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Which Lymph Nodes Should Be Exactly Removed during Breast Cancer Surgery to Prevent Metastasis?

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Abstract

The aim of the study was to identify the relationship between molecular subtypes of breast cancer (BC) and the morphological characteristics of axillary lymph nodes (ALN) and metastatic risk in BC patients to clarify danger degree and justification of removal before metastases appear. Material and methods. Tumor molecular subtypes of 116 female BC patients aged 24 - 75 (53.9 ± 0.8) were determined by tumor tissue immunohistological examination (obtained by tru-cut biopsy), and the BC was classified as Luminal A, Luminal B/HER2−, Luminal B/HER2+, TNBC, and HER2+ subtypes. To interpret the results for the BC receptor status, immunohistochemical analysis was performed and interpreted according to the Allred scale. Lymph node size, shape, structure and conglomerates availability were recorded according to ultrasonography (USG) examination evaluated on "LOGIQ C5-Premium" (2012). Blood CA-15-3 levels were analyzed using a COBAS-e 411 automated analyzer. Statistical analysis of the obtained results was carried out using the SPSS-26 software package, and based on the t-Student-Bonferroni and H-Kruskal-Wallis criteria. The sensitivity and specificity of the indicators studied were determined using ROC statistical analysis. Results showed a significant association of some subtypes, as well as receptor expression, with tumor metastasis to ALN. Conclusion: 1) The HER2+ subtype is the most aggressive in terms of ALN metastasis. Although TNBC is the most aggressive subtype in general, it is characterized by fewer metastases to the ALN than the HER2+ subtype. 2) Metastatic ALNs can be distinguished based on their cortical structure before tumor tissue biopsy, which is economically profitable. These LNs can be removed without biopsy.

Keywords

Axillary Lymph Nodes (ALN), Breast Cancer Subtypes, Cortical Structure, Metastasis Prediction, Receptors, USG Examination, Surgical Removal

1. Introduction

The risk of tumors development is currently increasing due to drug resistance and high consumption of alcohol and tobacco worldwide [1]. Breast cancer (BC) is the most pressing issue in modern oncology due to its high morbidity rate [2] [3]. Although surgery is still considered the main method in BC treatment, organ-sparing strategies have recently become leading in medicine, and surgeon-oncologists have been trying to perform operations to reduce the axillary lymph dissection volume [4]-[8], because in most cases, metastases are not identified during surgically removed axillary lymph nodes (ALN) histological examination. Currently in practice, signal lymph node (LN) biopsy is considered an alternative to surgery for preventing postoperative complications; however, the literature lacks sufficient information on the impact of molecular subtypes on tumor lymphogenic differentiation. In this regard, the LN clinical and morphological parameters study is of great practical importance. Guo Q. (2022) and co-authors showed that BC directly affects ALN, causing morphological changes in the ultrasonography (USG) pattern due to changes in sonography sensitivity. It is believed that in ALN metastatic BC, the application of additional USG tests may increase the diagnostic value of the examination [9]. Bedi R.G. *et al.* (2008) detected USG examination values for malignant and benign LNs according to their morphological classification [10]; however, the data on this issue in the literature are contradictory [11] [12]. Therefore, we studied the relationship between changes in the morphological parameters of the ALN along with the extent of breast cancer dissemination and described the lymph nodes that can and should be removed during the elimination of primary lesions without the need for a rather expensive procedure, namely, biopsy.

We confirmed that clarification of the tumor histological subtype exerts clinical significance in predicting metastases to ALN. Some studies have been conducted to determine BC molecular subtypes based on the characteristics of metastatic LNs; however, the results obtained in this field remain ambiguous [13] [14]. Initially, depending on receptor expression, luminal A, luminal B, HER2+, and triple-negative subtypes of BC were distinguished. Further, the St. Gallen International Commission experts identified BC subtypes as luminal A (estrogen receptor positive (ER+) with progesterone receptor positive or negative (PR+/−), HER2− (human epidermal growth factor receptor 2 negative), Ki-67 < 14%; luminal B/HER2−; luminal B/HER2+; HER2+; and triple negative subtype (TNBC) [15]-[18]. Further investigations of the relationship between LN indicators ultrasonography (USG) and cancer histological characteristics serve to minimize costs for

detecting metastatic foci before biopsy, and therefore, reduce expenses for tumor treatment.

2. Material and Methods

Object of study: A total of 184 female patients with BC aged 24 - 75 (53.9 ± 0.8) were examined and treated at the Azerbaijan Medical University Oncology Clinic from 2023 to 2024.

