CLINICAL STUDY

Factors affecting sentinel lymph node metastasis in Turkish breast cancer patients: Predictive value of Ki-67 and the size of lymph node

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ABSTRACT

OBJECTIVES: We aimed to analyze the factors that affect the axillary lymph node involvement in Turkish breast cancer patients with clinically non-palpable axillary lymph node.

BACKGROUND: Sentinel lymph node biopsy is the gold standard technique to evaluate the axillary lymph node status that directly influences the prognosis and the treatment options in breast cancer.

METHODS: Breast cancer patients without axillary lymph node involvement in clinic examination were enrolled the study. Patients were categorized into the two groups according to existence of axillary lymph node metastasis or not. Demographic, histopathological and clinical data of patients were revealed retrospectively.

RESULTS: One-hundred and eighty-seven patients were analyzed and 101 of patients fulfilled the criteria and were included the study. Metastatic lymph node was detected in 38 (37.6 %) patients (Group 1), and was negative in 63 (62.4 %) patients (Group 2). Sentinel lymph node metastasis were statistically significant higher in patients with Ki-67 ≥ 14 % than patients with Ki-67 < 14 % (51.9 % vs 22.4 %; p < 0.01). Likewise, the mean size of the sentinel lymph node was statistically significant higher in Group 1 compared to Group 2 (p < 0.01).

CONCLUSION: Ki-67 proliferation index and sentinel lymph node size may provide a higher prediction about the sentinel lymph node involvement in patients with clinically negative axillary lymph nodes *(Tab. 3, Fig. 1, Ref. 31)*. Text in PDF *www.elis.sk.*

KEY WORDS: breast cancer, Ki-67, sentinel lymph node biopsy, lymph node size.

Introduction

Clinical and histopathological factors such as age, tumour size, tumour grade, estrogen receptor (ER) and progesterone receptor (PR) status, HER2 status and especially lymph node status directly influences the prognosis and the treatment option in patients with breast cancer (1). In the 1800s, axillary lymph node dissection (ALND) was introduced as the standard surgical procedure for breast cancer and played an important role in patients' prognosis assessment and regional control of the disease (2). As the first site of tumour cell infiltration via lymphatic vessels, sentinel lymph node (SLN) is seen as a safety indicator to determine the spread of the disease at axillary region and to avoid the ALND in patients

with SLN-negative breast cancer (3). SLN biopsy has become the standard procedure in diagnostic management of patients with non-palpable axillary lymph nodes, since it was defined (4, 5). Histopathological evaluation of SLN can be performed using touch imprint cytology, frozen section (FS) analysis, or both. The reported sensitivity of frozen section analysis of SLN in literature ranges from 70–98 % (6). Axillary ultrasound examination reduces the false negativity of preoperative assessment at the level of 53 % compared to only clinical examination (3). On the other hand, Ki-67 has a great potential as a prognostic and predictive factor in early breast cancer (7). Therefore, clinical, radiological and histopathological findings have a significant role to predict the SLN metastasis preoperatively. The study reported here analyzed the clinical, radiological and pathological data of 101 breast cancer patients with clinically negative axillary lymph nodes to reveal the risk factors for SLN metastasis.

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Methods

Patient selection

This retrospective study was carried out in patients with breast cancer without axillary lymph node involvement at Istanbul Medeniyet University, Department of General Surgery, between April 2010 and September 2014. Patients with inflammatory

cancers, pregnant patients, those who had axillary surgery, male patients, patients with multi-centric tumours and patients treated with neo-adjuvant chemotherapy and patients with unidentified sentinel node were excluded from the study. The informed consent was obtained from participants in this study.

Sentinel lymph node biopsy

Sentinel lymph node biopsy was performed through the methylene blue dye injection to the subareolar zone after general anesthesia. Eight minutes after the injection, 3 cm incision was made in the axillary region. Blue node was identified and excised with its lymphatic channel. Axillary region was examined for the presence of any other palpable rigid lymph node. Sentinel node was sent

to the pathology laboratory for frozen section examination. Axillary dissection was performed in case of detection of metastasis in sentinel node. The surgeon did the appropriate breast surgery such as lumpectomy or mastectomy.

Data analysis

The patients were divided into the two groups according to the existence of sentinel lymph node metastasis in the definitive histopathology reports or not. Patients with metastasis in the sentinel lymph node were named as Group 1. Patients with the benign sentinel lymph node were named as Group 2. Patients' ultrasound reports and histopathological data including frozen section reports, tumour type, tumour size, and axillary lymph node status were

Tab. 1. Clinical and pathological characteristics of patients.

