Original Article

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Sentinel Lymph Node Study and Its Relationship with Molecular Profile in Breast Cancer After Neoadjuvant Therapy

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Aim: Lymphoscintigraphy (LS)/sentinel lymph node (SLN) study is one of the controversial issues in patients with breast cancer who have undergone neoadjuvant therapy (NAT). The aim of our study is to investigate the value of LS/SLN study after NAT and to evaluate its relationship with the molecular profile in breast cancer.

Methods: This retrospective study included 59 female patients diagnosed with breast cancer who received NAT. Tumor biopsy pathology results were recorded. Following NAT, LS was performed, and SLN biopsy (SLNB) was conducted intraoperatively using a dual-tracer technique (methylene blue and radionuclide). Axillary lymph node dissection (ALND) was performed in selected cases. Intraoperative frozen results and ALND findings were documented. Logistic regression analysis was performed to identify the factors predicting SLN positivity in the study.

Results: Among patients with SLN detected in the SLN study, 15 (28.8%) had positive results on intraoperative frozen pathology. A significant relationship was found between SLNB positivity and human epidermal growth factor receptor-2 (HER2) status (p=0.003). Both univariate and multivariate analyses conducted to identify the factors predicting SLNB positivity; HER2 negativity (p=0.004) was determined to be an independent risk factor for SLNB positivity. HER2 negativity increased the risk of SLNB positivity by a factor of 34.1.

Conclusion: In the HER2 negative group, LS/SLN study alone may be insufficient, and ALND should be considered in selected cases. On the other hand, it has been shown that LS/SLN study can avoid unnecessary ALND in patients with HER2 positivity.

Keywords: Breast cancer, sentinel lymph node, lymphoscintigraphy, neoadjuvant therapy

Introduction

The incidence of early-diagnosed breast cancer is increasing worldwide, including in our country, largely due to advances in diagnostic methods. According to 2020 data, breast cancer is the most commonly diagnosed cancer among women and ranks as the fifth cause of cancer-related deaths [1]. Breast cancer is a heterogeneous and complex disease, a spectrum of many subtypes with distinct molecular-biological features that lead to differences in response patterns to various

treatment modalities [2]. For this reason, various diagnostic techniques and novel treatment modalities for breast cancer are continually being developed.

Locally advanced breast cancer (LABC) is a subset of breast cancer characterized by T3-4 tumours in the absence of distant metastasis with/without regional lymphadenopathy involvement [3]. Regional metastatic lymph nodes (MLN) are very important for the prognosis of LABC. In a retrospective study, it was demonstrated that LABC patients with 10 or more regional MLN have a poor prognosis [4].

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Therefore, neoadjuvant therapy (NAT) in LABC aims to not only reduce the size of the primary tumor but also convert the regional lymph node-positive disease to a negative state. According to the current National Comprehensive Cancer Network guideline, sentinel lymph node biopsy (SLNB) may be considered after NAT for a selected group of patients. These are patients with clinically positive lymph nodes (cN+) at baseline that become cNO after NAT. Highly selected patients with biopsy-proven axillary metastases, who convert to clinically node negative after preoperative systemic therapy, may undergo SLNB with removal of the clipped lymph node (category 2B recommendation) [5]. Although different treatment response rates are observed in tumors with different molecular profiles [estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and Ki67 proliferation index], both in tumor and lymph nodes after NAT, an overall pathological complete response (pCR) rate of 40% has been observed [6,7]. In a study, it was found that patients with HER2 positive breast cancer exhibited the highest rates of breast-conserving surgery and pCR after NAT when compared to patients with other molecular profiles [8].

The sentinel lymph node (SLN) is defined as the first lymph node or group of nodes to which cancer cells are most likely to spread from the primary tumor via lymphatic channels [9]. SLNB is a reliable method for detecting metastatic disease in regional lymph nodes and avoiding patients the potential morbidity associated with axillary lymph node dissection (ALND). In the GANEA-2 study, breast cancer patients with negative SLN treated with NAT could safely be spared an unnecessary ALND after NAT with a low-risk of relapse [10]. SLNB can be effectively performed through a combination of preoperative lymphoscintigraphy (LS) and intraoperative methylene blue injection. The decision to proceed with ALND is typically based on frozen section results and intraoperative assessment [11].

Although it is known that tumors with different molecular profiles have varying rates of pCR after NAT, there is limited research on the role of SLN study and its relationship with the molecular profile after NAT. Our study aimed to investigate the role of LS/SLN study, and its relationship with the molecular profile in LABC patients after NAT.

Methods

This retrospective study was conducted according to the principles of the Declaration of Helsinki, and approval was obtained from the Ethics Committee of University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (decision no: 2022-02/46, date: 10.02.2022).

