PREDICTIVE FACTORS FOR NON-SENTINEL LYMPH NODE INVOLVEMENT IN PATIENTS WITH BREAST CANCER

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Abstract. Introduction: Sentinel lymph node biopsy (SLNB) is accepted as a substitute method for axillary staging instead of axially lymph node dissection in patients with negative lymph node. The NSLN involvement is correlated with primary tumor and SLN characteristics. So, the diagnosis of the patients with lower risk of NSLN metastasis will save them from axillary lymph node dissection (ALND)-related morbidities. This study aimed to determine the predictive factors for NSLN involvement in breast cancer patients with positive SLN. **Materials and Methods**: This cross-sectional study was performed on 482 patients with breast cancer and SLN involvement, referring to Rasoul Akram and Khatam-al-Anbia Hospital hospitals, Tehran, Iran, during 2010-2017. 6 hours up to one day before the surgery, the patients received a periareolar injection of radioactive materials. Then, all hot lymph nodes with suspected cancer invasion were resected. The SLN radionuclide-avid were subjected to frozen section analysis. Axillary dissection was performed if metastases or even micrometastases were reported in nodes. **Results**: Overall, 66.5% and 33.5% of the patients had positive and negative SLNs, respectively. The results showed a significant relationship between lymph node involvement in the patients with breast cancer and SLN in term of non-SLN invasion ($\chi 2=4.62$; $P<0.005$). There was also a significant relationship with lymphovascular invasion (LVI) ($\chi 2=107.4$; $P<0.005$), perineural involvement ($\chi 2=32.27$; $P<0.005$), and extra capsular lymph node involvement ($\chi 2=48.01$; $P<0.005$). 59.3% of the patients with negative HER2-enriched (16 out of 27 cases) had lymphovascular involvement. there was no significant relationship between positive/negative Ki67 with gender and age ($P>0.005$). **Conclusion:** As the findings indicated, the patients with involved lymph node, a tumor size of > 2 cm, and high tumor stage have a significantly higher probability of metastasis progression to NSLN; therefore, they are suggested to undergo ALND.
Keywords: SLNB; Axillary dissection; Non-sentinel

Introduction. The SLNB is the standard of care for the axillary staging of breast cancer with negative node [1]. Today, breast cancer screening methods contribute to the early diagnosis of lymph node involvement prior to clinical examinations. Axillary dissection provides data about axillary node involvement and is considered as a trustable marker for the prediction of the prognosis and staging of breast cancer [1]. However, this approaches accompanied by some complications, such as seroma, lymph edema, and shoulder function disruption, in 6-30% of patients. In 1991, sentinel lymph node biopsy (SLNB) was accepted as an alternative method for axillary staging in patients with negative lymph node [2].

Based on the evidence, in 40-70% of breast cancer patients, SLNs are the only involved nodes without the interference of non-sentinel lymph nodes (NSLNs) [3]. The node-negative breast cancer patients undergo axillary lymph node dissection (ALND) without gaining any benefits or obtaining data on their disease prognosis; moreover, they are affected by surgical complications [4]. The diagnosis of the patients with lower risk of NSLN metastasis will save them from ALND-related morbidities. The NSLN involvement is correlated with primary tumor and SLN characteristics [5].

Breast cancer surgeries can even facilitate the reduction of primary tumor with lymphatic invasion. Many researchers suggest nomograms and scoring systems (with the factors affecting tumor and sentinel node) for the prediction of NSLN metastasis probability. Some of the most widely used methods in this regard include Memorial Sloan Kettering Cancer Center (MSKCC)[6], Cambridge [7], Stanford [7], and Ten on [8] nomograms, as well as Anderson Cancer Center scoring [9]. With this background in mind, the present study aimed to determine the predictive factors for NSLN involvement in breast cancer patients with positive SLN.

