Predicting the likelihood of non sentinel lymph node metastases after a positive sentinel lymph node biopsy in a group of breast cancer patients treated at Tanta University Hospital

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Abstract: Background/Aim: Reliable predictors of axillary nodal involvement would enable a better selection of candidate patients for sentinel lymph node biopsy (SLNB) and possibly allow identification of patients with such a low risk of axillary sentinel lymph node (SLN) involvement to be even spared SLNB. In this study, our aim was to evaluate the factors associated with SLN involvement with estimation of the predictability of various clinicopathologic factors on non-sentinel lymph node metastases (NSLNM) in breast cancer (BC) patients with positive SLNB to spare a subgroup of BC patients and a positive SLNB completion of axillary lymph node dissection (ALND). **Patients & Methods:** A total of 851 patients with clinically node-negative early invasive breast carcinomas underwent breast conserving surgery or modified radical mastectomy and SLNB followed by ALND were included in this study during the period between December 2005 and September 2014. Characteristics of the patients, tumors and SLNs were studied and analyzed both Histopathologically and immunohistochemically. **Results:** Sentinel lymph nodes showed positivity in 366 cases (43%). In univariate analysis, there was statistical significant correlation with the proliferative fraction ≥16%, multicentric disease, HER-2 status, lympho-vascular invasion, tumour histology, tumor status, tumor grade, epithelial hyperplasia, and perineural invasion. There was no statistical significant correlation when looking at the age of the patients, estrogen receptor (ER) status, progesterone receptor (PR) status, ER/PR +ve tumors, menopausal status, triple-negative tumors, microcalcification and family history. In multivariate analysis, only the occurrence of peritumoral vascular invasion was independently related to this end point. Overall, 166 of 366 patients (45.4%) had additional NSLNM. In univariate analysis, there was a significant correlation between NSLNM and the size of SLN metastases (SLNM), multicentric disease, tumor status, tumor grade, perineural invasion, epithelial hyperplasia, lympho-vascular invasion, and patients with 2 or more involved SLNs. There was no statistical significant correlation when looking at the age of the patients, multifocality, ER status, PR status, ER/PR +ve tumors, menopausal status, histologic type, Her2/neu expression, proliferative fraction, triple-negative tumor, microcalcification and family history. In multivariate analysis, only, the size of SLNM, and the occurrence of peritumoral vascular invasion in the primary breast carcinoma were independently related to this end point. **Conclusion**: In patients with early invasive breast carcinomas, histopathologic features of the primary tumor and SLNs status might be used to tailor the loco-regional treatment. However caution is required as patients with the most favorable combination of predictive factors still have a risk for NSLNM and should be offered completion ALND. Thus evaluation of additional molecular markers may further help to stratify patients to a risk-adapted approach.

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Key words: clinically node-negative early invasive breast carcinomas**,** sentinel lymph node biopsy, non-sentinel lymph node metastases

1. Introduction

The status of the axilla is the single most important prognostic indicator of overall survival in patients with breast cancer[(1)]. Staging is based on tumor size and on the presence of lymph node metastases[(2)]. Axillary lymph node dissection is an important procedure in the staging of breast cancer patients. However, it is associated with a significant morbidity rate[(3)]. In addition, using early diagnosis a high number of cases with negative lymph nodes can be identified[(4)].

A lymph node defined as SLN would be the first to receive tumoral drainage[(5)]. Over the past years an increasing number of breast cancer patients have benefited from the use of axillary SLNB[(6-9)]. Sentinel lymph node biopsy is the minimally-invasive alternative to ALND, avoiding the complications of the latter[(7)]. However, it is only beneficial in node negative patients, as ALND in breast cancer patients with positive SLNs is under debate[10-15].

Axillary lymph node dissection is recommended by many investigators for patients with BC metastasis to a SLN[14,15]. However in a significant proportion of patients, the SLN is the only involved axillary node[16-18]. Predictive factors of NSLNM at ALND have been studied[1,4,19-21]. They could verify that in patients with a positive SLN, predictive factors for NSLNM include size of the primary tumor, lymphovascular invasion, the size of the SLN metastases, extracapsular extension, and the proportion of positive SLN's among all identified SLNs[4,17, 21].

In this study, our aim was to evaluate the factors associated with SLN involvement with estimation of the predictability of various clinicopathologic factors on NSLNM in BC patients with positive SLNB to spare a subgroup of BC patients and a positive SLNB completion of ALND.

2. Patients & Methods

Patient Characteristics & inclusion criteria:

A series of 851 patients with clinically node-negative early invasive breast carcinomas (AJCC 6th edition[22] Stage T1 and T2,N0, M0) treated surgically by either modified radical mastectomy or conservative breast surgery with SLN biopsies followed by ALND at Surgical Oncology Department, Tanta University Hospital. All cases received chemotherapy (CT), radiotherapy, and/or hormonal therapy at Clinical Oncology Department, Faculty of Medicine, Tanta University Hospital during the period between December 2005 and September 2014.

Breast cancer patients seen in our Clinical Oncology Department but who did not undergo SLN biopsies followed by ALND surgery were considered not eligible in this study. Patients were selected for SLNB if they did not have palpable axillary nodes, previous lymph node surgery, or contraindications for lymphazurin blue injection. Also patients received previous neoadjuvant treatments were considered not eligible.

Clinical factors assessed were: age at diagnosis, parity, menopausal status and family history of breast cancer.

Informed consents for the investigational research were fully obtained from all patients included in the study.

Treatment Protocol:

Surgery: Five hundred twenty one patients (61.2%) were eligible to conservative breast surgery. So, they were submitted to lumpectomy and SLNB followed by ALND, while 330 patients (38.8%) underwent modified radical mastectomy. Briefly, SLNs were identified using blue dye. All the SLNs were serially and completely sectioned and examined histopathologically for all patients. All patients had at least level II ALND with an average of 15 lymph nodes removed [figures 1&2].