Inclusion criteria: patients with BC

Exclusion criteria: patients with BC aggravated by non-cancerous diseases, such as diabetes mellitus, liver inflammation, pneumonia, congestive heart failure, cardiovascular disease, and previous history of cancer localized in foci other than the breast.

All patients were staged according to the TNM system of the American Joint Committee on Cancer 8th edition [19], and when determining cancer stages, we considered surgical and histopathological results. The identification of cancer molecular subtypes was based on tumor immunohistological examination using the tru-cut biopsy method. Ki-67 expression was determined by immunohistochemical nuclear staining. LN size, shape, structure, and presence of conglomerates were assessed and recorded according to the USG results (LOGIQ C5-Premium, 2012). We found the lymph node length as the longest axis and width perpendicular to the length, less than 12 mm across were accepted as normal nodes. During the ultrasound, we paid attention to such parameters as: loss of ultrasound texture, nodular cortex thickening, round shape. The shape was identified by Tensorflow graph in C++. Lymph node cortex thickening was considered if cortical layer exeeded 3 mm. Areas of lymph node cortex with varying thickening like 3, 2, 5 mm, etc. thickness from different sides was described as thickened unevenly. The device resolution allowed to determine the depth of the lymph node LI, LII, and LIII.

Blood CA-15-3 levels was analyzed on COBAS-e 411 automated analyzer and interpreted vs. control reference 16.4 (4 - 23) U/mL.

Statistical analysis of the results was carried out using the SPSS-26 software package and based on the t-Student-Bonferroni and H-Kruskal-Wallis criteria.

3. Results and Discussion

ALN metastases were found in 116 (63.0%) out of 184 BC patients examined at Oncology Clinic in 2023-2024. We found 116 cases of ALN metastases in Luminal A, Luminal B/HER2−, Luminal B/HER2+, TNBC, and HER2 + tumors (**Table 1**). Regarding cortex structure of total LN, 29,9 % (n = 55) of total ALN were thickened somewhat evenly (<3.0 mm), while 17,9% (n = 33) thickened unevenly (>3.0 mm)), and in 52,2% (n = 96) of ALNs, a poorly visualized cortex with complete disruption of structure ($P_H < 0.001$) was detected. Amid *metastatic* ALN, 1.7% (n = 2) had slightly uniformly thickened cortex (<3.0 mm), 16.4% (n = 19) an unevenly thickened (>3.0 mm), and 81, 9% (n = 95) had a complete disruption of cortex structure, so that the cortex layer was poorly visualized ($P_H < 0.001$) (**Table**

2). These data indicate that metastatic ALNs can be easily distinguished based on changes in their cortical structure before biopsy. Cortical structure USG simplifies the diagnosis of tumor metastasis to the ALN and should be considered during surgery. According to our results, vast majority—10 of 11 HER2+ subtype BC patients had metastatic ALN (90.9%), indicating that this subtype will most probably end with ALN metastasis; therefore, it is better to remove ALN in this type of cancer as soon as it is diagnosed. For comparison, even in TNBC subtype, ALN metastasis has been detected only in 16 out of 36 (57.1%) cases.

We also observed that subclavian lymph node metastasis was more common in HER2+ subtype, whereas Luminal B/HER2+ subtype metastasized mainly to supraclavicular LN (n = 13; 43.3%; $P_H = 0.066$).

Table 1. Lymph nodes (LNs) morphological indicators relationship with BC molecular subtypes.

