-micron-thick sections at a 200 micron interval for four levels (three intervals; using the first cut as the first level), each level with one HE cut and a subsequent immunostaining with cytokeratin antibodies [cytokeratin clone AE1/AE3 (Diagnostic BioSystems, USA) as a primary antibody; Horse Raddish Peroxidase (HRP) method; Tris-HCL pH7.6 as an antibody diluent; EnVision complex, DAKO as a secondary antibody and Sigma (D5637) as a DAB] at levels 1 and 4. Included in this study were ten cases of negative-SLN/positive-NSLN which were previously studied (unpublished) by one of the authors using multilevel sectioning (every 250 micron for five intervals), each with one HE and one subsequent section for AE1/AE3 stain. The sizes of the maximal metastatic deposits were classified into: tumor cluster (≤ 0.2 mm), micrometastasis ($> 0.2-2$ mm) and macrometastasis (> 2 mm), according to the pathological staging of involved axillary lymph node of the AJCC staging, 6th edition (17).

Statistical analysis

An independent sample t-test was used to test the difference in quantitative variables between subjects with and without metastasis. Fisher's exact test was employed to test the association between metastasis and unordered or binary qualitative variable, e.g., tumor size (≤ 2 , > 2 cm), location (upper outer, others). Linear-by-linear association test was used to assess the relationship between metastasis and ordered qualitative variable, e.g., six tumor size groups, location (others, inner, outer), three histologic grades. Independent variables with univariable P-value of less than 0.2 were included in a multiple logistic regression model to assess the effect of each variable on axillary lymph node metastasis after adjusting for effect of the others. For moderate sample size, 95% CI of adjusted OR was computed by profile likelihood method instead of maximum likelihood method. With small sample size,

Table 1 Comparison of SLN status and final axillary lymph node (LN) status using standard HE stain and multilevel sectioning

SLN	Final axillary LN status		
	Positive	Negative	Total
Standard HE stain			
Positive	59	0	59
Negative	15	121	136
Total	74	121	195
Multilevel sectioning			
Positive	63	0	63
Negative	20	112	132
Total	83	112	195

SLN, sentinel lymph node.

Table 2 Association between metastatic size in SLN-positive and NSLN-positive

True SLN positive	True NSLN positive			Total
	Tumor cluster	Micro-metastasis	Macro-metastasis	
Tumor clusters (n=5)	0	0	1*	1
Micrometastasis (n=15)	0	1	8	9
Macrometastasis (n=43)	0	7	21	28
Total (n=63)	0	8	30	38

*, this 52-year-old woman had a moderately differentiated invasive ductal carcinoma of 1.5 cm size. Multilevel sections and immunohistochemistry revealed tumor cluster on SLN (0.15 mm) and macrometastasis on NSLN (6 mm) using standard HE stain. The total number of SLN and NSLN were 3 and 16 nodes, respectively. SLN, sentinel lymph node; NSLN, non-SLN.

P-value, adjusted OR, 95% CI of adjusted OR from logistic regression were obtained from exact method. Statistical data analyses were performed using SAS 8.1 and StatXact 6. An exact 2-sided P-value of less than 0.05 was considered statistical significance.

Results

Of 195 patients studied, the mean age and tumor size was

48.9 years (range: 28-78 years) and 2.31 cm (SD =1.12, range =0.08-6.5), respectively. A total of 185 patients (95%) had invasive carcinomas with 166 patients (85%) having invasive ductal lesions, 46% of which were moderately differentiated. The other ten patients had carcinoma *in situ*. The number of the SLNs and NSLNs in each patient ranged from 1-8 nodes (mean =3, median =2) and 1-53 nodes (mean =21, median =20), respectively.

