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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

THE SIGNIFICANCE OF RADIOMORPHOLOGICAL INDICATORS OF AXILLARY LYMPHATIC METASTASES IN THE DIAGNOSIS OF BREAST CANCER SUBTYPES

Specialty: 3225.01− Radiation diagnostics and therapy

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GENERAL CHARACTERISTICS OF THE RESEARCH

Relevance of the topic. Breast cancer (BC) is one of the most pressing medical and social problems due to its widespread prevalence and increasing mortality rates. BC ranks first in the structure of morbidity from malignant neoplasms among women[1]. According to global statistics, various forms of breast tumors are detected in one out of every two women over the age of 30. In our republic, BC also ranks first in incidence among women, with a standardized morbidity rate of 21.23 per 100,000 female population.

Since more than 70% of all new cases and 81% of deaths are observed in women aged 50 and older, the global burden of BC remains concentrated in this age group.[2] Despite the introduction of new diagnostic methods, difficulties still persist in the early diagnosis of the disease and in determining its clinical and morphological forms. Therefore, there is a need to develop modern minimally invasive approaches in this field. Timely determination of the disease stage, tumor localization in the breast, and metastasis to regional lymph nodes are considered the main factors influencing the clinical course of the disease. One of the major clinical complications of BC is metastasis to regional lymph nodes, particularly the axillary lymph nodes. Lymph nodes affected by metastasis undergo changes in size, shape, and structure. In this regard, the study of the radiomorphological characteristics of axillary lymph node metastases is of great scientific and practical importance.

A number of studies have shown that different degrees of changes in the axillary lymph nodes are observed in certain molecular subtypes of BC.[3]Although the classification of BC based on immunohistochemical markers is highly sensitive, diagnostic errors may still occur in

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[1] Sung, H. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries CA / H. Sung, J. Ferlay, R.L. Siegel [et al.] // Cancer J Clin., -2021. 71(3), - p. 209–49.

[2] Arnold, M. Current and future burden of breast cancer: Global statistics for 2020 and 2040 / M. Arnold, E. Morgan, H. Rumgay [et al.] // Breast, - 2022. 66, - p. 17.

[3] Morkavuk, S.B. Relationship between lymphovascular invasion and molecular subtypes in invasive breast cancer / S.B. Morkavuk, M. Güner, Çulcu [et al.] // Int J Clin Pract., - 2021. 75(4), - e13897.

some cases. In the treatment of early-stage BC, the removal of axillary lymph nodes is of significant importance. However, this procedure may lead to complications such as upper limb edema, paresthesia, chronic pain syndrome, and limited mobility. Sentinel lymph node biopsy (SLNB) is less invasive compared to axillary lymph node dissection. These considerations justify the development of new, minimally invasive, more sensitive, and specific methods for determining BC phenotypes. One of such informative methods is ultrasound examination (US), as improving its diagnostic capabilities allows for the development of new radiomorphological criteria. Ultrasound is a safe, minimally invasive, highly informative, and practically contraindication-free diagnostic method for detecting pathological changes in the breast and regional lymph nodes. Compared to mammography, ultrasound enables real-time visualization of organs and tissues and can detect high-density areas that may not be identified by mammography.[4]

The molecular-biological classification of BC was first described by the WHO in the St. Gallen 2011 Consensus and was later updated in accordance with the St. Gallen 2021 recommendations, which reflect modern diagnostic and therapeutic standards.[5] According to this classifycation, malignant epithelial tumors of the breast are divided into five subtypes based on the expression of four antigen receptors (estrogen, progesterone, HER2/Neu, and Ki-67).

Determining the radiomorphological features of axillary lymph node metastases using US and, on this basis, the preliminary identification of BC subtypes may have significant practical importance in the early diagnosis and prognosis of the disease, as well as in selecting the most appropriate treatment strategy. The radiomorphological characteristics of axillary lymph node metastases in different BC subtypes have not yet been sufficiently evaluated using ultrasound. Clinical

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[4] Chen, X. Ultrasound as a replacement for physical examination in clinical staging of axillary lymph nodes in breast cancer patients / X. Chen, X. Li, Z. Fan [et al.] // Thorac Cancer, - 2020. 11(1), - p. 48-54.