Chemotherapy:

A total of 702 (82.5%) patients had received adjuvant chemotherapy. Chemotherapy was applied in 331(38.9%) patients in the form of FAC regimen which consisted of cyclophosphamide (600 mg/m^2, day 1), adriamycine (50 mg/m^2, day 1) and fluorouracil (600 mg/m^2, days 1), intravenously and the cycle was repeated every 3 weeks. In 181 (21.3%) patients the FEC regimen was received in the form of cyclophosphamide (500 mg/m^2, day 1), epirubicine (100 mg/m^2, day 1) and fluorouracil (500 mg/m^2, days 1), intravenously and the cycle was repeated every 3 weeks. Since 2008, a sequential regimen of 3 cycles FEC followed by 3 cycles of docetaxel (100 mg/m^2 intravenous infusion on day 1) repeated every 3 weeks, was applied to 190 (22.3%) high risk patients. Before docetaxel, standard premedication was administered with dexamethasone 20 mg orally, diphenhydramine 50 mg intravenously (IV) and cimetidine 300 mg IV (or ranitidine 50 mg IV) 24 hours before chemotherapy and again 6 hours and 30 minutes before chemotherapy and for 2 days after administration. Antiemetics were administered at the oncologist's discretion. Supportive care included blood transfusions, growth factors and the administration of analgesics, as appropriate. Prophylactic use of growth factors was not recommended.

Radiotherapy:

Six hundred and seventy patients (78.7%) were treated with radiotherapy megavoltage equipment. Radiotherapy was initiated about 2 weeks after the sixth cycle of CT. Radiotherapy was delivered to the whole breast in patients underwent conservative breast surgery & to the chest wall in patients underwent modified radical mastectomy with individually shaped portals and daily fractions of 1.8 to 2.0 Gy on 5 consecutive days a week. A median total dose of 50 Gy given in 25 fractions over a period of 5 weeks (range 33 - 40 days) was applied. A boost of 10 Gy in 5 fractions over 1 week was applied to the tumor bed in patients submitted to conservative breast surgery. The chest wall and internal mammary lymph nodes (if indicated) were irradiated through two tangential fields, and immobilization techniques were used as required. Supraclavicular and axillary nodes were treated with an anterior field to a total dose of 50 Gy prescribed at 3 cm to the supraclavicular area and to the midplane of the axilla.

Hormonal therapy:

Patients with positive hormonal receptors receiving only hormonal therapy in the form of either anti-estrogens or aromatase inhibitors were restricted to those with luminal A breast cancer type and those aging 70 years and older (149 cases (17.5%)), while 512 (60.2%) cases received adjuvant hormonal therapy in the form of either anti-estrogens or aromatase inhibitors with or without ovarian suppression after chemotherapy and/or radiotherapy.

Paraffin blocks collection:

Paraffin blocks of the eligible patients were retrieved from the archives of pathology department, Faculty of Medicine, Tanta University and private laboratories. H&E sections were prepared from all blocks and the tumors were classified and graded according to the WHO classification of breast and female genital system tumors[23]. Staging was performed according to the International Union against Cancer TNM classification[22].

Pathological factors evaluated were: pathologic size of primary invasive breast tumor measured microscopically, tumor grade, tumor histologic type, presence of epithelial hyperplasia, proliferative fraction (Ki-67 labeling index), multifocality, Her2/neu expression of the primary tumors, number of positive and negative SLNs, and number of positive NSLNs for each patient, lymphovascular invasion that was proved by immunostaining for VEGF and ER status, PR status,, as well as multicentricity and occurrence of perineural invasion and microcalcification were also recorded.

Histopathologic Examination of the SLNs and NSLNs and Estimation of the Size of Metastases:

All the SLNs were completely sectioned and examined histopathologically & immunohistochemically for CK to detect micrometastasis; for all patients. The original histologic slides of all positive SLNs were reviewed and the actual size of the metastases was assessed. The recorded largest size corresponded to the maximum diameter in the plane of the section or to the thickness of the metastatic foci, whichever was larger was recorded. If multiple but distinct metastases were identified in the same SLN, the size of the largest was recorded. According to the size of the SLN metastases, 4 categories were devised: ITC (according to the current TNM classification)[22], small micrometastases (>0.2–1 mm), larger micrometastases (1–2 mm), and macrometastases (> 2 mm).

Nonsentinel axillary lymph nodes were accurately isolated. All patients had at least level II ALND with an average of 15 lymph nodes removed and the pathological status was known. The total number of isolated lymph nodes, the number of metastatic lymph nodes, and the TNM classification of the nodal metastases were recorded.

Statistical analysis:

Statistical analysis was conducted using SPSS Statistical package (version 12.0) for data analysis. Chi-square/ Fischer exact were tests of proportion independence. Cox-regression analysis was used to estimate univariate level and to evaluate independent prognostic variables affecting axillary lymph node metastases. *P* value is significant at 0.05 levels.

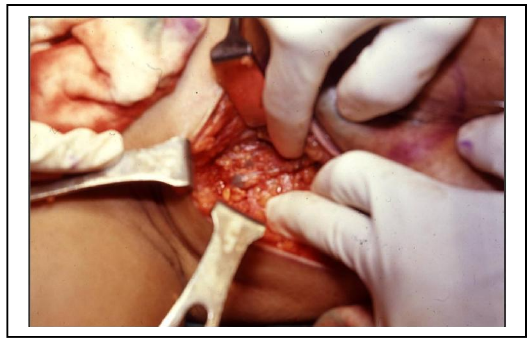

Fig (1): Intraoperative identification of sentinel lymph node (Blue).

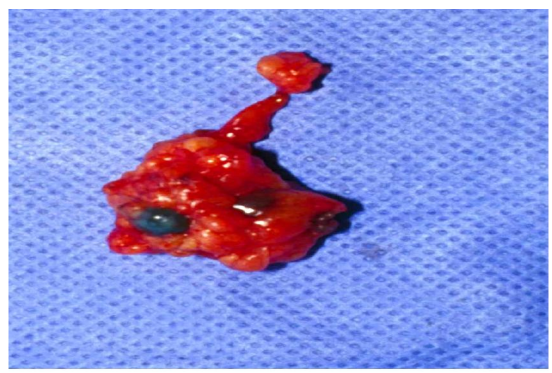

Fig (2): Resected specimen of axillary lymph nodes including SLN (Blue).

3. Results

Patient characteristics:

This study included 851 female patients with clinically node-negative early invasive BC, with their ages ranging from 26 to 72 years at the time of diagnosis (mean age 50.4 years), 51.1% of them were postmenopausal. Their tumors size ranged from 1.5 cm to 4.5 cm. The majority of cases were T2, node negative and grade II or greater. They showed ER positivity in 497 cases (58.4%) and HER-2 positivity in 181 cases (21.3%). Patients' and tumor characteristics were summarized in table (1).