The tumor-node-metastasis combined with tumor grading G		BC molecular subtypes					P_H
		Luminal A	Luminal B/HER2−	Luminal B/HER2+	TNBC	True HER2+	
Mts to ALN	none	17 (42.5%)	28 (37.3%)	10 (33.3%)	12 (42.9%)	1 (9.1%)	0.313
	available	23 (57.5%)	47 (62.7%)	20 (66.7%)	16 (57.1%)	10 (90.9%)*	
Frequency (number)of mts to ALN	none	17 (42.5%)	28 (37.3%)	10 (33.3%)	12 (42.9%)	1 (9.1%)	0.117
	small quantity	17 (42.5%)	24 (32.0%)	9 (30.0%)	13 (46.4%)	6 (54.5%)	
	numerous	6 (15.0%)	23 (30.7%)	11 (36.7%)	3 (10.7%)	4 (36.4%)	
ALN shape	elliptical	22 (55.0%)	36 (48.0%)	12 (40.0%)	17 (60.7%)	1 (9.1%)	0.018*
	irregular, imprecise	4 (10.0%)	6 (8.0%)	3 (10.0%)	4 (14.3%)	1 (9.1%)	
	spherical	14 (35.0%)	33 (44.0%)	15 (50.0%)	7 (25.0%)	9 (81.8%)	
ALN structure	ALN cortex equal/ somewhat equal (<3.0 mm)	16 (40.0%)	23 (30.7%)	9 (30.0%)	7 (25.0%)	0 (0.0%)	0.104
	ALN cortex uneven (>3.0 mm)	7 (17.5%)	11 (14.7%)	3 (10.0%)	10 (35.7%)	2 (18.2%)	
	ALN cortex damaged	17 (42.5%)	41 (54.7%)	18 (60.0%)	11 (39.3%)	9 (81.8%)	
Conglomerates in ALN	none	39 (97.5%)	74 (98.7%)	28 (93.3%)	27 (96.4%)	10 (90.9%)	0.519
	available	1 (2.5%)	1 (1.3%)	2 (6.7%)	1 (3.6%)	1 (9.1%)	
subclavian mts	none	32 (80.0%)	60 (80.0%)	23 (76.7%)	23 (82.1%)	7 (63.6%)	0.759
	available	8 (20.0%)	15 (20.0%)	7 (23.3%)	5 (17.9%)	4 (36.4%)	
supraclavicular mts	none	33 (82.5%)	53 (70.7%)	17 (56.7%)	24 (85.7%)	7 (63.6%)	0.066
	available	7 (17.5%)	22 (29.3%)	13 (43.3%)	4 (14.3%)	4 (36.4%)	
Tumor differentiation grade	G1	3 (7.7%)	10 (13.5%)	0 (0.0%)	4 (14.3%)	4 (36.4%)	0.008*
	G2	31 (79.5%)	53 (71.6%)	27 (90.0%)	8 (28.6%)	4 (36.4%)	
	G3	5 (12.8%)	11 (14.9%)	3 (10.0%)	16 (57.1%)	3 (27.3%)	

Note: LN: lymph node, ALN: axillary lymph node, mts: metastasis, ER: estrogen receptor, PR: progesteron receptor, HER2: receptor for human epidermal growth factor 2, TNBC: triple negative breast cancer, G: tumor grade indicator.

Table 2. Metastatic ALN morphological indicators relationship with primary cancer parameters and other metastases in BC.

		ALN mts				P_H
		none		available		
		N	N %	N	N %	
Mammary gland	right	34	50.0%	48	41.4%	0.257
	left	34	50.0%	68	58.6%	
Tumor number	single	58	85.3%	70	61.9%	<0.001*
	≥2	10	14.7%	43	38.1%	
Tumor shape	Regular/irregular	23	33.8%	23	19.8%	0.035*
	radioactive	45	66.2%	93	80.2%	
ALN mts	none	68	100.0%	0	0.0%	<0.001*
	available	0	0.0%	116	100.0%	
Number of ALN	none	68	100.0%	0	0.0%	<0.001*
	small quantity	0	0.0%	69	59.5%	
	numerous	0	0.0%	47	40.5%	
LN shape	elliptical	66	97.1%	22	19.0%	<0.001*
	irregular, imprecise	1	1.5%	17	14.7%	
	spherical	1	1.5%	77	66.4%	
LN structure	LN cortex equal/somewhat equal (<3.0 mm)	53	77.9%	2	1.7%	<0.001*
	LN cortex uneven (>3.0 mm)	14	20.6%	19	16.4%	
	LN cortex damaged	1	1.5%	95	81.9%	
LN conglomerates	none	68	100.0%	110	94.8%	0.057
	available	0	0.0%	6	5.2%	
Subclavian LN mts	none	68	100.0%	77	66.4%	<0.001*
	available	0	0.0%	39	33.6%	
Number of metastatic subclavian LN	none	68	100.0%	77	66.4%	<0.001*
	small quantity	0	0.0%	25	21.6%	
	numerous	0	0.0%	14	12.1%	
supraclavicular LN mts	none	60	88.2%	74	63.8%	<0.001*
	available	8	11.8%	42	36.2%	
Number of metastatic supraclavicular LN	none	60	88.2%	74	63.8%	<0.001*
	small quantity	8	11.8%	37	31.9%	
	numerous	0	0.0%	5	4.3%	
Mts to liver	none	64	94.1%	113	97.4%	0.261
	available	4	5.9%	3	2.6%	
Mts to lungs	none	66	97.1%	111	95.7%	0.641
	available	2	2.9%	5	4.3%	