Materials and Methods

This descriptive cross-sectional study was conducted on breast cancer patients with SLN involvement during 2010-2017. Out of 1,605 patients with breast cancer referring to Rasol-e Akram and Khatam-al-Anbia hospitals in Tehran, Iran, 505 cases with ultrasonography and clinical diagnosis of axillary lymph node involvement were subjected to ALND. The rests

of the patients (n=1,080) underwent SLN biopsy, 482 (481 females and 1 male) cases of whom who had positive axillary lymph node were included in the study.

The data recorded for each patient included age, cancer stage, histology, multifocality, lymphovascular invasion (LVI), invasion to capsule, SLN size, SLN count, estrogen receptor, progesterone receptor, and NSLN involvement. The patients received a periareolar injection of radioactive materials 6hours uo to one day before the surgery. Then, all hot lymph nodes with suspected cancer invasion were subjected to resection. Then The SLN were subjected to frozen section analysis. Axillary dissection was performed in case metastases or even micrometastases were reported in those nodes.

Statistical analysis

The normally and non-normally distributed variables were analyzed using t-test and Mann Whitney U test, respectively. Furthermore, the Chi-square test or Fisher's exact test was adopted to analyze the qualitative variables. Multivariate logistic regression analysis was run for confounding data expressed in odds ratio. The analysis of the relationship between variables was accomplished through Pearson and Spearman's rank order correlation coefficients. All data analyses were performed in SPSS, version 22.P-value less than 0.05 was considered statistically significant.

Results

Based on the obtained results, 99.6% of the patients were female. The mean age of the participants was obtained as 52.06±11.5 years (age range: 13-86 years). Furthermore, 1.5%, 42.6%, 47.9, and 8% of the patients were in the age groups of < 30, 30-49, 50-69, and ≥ 70 years respectively. The mean tumor size was 2.72±1.52 cm, and the mean number of positive lymph nodes was 2.59±4.64. Moreover, the mean number of lymph nodes was 9.31±6.18, which ranged within 0-33.

Overall, 66.5% and 33.5% of the patients had positive and negative SLNBs, respectively. The non-specified tumor was the most common type of tumors as observed in 62.7% (n=1,007) of the patients. Furthermore, classic tumor was the second common tumor type, which was observed in 9.8% (n=158) of the patients. Invasive ductal carcinoma (IDC) was the most commonly diagnosed pathology (58.7%) in breast cancer patients with SLN involvement, followed by IDC plus DCIS and invasive lobular carcinoma (ILC), respectively. 480 cases (56.8%) were positive in term of Ki67 while 365 ones (43.2%) were negative (table 1).

Table 1. Tumor characteristics

Variables		No	Percent	Variables		No	Percentage
Type of surgery	Subductal mastectomy	76	4.8	ANC	Yes	46	11.2
	MRM*	324	20.5		Unclear	23	5.6
	Breast preserving	1178	74.7		Not done	186	45.1
Tumor grade	None	53	3.5		Free	157	38.1
	One	128	8.5	Residual breast cancer	yes	15	4.4
	Two	721	48		Unclear	52	15.1
	Three	601	40		Not done	186	54.1
Nuclear grade	None	157	10.9		Free	91	26.5
	One	29	2.0	Fine needle aspiration	No	42	2.7
	Two	208	14.4		yes	1381	90.1
	Three	1051	72.7		Unclear	110	7.2
Multicentric tumors	No	1395	96.2	Side	Unclear	34	2.2
	Yes	55	3.8		Left	759	48.2

