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J.N. Akhundova, M.F. Amirova
Azerbaijan Medical Unıversity, Baku, Azerbaijan

LYMPH NODES MORPHOLOGICAL CHANGES AND BREAST CANCER SUBTYPES IN PREDICTION OF METASTASES

e-mail: gerayelmira@gmail.com

In this study, breast cancer patients aged 24–75 (53.9±0.8) were fully examined, and breast cancer receptor status immunohistochemical analysis was made by Allred scale. Lymph node's size, shape, structure were recorded according to an ultrasound exam on "LOGIQ C5-Premium" (2012). The statistical analysis of obtained results was carried out using SPSS-26 software package. The sensitivity and specificity of indicators studied were shaped by ROC statistical analysis. The study results showed a significant association of subtypes as well as receptor expression with tumor metastasis to axillary lymph nodes. We found that HER2+ is the most aggressive breast cancer subtype in terms of tumor malignancy and morphological changes in axillary lymph nodes, leading to complete destruction of the node cortex structure, and therefore axillary lymph nodes in this subtype must be removed during surgical resection of primary cancer foci without fail.

Key words: lymph nodes, breast cancer, cancer subtypes, lymph cortex, receptors.

Дж.Н. Ахундова, М.Ф. Амірова

ВЗАЄМОЗВ'ЯЗОК МОРФОЛОГІЧНИХ ЗМІН ЛІМФАТИЧНИХ ВУЗЛІВ І ПІДТИПІВ РАКУ МОЛОЧНОЇ ЗАЛОЗИ У ПРОГНОЗУВАННІ ВИНИКНЕННЯ МЕТАСТАЗІВ

У цьому дослідженні обстежено пацієнтів з раком молочної залози віком 24–75 (53,9±0,8), та проведено імуногістохімічний аналіз рецепторного статусу раку молочної залози за шкалою Allred. Розміри, форма та структура лімфовузлів реєструвалися за даними УЗД, отриманими на апараті "LOGIQ C5-Premium" (2012). Статистичний аналіз одержаних результатів проводився з використанням програми SPSS-26. Чутливість і специфічність показників, що вивчаються, визначалися за допомогою ROC-аналізу. Отримані результати показали значний взаємозв'язок підтипів, експресії рецепторів з метастазами пухлини в пахвові лімфатичні вузли. Ми виявили, що HER2+ підтип пухлини молочної залози є найбільш агресивним з точки зору злоякісності та морфологічних змін, які виникають в пахвових лімфатичних вузлах, що призводить до повного руйнування структури коркової речовини вузла, у зв'язку з чим при хірургічній резекції первинних вогнищ раку, пахвові лімфатичні вузли при HER2+ підтипі мають видалятися обов'язково.

Ключові слова: лімфатичні вузли, рак молочної залози, підтипи, кіркова речовина, рецептори.

Due to high morbidity rate breast cancer (BC) is most pressing issue of modern oncology. Although the surgery is considered the main method in BC treatment, recently organ-sparing strategy becomes leading in medicine, and surgeon-oncologists have been trying to perform operations reducing the axillary lymphodissection volume. Thus, in most cases, metastases are not identified during surgically removed axillary lymph nodes (ALN) histological examination, therefore currently in practice, signal lymph node (LN) biopsy is considered an alternative to surgery method preventing postoperative complications. However, biopsy is quite expensive, in this regard, metastatic LN clinical and morphological indicators study is of great practical importance. Some research works have being conducted to determine the BC molecular subtypes based on the characteristics of metastatic LNs; however the results obtained in this field remain ambiguous [5, 7, 11]. Initially, depending on receptors expression, luminal A, luminal B subtypes, HER2+ and triple negative BC subtypes were distinguished [3, 6, 10]. Further, the experts of the St. Gallen International Commission identified BC subtypes as luminal A (ER+ and/or PR+/-, HER2-, Ki67 < 14 %), luminal B/HER2-, luminal B/HER2+, HER2+, and triple negative subtype (TNBC) [3]. Further investigations of relationship between LN indicators ultrasonography (USG) and cancer histological characteristics serve to minimize costs of detecting metastatic foci before biopsy and and therefore reduce costs during tumor treatment [3, 4, 9, 12].