Standard HE and multilevel study

The standard HE stain on the initial sections identified 59 (30.2%) of 195 cases with SLN metastases. Among 136 SLN-negative cases, 15 cases showed NSLN metastasis (Table 1). Multilevel sections and immunohistochemistry on negative SLNs revealed 4 additional cases of SLN-positive (6.3% of all 63 patients with true positive SLN). Five NSLN-negative cases turned to be positive; 2 had tumor clusters and 3 had micrometastases (Table 1). The sensitivity of the SLN technique using standard method was 79.7% (95% CI: 68.8%, 88.2%) compared to 75.9% (95% CI: 65.3%, 84.6%) using multilevel HE study. The specificity of the SLN technique for both standard and multilevel method was 100% (95% CI: 97%, 100%). The positive predictive value was 100% (63/63). The negative predictive value of standard method and multilevel HE study were 88.9% and 84.8%, respectively. Concordance between the sentinel and the final pathologic lymph node status was 92.3% using standard method and 89.7% using multilevel HE study. A false-negative rate increased from 20.3% (15/74) for standard method to 24.1% (20/83) for multilevel HE study. Multilevel sectioning could detect positive axillary lymph node 10.8% (9/83) more than standard HE stain. Of all 63 SLN-positive cases, 5 (7.9%) were tumor cluster, 15 (23.8%) micrometastasis and the remaining 43 (68.2%) macrometastasis. Association between the size of the positive-SLN and positive-NSLN was shown in Table 2. The mean primary tumor size in SLN cases with tumor clusters, micrometastasis and macrometastasis were 1.7, 2.2 and 2.9 cm, respectively.

Factors determining axillary lymph node metastasis

Univariable analysis showed that factors significantly associated with axillary lymph node metastasis were tumor size, histologic type [invasive ductal carcinoma, not otherwise specified (IDC, NOS) and non IDC, NOS] and lymphovascular space invasion (Table 3). Patients with

Table 3 Clinical and pathological variables for axillary lymph node (SLN and NSLN) metastasis

	Mean \pm SD or number [%]		Crude OR (95% CI)	P-value
	No metastasis (n=112)	With metastasis (n=83)		
Age (yrs)	48.2 \pm 9.8	49.9 \pm 10.1	–	0.202
Tumor size (cm)	2.1 \pm 1.0	2.6 \pm 1.2	–	0.001
\leq 0.1	4 [100]	0	–	0.002
0.2-0.5	2 [100]	0		
0.6-1.0	10 [71]	4 [29]		
1.1-2.0	50 [62]	31 [38]		
2.1-5.0	44 [51]	43 [49]		
>5.0	1 [25]	3 [75]		
\leq 2.0	66 [65]	35 [35]	1	
>2.0	45 [49]	46 [51]	1.93 (1.04, 3.59)	0.037
\leq 2.2	71 [66]	37 [34]	1	
>2.2	40 [48]	44 [52]	2.11 (1.13, 3.95)	0.018
Location: outer/inner				
Others	13 [68]	6 [32]	1	0.065
Inner	43 [63]	25 [37]	1.26 (0.38, 4.56)	
Outer	54 [51]	52 [49]	2.09 (0.67, 7.18)	
Location: upper outer/others				
Others	67 [59]	46 [41]	1	
Upper outer	43 [54]	37 [46]	1.25 (0.67, 2.33)	0.464
Histologic type				
Mucinous CA, papillary CA, others	10 [91]	1 [9]	–	0.004
DCIS, DCIS with microinvasion, LCIS	9 [90]	1 [10]		
IDC, IDC with Paget's, IDC with predominant DCIS, ILC, mixed	93 [53]	81 [47]		
Histologic type				
Non-IDC	24 [83]	5 [17]	1	
IDC	88 [53]	78 [47]	4.26 (1.49, 14.87)	0.004
Grade				
Well differentiated	17 [77]	5 [23]	1	0.099
Moderately differentiated	49 [55]	40 [45]	2.78 (0.87, 10.39)	
Poorly differentiated	41 [53]	37 [47]	3.07 (0.95, 11.59)	
Lymphovascular invasion				
No	83 [72]	32 [28]	1	
Yes	22 [31]	50 [69]	5.90 (2.95, 11.87)	<0.001
Estrogen receptor*				
Positive	15 [60]	10 [40]	1	
Negative	9 [53]	8 [47]	1.33 (0.32, 5.49)	0.890
Progesterone receptor*				
Positive	13 [68]	6 [32]	1	
Negative	11 [48]	12 [52]	2.36 (0.57, 10.29)	0.304
HER-2/neu*				
Positive	1 [33]	2 [67]	–	–
Negative	2 [67]	1 [33]		

*, HER-2/neu and hormonal receptor immunoreactivities were performed in only a small number of patients. SLN, sentinel lymph node; NSLN, non-SLN; DCIS, ductal carcinoma *in situ*; LCIS, lobular carcinoma *in situ*; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