Table (1): Patients' and tumor characteristics of the 851 patients with invasive breast carcinomas.

Characteristic	No. patients (%)
Age (years)	
Mean	50.4 years
Range	(26-72)
≤ 35 years	331(38.9%)
> 35 years	520 (61.1%)
Family history	
+ve	24 (2.8%)
-ve	827 (97.2)
Tumor Status	
T1	392 (46.1%)
T2	459(53.9%)
ER	
-ve	354 (41.6%)
+ve	497 (58.4%)
PR	
+ve	430 (50.5%)
-ve	421 (49.5%)
Her-2-neu	
+ve	181 (21.3%)
-ve	670 (78.7%)
ER/PR +ve	
Yes	330 (38.8%)
No	521 (61.2%)
Triple –ve	
Yes	190 (22.3%)
No	661 (77.7%)
Menopausal status	
Premenopausal	416 (48.9%)
Postmenopausal	435 (51.1%)
Tumor Grade	
G1	330(38.8%)
G2	361(42.4%)
G3	160 (18.8%)
Histology	
Invasive ductal	500 (58.8%)
Invasive lobular	111 (13%)
Others	240 (28.2%)
Multicentricity	
Positive	221 (26%)
Negative	630 (74%)
Perineural invasion	
Positive	301 (35.4%)
Negative	550 (64.6%)
Lymphovascular invasion	
Positive	149 (17.5%)
Negative	702 (82.5%)
Epithelial hyperplasia	
Positive	400 (47%)
Negative	451 (53%)
Microcalcification	
Positive	300 (35.3%)
Negative	551 (64.7%)
Lymph nodes dissected (median, range)	15 (10-25)
Involved lymph node Median, (range)	7 (0-24)
Sentinel nodal status	
Positive	366 (43%)
Unifocal	320
Multifocal	46
Negative	485 (57%)
Ki_{67} (proliferative fraction)	
Mean	16
Range	0-80
< 16	421 (49.5)
≥ 16	430 (50.5%)

Correlation between sentinel nodal status, and patient and tumor characteristics

Table (2) summarizes the relation of sentinel nodal status and the patient and tumor characteristics.

The table shows SLNs positivity in 366 cases (43%). There was statistical significant correlation with the proliferative fraction ≥16% (16% representing the median value of Ki-67 immunostaining in the current series), with a higher frequency of Ki_{67} ≥16% cancers being node positive (p<0.001). Sentinel axillary lymph node status was also significantly correlated with multicentric disease (p = 0.030), HER-2 status (p = <0.001), lympho-vascular invasion (p = <0.001), tumour histology (p = <0.001), tumor status (p = <0.001), tumor grade (p = <0.001), epithelial hyperplasia (p = 0.002), and perineural invasion (p = 0.003). There was no statistical significant correlation when looking at the age of the patients (p =0.082), ER status (p =0.061), PR status (p =0.512), ER/PR +ve tumors (p =0.081), menopausal status (p=0.071), triple-negative tumor (p =0.765), microcalcification (p =0.087) and family history (p = 0.912).

In multivariate analysis, only the occurrence of peritumoral vascular invasion in the primary breast carcinoma was independently related to this end point (p <0.001).

Correlation between size of sentinel lymph node metastases and incidence of additional metastases to non-sentinel nodes

Among 366 patients who had evidence of disease at SLN biopsy, 549 SLNs (mean, 2 SLN per patient; range, 1–4) were obtained, with 5490 NSLN (mean, 15 lymph nodes per patient; range, 10–22). In the subgroup of patients who shows SLN positivity, 36 patients (9.8%) had ITC only in the SLNs, while, 92 patients (25.1%) had micrometastases (Figure 3), of them, 52 and 40 patients had tumor deposits up to 1 mm or larger, respectively, whereas 238 (65%) had macrometastases, as shown in Table3.

Overall, 166 of 366 patients (45.4%) had additional metastases to NSLN, with a mean number of 4 (range, 1–22) involved lymph nodes. The prevalence of additional metastases, according to the size of the SLN metastasis, is reported in Table 3. Of the 36 patients with ITC only in the SLN, 5 (13.9%) had further axillary involvement, as well as 22 (23.9%) of the 92 patients with SLN micrometastases (0.2–2 mm in size) ($P = 0.3$). However, additional lymph node metastases were detected in 58.4% (139 of 238) of the patients with macrometastatic disease in the SLNs, and this correlation was statistically significant ($P < 0.001$), (Table 3).

Interestingly, classification of patients with micrometastasis to the SLN according to the metastasis size (up to 1 mm vs. >1–2 mm), the percentages of patients sustaining non-sentinel lymph node involvement was 19.2% (10 of 52) and 30% (12 of 40), respectively ($P = 0.04$). Thus, patients with a positive SLN biopsy could be stratified in 3 groups at significantly different risk for metastases to non-sentinel axillary lymph nodes. Patients with ITC only or SLN micrometastases up to 1 mm had the lowest risk of additional metastases, compared with those with micrometastases >1 to 2 mm in size and with those with SLN macrometastases ($P < 0.001$), (Table3).

Table (2): Correlation between sentinel nodal status and patient and tumor characteristics

		Sentinel lymph node status				***p*-value**
		Negative (n=485)		Positive (n=366)		
		N	%	N	%	
Age	≤35	176	36.3%	155	42.3%	
	>35	309	63.7%	211	57.7%	0.082
Estrogen receptor status	Negative	189	39%	165	45.1%	
	Positive	296	61%	201	54.9%	0.061
Menopausal status	Pre- menopausal	210	43.3%	206	56.3%	
	Postmenopausal	275	56.7%	160	43.7%	0.071
Tumor Status	T1	301	62.1%	91	24.9%	
	T2	184	37.9%	275	75.1%	<0.001*
Tumor Grade	Grade I	300	61.9%	30	8.2%	
	Grade II	180	37.1%	181	49.5%	<0.001*
	Grade III	5	1%	155	42.3%	
Progesterone receptor Status	Negative	230	47.4%	191	52.2%	0.512
	Positive	255	52.6%	175	47.8%	
HER-2	Positive	21	4.3%	160	43.7%	
	Negative	464	95.7%	206	56.3%	<0.001*
ER/PR +ve	Yes	180	37.1	150	41	
	No	305	62.9	216	59	0.081
Multicentricity	Negative	430	88.7	200	54.6%	
	Positive	55	11.3	166	45.4	0. 030*
Epithelial hyperplasia	Negative	291	60	160	43.7	
	Positive	194	40	206	56.3	0.002*
Microcalcification	Negative	329	67.8	222	60.7	
	Positive	156	32.2	144	39.3	0.087
Triple-negative tumor	Yes	100	20.6	90	24.6	
	No	385	79.4	276	75.4	0.765
Perineural invasion	Negative	344	70.9	206	56.3	
	Positive	141	29.1	160	43.7	0.003*
Lympho-vascular invasion	Positive	5	1%	144	39.3%	
	Negative	480	99%	222	60.7%	<0.001*
Tumour histology	Invasive ductal	230	47.4%	270	73.8%	
	Invasive lobular	75	15.5%	36	9.8%	<0.001*
	Others	180	37.1%	60	16.4%	
Ki76	<16%	280	57.7	141	38.5	<0.001*
	≥16%	205	42.3	225	61.5	
Family history	Present	14	2.9%	10	2.7%	
	Absent	471	97.1%	356	97.3%	0.912