The purpose of the study was to consider factors influencing tumor dissemination to lymph nodes concerning receptor expression and morphological changes in breast cancer patients' nodal cortex.

Materials and methods. LNs of 184 female BC patients treated at the Azerbaijan Medical University Oncology Clinic during 2020–2022 aged 24–75 (53.9±0.8) were examined and data included in the research work. The study was approved by Azerbaijan Medical University Ethics Committee, Protocol No. 26 dated April 14, 2023. The patients agreed for examination, were divided into the following groups: a control group (non-cancerous), patients with metastases in the ALN, and patients without metastases to ALN. Patients with comorbid conditions were excluded from the study, included ones were informed about investigation and provided written consent to participate in the study. Therefore, inclusion criteria: BC, exclusion criteria: BC aggravated by non-cancerous diseases.

Identification of cancer molecular subtypes was based on tumor immunohistological examination by tru-cut biopsy method. Ki67 was determined by Immunohistochemical nuclear staining method. All patients' cancer were staged according to TNM system according to American Joint Committee on Cancer 8th edition published in 2002. LN size, shape structure and presence of conglomerates were assessed and recorded according to the ultrasound study ("LOGIQ C5-Premium", 2012).

The statistical analysis of results obtained was carried out using SPSS-26 software package and based on t-Student-Bonferroni and H-Kruskal-Wallis criteria. Values with a p-value of less than 0.05 were considered statistically significant.

Results of the study and their discussion. ALN metastases were found in 116 (63.0 %) out of all 184 BC patients examined. The following are the patients with metastatic and non-metastatic ALNs analysis results. The LNs morphological characteristics are presented in relation to the molecular subtypes of BC. The table also shows the percentage of spherical (non-viable) lymph nodes for each molecular subtype of breast cancer (BC). According to ultrasound examination, 116 cases with ALN metastases have been sorted as Luminal A, Luminal B/ HER2-, Luminal B / HER2+, TNBC and HER2 + (Table 1).

Table 1

Metastatic and non-metastatic lymph node morphological indicators related to tumor grade according to breast cancer molecular subtypes

Parameters		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 %)	
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, G – tumor grade index.

The highest number of spherical shaped ALNs was found in the HER2+ subtype. In terms of tumor progression (G3), HER2+ was also the most aggressive after axiomatically accepted as the most aggressive subtype, TNBC.

We also examined the metastatic ALN morphological parameters relationship with other cancer parameters in breast cancer (Table 2).

In 29.9 % (n=55) of their total number it was thickened in structure somewhat evenly (<3.0 mm), while in 17.9 % (n=33) – unevenly (>3.0 mm)), and in 52.2 % (n=96) of ALNs a poorly visualized cortex with complete disruption of structure (pH<0.001) was detected, while amid metastatic ALN 1.7 % (n=2) had slightly uniformly thickened cortex (≤3.0 mm), 16.4 % (n=19) – unevenly thickened (>3.0 mm), and 81, 9 % (n=95) – a complete disrupted cortex structure, so that in last case the cortex layer was poorly visualized (P_H <0.001). Thus, the lymph nodes cortical layer is an informative factor in predicting metastasis and detecting it. LN cortical substance thickening indicates a disruption in the structure and function of vital for the immune system's functioning cells, and alteration of their structure contributes to the further tumor process development and spread to nearby and distant tissues and organs in the body. According to the results, vast majority – 10 out of 11 BC patients with HER2+ subtype had metastatic ALN (90.9 %), indicating that this subtype will most probably end with ALN metastasis. The high frequency of morphologically altered ALN in this subtype indicates that, upon its detection in a patient, metastasis to the ALN should be anticipated above all. To prevent tumor spread to nearby tissues, it is likely that ALNs should be removed when HER2+ subtype tumors are excised. For comparison, even in TNBC subtype, only in 16 out of 36 (57.1 %) cases, ALN metastasis had been detected.