Table 4 Multiple logistic regression analysis of factors associated with axillary lymph node metastasis

	P-value	Adjusted OR	Profile likelihood 95% CI of OR
Logistic model with 5 independent variables			
Tumor size (cm)			
≤2.2		1	
>2.2	0.051	1.99	1.00, 4.00
Location			
Others		1	
Outer	0.325	1.83	0.56, 6.45
Inner	0.544	1.47	0.44, 5.32
Histologic group			
Non-IDC		1	
IDC, NOS	0.044	3.29	1.11, 11.59
Grading			
Well differentiated		1	
Moderately differentiated	0.607	1.37	0.43, 4.87
Poorly differentiated	0.664	1.31	0.41, 4.67
Lymphovascular invasion			
No		1	
Yes	<0.001	4.70	2.37, 9.57
Logistic model with 2 independent variables			
Tumor size (cm)			
≤2.2		1	
>2.2	0.004	2.46	1.34, 4.57
Histologic group			
Non-IDC		1	
IDC, NOS	0.003	4.85	1.84, 15.38

OR, odds ratio; IDC, invasive ductal carcinoma; NOS, not otherwise specified.

tumor size greater than 2.2 cm had 2.11 higher risk of metastasis than those with tumor size less than or equal to 2.2 cm. Histologic type of IDC, NOS and LVI were associated with a higher risk of metastasis with crude odds ratios of 4.26 and 5.90, respectively. Location (inner/outer/others) and primary tumor grade (well/moderately/poorly differentiated) seemed to be related to metastasis but they were not statistically significant ($P=0.065$, 0.099 , respectively). There was no statistically significant difference in age, ER and PR between patients with and without metastasis. A multiple logistic regression model was fitted using five independent variables with P-value of less than 0.2 from univariable analysis (*Table 4*). Analysis revealed three statistically significant factors for metastasis: tumor size of >2.2 cm (OR =1.99, $P=0.051$), histologic type of IDC, NOS (OR =3.29, $P=0.044$) and LVI (OR =4.70,

$P<0.001$). *Table 4* revealed another multiple logistic model with only two independent variables of tumor size of >2.2 cm. and histologic type of IDC, NOS due to their availability in cytological or biopsy reports. Analysis showed adjusted OR of 2.46 ($P=0.004$) and 4.85 ($P=0.003$) for tumor size >2.2 cm and histologic type of IDC, NOS respectively.

Univariable analysis of predictive factors for NSLN metastasis (after SLN metastasis) based on standard HE staining group (*Table 5*) demonstrated that outer (*vs.* inner) location and perinodal invasion of SLN seemed to be related to metastasis with OR of 3.29 ($P=0.072$) and 3.24 ($P=0.062$), respectively. Due to small sample size ($n=32$ and 27 for with and without metastasis, respectively), a multiple logistic regression model was fitted using exact method with only three independent variables, i.e., location (inner/outer), grade (moderate/poor) and perinodal invasion of SLN

Table 5 Variables for NSLN metastasis after positive SLN using standard method: univariable analysis

	Mean \pm SD or number [%]		Crude OR (95% CI)	P-value
	No metastasis (n=27)	With metastasis (n=32)		
Age (yrs)	48.3 \pm 10.9	50.9 \pm 10.1	–	0.357
Tumor size (cm)	2.4 \pm 1.0	3.0 \pm 1.3	–	0.076
\leq 2.0	12 [52]	11 [48]	1	
>2.0	15 [43]	20 [57]	1.46 (0.44, 4.77)	0.669
\leq 2.2	13 [54]	11 [46]	1	
>2.2	14 [41]	20 [59]	1.69 (0.52, 5.52)	0.478
Location: outer/inner				
Others	1 [33]	2 [67]	–	–
Inner	13 [65]	7 [35]	1	
Outer	13 [36]	23 [64]	3.29 (0.92, 12.21)	0.072
Location: upper outer/others				
Others	16 [50]	16 [50]	1	
Upper outer	11 [41]	16 [59]	1.46 (0.46, 4.65)	0.654
Histologic type				
Mucinous CA, papillary CA, others	0	1 [100]	–	–
IDC, IDC with Paget's, IDC with predominant DCIS, ILC, mixed	27 [47]	31 [53]		
Histologic type				
Non-IDC	0	4 [100]	–	–
IDC	27 [49]	28 [51]		
Grade				
Well differentiated	2 [67]	1 [33]	–	–
Moderately differentiated	17 [57]	13 [43]	1	
Poorly differentiated	8 [32]	17 [68.0]	2.78 (0.81, 9.82)	0.118
Lymphovascular invasion				
No	11 [58]	8 [42]	1	
Yes	15 [39]	24 [62]	2.20 (0.63, 7.84)	0.265
Perinodal invasion of SLN				
No	20 [57]	15 [43]	1	
Yes	7 [29]	17 [71]	3.24 (0.95, 11.56)	0.062
Estrogen receptor				
Positive	4 [57]	3 [5]	–	–
Negative	3 [43]	4 [57]		
Progesterone receptor				
Positive	5 [100]	0	–	–
Negative	2 [22]	7 [78]		
HER-2/neu				
Positive	0	1	–	–
Negative	0	1		