* Significant relation

Table (3): Distribution of metastases to the sentinel and non-sentinel lymph nodes according to size of SLN metastases

Sentinel Lymph Nodes Metastases	Sentinel Lymph Node Metastases Size	No. of cases with positive Sentinel axillary Lymph Node	Number of Additional positive axillary Lymph Node
All Metastases	Any size	366	166
ITC	<0.2 mm	36	5
Micrometastases	≤ 2 mm	92	22
	≤ 1 mm	*52*	*10*
	> 1 mm	*40*	*12*
Macrometastases	>2 mm	238	139

Correlation between non-sentinel lymph node metastases and patient and tumor characteristics of the 366 patients with sentinel axillary lymph node involvement

In univariate analysis, there was a significant correlation between NSLNM and the size of SLN metastases, with a higher frequency in those with micrometastases >1 to 2 mm in size and with those with SLN macrometastases ($P < 0.001$). In addition, NSLNM was significantly correlated with multicentric disease ($p = 0.011$), tumor status ($p < 0.001$), tumor grade ($p < 0.001$), perineural invasion ($p = 0.041$), epithelial hyperplasia ($p = 0.010$), lympho-vascular invasion ($p < 0.001$) (Figure 4), and patients with 2 or more involved SLNs ($p < 0.001$).There was no statistical significant correlation when looking at the age of the patients ($p = 0.061$), multifocality ($p = 0.712$), ER status ($p = 0.642$), PR status ($p = 0.923$), ER/PR +ve tumors ($p = 0.713$), menopausal status ($p = 0.701$), histologic type ($p = 0.823$), Her2/neu expression ($p = 0.631$), proliferative fraction ($p = 0.810$), triple-negative tumors ($p = 0.912$), microcalcification ($p = 0.072$) and family history ($p = 0.512$) as shown in table 4.

In multivariate analysis, only, the size of SLN metastases, and the occurrence of peritumoral vascular invasion in the primary breast carcinoma were independently related to this end point, (all $p < 0.001$).

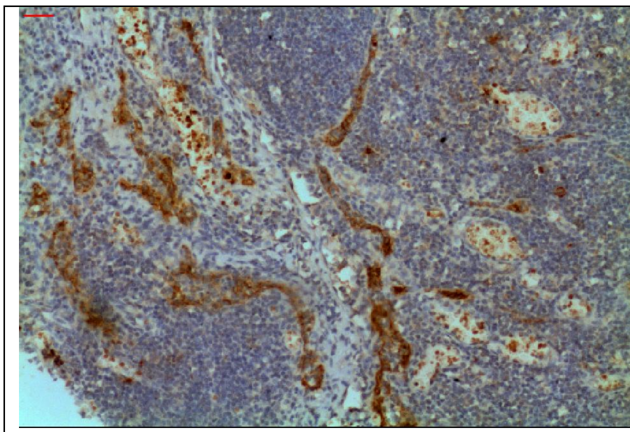

Fig (3): Sentinel lymph node with sinusoidal micrometastasis, CK stain (Strepavidin Biotin X200).

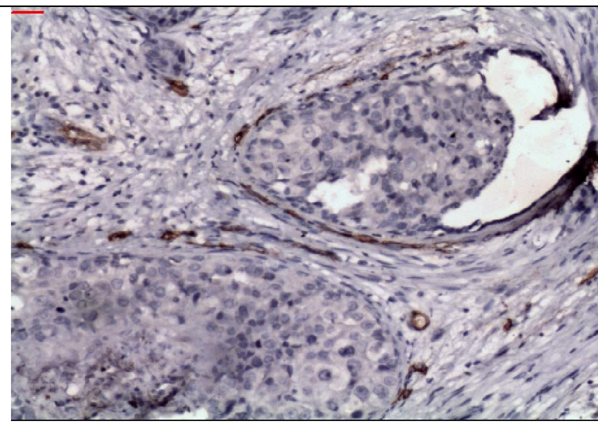

Fig (4): Mastectomy specimen, IDC with lymphovascular emboli, attached to the wall with marking endothelial cells with VEGF stain (Streptavidin Biotin X 400).

4. Discussion

Axillary lymph node metastasis (ALNM) is one of the most important prognostic determinants in breast carcinoma[24]; however, the reasons why tumors vary in their capability to result in ALNM remain unclear. Identifying breast carcinoma patients at risk for ALNM would improve treatment planning [25-27]. Node negative patients do not benefit from ALND but may suffer its attendant complications [28,29]. This has led to calls for more conservative management of the axilla in early breast cancer.

The current investigation emphasizes the most powerful predictive features of the primary tumor related to the metastatic involvement of the axillary SLN in a definite subgroup of patient candidates for SLNB.

Our results showed that SLNs positivity was significantly associated with the presence of proliferative fraction ≥16%, high tumor grade, HER2 over-expression, large tumor size, multicentric disease, lympho-vascular invasion, tumour histology, epithelial hyperplasia, and perineural invasion. There was no statistical significant correlation when looking at the age of the patients, ER status, PR status, ER/PR +ve tumors, menopausal status, triple-negative tumor, microcalcification and family history. In multivariate analysis, only the occurrence of peritumoral vascular invasion in the primary breast carcinoma was independently related to this end point.

