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Original Article

Sentinel Lymph Node Biopsy in Breast Cancer: Predictors of Axillary and Non-Sentinel Lymph Node Involvement

Hakan Postacı[2], Baha Zengel[1], Ülkem Yararbaş[3], Adam Uslu[1], Nuket Eliyatkın[2], Göksever Akpınar[1], Fevzi Cengiz [1], Raika Durusoy[4]

[1]*Department of General Surgery, Turkish Ministry of Health İzmir Bozyaka Research and Training Hospital, İzmir, Turkey*
[2]*Department of Pathology, Turkish Ministry of Health İzmir Bozyaka Research and Training Hospital, İzmir, Turkey*
[3]*Department of Nuclear Medicine, Ege University Medical Faculty, İzmir, Turkey*
[4]*Department of Public Health, Ege University Medical Faculty, İzmir, Turkey*

ABSTRACT

Background: Sentinel lymph node biopsy is a standard method for the evaluation of axillary status in patients with T1-2N0M0 breast cancers.

Aims: To determine the prognostic significance of primary tumour-related clinico-histopathological factors on axillary and non-sentinel lymph node involvement of patients who underwent sentinel lymph node biopsy.

Study design: Retrospective clinical study.

Methods: In the present study, 157 sentinel lymph node biopsies were performed in 151 consecutive patients with early stage breast cancer between June 2008 and December 2011.

Results: Successful lymphatic mapping was obtained in 157 of 158 procedures (99.4%). The incidence of larger tumour size (2.543±1.21 vs. 1.974±1.04), lymphatic vessel invasion (70.6% vs. 29.4%), blood vessel invasion (84.2% vs. 15.8%), and invasive lobular carcinoma subtype (72.7% vs. 27.3%) were statistically significantly higher in patients with positive SLNs. Logistic stepwise regression analysis disclosed tumour size (odds ratio: 1.51, p=0.0021) and lymphatic vessel invasion (odds ratio: 4.68, p=0.001) as significant primary tumour-related prognostic determinants of SLN metastasis.

Conclusion: A close relationship was identified between tumour size and lymphatic vessel invasion of the primary tumour and axillary lymph node involvement. However, the positive predictive value of these two independent variables is low and there is no compelling evidence to recommend their use in routine clinical practice.

Key Words: Breast neoplasms, lymphatic metastasis, sentinel lymph node biopsy.

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Introduction

Subsequent to breast-conserving surgery, sentinel lymph node biopsy (SLNB) has emerged as another major step in the surgical treatment of breast cancer. In the meantime, it is accepted as the standard method for the evaluation of axillary status in patients with T1-2N0M0 breast cancers. Completion of axillary lymph node dissection (ALND) is the standard surgical procedure for patients with metastatic SLNs (1). The prognostic significance of micrometastasis in SLN or non-SLN is still a matter of debate. A meta-analysis by Dowlatshahi et al. indicated a statistically significant reduction in survival of patients with occult micrometastatic nodal disease (2). Recent data from studies investigating the prognostic significance of lymph node micrometastases compared with node-negative disease revealed poorer disease-free survival (DFS) (3), which was close to 40% (4), or overall survival (OS) rates in the micrometastatic group (5, 6). Thus, the data emphasised the risk of observational protocols in this subgroup of patients (7). On the contrary, a novel conservative approach omitting ALND in SLN-positive patients has been suggested recently (8). In view of the literature mentioned above, we have so far accepted both macro- and micrometastatic disease essentially as the stages of a disease process and have treated them with the same surgical methods and medical approach.

Following the evolution of axillary conservation concept, determination of risk factors influencing axillary lymph node involvement aroused interest in many investigators (9, 10). Moreover, estimation of the risk of positive non-SLN in SLN-positive patients has been an area of research and several mathematically designed nomograms that are essentially based on information about pathological features and the method of detection were developed (11-14).

In this retrospective analysis, we reviewed our results in SLNB and elucidated the association between primary tumour-related histopathological factors and axillary lymph node involvement. In addition, we systematically reviewed the current evidence on this issue and evaluated the positive predictive value (PPV) of primary tumour-related factors on SLN involvement in a meta-analysis. As a secondary objective, we employed patient characteristics and primary tumour-related histopathological factors to evaluate their effectiveness in predicting the involvement of non-SLN metastasis.