Continued

Mts to bones	none	57	83.8%	99	85.3%	0.782
	available	11	16.2%	17	14.7%	
T	T1	4	5.9%	3	2.6%	<0.001*
	T2	44	64.7%	42	36.8%	
	T3	8	11.8%	12	10.5%	
	T4	12	17.6%	57	50.0%	
N	N0	18	26.5%	9	7.9%	<0.001*
	N1	32	47.1%	37	32.5%	
	N2	8	11.8%	22	19.3%	
	N3	10	14.7%	46	40.4%	
M	M0	51	75.0%	87	76.3%	0.984
	M1	16	23.5%	20	17.5%	
	Mx	1	1.5%	7	6.1%	
Molecular-histological subtypes	İnvasive lobular	15	22.1%	23	19.8%	0.596
	Ductal lobular	7	10.3%	10	8.6%	
	İnvasive ductal	46	67.6%	83	71.6%	
Tumor grade	G1	6	9.0%	15	13.0%	0.194
	G2	44	65.7%	79	68.7%	
	G3	17	25.4%	21	18.3%	
ER+/−	ER negative	14	20.6%	28	24.1%	0.581
	ER positive	54	79.4%	88	75.9%	
PR+/−	PR negative	22	32.4%	47	40.5%	0.271
	PR positive	46	67.6%	69	59.5%	
Ki-67+/−	Ki-67 < 14	21	30.9%	26	22.4%	0.205
	Ki-67 ≥ 14	47	69.1%	90	77.6%	
HER-2+/−	HER-2 negative	57	83.8%	86	74.1%	0.129
	HER-2 positive	11	16.2%	30	25.9%	
5 Subtypes	Luminal A	17	25.0%	23	19.8%	0.281
	Luminal B/HER-2-	28	41.2%	47	40.5%	
	Luminal B/HER2+	10	14.7%	20	17.2%	
	TNBC	12	17.6%	16	13.8%	
	True HER2+	1	1.5%	10	8.6%	

Note: N: number of cases, N%: percentage of cases, ALN: axillary lymph node, LN: lymph node, mts: metastasis, TGR: tumor growth rate, N: cancer spread to LN, M: metastasis type, G: tumor maldifferentiation grade, ER: estrogen receptor, PR: progesteron receptor, Ki-67: cancer prognostic factor antibody protein, HER2: receptor for human epidermal growth factor 2.

In general, luminal A subtype covered 19.8%, luminal B/HER2− subtype 40.5%, luminal B/HER2+ subtype – 17.2%, HER2+ subtype – 8.6% of all subtypes with

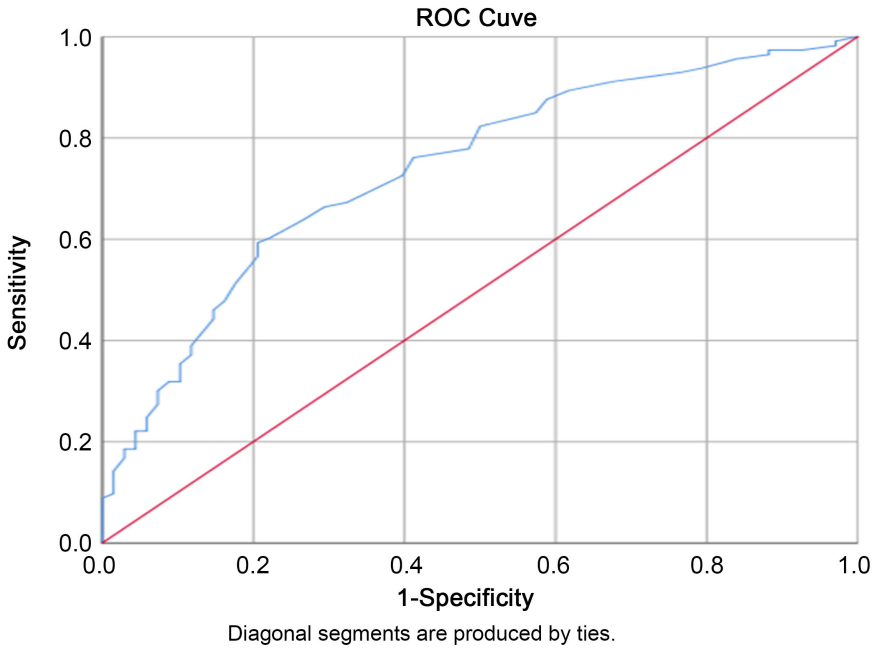
ALN, while TNBC subtype was spread in 13.8% of our BC patients with ALN metastasis (**Table 2**). Some researchers have also studied the relationship between ALN metastasis and various molecular subtypes of BC [13] [14] [16] [17] and discovered that BC histological subtypes are noteworthy predictors of LN metastasis. According to these studies, the HER−/HER2− subtype is least likely to cause LN metastasis [20]. Results obtained by Ahmed R.H. (2016) showed that patients with the True HER2+ subtype exhibited the highest risk of ALN metastatic lesions vs other subtypes. We also observed multiple ALN metastases in HER2+ cases, but interestingly, the incidence of ALN metastases was highest in both luminal B/HER2+ and luminal B/HER2− subtypes, whereas luminal A and TNBC subtypes were characterized by the lowest risk of ALN metastasis. According to our results, in Luminal A and TNBC subtypes, ALN metastasis can be expected with an accuracy 57%, in Luminal B/HER2− subtype, in 62.7% of patients, while in the case of Luminal B/HER2+ with an accuracy of 66.7%, and in True HER2+ with probability of 90.9%.