Table (4): Correlation between non-sentinel lymph node metastases, and patient and tumor characteristics of the 366 patients with sentinel axillary lymph node involvement

		Number of patients with sentinel lymph nodes metastases	**Non-sentinel lymph node metastases**		***p*-value**
			Number	**%**	
Age	≤35	155	80	51.6 %	
	>35	211	86	40.8%	0.061
Estrogen receptor status	Negative	165	80	48.5%	
	Positive	201	86	42.8%	0.642
Menopausal status	Pre- menopausal	206	91	44.2%	
	Postmenopausal	160	75	46.9%	0.701
Sentinel lymph nodes metastases size	<0.2 mm	36	5	13.9%	
	0.2– 2 mm	92	22	23.9%	<0.001*
	> 2 mm	238	139	58.4%	
Number of positive sentinel lymph nodes	1	189	49	25.9%	
	≥2	177	117	66.1%	<0.001*
Multifocality	Unifocal	320	144	45%	
	multifocal	46	22	47.8%	0.712
Tumor Status	T1	91	20	22%	
	T2	275	146	53%	<0.001*
Tumor Grade	Grade I	30	5	16.7%	
	Grade II	181	70	38.7%	<0.001*
	Grade III	155	91	58.7%	
Progesterone receptor Status	Negative	191	86	45%	
	Positive	175	80	45.7%	0.923
ER/PR +ve	Yes	150	66	44	
	No	216	100	46.3	0.713
Perineural invasion	Negative	206	66	32%	
	Positive	160	100	62.5	0.041*
HER-2 neu	Positive	160	81	48.8%	
	Negative	206	85	41.3%	0.631
Triple-negative tumor	Yes	90	40	44.4	
	No	276	126	45.7	0.912
Epithelial hyperplasia	Positive	206	120	58.3	
	Negative	160	46	28.8	0.010*
Lympho-vascular invasion	Positive	144	95	65.9%	
	Negative	222	71	31.9%	<0.001*
Micro-calcification	Positive	144	60	41.7	
	Negative	222	106	47.7	0.072
Tumor histology	Invasive ductal	270	125	46.3%	
	Invasive lobular	36	16	44.4%	0.823
	Others	60	25	41.7%	
Multicentricity	Positive	166	120	72.3%	
	Negative	200	46	23%	0.011*
Ki76	<16%	141	66	46.8%	
	≥16%	225	100	44.4%	0.810
Family history	Present	10	4	40%	
	Absent	356	162	45.5%	0.512

Attempts have been made to identify factors that may predict an increase risk of nodal involvement in patients with clinically node-negative early invasive breast carcinomas. This study found a statistical significant correlation between tumor size and the incidence of SLNM, concurring with data from several other studies[30-32].

In this study, both univariate and multivariate analysis identified lymphovascular invasion as a significant predictor for SLN involvement.

Lymphovascular invasion has been proved to be a significant independent predictor of nodal involvement in other series, which considered lymphovascular tumor embolus as the most powerful predictor of axillary lymph node metastases[33-35]. As a result, pathologists should accurately assess the lymph-vessel tumor emboli as part of a histological prognostic and predictive classification.

Studies in breast cancer have shown conflicting data about the predictive significance of different histologic types on SLNM, ranging from no predictive significance[27], to a statistical significant correlation[36]. The findings of the present study point to the statistically significant difference in the distribution of SLNM frequency among the different histologic types compared to infiltrative ductal carcinoma.

Our study, identified tumor grade as a significant predictor for SLN involvement, confirming the data obtained in other studies[31,37]. Contrary to previous findings[38,39], in which, tumor grade did not retain any significant association with SLN status.

The predictive role of PR and ER status in previous studies is controversial, with some studies pointing to higher risk of axillary lymph node metastases for tumors negative for either receptor[37], or for PR only[30,35,40]. In contrast, similar to the findings of this study, Fein *et al.* [41], Chua *et al.* [31], Barth *et al.* [39], and Gajdos *et al.* [42], could not verify the same relation and found that ER and PR status did not appear to be a helpful predictor for SLNM. Therefore, the relationship between hormonal receptors and lymph node status warrants further studies.

Our finding of a significant relationship between multicentricity and SLNM in breast carcinoma patients has also been observed by Yenidunya *et al.* [27] and Coombs *et al.* [43].

Contradictory results have been reported in the literature concerning the role of age in breast cancer as a predictive factor of SLNM. Many investigators claimed that young patients tend to have more positive lymph nodes than their older counterparts[44-46], while our finding of a lack of a significant relationship between patient age and SLNM in breast carcinoma patients has also been observed by other investigators[12,38,39,47]. Therefore, young age, by itself, should not be considered as an indicator for more aggressive treatments.

Univariate analysis of this study identified proliferative activity of tumor cells assessed by immunohistochemical Ki-67 expression as a significant indicator in breast cancer for SLNM. This finding was agreed upon by Wrba *et al.* [48] who found that high Ki-67 index breast cancer showed a significant association with SLNM. However other study have shown that Ki-67 did not appear to be a helpful predictor[49].

Fewer than half of women (166 of 366 patients {45.4%}) with positive SLNB undergoing completion ALND for breast cancer had NSLNM. One of our goals in this study was to identify patients with tumor-free NSLNs who, with near certainty, may be spared completion ALND.

The very high negative predictive value of SLN biopsy in staging patients with clinically node-negative breast carcinoma allows almost 65% to 70% of patients to be spared ALND and its associated morbidity because of a metastasis-free SLN[50]. Conversely, in case of a positive SLNB, the standard of care remains completion ALND for a more exhaustive staging[51-54]. Further axillary involvement, however, will not be identified in the majority of these patients, who will not derive any benefit from ALND. Thus, a predicted small chance of additional axillary metastasis after a positive SLNB might justify avoiding ALND also in a selected cohort of patients with positive SLNB[55,56].

Our results showed that of the 366 patients subjected to ALND because of a positive SLN biopsy, 9.8% (36 of 366), had ITC only in the SLN. Thus, our population contains a minority of limited SLN disease burden relative to prior reports, including van Rijk's[57] reported rate of 16% for ITC[57]. Of our patients with positive SLNs, 92 patients (25.1%) presented with micrometastasis. The reported incidence of SLN micro-metastases markedly varies from institution to institution, ranging between 0 and 25%[9,57-61].