Address for Correspondence: *Dr. Baha Zengel, Department of General Surgery, Turkish Ministry of Health İzmir Bozyaka Research and Training Hospital, İzmir, Turkey. Phone: +90 232 250 50 50 e-mail: bahazengel@gmail.com*

Material and Methods

The study group consisted of 157 breast cancer patients (155 female and 2 male) who underwent SLNB between June 2008 and December 2011. All patients had unilateral lesions. A total of 158 SLNBs were performed. The age range was 24–86 years (mean: 56.8±13.8). Thirty-three patients had prior excisional biopsy. Patient characteristics such as age, menopausal status, primary tumour characteristics including histological type (HT), histological grade (HG) via the modified Bloom and Richardson system, tumour size (TS), lymphovascular invasion (LVI), and blood vessel invasion (BVI), and ER, PR, cerb-B2, p53, and Ki67 status were assessed as potential predictive factors of axillary lymph node involvement.

Lymphatic mapping was performed by a combined method (blue dye and radiocolloid) in 87 procedures and only by radiocolloid in the remaining 75 procedures due to temporary blue-dye shortage. Three different radiocolloids, with respect to colloid diameter and chemical composition, were used. Of the commercially available products; Tc^{99m}-tin colloid (TC) (Amerscan Hepatate II; Amersham International, Amersham, U.K.) was used in 17, Tc^{99m} nanocolloid of serum albumin (NC) (Nanocoll; Nycomed Amersham Sorin s.r.l., Saluggia, Italy) was used in 43, and Tc^{99m} colloidal rhenium sulphide (CS) (Nanocis; CIS Bio International, Gif-sur-Yvette, France) was used in 89 procedures. Mean colloid diameter was 72–88 nm in TC, 8 nm in NL, and 22–29 nm in NC. Radiocolloid was injected subdermally in the four quadrants of the periareolar region on the day (18–24 hr) before surgery in all 149 procedures. For each injection, 0.25 mCi of radiocolloid was prepared in 0.1 mL volume. Before the operative procedure, lymphatic images were collected at anterior and lateral projections within an hour following injection. Isosulphan blue 1% (Lymphazurin® Tyco Healthcare Group LP, Norwalk, CT 06856 USA) was injected into the subareolar space in 5 mL volume following induction of anaesthesia. Five minutes of efficient massage was performed to stimulate lymphatic drainage. In group A, all hot and/or blue nodes and in group B, all hot nodes were accepted as SLNs and were harvested. Hot nodes were defined as nodes bearing radioactivity fourfold of the background activity and were localised using a hand-guided gamma probe (Navigator® GPS, Tyco Healthcare, Group LP, Norwalk, CT USA). 'Successful lymphatic mapping' was defined as localisation of one or multiple SLN(s) by radiocolloid and/or blue dye. SLNs were evaluated with frozen section analysis intraoperatively. At least two sections were prepared and examined. In case of suspicion, an additional two sections from the same SLN were evaluated. The remaining tissue fragments of the SLNs were formalin-fixed, paraffin-embedded, and sectioned. Haematoxylin&Eosin (HE) staining was used for histological evaluation. Immunohistochemistry using cytokeratin antibody was used for lymph nodes that were negative with HE staining. The detection of metastatic cells in one of these steps was defined as a 'positive SLN'. Metastatic lymph nodes were classified according to the size of metastatic deposit as macrometastasis (>2 mm) and micrometastasis (0.2-2 mm). Cell clusters or isolated tumour cells of <0.2 mm diameter were described as submicrometastasis. Patients having macro- or micrometastasis in sentinel nodes underwent axillary dissection (ALND). No additional axillary clearance was performed in patients with submicroscopic deposits because of unknown biological relevance of these cells.

The precise effect of patient and primary tumour characteristics in different studies were qualitatively examined in a meta-analysis. The primary objective was to determine the PPV of the statistically significant prognosticators of SLN involvement in independent studies (9, 15-19).

Statistical analysis

Statistical analysis was performed using SPSS 18.0 (SPSS, Chicago, IL, USA) for Windows. The relationship between clinico-pathological variables and axillary lymph node involvement was initially evaluated using univariate analysis. Following this, multivariate logistic regression analysis was performed in order to demonstrate the relationship between significant dependent variables and their relevance to metastatic involvement of SLNs or non-SLNs. Two-sided p values were calculated for all tests and a p value less than 0.01 was considered statistically significant.