In 82 (44.6%) out of 184 patients, neoplasms were localized in the right mammary gland, while in 102 (55.4%) in the left one, and 116 (63.0%) of these patients had metastases to ALN (**Table 2**). Seven (3.8%) of total BC patients exhibited metastases to liver, seven (3.8%) to the lungs, and 28 (15.2%) to the bones. Depending on the number of metastatic LN number, groups with few (1 - 3) and multiple (> 4) metastases were distinguished. In 69 (37.5%) of them, only few LN metastases were observed, while in 47 (25.5%) cases, multiple metastases have been detected. 39 (21.3%) ALN metastatic patients had metastases to the subclavian and 42 (31.9%) to the supraclavicular LN. In 25 (13.6%) patients few, while in 14 (7.6%) multiple metastases to the subclavian LN were registered. In BC with metastases to supraclavicular LN, 37 (31.9%) patients were diagnosed with few, and 5 (2.7%) with multiple metastases.

According to our study results, in BC patients with metastasis to the ALN, the primary tumor was more likely localized in the left mammary gland (n = 68; 58.6%; $P_H = 0.257$). Tumor foci number was also found to be a risk factor for ALN metastasis: availability of multiple foci in primary tumor more likely to end with metastasis (n = 43; 38.1% vs. n = 10; 14.7%, $P_H < 0.001$). Examinations showed that BC patients with tumor radiation type (n = 93; 80.2%; $P_H = 0.035$) had significantly more ALN metastases.

Englander K. *et al.* found a correlation between tumor size and the risk of ALN metastasis [21]. According to our study, the invasive ductal (infiltrative) tumors (n = 83; 71.6%; $P_H = 0.596$) showed a tendency for LN metastasis, and the risk of metastasis to ALN increased with tumor size (**Table 2**). The results of our study show that the tumor size (37.5 ± 1.6 mm; 10.0 - 106.0 mm) in BC *with* ALN metastasis is significantly greater vs BC without metastasis (25.6 ± 1.3 mm; 10.0 - 56.0 mm; $P_H < 0.001$). This result was confirmed by the Receiver Operating Characteristic (ROC) analysis. Thus, according to ROC statistical analysis (AUC = 0.737, 95EI: 0.664 - 0.811; $P_H < 0.001$), tumor size is important in terms of specificity and

sensitivity for predicting ALN metastasis (Graph 1), since the maximum AUC was 0.737.



Graph 1. Receiver operating characteristic (ROC) curve analysis between tumor size and ALN metastasis.