Fifty-nine female patients who were diagnosed with LABC through clinical-radiological and histopathological evaluations were included in this study.

The molecular profiles of all patients were documented and they underwent NAT (chemotherapy and/or immunotherapy)

between January 2019 and December 2021. The demographic characteristics, clinical-radiological findings, results of axillary lymph node biopsy prior to NAT, and pre-NAT tumor biopsy pathology (grade, Ki67 proliferation index, ER-PR, and HER2 expression status) of the patients were recorded. The patients received neoadjuvant treatment consisting of alkylating agents (cyclophosphamide), anthracycline derivatives (doxorubicin), and taxane group agents (paclitaxel or docetaxel) chemotherapeutic agents. In cases of HER2 positivity, monoclonal antibodies (trastuzumab or trastuzumab+pertuzumab) were added to the treatment regimen.

Lymphoscintigraphy

LS was performed in patients at least 2-3 hours before the surgery or on the day prior to the surgery to identify the SLN. For LS, 37 MBq of 99mTc-labeled nanocolloid was injected intradermally into four quadrants around the tumor or the periareolar area. Additionally, one deep injection was administered intraparenchymally or peritumorally. After the injections, dynamic imaging was performed in two planes (anterior and lateral) using a 64x64 matrix, with a frame duration of 30 seconds , and lasting a total of 15 minutes. Subsequently, static or planar imaging was performed in two planes using a 256x256 matrix. Additional images were acquired if necessary.

Sentinel Lymph Node Study

At the beginning of surgery, methylene blue was injected into the subareolar region. SLNB was performed using both methylene blue dye and a gamma probe. Excised SLNs were referred to the pathology department for frozen examination. Axillary dissection was performed in selected patients based on the MLN' frozen section examination results (SLNB positivity), in cases where the SLN could not be identified and/or intraoperative suspicion. Frozen/biopsy results, ALND outcomes, and pathological nodal (pN) stages of the patients were recorded.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). The conformity of numerical variables to the normal distribution was evaluated with the Kolmogorov-Smirnov test. Descriptive statistics in the analyses are presented as numbers and percentages; distribution statistics were expressed as mean, standard deviation, median, minimum, and maximum values. The Pearson chi-square test was used to evaluate whether there was a difference in clinicopathological features between SLNB positive/negative groups. The Youden index (sensitivity+specificity-1) was used in the receiver operating characteristic (ROC) analysis to determine the threshold value for primary tumor size and Ki67 index. Logistic regression analysis was performed to determine the factors predicting SLNB positivity in the LS/SLN study. Logistic regression analysis was performed using the forward method. An overall 5% type I error level was used to infer statistical significance (p<0.05).

Results

In our study, the mean age of the patients was 47.9±10.5 years (range 25-66). The clinical-pathological characteristics of the patients are presented in Table 1.

Eleven of the patients (18.6%) received injections around the lesion, while the remaining 48 (81.4%) received superficial and deep injections in the periareolar area. On LS, axillary drainage was observed in 34 patients (57.6%) on dynamic imaging. Focal activity involvement was observed in 40 patients (67.7%) in the early static images and in 12 patients (10.3%) in the late static images. SLN was detected in 52 (88.1%) patients, while it could not be identified in 7 (11.9%) patients either at LS or during operation. The average number of SLNs extracted was 4 (range 3-12). The SLNB results showed that 15 (28.8%) patients had positive SLN, while 37 (71.2%) patients had a negative SLN. ALND was performed in 31 (52.5%) patients. These were patients who had SLNB positivity or who could not have their SLN identified, or who had intraoperative suspicion. The rate of ALND was 23% in HER2 positive patients and 83% in ER positive/HER2 negative patients. Among the patients who underwent ALND, 19 (61.2%) had positive lymph nodes and 12 (38.8%) had negative lymph nodes. The pathological N stage was determined as pN0 in 35 (59.3%) patients, pN1 in 12 (20.3%) patients, pN2 in 10 (16.9%) patients, and pN3 in 2 (3.4%) patients.

A statistically significant association was found between SLNB positivity and HER2 receptor status (p=0.003). Among the 15 patients with positive SLN, only 1 (6.6%) patient was HER2 positive, 12 (80%) patients were ER positive/HER2 negative, and 2 (13.4%) patients were triple negative (Table 2).

ROC curve analysis revealed that Ki67 proliferation indices have a statistically significant diagnostic value in predicting SLNB positivity (area under the curve: 0.650, 95% confidence interval: 0.486-0.815, p=0.042). A statistically significant difference was found between SLNB positivity and Ki67 index with a cut-off of 55% (p=0.034) (Table 2). There was no statistically significant difference among SLNB positivity, tumor localization (p=0.513), tumor size (p=0.404), clinical axilla status (p=0.097), ER status (p=0.198), PR status (p=0.070), and tumor grade (p=0.154).