SLN, sentinel lymph node; NSLN, non-SLN; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

Table 6 Variables for NSLN metastasis after positive SLN using standard method: exact multiple logistic regression analysis

	P-value	Exact adjusted OR	Exact 95% CI of OR
Location			
Inner		1	
Outer	0.412	1.99	0.50, 8.25
Grading			
Moderately differentiated		1	
Poorly differentiated	0.120	3.00	0.79, 12.58
Perinodal invasion of SLN			
No		1	
Yes	0.113	3.08	0.81, 13.43

SLN, sentinel lymph node; NSLN, non-SLN; OR, odds ratio.

(no/yes). Result showed no statistically significant predictors for metastasis (*Table 6*).

Table 7 displayed factors associated with NSLN metastasis based on multilevel sectioning. Outer location significantly increased the risk of metastasis (OR =2.66, P=0.040) compared to the inner location. LVI and perinodal invasion seemed to increase the risk of metastasis as well with OR of 3.15 (P=0.058) and 3.32 (P=0.056) respectively. An exact multiple logistic regression analysis on three independent variables, i.e., location (inner/outer), LVI (no/yes) and perinodal invasion of SLN (no/yes) demonstrated that only outer location increased the risk with adjusted OR of 3.90, P=0.036 (*Table 8*).

IHC technique increased detection of metastatic deposits. Eight cases were detected by IHC (four cases in SLN and four in NSLN) but not by initial examination of the corresponding HE slides using 4× objective (*Figure 1A,B*). Among these eight cases, three were tumor clusters (range, 0.1-0.15 mm; *Figure 2A,B*) and the other five were micrometastases (range, 0.25-1 mm). There was only one case that the IHC identified the metastatic deposit without tumor in HE section.

From the 19 false negative SLN almost all had the primary tumors in the upper outer location, histologic type of IDC, NOS and moderate grade (*Table 9*). The average primary tumor size was 2.6 cm (range, 1-5.5 cm) and numbers of the SLN and NSLN were 2.5 nodes (range, 1-7 nodes) and 22.8 nodes (range, 8-47 nodes), respectively. The metastatic lesions comprised ten macrometastases, eight micrometastases and one tumor cluster. Eleven cases were performed by one surgeon. No recurrent tumor following initial operations was found during the average three-year follow-up period (4.8 months-5.7 years). Among the

19 false negative cases, seven patients who have more than ten years follow up still are free of recurrent disease.

Discussion

Previous investigations on SLN have been shown to accurately reflect the presence or absence of metastases in the axilla in patients with breast cancer. The incidence of 32.3% positive SLN in this study was within the range of 21.3-46% as studied by others (2,6-9,15). The patients in this study comprised a little more T and N staging since it was an initial study intended to perform dissection of all axillary lymph nodes. Multiple studies showed that SLN examination had a sensitivity of 83.4-100% for the detection of axillary lymph node disease (3,5-9). In a number of large studies using different histopathologic techniques, the SLN false-negative rates varied between 0-11% (2,3,6,8,9). In the present study, the SLN examination had a sensitivity of 75.9%, and a false-negative rate of 24.1%. These results are different from others as the sensitivity was lower and the false negative rate was quite high. As mentioned above, this study was a retrospective study using the same population from the initial study (learning curve) of SLN biopsy in Siriraj Hospital. The SLN identification rate varied depending upon the experience of the surgeons and the techniques used. Most investigators reported that learning these techniques required time and experience (1,7,18). Another reason which might affect the detection of SLN was the technique used, we performed peritumoral dye injection only. Combined dye and radioisotope injection techniques used intraoperatively has been addressed to be superior to using dye alone by comparing the false-negative rate which was higher in the dye alone group (21%