A positive correlation was found between the *shape* ($\rho = 0.731$; $P_H < 0.001$) and *structure* ($\rho = 0.854$; $P_H < 0.001$) of metastatic ALN. Metastatic ALN with spherical shape ($\rho = 0.172$; $P_H = 0.019$) and abnormal drainage ($\rho = 0.167$; $P_H = 0.023$), as well as subclavian ($\rho = 0.279$; $P_H < 0.001$) and supraclavicular ($\rho = 0.232$; $P_H = 0.002$) LNs joined to the process, had a high risk of conglomerate formation. In general, 142 (77.2%) of BC patients, 88 (75.9%) of them with ALN were found to be estrogen positive (ER+), while 115 (62.5%) of total number, 69 (59.5%) with ALN – progesterone positive (PR+), and only 41 (22.3%) out of 184 with ALN were positive for epidermal growth factor receptors (HER2+) (Table 2). According to ER, PR, and HER2/neu receptor numbers, *in general* 40 (21.7%) *of all* examined BC patients were of luminal A, 75 (40.8%)—luminal B/HER2–, 30 (16.3%)—luminal B/HER2+, 11 (6.0%) HER2+, and 28 (15.2%) TNBC molecular subtypes.

Metastasis to ALN was more often seen in patients with Ki-67 ≥ 14 (n = 90; 77.6%) vs non-metastatic cases (n = 47; 69.1%; $P_H = 0.205$) and HER2+ (n = 30; 25.9%) vs non-metastatic cases (n = 11; 16.2%; $P_H = 0.129$). These data coincide with given in other resources, claiming that distant metastases or death occur in Ki-67 ≥ 14 [22] [23], but Ki-67 < 14 does not exclude availability of ALN metastasis. ER+ and PR+ tumors showed lower ALN metastatic potential than ER– ($P_H = 0.581$) and PR– ($P_H = 0.271$) tumors, respectively.

Elliptic-shaped LN were mainly observed in luminal A (n = 22; 55%) and TNBC (n = 17; 60.7%) subtypes with less metastasis to ALN. LN with irregular and

imprecise contours were more common in TNBC subtype (n = 4; 14.3%). In addition, HER2+ subtype (n = 9; 81.8%) had more spherical shaped ALN than other subtypes. Conglomerates in LN were more commonly seen in luminal B/HER2+ (n = 2; 6.7%) and True HER2+ (n = 1; 9.1%) subtypes. Although we found a statistically significant difference in the metastatic potential of molecular subtypes depending on the shape of metastatic LN ($P_H = 0.018$), but no significant difference in metastatic potential depending on conglomerates availability ($P_H = 0.519$).

Correlation analysis revealed a relationship between tumor size ($\rho = 0.232$; $P_H = 0.002$), and number of primary foci with risk of ALN metastasis ($\rho = 0.399$; $P_H < 0.001$). A positive correlation between ALN metastasis and lymph node *shape* ($\rho = 0.282$; $P_H < 0.001$) along with LN structure ($\rho = 0.408$; $P_H < 0.001$), metastatic multiplicity ($\rho = 0.178$; $P_H = 0.018$), and subclavian LN metastases ($\rho = 0.178$; $P_H = 0.017$) was detected. A direct correlation was found between pimary tumor *size* and Ki-67 expression level ($\rho = 0.155$; $P_H = 0.038$).

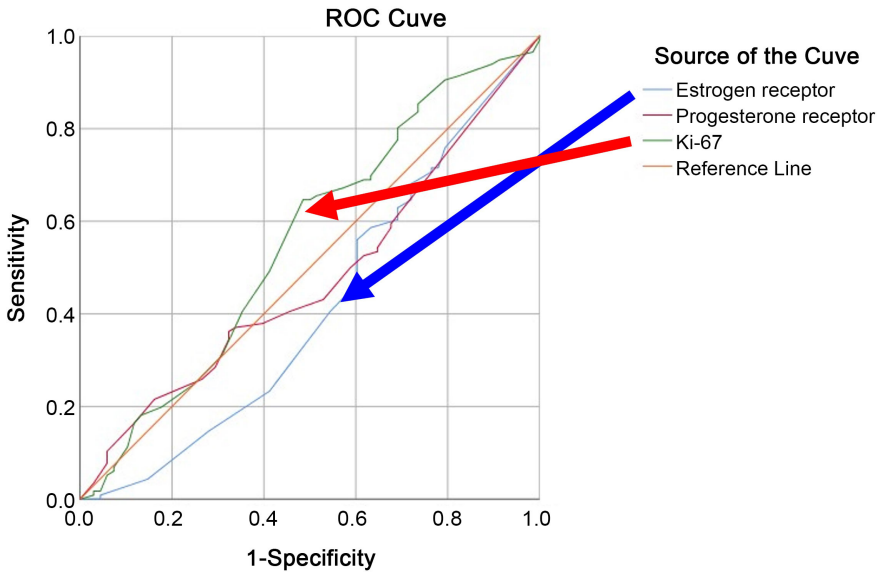
With an increase of tumor cells number, the tumor became more radial-shaped ($\rho = 0.196$; $P_H = 0.008$) and had multiple metastases to ALN ($\rho = 0.265$; $P_H < 0.001$); metastatic LNs changed to spherical ($\rho = 0.323$; $P_H < 0.001$) and were characterized by abnormal cortex structure ($\rho = 0.268$; $P_H < 0.001$). With an increase in tumor grade, the detection of conglomerates in LN ($\rho = 0.241$; $P_H = 0.001$), the risk of subclavian ($\rho = 0.280$; $P_H < 0.001$), as well as supraclavicular LN metastases also increased ($\rho = 0.320$; $P_H < 0.001$).