Despite the seemingly low sentinel node tumor burden, the prevalence of additional NSLNM upon completion ALND in this cohort of our patients with ITC only was 13.9% (5 of 36 patients), which had no statistically significant difference from the distribution of NSLNM frequency in 23.9% (22 of 92) of patients with SLN micrometastases (0.2–2 mm in size). However, this figure is significantly lower than the 58.4% (139 of 238 patients) obtained in the cohort of patients with SLN macrometastasis (>2 mm).

Findings of the reported prevalence of NSLNM in the cohort of patients with ITC only ranged from 4.7% to 20%[52,58,62-65], while that in patients with SLN micrometastases, documented after ALND, was between 15 and 20%[9,58-61].The results of these studies were comparable with our findings.

In our study no significant difference in the risk for additional NSLNM in patients with either ITC or micrometastases in the SLN was found. Interestingly, classification of patients with micrometastasis to the SLN according to the metastasis size (up to 1 mm vs. >1–2 mm), the percentages of patients sustaining NSLNM was 19.2% (10 of 52) and 30% (12 of 40), respectively (P = 0.04). Thus, patients with a positive SLNB could be stratified in 3 groups at significantly different risk for NSLNM. Patients with ITC only or SLN micrometastases up to 1 mm had the lowest risk of additional metastases, compared with those with micrometastases >1 to 2 mm in size and with those with SLN macrometastases ($P < 0.001$), these findings had also been observed by Turner *et al.* [66], Rahusen *et al.* [67], Weiser *et al.* [68], Reynolds *et al.* [69], Cserni *et al.* [70], Viale *et al.* [71], and Hwang *et al.* [72].

In addition to the potential predictive value of the size of SLNM, univariate and multivariate analysis of our data demonstrated also that the occurrence of peritumoral vascular invasion in the primary breast tumor was an independent predictor for NSLNM. Additionally, many studies had found that peritumoral vascular invasion is as strong a predictor of NSLNM as is size of SLNM[62,66,72].

In our study, the number of positive SLNs significantly correlated with further axillary involvement. These findings had also been observed by Viale *et al.* [73].

We identified tumor size as one of the tumor characteristics that significantly optimized stratification of NSLN status, perfectly in line with Kohrt *et al.* [62], findings.

Multifocality, microcalcification, age of the patients, menopausal status, histologic type, family history and biologic features (ER and PR status, ER/PR +ve tumors, Her2/neu expression, triple-negative tumor, and proliferative fraction) of the primary tumor has been evaluated by Viale *et al.*[73], as potential predictive factors of NSLNM, and it was found that they did not correlate with the prevalence of NSLNM. This study agrees with the results of our study.

In conclusion, the current data about histopathologic features of the primary tumor and SLNs status should be reconsidered in this selected population of BC patients with relatively small primary tumors and clinically uninvolved axilla to tailor the loco-regional treatment with the aim of minimizing as much as possible diagnostic and therapeutic procedures and improving the quality of life of the patients without any adverse effect on their survival rates.

However caution is required as patients with the most favorable combination of predictive factors still have a risk for NSLNM and should be offered completion ALND, as the benefits of no further axillary dissection must be weighed against the risk of harboring axillary metastasis that may potentially seed occult metastatic disease. Thus, evaluation of additional molecular markers with better definition of their role in guiding clinical decision-making by validating accuracy in stratifying risk of NSLNM to refine in which populations it may be best used, may further help to stratify patients to a risk-adapted approach.

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References

1. Abreu EB, Martinez P, Betancourt L, et al. Treatment plan for breast cancer with sentinel node metastasis. E cancer 2014, 8: 383.
2. Amersi F, Hansen NM. The benefits and limitations of sentinel lymph node biopsy. Curr Treat Options Oncol. 2006; 7(2):141-51.
3. Sanuki N, Takeda A, Amemiya A, *et al.* Outcomes of clinically node-negative breast cancer without axillary dissection: can preserved axilla be safely treated with radiation after a positive sentinel node biopsy? Clin Breast Cancer. 2013; 13(1): 69-76.
4. Bargehr J, Edlinger M, Hubalek M, *et al.* Axillary Lymph Node Status in Early-Stage Breast Cancer Patients with Sentinel Node Micrometastases (0.2-2 mm). Breast Care 2013; 8 (3): 187–91.
5. Mustać E, Matusan-Ilijas K, Marijić B, *et al.* Predicting the likelihood of additional nodal metastases in breast carcinoma patients with positive sentinel node biopsy. Int J Surg Pathol. 2010; 18 (1): 36-41.
6. Qiu1 P-F, Liu J-J, Wang Y-S, *et al.* Risk Factors for Sentinel Lymph Node Metastasis and Validation Study of the MSKCC Nomogram in Breast Cancer Patients. Jpn. J. Clin. Oncol. 2012; 42 (11): 1002-7.
7. Veronesi U, Viale G, Paganelli G, *et al.* Sentinel lymph node biopsy in breast cancer: ten-year results of a randomized controlled study. Ann Surg. 2010; 251:595–600.
8. Bernier J. Do we really need to undergo any axillary treatment after sentinel node biopsy in patients with early breast cancer and micrometastasis? The Breast 2011; 20 (5): 385–8.
9. Erb KM, Shapiro-Wright HM, Julian TB. Axillary recurrences following positive sentinel lymph node biopsy with individual tumor cells or micrometastases and no axillary dissection. Breast Dis. 2010; 31:83–90.
10. Giuliano AE, Hunt KK, Ballman KV, *et al.* Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. JAMA. 2011; 305:569–75.
11. Tvedskov TF, Jensen MB, Lisse IM, *et al.* High risk of non-sentinel node metastases in a group of breast cancer patients with micrometastases in the

sentinel node. Int J Cancer. 2012; 131 (10): 2367–2375.