Table 7 Variables for NSLN metastasis after positive SLN using multilevel sectioning: univariable analysis

	Mean \pm SD or number [%]		Crude OR (95% CI)	P-value
	No metastasis (n=27)	With metastasis (n=37)		
Age (yrs)	50.2 \pm 9.9	49.4 \pm 10.7	–	0.767
Tumor size (cm)	2.3 \pm 1.0	2.8 \pm 1.3	–	0.113
\leq 2.0	13 [46]	15 [54]	1	
>2.0	14 [40]	21 [60]	1.30 (0.42, 3.99)	0.797
\leq 2.2	14 [48]	15 [52]	1	
>2.2	13 [38]	21 [62]	1.51 (0.49, 4.63)	0.584
Location: outer/inner				
Others	2 [50]	2 [50]	–	
Inner	13 [62]	8 [38]	1	
Outer	12 [31]	27 [69]	3.66 (1.05, 12.96)	0.040
Location: upper outer/others				
Others	18 [51]	17 [49]	1	
Upper outer	9 [31]	20 [69]	2.35 (0.75, 7.55)	0.164
Histologic type				
Mucinous CA, papillary CA, others	0	1 [100]	–	–
IDC, IDC with Paget's, IDC with predominant DCIS, ILC, mixed	27 [43]	36 [57]		
Histologic type				
Non-IDC	0	4 [100]	–	–
IDC	27 [45]	33 [55]		
Grade				
Well differentiated	3 [75]	1 [25]	–	
Moderately differentiated	16 [49]	17 [52]	1	
Poorly differentiated	8 [31]	18 [69]	2.12 (0.64, 7.24)	0.267
Lymphovascular invasion				
No	14 [58]	10 [42]	1	
Yes	12 [31]	27 [69]	3.15 (0.97, 10.38)	0.058
Perinodal invasion of SLN				
No	21 [53]	19 [48]	1	
Yes	6 [25]	18 [75]	3.32 (0.98, 12.22)	0.056
Estrogen receptor				
Positive	3 [38]	5 [63]	–	–
Negative	2 [29]	5 [71]		
Progesterone receptor				
Positive	3 [60]	2 [40]	–	–
Negative	2 [20]	8 [80]		
HER-2/neu				
Positive	0	1	–	–
Negative	0	1		

SLN, sentinel lymph node; NSLN, non-SLN; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

Table 8 Variables for NSLN metastasis after positive SLN using multilevel sectioning: exact multiple logistic regression analysis

	P-value	Exact adjusted OR	Exact 95% CI of OR
Location			
Inner		1	
Outer	0.036	3.90	1.08, 15.64
Lymphovascular invasion			
No		1	
Yes	0.156	2.72	0.73, 10.80
Perinodal invasion of SLN			
No		1	
Yes	0.311	2.17	0.58, 8.97

SLN, sentinel lymph node; NSLN, non-SLN; OR, odds ratio.

versus 2.8%) (19). In addition, variability of lymph flow and rerouting of lymphatic flow by tumor blockage of the lymph node could also affect the false negative rate (1,18).

The 6.3% increased detection of metastases in SLN on multilevel sectioning and IHC was similar to those studied by others which ranged from 3-20% (6,11,12,14,15). In our series with a rather high false-negative rate and a small number of studied cases, the percentage of the SLNs with tumor cluster or micrometastasis that had macrometastasis in the NSLNs were quite high (25% and 53%, respectively) in comparison with other study (20). The finding of tumor cluster or micrometastasis in multilevel sectioning and IHC is significant in false negative cases.