A positive correlation was detected between CA 15-3 high levels and tumor size ($\rho = 0.187$; $P_H = 0.017$), risk of *metastasis to* ALN ($\rho = 0.168$; $P_H = 0.031$), number of metastases ($\rho = 0.185$; $P_H = 0.017$), structure ($\rho = 0.211$; $P_H = 0.006$), and shape ($\rho = 0.226$; $P_H = 0.003$) of metastatic axillary nodes. In other words, patients with higher CA 15-3 levels have a high risk of metastasis to ALN, the shape of which changes predominantly to spherical with an almost complete disruption of the cortex structure during malignancy. In studies conducted by other authors, the relationship between the CA-15-3 oncomarker and BC molecular subtypes was also determined. According to Ruswendro.D. and co-authors (2021), CA 15-3 levels were significantly higher in Luminal B/HER2(+) and TNBC subtypes vs other subtypes [24]. We also tried to find a relationship between BC subtypes and CA-15-3 levels, butin our study, CA 15-3 expression was highest in the Luminal HER2 + subtype (51.8 ± 6.9, $p = 0.031$). According to our results, the tumor was denser and larger in True HER2(+) BC, which, according to our results, can be considered the most aggressive in terms of metastasis to ALN.

The risk of ALN metastasis was also associated with tumor shape ($\rho = 0.156$; $p = 0.034$), LN shape ($\rho = 0.207$; $p = 0.005$), as well as metastasis to the subclavian ($\rho = 0.146$; $p = 0.048$) and supraclavicular ($\rho = 0.155$; $p = 0.035$) LNs.

Investigations of the relationship between LNs USG indicators and cancer histological characteristics have helped to establish that hormonal receptor type can also be used in predicting the tumor spread and metastasis [25] [26]. According to the data obtained (**Graph 2**), the risk of tumor spread to ALN *has not been*

associated with progesterone receptors (ROC for PR: AUC = 0.474, 95EI: 0.389 - 0.559; p = 0.556), but showed dependence on Ki-67 (AUC = 0.557, 95EI: 0.469 - 0.646; p = 0.195); the higher the Ki-67 value, the greater the tendency for ALN metastasis (Graph 2). As for estrogen receptors (ER), their correlation is rather inverse: the fewer the receptors, the greater ALN metastasis risk. Therefore, according to our results, *ER* level (AUC = 0.412, 95EI: 0.324 - 0.500; p = 0.046) can be considered as a negative indicator of tumor malignancy (p = 0.046) and metastases to ALN. A negative correlation between ALN metastasis and ER availability on the tumor surface explains the lowest tumor spread, best life quality and higher survival of this group amid all BC patients. As for TNBC, it was found to be less ALN invasive, and these data are consistent with the results of Liu N. *et al.* [27]-[29].



Graph 2. ROC analysis of hormonal receptors and Ki-67 impact on ALN metastasis in BC patients. Arrows show the risk of metastasis to the ALN: red—direct dependence on the Ki-67 protein, blue—inverse dependence on the estrogen receptors availability.

Our data allow us to conclude that the absence or low PR is characterized by higher expression of the growth factor receptors HER2 (ρ = −0.221; p = 0.003) and tumor spread to the bone (ρ = −0.201; p = 0.006). Ki-67 high expression and levels above the reference line were also found to be negatively correlated with ER availability on the tumor surface (ρ = −0.350; P_H < 0.001), indicating that the less ER, the more Ki-67, and the more aggressive the tumor is. We claim negative correlation between Ki-67 expression and PR (ρ = −0.395; P_H < 0.001) as well. Besides, in patients with ER+ and PR+ tumors, Ki-67 expression protein was below the reference line, namely 14 (ρ = −0.259; P_H < 0.001). However, ER+ patients tended to develop *multiple* tumors (ρ = 0.192; P_H = 0.009) and *bone* metastases (ρ = 0.194; P_H = 0.008). In these patients, predominantly G2 differentiation rate (ρ = −0.204; p = 0.006) and high PR expression (ρ = 0.822; P_H < 0.001) were detected. PR+

tumors also exerted a higher risk of metastases to bone tissue (ρ = 0.204; P_H = 0.005) and weakened expression of Ki-67 (ρ = −0.324; P_H < 0.001) and HER2+ (ρ = -0.264; P_H < 0.001).