[5] Burstein HJ, Curigliano G, Thürlimann B, Weber WP, Poortmans P, Regan MM, et al. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021 // Ann Oncol. – 2021.32(10), - p. 1216–1235

observations in this field remain fragmented, highlighting the need for comprehensive and comparative studies.

Object of the study. The study was conducted prospectively and included 184 female patients with breast cancer who were examined and treated at the Oncology Clinic of Azerbaijan Medical University. US examination of the mammary glands and axillary lymph nodes was performed.

Aim of the study: To investigate the relationships between different subtypes of breast cancer (BC) and the radiomorphological features of axillary lymph nodes, and to predict the molecular subtypes of BC based on these parameters.

Objectives of the study:

1. To investigate the US features of tumors in different molecular subtypes of breast cancer (BC);
2. To develop US criteria for detecting axillary lymph node metastases in patients with BC and to determine these criteria according to molecular subtypes;
3. To determine the correlation between the molecular characteristics of BC and the US features of metastatic axillary lymph nodes;
4. To assess the prognostic significance of US criteria for axillary lymph node metastases in different subtypes of BC;
5. To perform a comparative analysis of axillary lymph node metastases based on US findings of BC and the results of histological, immunohistochemical, and laboratory examinations.

Research methods: Examinations were performed using a "LOGIQ C5-Premium" (2012) US device. The level of the CA 15-3 tumor marker was measured in a clinical laboratory using an Integra-411 fully automated immunoassay analyzer. Immunohistochemical analyses were carried out in the pathohistology laboratory of the Oncology Clinic of Azerbaijan Medical University, as well as in other commercial clinical laboratories.

Main provisions submitted for defense:

1. In patients with breast cancer, the level of estrogen receptors (ER) is considered a highly sensitive and specific indicator for the detection of axillary lymph node metastases.

2. There is a correlation between the radiomorphological subtypes of BC and the US features of metastatic axillary lymph nodes. In patients with luminal subtypes of BC, uneven thickening of the cortical layer of axillary lymph nodes is more frequently observed.

3. The radiomorphological appearance of axillary lymph node metastases varies depending on the biological subtypes of BC, as well as on the expression of hormonal receptors on the surface of tumor cells, including the HER2 oncogene and Ki-67 proliferation index. In this regard, US- determined radiomorphological features of axillary lymph node metastases may allow prediction of the molecular characteristics of BC.

4. The interrelationship between the US features of axillary lymph node metastases and the results of histological, immunohistochemical, and laboratory examinations reflects the pathogenetic mechanisms of lymph node involvement. Based on the molecular-biological subtypes of the primary tumor, criteria for metastatic involvement of axillary lymph nodes have been developed.

Scientific novelty of the study: In this study, relationships were identified between the radiomorphological characteristics of axillary lymph nodes, an area of ongoing debate and interest, and the expression of antigen receptors (estrogen, progesterone, HER2/neu, and Ki-67). The possibility of predicting the molecular subtypes of breast cancer (BC) based on the US radiomorphological features of breast lesions was investigated. Additionally, it was demonstrated that, based on the US- determined radiomorphological features of axillary lymph nodes, it is not possible to definitively determine the molecular subtypes of BC. However, it is possible to predict the expression of antigen receptors.