Results

Successful lymphatic mapping was achieved in 157 of 158 procedures (99.4%). Radiocolloid uptake was observed in all 148 cases. The single patient with unsuccessful lymphatic mapping had a prior excisional biopsy in the upper outer quadrant and was treated by the combined method.

SLNs were metastatic in 59 (37.6%) cases. Forty-seven of them were diagnosed during frozen section analysis and 12 during the evaluation of H&E-stained paraffin sections or immunohistochemistry. In this latter group, 10 SLNs had micrometastasis and one had macrometastasis. In a single patient with three consecutive negative SLNs, we harvested a parasentinel node with suspicious macroscopic appearance and demonstrated micrometastatic deposits in paraffin sections. Among 47 patients, one had micrometastasis and 46 had macrometastasis. Complementary axillary dissection was performed in all patients with positive SLNs and in the particular patient with positive parasentinel node.

No additional metastatic lymph nodes were identified in paraffin section analysis of the ALND specimens of 12 patients with micrometastatic nodal disease. However, additional metastatic lymph nodes were detected in 19 of 47 (40.4%) patients with macrometastasis in SLNs (range: 1-25, median: 2). Of the 12 patients with micrometastatic nodal involvement, only one (8.3%) was detected by frozen section analysis.

Patient characteristics and results of the histopathological evaluation of the primary tumour are shown in Table 1. Patient demographics and histopathological features of the primary tumour that were likely to predict metastatic involvement of SLNs are shown in Table 2. Any potential confounder that had a $p<0.01$, which reflected a relationship with the outcome was included in the multiple logistic regression analysis. TS ($p=0.002$), tumour histology (presence of ILC, $p=0.001$), and blood and lymphatic vessel invasion ($p<0.0001$) were statistically significantly associated with tumour involvement of SLNs

Table 1. Patient and tumour characteristics

Characteristics	Number of patients (%)
All cases	151
Mean age, years (range)	56.8 (24–88)
Sex	
Female	149 (98.7)
Male	2 (1.3)
Menopausal Status	
Postmenopausal	97 (65.1)
Premenopausal	52 (34.9)
Breast Surgery	
Mastectomy	81 (51.6)
Lumpectomy	76 (48.4)
Histology	
Invasive ductal carcinoma	99 (63.1)
Invasive lobular carcinoma	11 (7.0)
Mixed (invasive ductal + invasive lobular carcinoma)	16 (10.2)
Ductal carcinoma in situ	10 (6.4)
Others	21 (13.4)
Histological Grade	
1	14 (8.9)
2	89 (56.7)
3	36 (22.9)
Mean Tumour Size (cm) (range)	2.192 (0.2–6)
SLN Metastasis	
None	97 (61.8)
Metastatic	47 (29.9)
Micrometastatic	13 (8.3)
ER	
Positive	110 (70.1)
Negative	44 (28.0)
Unknown	3 (1.9)
PR	
Positive	109 (69.4)
Negative	45 (28.7)
Unknown	3 (1.9)
c-erbB2	
Positive	29 (18.5)
Negative	116 (73.9)
Unknown	12 (7.6)
p53	
Positive	34 (21.7)
Negative	91 (58.0)
Unknown	32 (20.4)
Ki67	
Positive	31 (19.7)
Negative	95 (60.5)
Unknown	31 (19.7)

SLN: sentinel lymph node; ER: oestrogen receptor; PR: progesterone receptor

Table 2. Comparison of clinical and histopathological characteristics in SLN (+) and SLN (-) patients and their statistical significance