12. Mansel RE, Fallowfield L, Kissin M, *et al.* Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC. Trial J Natl Cancer Inst. 2006; 98:599–609.
13. Kwon Y, Ro J, Kang HS, *et al.* Clinicopathological parameters and biological markers predicting non-sentinel node metastasis in sentinel node-positive breast cancer patients. Oncol Rep. 2011; 25 (4):1063-71.
14. Lyman GH, Giuliano AE, Somerfield MR, *et al.* American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. J Clin Oncol. 2005; 23:7703–20.
15. Carlson RW, McCormick B. Update: NCCN breast cancer Clinical Practice Guidelines. J Natl Compr Canc Netw. 2005; 3 (1):S7–11.
16. Boler DE, Uras C, Ince U, *et al.* Factors predicting the non-sentinel lymph node involvement in breast cancer patients with sentinel lymph node metastases. The breast 2012. doi:10.1016/j.breast.2012.02.012.
17. Coutant C, Morel O, Antoine M, *et al.* Is axillary lymph node dissection always necessary in breast cancer patients with a positive sentinel node? J Chir (Paris). 2007; 144 (6): 492-501.
18. Chue KM, Yong WS, Thike AA *et al.* Predicting the Likelihood of Additional Lymph Node Metastasis in Sentinel Lymph Node Positive Breast Cancer. J Clin Pathol. 2014; 67(2): 112-19.
19. Cody HS, Houssami N. Axillary management in breast cancer: What's new for 2012? The breast 2012. doi:10.1016/j.breast.2012.01.011.
20. Houvenaeghel G, Nos C, Giard S, *et al.* A nomogram predictive of non-sentinel lymph node involvement in breast cancer patients with a sentinel lymph node micrometastasis. Eur J Surg Oncol. 2009; 35(7):690-5.
21. Eldweny H, Alkhaldy K, Alsaleh N, *et al.* Predictors of non-sentinel lymph node metastasis in breast cancer patients with positive sentinel lymph node Pilot study. Journal of the Egyptian National Cancer Institute 2012; 24 (1): 23-30.
22. Greene FL, Pgae DL, Felming D *et al.* American Joint Comettee on Cancer (AJCC) cancer staging manual. 6th edition. Springer-Verlag; New York, 2002.
23. Tavassoli FA, Devilee P. World Health Organization Classification of Tumors: Pathology and Genetics of Tumors of the Breast and Female Genital Organs. IARC Press: Lyon, France, 2003.
24. Andersson Y, Frisell J, Sylvan M, *et al.* Breast cancer survival in relation to the metastatic tumor burden in axillary lymph nodes. J Clin Oncol. 2010; 28: 68–2873.
25. Galimberti V, Cole BF, Zurrida S, *et al.* International Breast Cancer Study Group Trial 23-01 Investigators. S3-1. Update of international breast cancer study group trial 23-01 to compare axillary dissection versus no axillary dissection in patients with clinically node negative breast cancer and micrometastases in the sentinel node. CTRC-AACR San Antanio breast cancer symposium, San Antanio, Texas, USA; December 6–10, 2011.
26. Wernicke AG, Goodman RL, Turner BC, *et al.* A 10-year follow-up of treatment outcomes in patients with early breast cancer and clinically negative axillary nodes treated with tangential breast irradiation following sentinel lymph node dissection or axillary clearance**.** Breast Cancer Res Treat. 2011; 125:893–902.
27. Yenidunya S, Bayrak R, Haltas H. Predictive value of pathological and immunohistochemical parameters for axillary lymph node metastasis in breast carcinoma. Diagn Pathol. 2011; 6: 18.
28. Rietman JS, Dijkstra PU, Geertzen JH, *et al.* Short-term morbidity of the upper limb after sentinel lymph node biopsy or axillary lymph node dissection for Stage I or II breast carcinoma. Cancer. 2003; 98:690–6.
29. Hack TF, Cohen L, Katz J, *et al.* Physical and psychological morbidity after axillary lymph node dissection for breast cancer. J Clin Oncol. 1999; 17:143–9.
30. Silverstein MJ, Skinner KA, Lomis TJ. Predicting axillary nodal positivity in 2282 patients with breast carcinoma. World J Surg. 2001; 25**:** 767–72.
31. Chua B, Ung O, Taylor R, *et al.* Frequency and predictors of axillary lymph node metastases in invasive breast cancer. ANZ J Surg 2001; 71:723-8.
32. Lee JH, Kim SH, Suh YJ, *et al.* Predictors of axillary lymph node metastases (ALNM) in a Korean population with T1-2 breast carcinoma: triple negative breast cancer has a high incidence of ALNM irrespective of the tumor size. Cancer Res Treat. 2010; 42: 30–6.
33. Hasebe T, Okada N, Iwasaki M, *et al.* Grading system for lymph vessel tumor emboli: significant outcome predictor for invasive ductal carcinoma of the breast. Hum Pathol. 2010; 41: 706–15.
34. Hasebe T, Tamura N, Iwasaki M, *et al.* Grading system for lymph vessel tumor emboli: significant outcome predictor for patients with invasive ductal carcinoma of the breast who received neoadjuvant therapy. Mod Pathol. 2010; 23: 581–92.