As can be seen, 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 metastases, while TNBC subtype was revealed in 13.8 % of BC patients with ALN metastasis. Patients with True HER2+ subtype showed highest risk of ALN metastatic lesions vs other subtypes. This subtype also accounts for

the largest number of metastases in ALNs. This sheds light on the cortical substance structure disruption high percentage in ALN identified in this subtype.

Table 2

Relationship of metastatic ALN morphological changes with main BC parameters

		ALN mts				P_H
		none		available		
		N	N %	N	N %	
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 %	
	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 %	
	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 %	
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 %	
HER2 +/-	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, T – tumor size and extent, N – cancer spread to LN, M – metastasis type, ER- estrogen receptor, PR – progesteron receptor, KI-67 – cancer prognostic factor antibody protein, HER2 – receptor for human epidermal growth factor 2.

As can be seen, multiple metastases to the ALN were also observed in the luminal B/HER2- subtype. Data indicate B/HER2- subtype as quite aggressive in terms of metastasis to ALN, as the percentage of metastases for this subtype is the highest. However, considering the data, it can be noted that although the number of affected ALNs is highest in the B/HER2- subtype, the severity of functional and structural alterations in this subtype is less pronounced compared to the HER2+ subtype.

In 82 (44.6 %) out of 184 patients, neoplasm was 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. 7 (3.8 %) of total BC patients exhibited metastases to liver, 7 (3.8 %) – to the lungs, and 28 (15.2 %) – to the bones. Depending on metastatic LN number, groups with few (1–3) and multiple (more than 4) metastases were distinguished. In 69 (37.5 %) of them, few LN metastases were observed, while in 47 (25.5 %) cases multiple metastases were detected. 39 (21.3 %) ALN metastatic patients had metastases to the subclavian, and 42 (31.9 %) – to the supraclavicular LNs. In 25 (13.6 %) patients few, and 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.

More metastases to LN were observed in invasive ductal (infiltrative) tumors (n=83; 71.6 %; P_H =0.596), and the risk of metastasis to ALN increased with tumor size.

It is generally accepted that the receptors expression in primary tumor foci affects tumor spread. but there is debate, as to which receptor is more responsible for the malignant process.

According to the data obtained (Fig.1), the risk of tumor spread on ALN has not been positively associated with progesterone receptors (ROC for PR: AUC=0.474, 95EI: 0.389-0.559; p=0.556), but shows dependance on Ki-67 (AUC=0.557, 95EI: 0.469-0.646; p=0.195): the higher Ki-67, the more risk of metastasis to ALN.

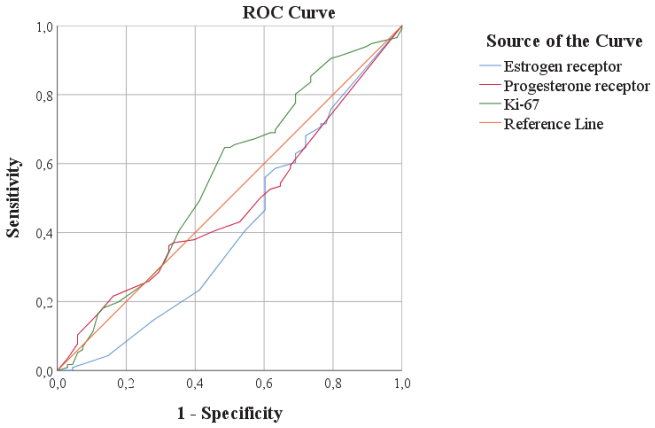

Fig. 1. ROC analysis of hormonal receptors and Ki-67 impact on ALN metastasis in BC patients.