	SLN (+) (n=38)	SLN (-) (n=63)	p value
Mean age (years)†	54.3±12.1	52.9±12.9	0.575
Age §			
– <40 years (n=15)	4 (10.5%)	11 (17.5%)	0.342
– >40 years (n=86)	34 (89.5%)	52 (82.5%)	
Tumour size (cm)‡	2.31±1.07	1.92±0.98	0.086
SLN size (cm)‡	1.57±0.75	1.35±0.64	0.044*
Tumour site§			
– left	25 (65.7%)	28 (44.4%)	0.037*
– right	13 (34.3%)	35 (55.6%)	
Histologic type§			
– invasive Ductal CA	35 (92.1%)	44 (69.8%)	0.029*
– invasive Lobular CA	3 (7.9%)	7 (11.1%)	
– invasive Mucinous CA	–	3 (4.8%)	
– DCIS	–	9 (14.3%)	
SLN Frozen section‖,§			
– positive	35 (92.1%)	2 (3.2%)	<0.01**
– negative	3 (7.9%)	61 (96.8%)	r:0.894
ER status§			
– positive	33 (86.8%)	53 (84.1%)	0.710
– negative	5 (13.2%)	10 (15.9%)	
PR status§			
– positive	33 (86.8%)	47 (74.6%)	0.142
– negative	5 (13.2%)	16 (25.4%)	
HER-2 status§			
– Positive	14 (36.8%)	26 (41.3%)	0.659
– Negative	24 (63.2%)	37 (58.7%)	
ER percentage §			
– 0	5 (13.2%)	10 (15.9%)	0.800
– 1–10%	5 (13.2%)	6 (9.5%)	
– 11–33%	2 (5.3%)	6 (9.5%)	
– 34–66%	8 (21%)	9 (14.3%)	
– 67–100%	18 (47.3%)	32 (50.8%)	
Allred Score (0–8) (Mean±SD)‡	5.1±2.5	5.2±2.7	0.514
Molecular Subtypes§			
– luminal A/B group	33 (86.9%)	53 (74.2%)	0.839
– HER-2 group	2 (5.3%)	8 (12.6%)	0.079
– triple negative group	3 (7.8%)	2 (3.2%)	0.823
Ki-67 percentage (Mean±SD)‡	20.5±13.1	16.4±18.8	0.01*
Ki-67 Classification§			
– >14%	27 (71.1%)	25 (39.7%)	0.002**
– <14%	11 (28.9%)	38 (60.3%)	

SLN – Sentinel lymph node, ER – Estrogen receptor, PR – Progesterone receptor, r – Spearman's rho correlation value, ‡ – Mann–Whitney Utest, § – Pearson Chi-Square, † – T-Test, ‖ – Sperman's rho correlation, * – significant at the 0.05 level, ** – significant at the 0.01 level.

reviewed retrospectively. Immunohistochemical (IHC) staining was performed for ER, PR, HER-2 and Ki-67 for each patient. The Allred scoring system was used to evaluate ER status (8). The percentage and intensity scores were revealed, and tumour cells with a total score of 3–8 were considered ER and PR positive. Scores of 0 and 1 were considered negative for HER-2 expression according to IHC and score 3 was considered as positive. In case of HER-2 score 2, FISH analysis were performed. While assessing the Ki-67 expression, values > 14 % were considered as positive as suggested by St. Gallen (4, 9).The mean tumour size, mean size of sentinel lymph node and number of total metastatic lymph nodes was compared according to Ki-67 proliferation index of patients. We also performed subtype classification by IHC based on ER, PR and HER-2 status and Ki-67 expression.

SPSS software version 20.0 (SPSS Inc., Chicago, IL) was used to analyze the data of this study. Descriptive statistics was applied in relevant parameters. Distributions of the numerical variables were examined by histograms and Shapiro–Wilk test. Where appropriate, comparisons of categorical variables were performed using the chi-squared test and continuous variables with median or mean values were compared using the Mann–Whitney U test. Spearman's rho correlation coefficient was used for the correlation analysis between the parameters. The results were reviewed in the confidence interval of 95 %, and the value $p < 0.05$ was considered statistically significant.

This study was approved by the Scientific Ethics Committee of the Istanbul Medeniyet University, School of Medicine.