Practical significance of the study:

The US demonstrates high effectiveness in the radiomorphological evaluation of metastases to the axillary lymph nodes. The newly proposed criteria for axillary lymph node metastases in breast cancer (BC) are of significant practical importance for the early diagnosis of the disease. Monitoring the progression dynamics of axillary lymph node metastases in BC using US, as well as identifying the underlying

patterns, may contribute to improved treatment outcomes. Furthermore, the determination of antigen receptor status based on the radiomorphological characteristics of axillary lymph nodes is of considerable practical importance for the development of both endocrine therapy and chemotherapy (CT) protocols. Early diagnosis enables prediction of disease progression, supports the appropriate selection of treatment strategies, and facilitates the development of diagnostic algorithms based on the radiomorphological parameters of axillary lymph nodes.

Approbation of the dissertation. The main materials and obtainned results of the dissertation were presented and discussed at the XIII and XIV Congresses of Oncologists and Radiologists of the CIS and Eurasian countries (Kazakhstan, 2022; Tajikistan, 2024), the International Scientific-Practical Conference dedicated to the 270th anniversary of Shusha titled "Actual Problems of Medicine" (Baku, 2022), the International Scientific-Practical Congress dedicated to the 100th anniversary of National Leader H.A. Aliyev (Baku, 2023), the Scientific-Practical Conference dedicated to the 80th anniversary of Professor A.M. Afandiyev (Baku, 2023), the I and II International Breast Cancer Congresses (Baku, 2023), the Scientific-Practical Conference dedicated to the birthday of Aziz Mammadkarim oglu Aliyev (Baku, 2024), the Scientific-Practical Conference dedicated to the 80th anniversary of V. Shadlinski (Baku, 2025), the First Congress of Oncologists of the Union of Turkic-Speaking States (Baku, 2025), and the 19th St. Gallen International Breast Cancer Conference (Vienna, 2025).

The results of the dissertation were presented and discussed at the interdepartmental conference of the Educational Surgery Clinic of Azerbaijan Medical University (ATU), Meeting No. 09, held on June 23, 2025, as well as at the scientific seminar of the Dissertation Council FD 1.02 operating under the National Oncology Center of the MH of AR on October 30, 2025 (Protocol No. 3).

A total of 23 scientific works on the topic of the dissertation have been published, including 7 articles and 9 theses in national journals, and 4 articles and 3 theses in international journals.

Application of the results in practice. The results of the study

have been applied in the treatment base of the Oncology Clinic of Azerbaijan Medical University and incorporated into the curriculum of the Department of Oncology.

Institution where the dissertation was conducted. The dissertation research was carried out at the Oncology Clinic of Azerbaijan Medical University.

Volume and structure of the dissertation:

The dissertation is presented on 163 computer-typed pages (272,868 characters) and consists of an introduction (20,051 characters), Chapter I – literature review (73,897 characters), Chapter II – materials and methods (10,378 characters), Chapter III – personal research results (63,734 characters), conclusion (67,317 characters), results and practical recommendations (2,959 characters), and a list of references (34,532 characters).The reference list includes 190 scientific sources (16 in Azerbaijani, 30 in Russian, and 144 in foreign languages).The dissertation is illustrated with 14 tables, 9 figures, and 7 graphs.

MATERIALS AND METHODS

The study included 184 female BC patients aged 24–75 years (53.9 ± 0.8; Me = 55; Q1 = 46; Q3 = 62) who underwent examination and treatment at the Oncology Clinic of Azerbaijan Medical University between 2020 and 2022. Clinical and anamnesis data were collected, and the results of radiological imaging and immunohistochemical analyses were comparatively evaluated. Among these patients, 3 cases of bilateral BC were recorded. Of the detected breast cancers, 82 cases (44.6%) were localized in the right breast, and 102 cases (55.4%) in the left breast. The breast lesions of the patients included in the study were evaluated according to the BI-RADS classification based on US results, and patients with lesions classified as BI-RADS 4A, 4B, 4C, and 5 were included in the study. Among the included patients, 110 (59.8%) had not received CT, while 74 (40.2%) had undergone CT.

Pathohistological examinations of BC patients

The molecular subtypes of tumors in patients were determined based on immunohistochemical examination of tumor tissue obtained by the Tru-Cut biopsy method, performed according to standard procedures.