Perineural invasion	No	1228	80.0		Right	781	49.6
	Yes	253	16.5	Neoadjuvant therapy	No	1351	90.9
	Unclear	54	3.5		Yes	136	9.1
Lymphatic system invasion	No	965	62.0	Type of diagnosis	Unclear	57	3.7
	Yes	557	35.8		Open biopsy	51	3.3
	Unclear	34	2.2		frozen	268	17.4
Calcification	No	1184	77.4		FNA	10	0.6
	Yes	250	16.3		Core biopsy	1155	75
Necrosis	No	1010	66.1	P53	Ser[1]	67	15
	Yes	424	27.7		Ser[6]	242	54
	Unclear	94	6.2		Ser[9]	139	31
Stage of cancer	0	10	0.9	Tumor/node/metastasis	T0	10	0.9
	1	252	23.5		T1N0	226	21.4
	2A	325	30.3		T1N1	80	7.6
	2B	172	16.1		T1N2	33	3.1
	3A	144	13.4		T1N3	13	1.2
	3B	28	2.6		T2N0	270	25.6
	3C	124	11.6		T2N1	162	15.3
	4	16	1.5		T2N2	107	10.1
Ki67	Yes	480	56.8		T2N3	49	4.6
	No	365	43.2		T3N0	31	2.9
ER*	Positive	960	65.4		T3N1	20	1.9
	Unclear	135	9.2		T3N2	20	1.9
	Negative	373	25.4		T3N3	26	2.5
Sentinel	Positive	1011	66.5		T4N1	2	0.2
	Negative	509	33.5		T4N2	3	0.3
HER2**	Positive	155	10.7		T4N3	4	0.4
	Unknown	150	10.4	PR***	Positive	940	64.1
	Negative	1141	78.9		Unknown	131	8.9
					Negative	395	26.9

* Estrogen-Receptor ** Human Epidermal Growth Factor Receptor 2*** Progesterone-Receptor

The results also demonstrated a significant relationship between lymph node involvement in the patients with breast cancer and SLN in terms of extra capsular lymph node involvement ($\chi2=1.36$; $P<0.005$), size ($\chi2=3.67$; $P<0.005$), multifocality/multicentricity ($\chi2=33.21$; $P<0.005$), number of lymph nodes ($\chi2=5.68$; $P<0.005$), invasion grade ($\chi2=2.47$; $P<0.005$), non-SLN invasion ($\chi2=4.62$; $P<0.005$), lymphatic system invasion ($\chi2=1.82$; $P<0.005$), histological condition ($\chi2=1.21$; $P=0.004$), and pathology of tumor ($\chi2=1.53$; $P<0.005$).
Table 2 demonstrates the comparison of patients with different age groups and genders in terms of various tumor characteristics.

Table 2: Comparison of patients with different age groups and genders in terms of various tumor characteristics

	Age		Gender	
Variables	χ^2	P-value	χ^2	P-value
Tumor grade	15.36	0.08	0.89	0.83
Nuclear grade	13.69	0.32	1.27	0.86
Tumor size	11.48	0.24	0.54	0.9
Being *multifocal*/multicentric	5.58	0.13	0.27	0.59
Number of lymph nodes	1.43	0.007	61.3	0.004
ER*	12.56	0.05	7.89	0.9
PR**	6.64	0.35	4.09	0.53
HER***	3.74	0.71	1.78	0.77
Ki 67	5.85	0.11	5.6	0.58
Invasion grade	56	0.12	15.73	0.4
ExCLNI****	70.57	0.78	38.81	0.084
Non-SLN invasion	6.82	0.96	4.44	0.48
Histological condition	43.8	0.27	9.107	0.69
Lymphatic System Invasion	8.17	0.22	1.46	0.48
Stage	26.904	0.17	5.6	0.58

* Estrogen-receptor; ** Progesterone receptor;*** Human epidermal growth factor receptor; **** Extra *capsular lymph node* involvement
In our study, the majority of the breast cancer cases were identified as luminal A group. Table 3 presents the percentage of tumor involvement in four subtype groups of positive or negative sentinel and non-sentinel nodes. Positive and negative SLNs showed a significant relationship with LVI ($\chi2=107.4$; $P<0.005$), perineural involvement ($\chi2=32.27$; $P<0.005$), and capsular lymph node involvement ($\chi2=48.01$; $P<0.005$). The LVI possibility was higher in negative human epidermal growth factor receptor 2(HER2)-enriched type compared with the other groups. The comparison of the HER2-enriched group with other three groups revealed that 59.3% (16 out of 27 patients) of the patients with no HER2-enriched had lymphovascular involvement. Based on the results, there was no significant difference among the subtypes (i.e., luminal A, luminal B/HER2 positive, HER2-enriched type, and triple negative) in terms of LVI ($\chi2=9.75$; $P=0.13$).