Results

Totally, 187 breast cancer patients underwent surgical treatment in this process. Clinically negative axillary lymph node was detected in 101 of the 187 patients and included in the study to evaluate the risk and predictive factors for SLN metastasis. The mean age of the patients was 53.4 ± 12.6 years and the mean tumour size was 2.07 ± 1.03 cm. In 38 (37.6 %) patients, the SLN metastasis was shown in the final pathological examination and so underwent axillary lymph node dissection (Group 1). SLN biopsy was detected benign in 63 (62.4 %) patients (Group 2). Range of harvested SLN was 1–4 lymph nodes in each surgery (mean; 1.51 ± 0.72).

The mean age of patients in Group 1 was 54.3 ± 12.1 years, while those in Group 2 was 52.9 ± 12.9 years (p = 0.575). We subcategorized the patients in terms of younger and older than 40 years. Four (10.5 %) of the patients in Group 1 and 11 (17.5 %) of the patients in Group 2 were younger than 40 years (p = 0.342). The mean tumour sizes of Group 1 and Group 2 were 2.31 ± 1.07 cm and 1.92 ± 0.98 cm, respectively (p = 0.086). Univariate analysis showed that there were no significant differences between Group 1 and Group 2 according to demographic data like age and tumour size (Tab. 1). Likewise, there was no significant difference between the groups according to IHC staining features, such as ER, PR and HER-2 receptor status, Allred scoring system, ER staining percentages and molecular subtypes (Tab. 1).

In fifty-three (52.5 %) of the patients, the tumour was on the left breast, and in 48 (47.5 %) on the right breast. Twenty-five (65.7 %) of the left side tumours and 13 (34.3 %) of the right side tumours had SLN metastasis. SLN metastasis were detected statistically significantly higher on the left side tumours than right side tumours (p = 0.037). SLN metastasis were detected statistically significantly higher in patients with invasive ductal carcinoma than other histologic types (p = 0.029).The mean size of the sentinel lymph node in Group 1 was statistically significantly higher than in Group 2 (1.57 ± 0.75 cm versus 1.35 ± 0.64 cm, respectively, p = 0.044). The mean Ki-67 proliferation index of patients in Group 1 was detected 20.5 ± 13.1, while it was detected 16.4 ± 18.8 in Group 2. The mean Ki-67 value was statistically significantly higher in Group 1 than in Group 2 (p = 0.01) (Tab. 1).

According to the suggestion of St Gallen, patients were subcategorized in terms of Ki-67 values that were higher or lower than 14 %. Twenty-seven (71.1 %) of the patients in Group 1 had > 14 % Ki-67 expression while it was 25 (39.7 %) in Group 2. SLN metastasis was detected statistically significant higher in patients with > 14 % Ki-67 (p = 0.002). The mean tumour size was determined statistically significantly higher in patients with > 14 % Ki-67 proliferation index (2.26 ± 0.90 cm versus 1.86 ± 1.12 cm, p = 0.01). Number of metastatic lymph nodes was compared and significant difference was detected between Ki-67 subgroups. The mean metastatic lymph node number was 2.15 ± 5.04 in patients with > 14 % Ki-67 proliferation index, while it was 0.51 ± 1.50 in patients with < 14 % Ki-67 proliferation index (p = 0.004). Ki-67 values higher than 14 % were statistically significant and positively correlated with SLN positivity (r = 0.258), tumour size (0.222) and number of metastatic lymph nodes (r = 0.224) (Tab. 2, Fig. 1).

We analyzed the sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of the ultra-

Tab. 2. Tumour and axillary lymph node features according to Ki-67 subgroups.

	Ki-67 > %14 (n:52)	Ki-67 < %14 (n:49)	p value	r value
SLN size†	1.59±0.79	1.27± .53	0.079	–
Tumour size†	2.26±0.90	1.86±1.12	0.01**	0.222*
SLN‡				
– Positive	27 (71.1%)	11 (28.9%)	0.002**	0.258*
– Negative	25 (39.7%)	38 (60.3%)		
Number of Metastatic Lymph Nodes†	2.15±5.04	0.51±1.50	0.004**	0.224**