Our study results confirm that Ki67 is a specific and sensitive indicator for tumor malignancy and can be considered as the best in ALN metastases predicting. According to our results, *ER* level (AUC=0.412, 95EI: 0.324-0.500; p=0.046) can be considered as the best negative index of tumor malignancy (p=0.046) and metastases to ALN. Estrogen receptors correlation with tumor malignancy is rather inverse: the fewer receptors, the greater ALN metastasis risk. A negative correlation between ALN metastasis and ER availability on tumor surface explains the lowest tumor spread, best life quality and higher survival of this group amid all BC patients [1].

Considering the pain and the likelihood of cancer spread due to tumor growth-stimulating factors washing into the bloodstream when the integrity of malignant structures is compromised, N Cabıoğlu et al. also supports avoiding lymph node biopsy whenever possible. To avoid further tumor progression in biopsy painful procedure, we assert that in HER2+ cases, lymph nodes are better removed rather than subjected to biopsy [2].

Our research sheds light on doubts raised in a number of articles regarding the primary tumor site impact on ALN in the HER2+ subtype [8, 12]. Iqbal N. and Iqbal N. [7] claim that in BC approximately 30 % cases HER2 amplification occurs, and observed in that case receptor dimerization leads to its cytoplasmic domain tyrosine residues' autophosphorylation, which initiates multiple signaling pathways including tumor progression.

Our data allow us to conclude that the absence or low PR is characterized by growth factor HER2 higher expression (ρ=-0.221; p=0.003). Ki67 high expression and level above reference line is also found to be negatively correlated with ER availability on tumor surface (ρ=0.350; p<0.001), indicating that the more ki67, the more aggressive is tumor. We can claime negative correlation between Ki67 and PR (ρ=-0.395; p<0.001) as well. Contrary, in patients with ER+ and PR+ tumors Ki-67 expression was below 14 (ρ= -0.259; p<0.001). ER+ patients tended to develop more likely multiple tumors (ρ=0.192; p=0.009) with bone metastases (ρ=0.194; p=0.008). In these patients, predominantly G2 differentiation rate (ρ=-0.204; p=0.006) and high expression of PR (ρ=0.822; p<0.001) were detected. PR+ tumors exerted a higher risk of metastases to bone tissue (ρ=0.204; p=0.005), as well as weakened expression of Ki-67 (ρ=-0.324; p<0.001) and HER2+ (ρ=-0.264; p<0.001).

In patients with HER2+ cancer, in vast majority of cases LN shape significantly changed (ρ= 0.182; p=0.013), risk of multiple metastases increased (ρ= 0.174; p=0.018), in particular to supraclavicular LN (ρ= 0.172; p=0.020) and lungs (ρ= 0.167; p=0.018) =0.024), while PR expression decreased (ρ= -0.233; p=0.001). With True HER2+, we did not observe a single case of ALN with a relatively normal cortical structure.

Conclusions

1. We identify True HER2+ breast cancer subtype as the most aggressive in terms of axillary lymph node (ALN) morphology damage, with 91% of cases showing alterations and 100% involvement of the cortical layer, leading to functional failure of the lymph nodes. Therefore, for this breast cancer subtype, all ALNs should be removed during the surgical removal of primary tumor foci.

2. Our study proved B/HER2- to be one of the most aggressive subtypes in terms of the extent of ALN involvement. However, it should be noted that in this subtype, the structure — and thus the function — of the lymph nodes can still retain relative integrity. Therefore, it may be advisable to preserve them when removing lesions from the mammary glands.

3. An increase in protein Ki-67 correlated with a decrease in progesterone and estrogen receptors, indicating an inverse relationship between malignancy progression and these receptors' levels.

4. With the exception of TNBC, the more solid the tumor, the higher the likelihood of its metastasis to ALNs.

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