Table 3: Percentage of tumor involvement in four subtype groups of positive or negative sentinel and non-sentinel nodes

		Luminal A		Luminal B/HER2 positive		HER2 enrich		Triple negative	
Subtype groups		No	Percent	No	Percent	No	Percent	No	Percent
LVI*	No	360	63.9	40	66.7	11	40.7	102	68.0
	Yes	198	35.2	20	33.3	16	59.3	48	32.0
	Unclear	5	0.9	--	--	--	--	--	--
Perineural involvement	No	472	85.5	45	78.9	20	74.1	132	89.2
	Yes	68	12.3	12	21.1	7	25.9	15	10.1
	Unclear	12	2.2	--	--	--	--	1	0.7
Tumor grade	0	5	0.9	--	--	--	--	--	--
	1	62	11.2	3	5.2	1	4.0	7	5.0
	2	320	57.6	17	29.3	11	44.0	44	31.2
	3	169	30.4	38	65.5	13	52.0	90	63.8

***** lymphovascular invasion

Discussion

In the present study, out of the patients with negative SLNB report undergoing axillary dissection based on the surgeon's decision by NSLN palpation and professionalism, five patients had positive lymph nodes, and the rate of false-negative SLNB was obtained as 0.8%. Some causes of false-negative results could be:

-Tissue attenuation and shine-through effect from the injection site

-Delayed tracer migration due to obesity or elderliness (that could be due to the increased fatty tissue with impending the flow of the tracer through the lymphatic or fatty degeneration of LNs reducing their capacity to concentrate the tracer)

-Pathologic examination of the samples to detect more micrometastasis and isolated tumor cells

It is supposed that after the lodging of tumor cells in sentinel nodes, the node losses its function; thereafter, venous drainage will bypass the involved SLN and reach a new lymph node (i.e., NSLN). Therefore, it is supposed that NSLN metastasis is detected, along with SLN metastasis. The majority of our patients had both NSLN and SLN involvement.Estrogen and progesterone receptors have been observed in tumor cells the growth of which depends on estrogen and progesterone. The evaluation of these receptors can help the doctors to choose between hormonal treatment or other treatments. Hormonal treatment includes the administration of drugs to reduce estrogen level in body or stop the estrogen impact on the growth and function of the breast tumor cells. The presence of at least one kind of these receptors in tumor cells would result in a very good response to hormone therapy. Regarding this, proving of existence of these receptors would contribute to the estimation of the disease prognosis and treatment success.

Our study indicated that 56.8% of tumors were positive in term of Ki67. According to Colleoni et al., PR, ER, and Ki67 receptors are more common in breast cancer, occurring in younger ages, and more invasive types[10]. Ki67 is a predictive marker of a patient's response to chemotherapy in those with lymph node non-involvement and early-diagnosed cancers. Another marker that was evaluated in this study was HER2. This marker was positive in just 14% of our patients with positive SLN. This is in line with the results reported in another study conducted with the purpose of determining the factors responsible for cancers occurring at young age and cancers of poor prognosis in the recurrence and invasion rate of which HER2 plays no role [10-12].

In our data analysis, a higher axillary node involvement showed a significant relationship with cancer stage, mass size (with a cut off value of2cm), and investigated biomarkers (i.e., PR, ER, and Ki67). Furthermore, there was a direct relationship between P53, HER2, tumor grade, vascular invasion, positive margins, and tumor subtypes. Additionally, an indirect relationship was observed between calcification rate, neoadjuvant therapy, and multi; however, this relationship was not statistically significant.

Based on the ROC curve analysis with a cut off value of30%, a significant relationship was obtained between Ki67 and axillary node involvement. Totally, ALND is necessary in cases with axillary node involvement; nonetheless, we should not

candidate patients with lower risk of axillary node involvement for this invasive surgery due to such complications as edema, pain, and swelling of the lower limb.