SLN – Sentinel lymph node, † – Mann Whitney Utest, ‡ – Pearson Chi-Square, r – Spearman's rho correlation coefficient, * – correlation is significant at the 0.05 level, ** – correlation is significant at the 0.01 level
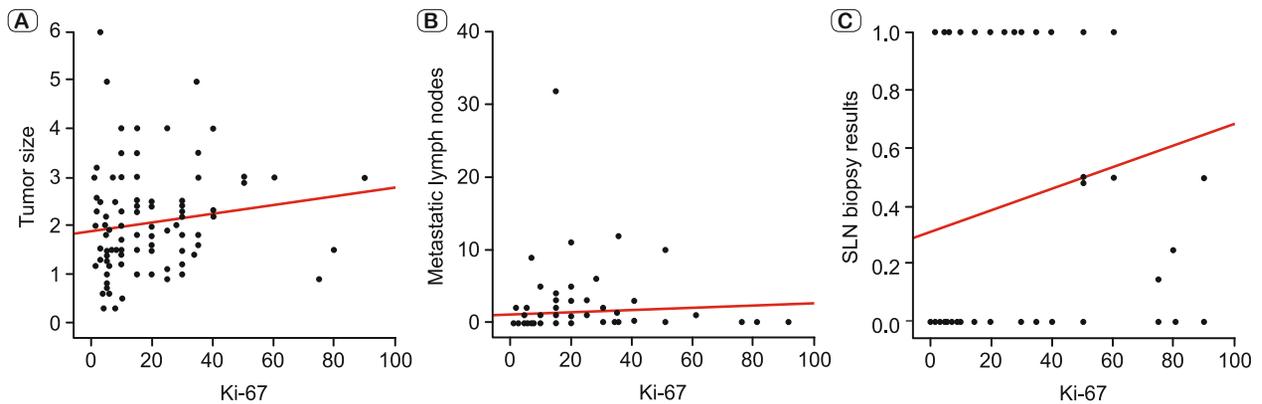
Fig. 1. Scatter-plot graphics; A – correlation between Ki-67 values and tumour size, B – correlation between Ki-67 values and number of metastatic lymph nodes, C – correlation between Ki-67 values and SLN biopsy results

sonography (USG), frozen section (FS), magnetic resonance imaging (MRI) and Ki-67 expression percentages to compare the diagnostic significance of these methods to detect the metastatic axillary lymph node in this specific patient group (Tab. 3). We revealed that FS had the highest diagnostic significance among these methods. Statistical analysis showed FS results had a statistically significant and positive correlation relationship (r = 0.894) with definitive histology reports of SLN (Tab. 1). We determined that Ki-67 expression ratio had similar predictive significance with USG and MRI examination in terms of detecting SLN metastasis in patients with clinically negative axilla.

Discussion

SLN biopsy has provided a decreased morbidity and it has become the standard of care for breast cancer management (10). It continues to be an issue of an extensive study in the management of breast cancer since it was first defined in 1997 (11). SLN metastasis rate is about 30–35 % in patients with early stage breast cancer (12), and it was reported that 40–70 % of patients with metastatic SLN were free of metastasis in non-SLNs (13). Therefore, prediction of SLN status preoperatively with biomarkers or histopathological features of the tumour may allow us to avoid the unnecessary SLN biopsies in selected patients. Nomogram is a predictive model used to identify sentinel or additional lymph node metastasis at several facilities. Nomograms and other scoring systems are based on patients' clinicopathological data and performed preoperatively (14, 15). In this study we analyzed the factors that were related to patients and tumour characteristics, and their predictive or causing effects on the SLN metastasis.

ALND and SLN biopsy provide postoperative staging of the disease, therefore preoperative diagnostic modalities have significance to assess the axilla in breast cancer patients, especially in patients being prepared for SNL biopsy. Nowikiewicz (3) et al revealed that USG scan is the most available, the cheapest, and the repeatable method if performed in the period directly preceding SLN biopsy. If performed more than 4 weeks before surgery, false negative results increased and diagnostic significance decreased. We found the false negative results in 10/44 (22.7 %) patients. Various studies showed that USG, computed tomography and MRI have sensitivities 72–78 %, 78 % and 67 % and specificities 77–78 %, 75 % and 78 %, respectively to detect the metastatic lymph node in breast cancer patients (16). Flurodeoxyglucose positron emission tomography (PET/CT) was evaluated in various studies

Tab. 3. Sensitivity, specificity, PPV and NPV of the diagnostic methods in terms of detecting the metastasis of SLN in patients with clinically negative axilla.

	Group 1	Group 2	Sensitivity	Specificity	PPV	NPV
USG						
– suspicious lymph metastasis	29	28	76.3%	55.5%	50.9%	79.5%
– benign lymph nodes	9	35				
MRI						
– suspicious lymph metastasis	16	23	72%	50%	41%	71.9%
– benign lymph nodes	9	23				
Frozen section						
– malign SLN	35	2	92.1%	96.8%	94.6%	95.3%
– benign SLN	3	61				
Ki-67 expression						
– >14%	27	25	71.1%	60.3%	51.9%	77.6%
– <14%	11	38				

USG – Ultrasonography, MRI – Magnetic resonance imaging, PPV – Positive predictive value, NPV – Negative predictive value, SLN – Sentinel lymph node

for detecting the axillar metastasis. It was revealed less sensitive (20–30 %) but more specific (> 95 %) than other modalities (17). In this study, USG and MRI sensitivity was similar to literature (76.3 % and 72 %, respectively), but specificities were lower than literature (55.5 % and 50 %, respectively).