Regarding the predictive factors of axillary node metastasis, Olivotto *et al.* (21) studied the multivariate

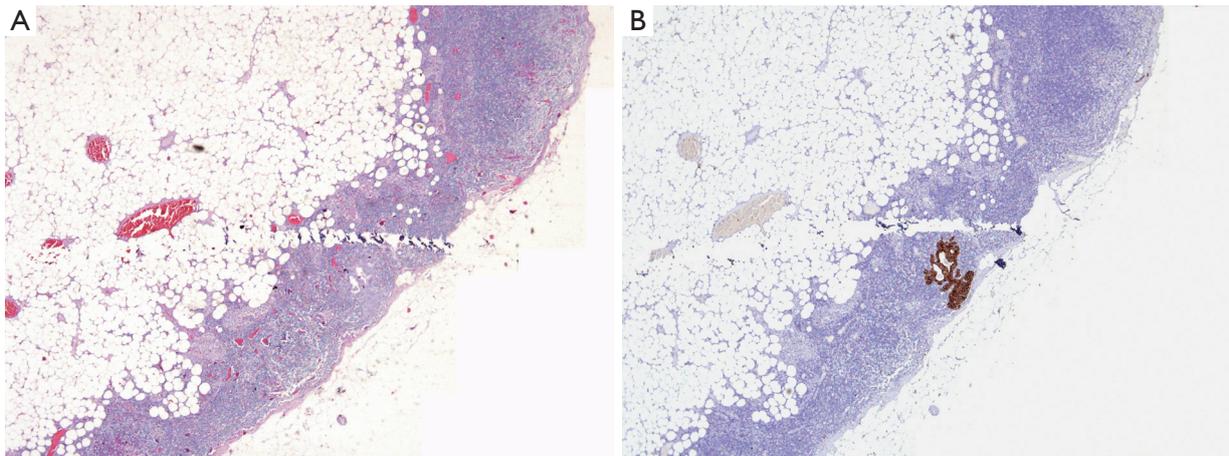


Figure 1 Good staining property is essential in diagnosing small metastatic lesion. Tumor cells are better visualized by AE1/AE3 immunostain: (A) H&E, 4x; (B) AE1/AE3, 4x.

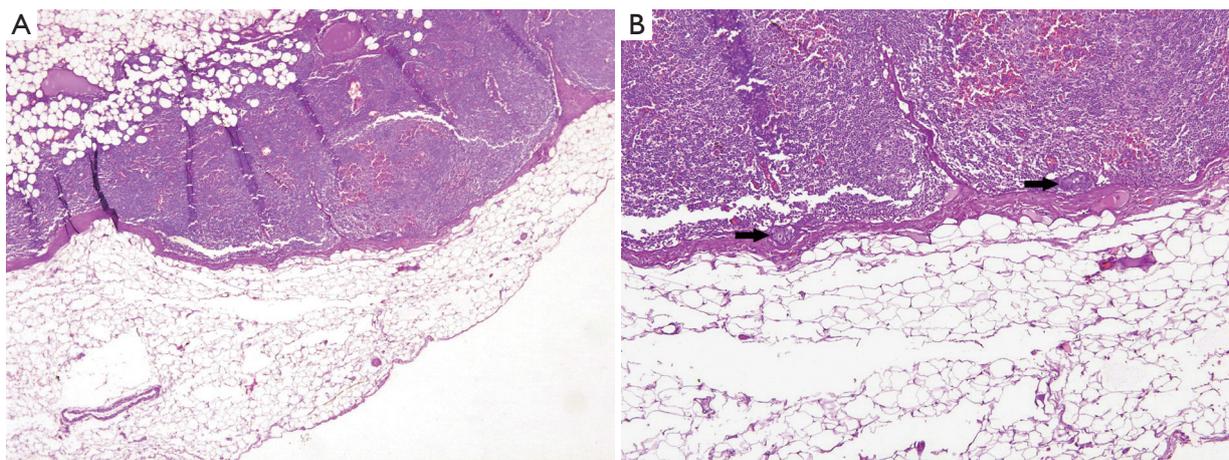


Figure 2 ‘Tumor cluster’ may be missed by screening with low power magnification: (A) H&E, 4x; (B) H&E, 10x. Arrows denote two tumor clusters.