	SLN (-) N (%)	SLN (+) N (%)	p
Age (years)			
>50	35 (57.4)	26 (42.6)	0.192
≤50	63 (65.6)	33 (34.4)	
Menopausal Status			
Premenopausal	31 (57.4)	23 (42.6)	0.396
Postmenopausal	65 (64.4)	36 (35.6)	
Tumour Histology			
IDC	58 (58.6)	41 (41.4)	0.001
ILC	3 (27.3)	8 (72.7)	
Mixed (IDC+ILC)	8 (50.0)	8 (50.0)	
DCIS	10 (100.0)	0 (0.0)	
Others	18 (85.7)	3 (14.3)	
Histological Grade			
HG1	12 (85.7)	2 (14.3)	0.09
HG2	51 (57.3)	38 (42.7)	
HG3	19 (52.8)	17 (47.2)	
Tumour Size			
(cm) (mean±SD)	1.974±1.038	22.543±1.2077	0.002
Lymphatic Vessel Invasion			
(-)	87 (70.7)	36 (29.3)	<0.0001
(+)	10 (29.4)	24 (70.6)	
Blood Vessel Invasion			
(-)	94 (68.1)	44 (31.9)	<0.0001
(+)	3 (15.8)	16 (84.2)	
Oestrogen Receptor			
ER (-)	27 (61.4)	17 (38.6)	0.958
ER (+)	67 (60.9)	43 (39.1)	
Progesterone Receptor			
PR (-)	29 (64.4)	16 (35.6)	0.578
PR (+)	65 (59.6)	44 (40.4)	
c-erbB2			
Negative or (1+) or (2++) and FISH (-)	72 (62.1)	44 (37.9)	0.309
(3+++) or (2++) and FISH (+)	15 (51.7)	14 (48.3)	
p53 (+) >10%			
(-)	51 (56.0)	40 (44.0)	0.780
(+)	20 (58.8)	14 (41.2)	
Ki67 (+) >14%			
(-)	56 (58.9)	39 (41.1)	0.474
(+)	16 (51.6)	15 (48.4)	

SLN: sentinel lymph node; IDC: ınvasive ductal carcinoma; ILC: ınvasive lobular carcinoma; DCIS: ductal carcinoma ın situ; FISH: fluorescence in situ hybridization

Table 3. Multivariate logistic regression analysis for predicting tumour-related variables having a potential influence on SLN metastasis

	Odds ratio	95% confidence interval	p value
Tumour size	1.511	1.065–2.144	0.0021
Lymphovascular invasion	4.680	1.865–11.743	0.001

in the univariate analysis. These four independent variables were further evaluated by logistic stepwise regression analysis, which disclosed tumour size (odds ratio: 1.51, p=0.0021) and LVI (odds ratio: 4.68, p=0.001) as the only significant primary tumour-related prognostic determinants of SLN metastasis (Table 3). However, the PPV of both tumour size (0.47) and LVI (0.70) in determining SLN involvement remained lower than expected.

The same variables were reevaluated to identify their probable impact on non-SLN involvement of patients with micro- or macrometastatic SLNs following ALND (Table 4). Here again, LVI of the primary tumour (p=0.002) appeared as the unique prognostic determinant of non-SLN metastasis. With regard to other covariates, the impact of tumour size and BVI (p=0.066) disclosed a statistical trend, but was not statistically significant.

The review of the literature revealed six methodologically sound studies with some risk factors being included in the assessment of metastatic involvement of SLNs (Table 5 and 6). Tumour size and/or LVI were invariably the significant two factors in determining SLN metastasis in multivariate regression analysis in all studies. We evaluated the PPV and NPV (negative predictive value) of these two determinants cumulatively and display the results in Tables 5 and 6.

Discussion

SLNB is currently the accepted standard method for the evaluation of axillary status in patients with stage 1 and 2 breast cancer (1, 20-23). Almost 75% of patients in this group benefit from the technique by avoidance of unnecessary axillary dissection and its related morbidity (24).

Many controversial issues have been disputed following the introduction of SLNB for the assessment of axillary status in early breast cancer. Some authors recommended complementary axillary dissection in patients with micrometastasis due to the relationship of subclinical nodal disease with worse survival figures (3, 5-7, 25).

On the contrary, this concept has been questioned even in patients with metastatic SLNs, due to the encouraging survival results in recent publications. The Z0011 trial, the only multicentre randomised phase 3 study, compared ALND with no further axillary treatment in patients with axillary lymph node metastasis. The primary objective of this study was OS with the determination of non-inferiority of SLND to ALND. The study prematurely closed due to enrolment of less than 50% of the targeted population (planned: 1900 patients, enrolled:

Table 4. Clinical and histopathological features of metastatic or tumour-free non-SLNs in SLN-positive patients who underwent axillary lymph node dissection