The Allred scoring system was used to interpret the results of immunohistochemical analysis of receptor status in breast tumors. In this system, the proportion of positive cells was scored from 0 to 5, while staining intensity was evaluated from 0 to 3. Tumors were considered ER-positive (ER+) and PR-positive (PR+) when ≥1% of tumor cells showed expression of estrogen receptors (ER) and progesterone receptors (PR) on the surface of tumor cells, respectively. A three-tier scoring system was used for HER2 classification. The HER2 receptor was considered positive in cases of HER2 gene amplification. The Ki-67 proliferation index was assessed based on the percentage of positively stained invasive tumor cells. Ki-67 expression <14% was considered low, whereas Ki-67 ≥14% was considered high.

Based on the expression levels of hormonal receptors and HER2 on the surface of tumor cells, as well as the Ki-67 proliferation index, five molecular subtypes of BC were identified: luminal A (ER+ and/or PR+/, HER2–, Ki-67 < 14%), luminal B/HER2– (ER+and/or PR+/–, HER2–, Ki-67 ≥ 14%), luminal B/HER2+ (ER+ and/or PR+/–, HER2+, Ki-67 ≥ 14%), HER2+ (ER–, PR–, HER2+, any Ki-67 level), triple-negative breast cancer (TNBC) (ER–, PR–, HER2–, any Ki-67 level).

Ultrasound examination of the breast and axillary lymph nodes in BC patients

The patients included in the study were examined using a "LOGIQ C-5 Premium" (2012) ultrasound system with a 5–14 MHz linear transducer in B-mode. The location of the detected lesion was initially recorded either according to the clock-face orientation or by breast quadrants (upper medial, lower medial, lower lateral, or upper lateral). Subsequently, the lesion size was measured in two or three dimensions (width, length), structure (homogeneous, heterogeneous), margins (well-defined, irregular, spiculated), and orientation relative to the breast tissue (parallel or vertical). The presence of calcifications and necrotic areas within the lesion, as well as the level of vascularization (central, peripheral, or mixed), were also assessed.

During the examination of the axillary region, the following characteristics of the detected lymph nodes were evaluated: size, structure (including the presence or absence of cortical thickening, with ≤3

mm considered normal), shape (round or oval), presence of calcifications or fluid-containing areas, number of pathological lymph nodes, depth, level of localization, and the ratio of short-axis to long-axis dimensions (<2 or ≥2).

In this study, the informativeness of US in detecting metastatic changes in the morphological structure of axillary lymph nodes in patients with BC was investigated. Based on metastatic involvement of axillary lymph nodes, negative results were obtained in 68 patients and positive results in 116 patients. However, among these results, 4 (5.9%) were false-negative and 64 (94.1%) were true-positive. While metastasis to axillary lymph nodes was confirmed in 115 (99.1%) patients, a false-positive result was obtained in 1 (0.9%) patient. Thus, ultrasonography can be considered an effective diagnostic method for detecting metastatic axillary lymph nodes, with high specificity (94.1%) and sensitivity (99.1%) ($\kappa = 0.850$; $p < 0.001$) (Table 1).

Table 1

The informativeness of the US in detecting metastatic axillary lymph nodes in patients with BC

Examination type		Number	Axillary lymph node metastasis		κ	P
			absent	present		
US	absent	N	64	1	0.850	<0.001
		%	94.1%	0.9%		
	present	N	4	115		
		%	5.9%	99.1%		

Results and Discussion

Changes in the radiomorphological characteristics of tumors according to molecular subtypes in patients with breast cancer

A certain relationship was observed between tumor size, contours, shape, and other biological characteristics and the molecular subtypes of BC. Based on the levels of estrogen (ER), progesterone (PR), and HER2 receptors determined by immunohistochemical analysis, the following subtypes were identified: 40 (21.7%) luminal A, 75 (40.8%) luminal B/HER2–, 30 (16.3%) luminal B/HER2+, 28 (15.2%) TNBC, and 11 (6.0%) HR–/HER2+ subtypes. As evident from the

results, luminal subtypes were more prevalent among BC cases, followed by HR–/HER2+ and TNBC subtypes.