Positive NSLN is associated with primary tumor size, the biggest SLN metastasis size, and lymphovascular aggression. Several studies have investigated the factors predicting SLN involvement in metastatic breast cancer patients [9]. Barranger et al. evaluated predictive factors for SLN involvement in the majority of the women undergoing axillary lymph node (ALND). Mono-variable analysis showed a significant relationship between NSLN involvement and primary histological tumor size, SLN macro-metastasis, diagnostic method of SLN metastasis, number of SLN-positive cases, SLN involvement cases, and LVI [8].

Gunay et al. reported that the risk of metastasis to NSLN is very low when SLN is free of tumor. They also revealed that peritumoral LVI and tumor size of> 2cm significantly increase the probability of NSLN metastasis. Overall, it is obvious that the patients with no SLN metastasis has a very good prognosis (close to that of the normal population). However, this prognosis is lower than that of the patients with LVI. In addition, LVI and tumor size of> 2cm were reported as the most important factors affecting NSLN metastasis. It is probable to have false-negative lymphoscintographic results for SLN metastasis in cases with NSLN involvement. Therefore, even negative-SLN patients with LVI and tumor size of > 2cm can take benefits of ALND because according to studies, they have axillary lymph node involvement [13].

Previously, the determination of lymph node involvement was accomplished by using ALND. Nonetheless, currently, SLNB is adopted to this end as the most standard and complication-free method. The SLNB is mostly used in recently diagnosed cancers and facilitates the evaluation of axillary lymph node involvement prior to the surgery, as well as the selection of the best surgical method [14,15]. In order to identify these factors and a tool to determine metastasis possibility in NSLN, some centers, such as MD Anderson cancer center, designed a nomogram that predicts the metastasis in NSLN. According to their view, the determining factors include the primary tumor size, histology, LVI, involved SNL number, metastasis size, and extracapsular invasion. Another nomogram has been designed by the MSKCC considering some factors, such as tumor size, LVI, assessment method, and number of positive nodes [16].

Edward et al. used a combined blue dye SLNB/ANS technique and reported positive SLN in 98.3% of cases. However, axillary NSLN metastasis was observed in few cases without SLN involvement [17]. In another study, Nowikiewicz et al. investigated the NSLN metastasis rate in patients with SLN involvement using artificial neural network. They reported SLN metastasis size asthe most important risk factor in this regard. In the mentioned study, the possibility of NSLN involvement in isolated tumor cells in SLN was obtained as4.7%, while this rate was estimated as 42%in SLN micrometastasis cases[18]. Not being SLNB-positive is not necessarily mean no metastasis to NSLN; however, residual disease will not affect the disease survival or recurrence [16].

After histochemical evaluation, it could be possible for patients with negative lymphoscintography SLNB to have no need to AD. There is a very low probability of NSLN involvement before SLN involvement; accordingly, surgeons need an algorithm to determine the NSLN involvement risk. Given the possibility of obtaining false-negative results for SLNB, it is essential to detect patients with a higher probability of having positive NSLN findings to prevent the recurrence caused by the remained positive posterior axillary nodes, which are not carefully explored (because of trusting the negative SLNB result) [14].

Conclusion

As the findings indicated, SLNB and evaluation of the factors determining NSLN involvement probability would facilitate the selection of the best therapeutic method. Moreover, these measures would save the patients with lower axillary node metastasis risk from the complications caused by undergoing ALND. Out of the biomarkers evaluated in this study, Ki67, ER, and PR showed a significant relationship with more invasions to lymph nodes. Nonetheless, HER2 and P53 markers demonstrated no significant relationship with the mentioned variable. According to our results, the patients with involved lymph node, tumor size of > 2cm, and high tumor stage had a significantly higher probability of the progression of the metastasis to NSLN; accordingly, they were suggested to undergo ALND.

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