	Non-SLNs without tumour N (%)	Non-SLNs with tumour N (%)	p
Age (years)			
≤50	17 (65.4)	9 (34.6)	0.470
>50	23 (69.7)	10 (30.3)	
Menopausal Status			
Premenopausal	14 (60.9)	9 (39.1)	0.363
Postmenopausal	26 (72.2)	10 (27.8)	
Tumour Histology			
IDC	27 (65.9)	14 (34.1)	NA
ILC	7 (87.5)	1 (12.5)	
Mixed (IDC+ILC)	5 (62.5)	3 (37.5)	
Others	2 (66.7)	1 (33.3)	
Histological Grade			
HG1	2 (100.0)	0 (0.0)	NA
HG2	29 (76.3)	9 (23.7)	
HG3	7 (41.2)	10 (58.8)	
Tumour Size			
(cm) (median±IR)	2.0±1.5	2.8±1.5	0.066
Lymphatic Invasion			
(-)	30 (83.3)	6 (16.7)	0.002
(+)	11 (45.8)	13 (54.2)	
Blood Vessel Invasion			
(-)	33 (75.0)	11 (25.0)	0.066
(+)	8 (50.0)	8 (50.0)	
Oestrogen Receptor			
ER (-)	11 (64.7)	6 (35.3)	0.704
ER (+)	30 (69.8)	13 (30.2)	
Progesterone Receptor			
PR (-)	11 (68.8)	5 (31.3)	0.967
PR (+)	30 (68.2)	14 (31.8)	
c-erbB2			
Negative or (1+) or (2++) and FISH (-)	29 (65.9)	15 (34.1)	0.486
(3+++) or (2++) and FISH (+)	10 (71.4)	4 (28.6)	
p53 (+) >10%			
(-)	30 (75.0)	10 (25.0)	0.083
(+)	7 (50.0)	7 (50.0)	
Ki67 (+) >14%			
(-)	29 (74.4)	10 (25.6)	0.123
(+)	8 (53.3)	7 (46.7)	

SLN: sentinel lymph node; IDC: ınvasive ductal carcinoma; ILC: ınvasive lobular carcinoma; FISH: fluorescence in situ hybridization

Table 5. Analysis of the impact of tumour size on sentinel lymph node metastasis (literature review) (9, 15-19)

Study	T≤2 cm/All	T>2 cm/All	p value	PPV	NPV	Sensitivity	Specificity	Accuracy
Rivadeneira	NA	NA	0.01	NA	NA	NA	NA	NA
Fan	NA	NA	NA	NA	NA	NA	NA	NA
Ozmen	65/218	83/161	<0.0001	0.52	0.70	0.56	0.66	0.62
Mustac	NA	NA	0.004	NA	NA	NA	NA	NA
Aitken	69/286	160/337	<0.01	0.47	0.76	0.70	0.55	0.61
Boler	69/222	67/110	0.0001	0.61	0.69	0.49	0.78	0.66
Present Study	29/91	31/66	0.002	0.47	0.68	0.52	0.64	0.59
Total	232/817	341/674	NA	0.51	0.72	0.60	0.64	0.62

T: tumour size; PPV: positive predictive value; NPV: negative predictive value

Table 6. Analysis of the impact of lymphovascular invasion on sentinel lymph node metastasis (literature review) (9, 15-19)

Study	LVI (-)/All	LVI (+)/All	p value	PPV	NPV	Sensitivity	Specificity	Accuracy
Rivadeneira	68/450	14/28	0.0001	0.50	0.85	0.17	0.96	0.83
Fan	81/336	32/47	NA	0.68	0.76	0.28	0.94	0.75
Ozmen	67/223	81/161	<0.001	0.50	0.70	0.55	0.66	0.62
Mustac	38/195	21/64	0.034	0.33	0.81	0.36	0.79	0.69
Aitken	127/455	97/168	<0.001	0.58	0.72	0.43	0.82	0.68
Boler	51/210	82/109	0.0001	0.75	0.76	0.62	0.85	0.76
Present Study	36/123	24/34	<0.0001	0.71	0.71	0.40	0.90	0.71
Total	468/1992	351/611	NA	0.57	0.77	0.43	0.85	0.72

LVI: lymphovascular ınvasion; PPV: positive predictive value; NPV: negative predictive value

891 patients). The definition of clinical non-inferiority was too lax and the assumption was 5-year survival in the SLND arm to be no less than 75% of that in the ALND arm. Initially, 500 deaths were required to reach 90% statistical power to confirm non-inferiority of SLND with the use of a two-sided 90% confidence interval (8). However, final survival analysis has been reported at a median follow-up of 6.3 years with 94 deaths (42 in the SLN group and 52 in the ALND group). Moreover, axillary recurrence rate in the SLN arm was double that in the ALND arm (26). With these drawbacks in mind, the conclusion of the authors that SLND was non-inferior to ALND in patients with SLN metastasis should be interpreted very cautiously.