For patients with Ki-67 > 14 tumors, the ALN shape in this group was found to be mostly spherical (ρ = 0.209; p = 0.004), with multiple (ρ = 0.149; P_H = 0.043), particularly supraclavicular (ρ = 0.162; P_H = 0.028) LN metastases. This group was characterized by decreased expression of ER (ρ = −0.259; P_H < 0.001) and PR (ρ = −0.325; P_H < 0.001), and increased expression of HER2 (ρ = 0.164; P_H = 0.026).

In patients with HER2+ cancer subtype, the LN shape changed (ρ = 0.182; P_H = 0.013), and the risk of multiple metastases (ρ = 0.174; P_H = 0.018), in particular to the supraclavicular LN (ρ = 0.172; P_H = 0.020) and lungs (ρ = 0.167; P_H = 0.018) = 0.024) increased, while PR expression decreased (ρ = −0.233; P_H = 0.001). We note again that when metastasis occurs, the ALN shape changes to sphericity (whereas the non-metastatic LN shape is elliptical) with diffused and/or completely damaged cortex structure (Table 1).

We also noticed that with ALN metastases, the tumor was transferred both to the supraclavicular (n = 42; 36.2%) and the subclavian (n = 39; 43.8%; with P_H < 0.001) regions, but in relation to malignancy and multiple lesions, the subclavian (n = 14; 12.1%) lesions significantly predominate over supraclavicular ones.

When True HER2+ developed, we did not find a single case of ALN with a relatively normal cortical structure. Moreover, in 91% of cases node cortical layer was found unevenly thickened, indicating malignancy. Further, the algorithm of ALN metastasis according to BC subtypes is presented (Figure 1).

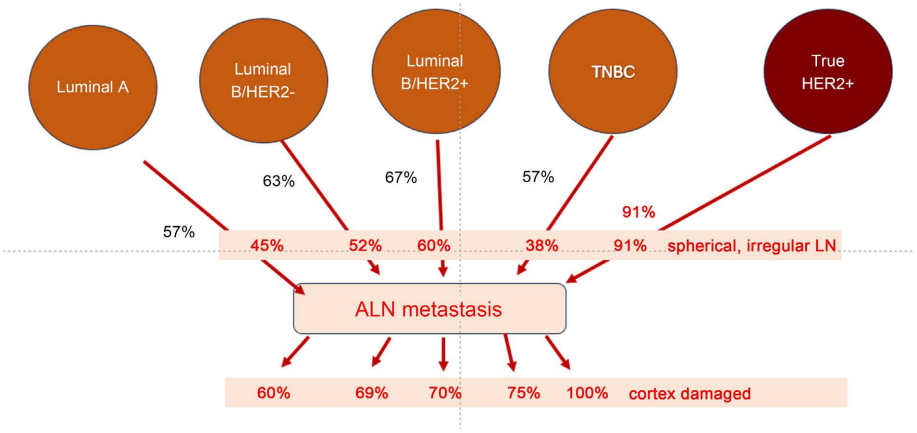


Figure 1. Prediction of metastases to axillary lymph nodes in breast cancer various subtypes by percentage of morphological changes in lymph nodes (shape and cortex structure).

According to our data, HER2+ is the type of lymph node that should be removed without any doubt during surgical removal of localized in mammary gland tumor.

4. Conclusions

1) We identified BC True HER2+ subtype as the most aggressive in terms of metastasis to ALN (91%) with 100% damaged lymph node cortex, which is why

in this BC subtype, ALN is better to be removed along with primary tumor foci.

2) According to our results, one of the main breast cancer indicators, CA 15-3 expression is also found to be highest in the Luminal HER2(+) subtype.

3) In cases observed, an increase in Ki-67 was associated with a decrease in ER/PR and increased expression of HER2, and vice versa.

Ethics Statement

The study was approved by Azerbaijan Medical University Ethics Committee, Protocol No. 26, 2023.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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