Morrison (18) et al revealed that young patients demonstrated more aggressive features and worse outcomes when compared to older patients, and Han (19) et al supported that young age was an independent risk factor for relapse. We analyzed and compared the mean ages and subgroups of young (< 40 years) and older (> 40 years) patients according to SLN metastasis, significant difference was not detected between Group 1 and Group 2.

There is no data available in literature that SLN metastasis is more common in left or right breast cancers. But in this study, we revealed a statistically significantly higher SLN metastasis in left side breast cancers (p < 0.05).

Wu (20) et al reported in their studies including 1325 patients, that the mean tumour size was 2.9 cm in SLN positive group, while it was 2.1 in SLN negative group. They revealed a statistically significant difference between the groups. In the present study, the mean tumour size of Group 1 was larger than in Group 2 without a statistically significance (p > 0.05).

HER-2-positive and triple negative breast cancers are more likely to exhibit higher metastatic lymph node involvement and worse survival, compared to luminal-like (luminal-A and luminal-B) tumours (21). Kumar (22) et al revealed that triple negative breast cancers are associated with younger age, higher grade and advanced stage at diagnosis. Dunnwald (23) et al compared ER+/PR+, ER+/PR–, ER–/PR+ and ER–/PR– breast cancer patients, and they found that SLN metastasis was statistically significantly higher in ER–/PR+ and ER–/PR– patients than in others. In the study by Crawford (24) et al, the authors reported the hormone receptor status of breast cancer patients. According to this study, 83.5 % of ER, 76.4 % of PR, and 34.4 % of HER-2 were positive in patients with SLN metastasis. Ratios were 83.0 %, 78.5 % and 21.8 %, respectively, in patients with negative SLN biopsy. They found statistically significantly higher HER-2 positivity in SLN positive group. We did not find significant difference between the groups according to molecular subtype categories or hormone receptor status in patients with clinical negative axilla.

Allred scoring system is a clinical instrument based on the percentage of cells that stain by IHC for ER (on a scale of 0 to 5) and the intensity of that staining (on a scale of 0 to 3, for a possible total score of 8). Mohammed (25) et al revealed that patients with higher Allred score have longer disease free survival. Water (26) et al found the similar results that higher Allred scores related with better survival rates. We compared the Allred scores according to existence of metastasis in SLN biopsy. There was no significant difference between the patients in Group 1 and Group 2.

Ki-67 was found to be universally expressed among proliferating cells and absent in quiescent cells, making it ripe for evaluation as a tumour proliferation biomarker (27). It is well documented that tumour cell proliferation by Ki-67 expression is strongly associated with breast cancer prognosis (7). Inic (28) et al considered the cut-off at 14 % for Ki-67, and they showed that higher Ki-67

values were related with the axillary lymph node involvement. Likewise, Matsonneuve (29) et al determined that higher Ki-67 values than 14 % were related to worse prognosis, axillary and distant metastasis. In our study, we revealed statistically significantly higher Ki-67 expression values in patients with metastatic SLN than in patients with benign SLN. Shokouh (30) et al. revealed that higher Ki-67 index tumours showed more HER-2 overexpression, larger size and more lymph node involvement. Also, we have performed the subgroup analysis in terms of Ki-67 proliferation index value. Higher Ki-67 values were statistically significantly related and positively correlated with larger tumour size and increased number of metastatic lymph nodes. Recent studies and some of the guidelines suggested omitting the axillary dissection in selective patients even if SLN biopsy was positive (31). Ki-67 proliferation index ratio and size of SLN can play an important role on decision-making to perform axillary dissection or not.

This study showed that larger size of the sentinel lymph node and higher Ki-67 values, especially > 14 % were related with metastatic SLN. We thought that addition of these criteria to USG examination might provide a higher prediction about the axillary lymph node involvement in patients with clinically negative axilla. Also, correlation between higher Ki-67 values and non-sentinel lymph node involvement is an important factor in the clinical management of this patient group.

Learning points

1) Ki-67 proliferation index higher than 14 % is an important cut-off point for Turkish breast cancer patients, likewise presented in some international studies.
2) The larger size of SLN and Ki-67 proliferation index > 14 % may be provide the prediction of existence of metastatic SLN and increased number of metastatic lymph nodes in breast cancer.

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