In patients with a single pathological lesion in the breast, the TNBC (n = 26, 92.9%) and HR–/HER2+ (n = 7, 70.0%) subtypes were more frequently observed. No statistically significant difference was found between molecular subtypes according to the number of breast tumors (p = 0.077) (Table 2).

Table 2

Variation in the radiomorphological characteristics of tumors according to molecular subtypes in patients with BC

Parameters		Molecular subtypes					P_H
		Luminal A	Luminal B/ HER2–	Luminal B/HER2+	HR–/ HER2+	TNBC	
Breast	right	17 (42.5%)	32 (42.7%)	13 (43.3%)	5 (45.5%)	15 (53.6%)	0.892
	left	23 (57.5%)	43 (57.3%)	17 (56.7%)	6 (54.5%)	13 (46.4%)	
Number of tumors	Single tumor	27 (67.5%)	49 (66.2%)	19 (65.5%)	7 (70.0%)	26 (92.9%)	0.077
	≥ 2	13 (32.5%)	25 (33.8%)	10 (34.5%)	3 (30.0%)	2 (7.1%)	
Tumor contours	well-defined, irregular	15 (37.5%)	15 (20.0%)	4 (13.3%)	3 (27.3%)	9 (32.1%)	0.123
	spiculated	25 (62.5%)	60 (80.0%)	26 (86.7%)	8 (72.7%)	19 (67.9%)	
Microcal-cifications	present	15 (37.5%)	28 (37.3%)	18 (60.0%)	6 (54.5%)	5 (17.9%)	0.017

As a result of US examination, tumors with indistinct, irregular, and spiculated contours were observed in patients with BC. In 45 (25.0%) patients, tumor margins were distinct and irregular, whereas in 138 (75.0%) patients, spiculated margins were present. Tumors with distinct and irregular margins were more frequently observed in the luminal A/HER2– subtype (n = 15; 37.5%) and the TNBC subtype (n = 9; 32.1%) compared with other subtypes. Spiculated tumors were mainly found in the luminal B subtypes: B/HER2– (n = 60; 80.0%) and luminal B/HER2+ (n = 26; 86.7%). According to statistical analysis, no statistically significant difference was found between BC

molecular subtypes in terms of tumor margins (p = 0.123).

Microcalcifications within the tumor were detected in 72 patients. Among them, 15 (37.5%) had the luminal A subtype, 28 (37.3%) had the luminal B/HER2– subtype, 18 (60.0%) had the luminal B/HER2+ subtype, 6 (54.5%) had the HR–/HER2+ subtype, and 5 (17.9%) had the TNBC subtype. As can be seen from the results, microcalcifications in breast tumors were most frequently observed in the luminal B/HER2+ subtype and least frequently in the TNBC subtype, and this finding was statistically significant (p = 0.017).

A number of studies have shown that, in patients with BC, lymph node involvement, molecular tumor subtypes, histological grade, lymphovascular invasion, and tumor size are important prognostic factors for metastasis. In our study, no statistically significant association was found between tumor size and the distribution of molecular subtypes (p = 0.461). Thus, among BC patients with metastatic axillary lymph nodes, the mean tumor size was 37.5 ± 1.6 mm (range: 10-106 mm), whereas in patients without metastasis it was 25.6 ± 1.3 mm (range: 10–58 mm) (p < 0.001). Larger tumors were mainly observed in the HR–/Hr- subtype (p = 0.461) (Table 3).