The results of the univariate and multivariate analyses conducted to identify factors predicting SLNB positivity are shown in Table 3. In the univariate analysis, a statistically

Clinical-pathological features			n (%)/Average±SD	
				29.6±12.2
Primary tumor size	≤27 mm		33 (55.9%)	
	>27 mm			26 (44.1%)
	Upper-outer quadrant			35 (59.3%)
	Lower-outer quadrant			12 (20.2%)
Localization of tumor	Upper-inner quadrant			5 (8.5%)
	Lower-inner quadrant			1 (1.7%)
	6 o'clock position			5 (8.5%)
	12 o'clock position			1 (1.7%)
		1		3 (5.2%)
	Grade	2		16 (27.6%)
		3		39 (67.2%)
	ER positivity			45 (77.6%)
Pathological features of primary tumors	PR positivity			38 (65.5%)
	HER2 positivity			21 (35.6%)
				45.5±23.7
	Ki67 prolife	ration index	≤55%	40 (67.7%)
			>55%	19 (32.2%)
	Clinical positive			19 (32.2%)
xillary LN status before NAT	Clinical negative-radiological positive			33 (56%)
	Clinical-radiological negative			7 (11.8%)
Result of axillary LN biopsy before NAT	Positive			18 (90%)
(n=20)	Negative			2 (10%)

n: The number of patients, LN: Lymph node, NAT: Neoadjuvant therapy, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor-2, SD: Standard deviation

significant association was found between SLNB positivity and patient age (p=0.027), HER2 negativity (p=0.013), and Ki67 index ≤55% (p=0.047). Additionally, due to the p value of PR positivity being nearly 0.05, it was included in the multivariate analysis. In the multivariate analysis, advanced age, PR positivity, and HER2 negativity were identified as independent risk factors for predicting SLNB positivity. PR positivity increases the risk of SLNB positivity by approximately 6.6 times, while HER2 negativity increases the risk of SLNB positivity by 34.1 times (Table 3).

The result of ALND was positive in 6 of 7 patients whose SLN could not be detected in the SLN study. Moreover, the result of ALND was positive in 3 patients whose SLN biopsy was negative (false negative). In these 9 patients with positive ALND, it was found that 2 (22.2%) were HER2 positive, and 7 (77.8%) were ER positive/HER2 negative. In addition, 8 patients (88.8%) had a Ki67 index ≤55%.

Discussion

The field of NAT in breast cancer is actively researched, and the literature is continually expanding with over 1,000 studies in the last year alone. As a result, our knowledge and insights about breast cancer are constantly evolving, with new discoveries and improvements being made every year. In certain cases of LABC, NAT can achieve pCR in the primary tumor and the axillary region. This successful response to NAT allows some patients to avoid ALND using the LS/SLN study and SLNB. However, in patients who do not achieve pCR, it can be challenging to detect the SLN and perform SLNB due to the potential blockage of lymphatic pathways by tumor cells. Although it is known that different pCR rates are seen in different molecular profiles after NAT, there are not enough published studies to provide definitive evidence regarding the relationship between the SLN study and the molecular profile.

Table 2. SLNB dis	tribution accor	ding to HER2 recepto	or status and Ki67 inc	lex	
Negative		SLNB		p value	
		Positive	Total		(Chi-square test)
LIED2 recentor	Negative	18 (56.3%)	14 (43.7%)	32 (100%)	p=0.003
HER2 receptor	Positive	19 (95%)	1 (5%)	20 (100%)	p=0.003
Ki67 index	≤55%	20 (60.6%)	13 (39.4%)	33 (100%)	- 0.034
	>55%	16 (88.9%)	2 (11.1%)	18 (100%)	p=0.034
SLNB: Sentinel lymph	node biopsy, HER2	: Human epidermal growtl	h factor receptor-2		

Clinical-pathological features	Univariant analysis			Multivari	Multivariant analysis		
	OR	95% CI	p value	OR	95% CI	p value	
Age	1.076	1.008-1.147	*p=0.027	1.087	1.007-1.172	p=0.032	
Right/left breast			p=0.686				
Quadrant of the tumor			p=0.972				
Radiologic size of the tumor			p=0.302				
BIRADS			p=0.166				
Axillary LN status before NAT			p=0.999				
Results of axillary LN biopsy before NAT			p=0.999				
ER positivity			p=0.212				
PR positivity	3.579	0.861-14.871	**p=0.079	6.653	1.181-37.494	p=0.032	
HER2 negativety	14.778	1.758-124.194	*p=0.013	34.154	3.171-367.832	p=0.04	
Grade			p=0.261				
Ki67 index (≤%55)	5.200	1.021-26.471	*p=0.047				
Pathologic size of the tumor, after NAT			p=0.825				

^{*}p<0.05: statistically significant parameters.