Improvements in imaging, surgical and radiation methods, and introduction of sophisticated pathological evaluation and effective systemic adjuvant chemotherapy entail revisiting of standard local therapy (27). In our series, none of the 11 patients with micrometastasis in SLNs had additional metastatic lymph nodes in dissection material, whereas 44.2% of patients with macrometastatic disease in SLNs had additional metastatic non-sentinel lymph nodes. These results are compatible with the current literature (12). With a conservative point of view, one might claim that all patients with micrometastasis and 55.8% of patients with macrometastasis in SLNs have been overtreated in our study group. In order to avoid unnecessary ALND, preoperative risk assessment for axillary status is of the utmost importance to estimate the metastatic involvement of the SLN and the likelihood of additional residual disease in the non-SLN (15, 28-30). In both situations, certain demographic characteristics of the patient and histological features of the tumour that are highly predictive of SLN and non-SLN status would be helpful in decision making.

As reported in the previous studies, the low identification rate of micrometastases by frozen section and conventional H&E staining (7) and the strong evidence about the impact of micrometastatic nodal disease on locoregional recurrence (31, 32) warrant identification of primary tumour-related histopathological factors with high predictive ability. Many studies have validated the MSKCC nomogram, which was proposed for predicting non-SLN metastases. Some of them did not find it trustworthy particularly for SLNs with micrometastatic involvement, while others have proposed a different procedure for improving predictive accuracy (11, 33). Given that the patients presented with a high frequency of micrometastasis, the predictive ability of non-SLN involvement of the Tenon and Stanford nomograms is the most important in this regard (14, 34). In two studies, the MSKCC nomogram did not prove to be reliable for identifying non-SLN metastasis in patients with micrometastasis-positive SLNs, with an area under the ROC curve of 54% and 59%, respectively (11, 34). However, the Cambridge model and a novel Turkish formula seem to be uninfluenced by SLN micrometastasis and non-SLN positivity

rates and have an area under the ROC curve of over 80% each, and thus deserve further validation in prospective trials (13, 14, 35). Age, menopausal status, HT, HG, size of the tumour, lymphatic and blood vessel invasion, and hormone receptor status are previously studied factors.

Despite the inhomogeneity of data concerning predictors of axillary positivity, the literature review and our results pointed to tumour size and lymphovascular invasion as factors having an influence on axillary metastatic rates (9, 15-19). Recently, in a prospective study of 177 patients concerning early invasive breast cancer treatment via tumour excision and SLNB, TS (p=0.003) and LVI (p=0.01) appeared to be the only histopathological characteristics having a significant association with lymph node positivity in multivariate analysis (36). However, the PPV of these two parameters in determining metastatic involvement of SLNs remained very low both in our study and in cumulative analysis of six studies including more than 4000 patients, thus hindering us from recommending their use in routine clinical practice and surgical decision making.

In our study, the low detection rate of micrometastatic deposits in SLNs by frozen section analysis was an interesting observation. Almost 92% percent (11 of 12 patients) of micrometastasis had been missed during intraoperative frozen section. There is a disparity of view on the contribution of complementary rapid IHC for improving the diagnostic ability of intraoperative FS in the subset of patients with micrometastatic disease (37-39). Our technique using H&E staining during frozen section analysis yielded a sensitivity of 97.9% for macrometastatic disease, which is comparable to prior reports based on the rapid-IHC technique (39).

In conclusion, frozen section analysis using H&E staining was very successful in detecting macrometastatic disease in SLNs; however, the technique failed to detect most of the micrometastasis. Among various factors, TS and lymphovascular invasion of the primary tumour were determined as predisposing factors for axillary SLN and non-SLN involvement. True-cut or excisional biopsy of primary tumour in patients with early stage breast cancer is a common clinical practice. Histopathological evaluation of paraffin-embedded but not frozen section specimens clearly demonstrated the prognosticators of axillary involvement. However, as the PPV of these prognosticators was unacceptably low, it is very premature to recommend the application of this information before proceeding to axillary dissection.

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Informed Consent: *Written informed consent was received from the participants of this study.*

Peer-review: *Externally peer-reviewed.*

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