Table 9 Nineteen false-negative cases

No	Surgeon	Location of tumor	Tumor size (cm)	Histologic		LVI	No. of SLN	No. of NSLN	NSLN metastatic size (mm)
				Type	Grade				
1	A	UO	4.0	IDC	2	Yes	1	24	19.00
2	B	LO	3.0	IDC	2	Yes	1	10	0.60 [®]
3	B	UO	3.0	IDC	2	No	7	21	0.50 [®]
4	B	Outer	3.5	IDC	2	Yes	1	23	25.00
5	B	UO	3.0	IDC	2	Yes	3	26	15.00
6	C	UO	1.8	IDC	2	Yes	2	18	1.80 [®]
7	C	UI	1.2	IDC	2	Yes	1	8	4.00
8	D	LO	>3.0	DCIS*	1	No	3	37	0.15 [#]
9	C	Central	4.0	IDC	2	Yes	2	21	0.60 [®]
10	C	UI	1.0	IDC*	2	No	1	25	0.30 [®]
11	C	UO	–	IDC	2	No	1	16	8.00
12	B	LI	3.0	IDC**	2	Yes	3	18	12.00
13	C	UO	5.5	IDC**	2	No	4	16	2.20
14	C	LO	2.0	IDC**	2	Yes	1	47	1.50 [®]
15	B	LO	1.5	IDC	2	Yes	3	23	0.60 [®]
16	C	UO	1.5	IDC	2	No	3	34	0.80 [®]
17	C	Inner	1.7	IDC*	2	No	4	21	2.60
18	C	UO	2.2	IDC*	2	Yes	5	14	4.00
19	C	Central	3.0	IDC	2	No	2	32	23.00

Location of tumor: UO, upper outer; UI, upper inner; LO, lower outer; LI, lower inner. Histologic type: DCIS*, DCIS with microinvasion; IDC*, IDC with predominant DCIS; IDC**, IDC with Paget. NSLN metastatic size: #, tumor cluster; ®, micrometastasis.

analysis of 6,052 patients and found that the readily available prognostic factors for axillary lymph node metastases were nodal palpability, tumor size and LVI. In a series by Joseph *et al.* (22) of 407 patients who underwent successful SLN dissection, 70 patients (17%) had positive SLN findings. The factors in their study that contributed to NSLN metastasis were tumor size and extranodal extension. Significant predictive factors of NSLN metastasis in the series of Abdessalam *et al.* (23) were LVI, extranodal extension and increasing size of metastatic focus within the SLN. Turner *et al.* (24) found that the predictive factors correlated with non SLN metastasis were primary tumor size and peritumoral LVI. In a study of 389 patients with positive SLN, Wong *et al.* (25) found that the likelihood of positive NSLN correlated with increasing tumor size. This result is similar to the study of Chu *et al.* (26), Reynolds *et al.* (27), and Kamath *et al.* (28). In the present study, we found that tumor size and LVI were significant predictors of axillary lymph node metastasis by multivariate analysis. By univariable analysis the outer location of primary tumor

and perinodal invasion were significantly associated with the presence of NSLN metastasis after positive SLN; LVI was also a predictor of NSLN status (from the multilevel study only). The findings were similar to the previously mentioned studies with an addition that a significant association between outer location of primary tumor and NSLN metastasis after positive SLN was also found.

For the SLN histologic examination, in order to decrease the metastasis detection error especially in micrometastasis and tumor cluster, we recommend careful examination of the standard HE section using an objective power 10× in screening the SLN and good quality of HE staining is mandatory. The size of metastatic deposits and perinodal invasion should be included in the report. Although multilevel and immunohistochemistry increased the detection of metastasis, all of them were micrometastases and tumor clusters. The study is costly and ensures a workload for the pathologist. To gain the maximal benefit of SLN biopsy, this intensive method may be considered in the patients at high risk for axillary node metastasis, e.g.,

those who had tumor size more than 2.2 cm, had IDC, NOS (non-papillary or non-mucinous carcinoma), and lymphovascular space invasion, since 19.5% of the patients with these characters had 77.8% metastasis in axillary node according to our series. However, more studies are needed to find a consensus on which patients are the high risk group to perform the intensive study which may also include other more sensitive test and if the multilevel study is considered, a standard protocol should be proposed.

In summary, a multilevel study of SLN and NSLN was performed and compared to the standard HE method. Caution should be used in using SLN biopsy technique with dye peritumoral injection alone especially in the early performing phase as there might be a high false-negative rate. However, the concordance rate of 89.7% confirmed that SLN biopsy is a reliable factor to determine the axillary nodal status.

Identification and pathological study of the SLN play an important role in the SLN procedure for staging of breast cancer. In breast cancer patients with positive SLN, the outer location of the primary tumor, LVI and perinodal extension significantly increased the frequency of additional positive nodes.

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