SLNB: Sentinel lymph node biopsy, OR: Odd ratio, CI: Confidence interval, BIRADS: Breast imaging reporting and data systems, LN:Lymph node, NAT: Neoadjuvant therapy, ER:Estrogen receptor, PR: Progesterone receptor, HER2: Human epitelyal growth factor receptor-2

^{**}It was included in the multivariate analysis because the p value was 0.05.

Furthermore, there are no recommendations associated with the molecular profile in the current SLNB guidelines. In our study, we investigated the value of LS/SLN study after NAT in breast cancer and its relationship with the molecular profile.

In breast cancer, several studies have demonstrated high rates of pCR in HER2 positive tumors after NAT, while lower pCR rates have been observed in ER positive/HER2 negative tumors [12-18]. In a recent study, the total pCR rate in HER2 positive and ER positive/HER2 negative profiles was 32.3% and 6.9%, respectively, while the ALND rate was 13.3% and 28.8%. The study concluded that SLNB can be performed before NAT in ER positive/HER2 negative tumors, while HER2 positive tumors show a good response to targeted therapy, and SLNB can be performed after NAT to avoid unnecessary morbidity associated with ALND [12]. In our study, we found that the rate of ALND was 23% in patients with HER2 positive breast cancer, while it was 83% in patients with ER positive/HER2 negative breast cancer. We observed low rates of pCR and high rates of SLNB positivity in the ER positive/HER2 negative patient group. Conversely, we found high rates of pCR and SLNB negativity in the HER2 positive patient group. Furthermore, it was noted that HER2 negativity increased the risk of SLNB positivity by approximately 34.1 times.

In our study, we found that PR positivity was an independent risk factor for SLNB positivity, increasing the likelihood of SLNB positivity by 6.6 times. Davey et al. [19] reported pCR rates of 10.1% in patients with a PR positive profile and 18% in patients with a PR negative profile. Other studies have also observed that PR negativity is associated with higher pCR rates in the ER positive/HER2 negative patient group [17,20,21]. In PR positivity, (considering the increased probability of SLNB positivity and the lower pCR rates after NAT), ALND should not be disregarded in selected patients.

In our study, we found a significant correlation between Ki67 index ≤55% and SLNB positivity, with the majority of false negatives observed in patients with Ki67 index ≤55%. Wang et al. [22] demonstrated that tumors with a high Ki67 index (≥14%) had a higher likelihood of achieving pCR compared to those with a low Ki67 index (<14%). In another study, Ki67 index <50% predicted higher risk of residual lymph node disease [18]. Based on our study findings, it was suggested that an LS/SLN study should be performed in patients with a Ki67 index ≤55%, and ALND should be considered even if SLNB results are negative.

In elderly patients, visualizing the SLN in LS can be challenging due to potentially slower lymphatic drainage. Studies have indeed demonstrated a lower rate of SLN detection in advanced-age patients [23-25]. In our study, we found that SLNB positivity increased with patients' age. Another study highlighted the importance of individualizing SLNB in patients over 70 years of age, considering that axillary staging may not always be beneficial for survival. However, if performed, SLNB can help improve local control, provide prognostic information, and guide decisions regarding adjuvant therapies like chemoradiotherapy [26]. Therefore, even though it can be difficult to detect the SLN in advanced age patients, SLNB is believed to

have potential benefits for patient management where it may impact survival outcomes.

Study Limitations

Several limitations should be considered in our study. First, this study was a retrospective analysis, and it was limited by the small size of our study population. Secondly, not perform axillary dissection to all patients. Thirdly, due to the absence of follow-up data for the patients, the long-term impact of the study remains unknown.

Conclusion

In conclusion, our study demonstrated that SLNB may not reduce the need for ALND in patients with an ER+/HER2 negative profile. However, in patients with a HER2 positive profile, SLNB can potentially reduce unnecessary ALND. Further prospective studies with larger patient populations can provide valuable insights into optimizing the use of SLNB in managing breast cancer patients undergoing NAT and its relationship with clinical, histopathological, and molecular profiles.

Ethics

Ethics Committee Approval: This retrospective study was conducted according to the principles of the Declaration of Helsinki, and approval was obtained from the Ethics Committee of University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (decision no: 2022-02/46, date: 10.02.2022).

Informed Consent: The study was retrospective, consent was not obtained from the patients.

Footnotes

Authorship Contributions

Design: G.U., B.B.D., Desing: G.U., B.B.D., Data Collection or Processing: S.A., Analysis or Interpretation: S.A., S.G.A., Literature Search: S.A., Writing: G.U., S.A., B.B.D., S.G.A., E.B., C.Ö.

Conflict of Interest: The authors declare that they have no conflict of interest.

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