35. Viale G, Zurrida S, Maiorano E, *et al.* Predicting the status of axillary sentinel lymph nodes in 4351 patients with invasive breast carcinoma treated in a single institution. Cancer 2005; 103:492-500.
36. Tan LGL, Tan YY, Heng D, *et al.* Predictors of axillary lymph node metastases in women with early breast cancer in Singapore. Singapore Med J 2005; 46 (12): 693-97.
37. Gann PH, Colilla SA, Gapstur SM, *et al.* Factors associated with axillary lymph node metastasis from breast carcinoma. Cancer. 1999; 86:1511–19.
38. Olivotto IA, Jackson JS, Mates D, *et al.* Prediction of axillary lymph node involvement of women with invasive breast carcinoma: a multivariate analysis. Cancer 1998; 83: 948-55.
39. Barth A, Craig PH, Silverstein MJ. Predictors of axillary lymph node metastases in patients with T1 breast carcinoma. Cancer 1997; 79:1918-22.
40. Ravdin PM, De Laurentiis M, Vendely T, *et al.* Prediction of axillary lymph node status in breast cancer patients by prognostic indicators. J Natl Cancer Inst. 1994; 86:1771–5.
41. Fein DA, Fowble BL, Hanlon AL, *et al.* Identification of women with T1-T2 breast cancer at low risk of positive axillary nodes. J Surg Oncol. 1997; 65:34–9.
42. Gajdos C, Tartter PI, Bleiweiss IJ. Lymphatic invasion, tumor size, and age are independent predictors of axillary lymph node metastases in women with T1 breast cancers. Ann Surg. 1999; 220:692–96.
43. Coombs NJ, Boyages J. Multifocal and multicentric breast cancer: does each focus matter? J Clin Oncol. 2005; 23: 7497–502.
44. Rakha EA, Putti TC, Abd El-Rehim DM, *et al.* Morphological and immunophenotypic analysis of breast carcinomas with basal and myoepithelial differentiation. J Pathol. 2006; 208: 495–506.
45. Cianfrocca M, Goldstein LJ. Prognostic and predictive factors in early-stage breast cancer. Oncologist. 2004; 9: 606–16.
46. Wolff AC, Hammond ME, Schwartz JN, *et al.* American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med. 2007; 131:18–43.
47. Rakha EA, El-Rehim DA, Paish C, *et al.* Basal phenotype identifies a poor prognostic subgroup of breast cancer of clinical importance. Eur J Cancer. 2006; 42: 3149–56.
48. Wrba F, Chott A, Reiner A, *et al.* Ki-67 immunoreactivity in breast carcinomas in relation to transferrin receptor expression, estrogen receptor status and morphological criteria. An immunohistochemical study Oncology. 1989; 46:255–9.
49. Kwon Y, Ro J, Kang HS, *et al.* Clinicopathological parameters and biological markers predicting non-sentinel node metastasis in sentinel node-positive breast cancer patients. Oncol Rep. 2011; 25 (4):1063-71.
50. Schwartz GF, Giuliano AE, Veronesi U, *et al.* Proceedings of the consensus conference on the role of sentinel lymph node biopsy in carcinoma of the breast, April 19–22, 2001, Philadelphia, Pennsylvania. Cancer. 2002; 94: 2542–51.
51. Sachdev U, Murphy K, Derzie A, *et al.* Predictors of nonsentinel lymph node metastasis in breast cancer patients. Am J Surg. 2002; 183: 213–217.
52. Mignotte H, Treilleux I, Faure C, *et al.* Axillary lymph-node dissection for positive sentinel nodes in breast cancer patients. Eur J Surg Oncol. 2002; 28: 623–6.
53. Nos C, Harding-MacKean C, Freneaux P, *et al.* Prediction of tumour involvement in remaining axillary lymph nodes when the sentinel node in a woman with breast cancer contains metastases. Br J Surg. 2003; 90:1354–60.
54. de Widt-Levert LM, Tjan-Heijnen VCG, Bult P, *et al.* Stage migration in breast cancer: surgical decisions concerning isolated tumor cells and micro-metastases in the sentinel lymph node. Eur J Surg Oncol. 2003; 29:216–20.
55. Liang WC, Sickle-Santanello BJ, Nims TA. Is a completion axillary dissection indicated for micrometastases in the sentinel lymph node? Am J Surg. 2001; 182: 365–8.
56. Guenther JM, Hansen NM, DiFronzo LA, *et al.* Axillary dissection is not required for all patients with breast cancer and positive sentinel nodes. Arch Surg. 2003; 138:52–6.
57. van Rijk MC, Peterse JL, Nieweg OE, *et al.* Additional axillary metastases and stage migration in breast cancer patients with micrometastases or submicrometastases in sentinel lymph nodes. Cancer. 2006; 107:467–71.
58. Bolster MJ, Peer PG, Bult P, *et al.* Risk factors for non-sentinel lymph node metastases in patients with breast cancer. The outcome of a multi-institutional study. Ann Surg Oncol. 2007; 14:181–9.
59. Straver ME, Meijnen P, van Tienhoven G, *et al.* Sentinel node identification rate and nodal involvement in the EORTC 10981-22023 AMAROS trial. Ann Surg Oncol. 2010; 17:1854–61.
60. Galimberti V. International breast cancer study group trial of sentinel node biopsy. J Clin Oncol. 2006; 24:210–11.

61. Giuliano AE, McCall L, Beitsch P, *et al.* Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: the American College of Surgeons Oncology Group Z0011 randomized trial. Ann Surg. 2010; 252:426–32.
62. Kohrt HE, Olshen RA, Bermas HR, *et al.* New models and online calculator for predicting non-sentinel lymph node status in sentinel lymph node positive breast cancer patients. BMC Cancer. 2008; 8: 66.
63. Calhoun KE, Hansen NM, Turner RR, et al. Nonsentinel node metastases in breast cancer patients with isolated tumor cells in the sentinel node: implications for completion axillary node dissection. Am J Surg. 2005; 190: 588–91.
64. Cserni G, Gregori D, Merletti F, *et al.* Meta-analysis of non-sentinel node metastases associated with micrometastatic sentinel nodes in breast cancer. Br J Surg. 2004; 91:1245–52.
65. Kamath VJ, Giuliano R, Dauway EL, *et al.* Characteristics of the sentinel lymph node in breast cancer predict further involvement of higher-echelon nodes in the axilla: a study to evaluate the need for complete axillary lymph node dissection. Arch Surg. 2001; 688–92.
66. Turner RR, Chu KU, Qi K, *et al.* Pathologic features associated with nonsentinel lymph node metastases in patients with metastatic breast carcinoma in a sentinel lymph node. Cancer. 2000; 89:574–81.
67. Rahusen FD, Torrenga H, van Diest PJ, *et al.* Predictive factors for metastatic involvement of nonsentinel nodes in patients with breast cancer. Arch Surg. 2001; 136:1059–63.
68. Weiser MR, Montgomery LL, Tan LK, *et al.* Lymphovascular invasion enhances the prediction of non-sentinel node metastases in breast cancer patients with positive sentinel nodes. Ann Surg Oncol. 2001; 8:145–9.
69. Reynolds C, Mick R, Donohue JH, *et al.* Sentinel lymph node biopsy with metastasis: can axillary dissection be avoided in some patients with breast cancer? J Clin Oncol. 1999; 17:1720–6.
70. Cserni G. Sentinel lymph-node biopsy-based prediction of further breast cancer metastases in the axilla. Eur J Surg Oncol. 2001; 27:532–8.
71. Viale G, Maiorano E, Mazzarol G, *et al.* Histologic detection and clinical implications of micrometastases in axillary sentinel lymph nodes for patients with breast carcinoma. Cancer. 2001; 92:1378–84.
72. Hwang RF, Krishnamurthy S, Hunt K, *et al.* Clinicopathologic factors predicting involvement of nonsentinel axillary nodes in women with breast cancer. Ann Surg Oncol. 2003; 10:248–54.
73. Viale G, Maiorano E, Pruneri G, *et al.* Predicting the Risk for Additional Axillary Metastases in Patients With Breast Carcinoma and Positive Sentinel Lymph Node Biopsy. Ann Surg. 2005; 241(2): 319–25.

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