Table 3

Variation in tumor size according to different molecular subtypes of BC

Parameters		Subtypes					P_H
		Luminal A	Luminal B HER2–	Luminal B HER2+	HR–/ HER2+	TNBC	
Tumor size, mm	N	40	74	29	10	28	0.461
	Mean (M)	31.0	33.9	30.0	43.7	32.8	
	Median (Me)	28.5	32.0	26.0	33.0	27.5	
	(Q1) 25	20	24	22	18	19.5	
	(Q3) 75	38	43	33	62	42	

In the patients with BC included in the study, a significant association was found between molecular tumor subtypes and the histological grade of the disease (p = 0.008) as well as the T classification (p = 0.040). Among tumors with G1 differentiation, the HR–/HER2+ subtype (n = 4;

36.4%) was most frequently observed. In tumors with G2 differentiation, the luminal B/HER2+ subtype (n = 27; 90%) predominated, whereas in G3 tumors, the TNBC subtype (n = 16; 57.1%) was more common compared with other subtypes. Regarding T classification, the luminal A subtype was more frequently observed in T1 tumors (n = 4; 10.0%), the TNBC subtype in T2 (n = 17; 63.0%) and T3 (n = 6; 22.2%) tumors, and the HR–/HER2+ subtype in T4 tumors (n = 7; 63.6%).

Variation of CA 15-3 tumor marker levels according to molecular subtypes of BC

In recent years, the level of the CA 15-3 tumor marker has been of great importance in the diagnosis of BC and in dynamic monitoring of treatment response. The European group on tumor markers has recommended CA 15-3 for early detection of disease progression, prognosis assessment, and follow-up of BC patients.

As can be seen from the results, the mean concentration of CA 15-3 was higher in the luminal B/HER2– and HR–/HER2+ subtypes. The lowest concentration of this tumor marker was observed in the luminal A subtype. Thus, the CA 15-3 concentration was 57.4% higher in the luminal B/HER2– subtype compared with the luminal A subtype; however, this difference was not statistically significant (p = 0.471) (Table 4).

Table 4

Variation in CA 15-3 concentration according to different molecular subtypes of breast cancer

Parameters		Subtypes					P_H
		Luminal A	Luminal B HER2–	Luminal B HER2+	HR–/ HER2+	TNBC	
CA 15-3, ng/ml	Valid N	34	69	29	10	24	0.273
	Mean (M)	48.9	53.1	61.3	45.3	25.8	
	Median (Me)	18.8	26.3	29.6	29.1	22.3	
	(Q1) 25	12.3	16.9	17.4	17.5	15.3	
	(Q3) 75	36.2	48.1	47.0	36.1	34.1	

Note: P_H – Kruskal–Wallis H test

Variation in the radiomorphological characteristics of axillary lymph nodes according to hormonal receptor status in patients with breast cancer

In 142 patients with ER-positive tumors, axillary lymph node metastasis was detected in 88 (62.0%) cases (p = 0.581). Among these patients, metastases were classified as low in number in 49 (34.5%) cases and high in number in 39 (27.5%) cases (p = 0.849). Regarding lymph node morphology, 68 (47.9%) patients had oval lymph nodes, 13 (9.2%) had irregularly shaped nodes, and 61 (43.0%) had round-shaped nodes (p = 0.901). In 47 (33.1%) patients, the cortical thickness of axillary lymph nodes was slightly increased and/or equal (< 3.0 mm), in 20 (14.1%) patients it was asymmetrically and/or focally increased (> 3.0 mm), and in 75 (52.8%) patients the normal dual-layer structure was completely lost, with absence of corticomedullary differentiation and non-visualization of the hilus (p = 0.608). In addition, lymph node conglomerates were detected in 4 (2.8%) ER-positive BC patients (p = 0.534).

Thus, in patients with ER-positive BC, no statistically significant differences were identified compared to ER-negative tumors in terms of the presence and number of axillary lymph node metastases, as well as the shape and bilayer structure of metastatic lymph nodes.

Among 115 patients with PR-positive tumors, axillary lymph node metastases were detected in 69 (60.0%) patients (p=0.271). A small number of metastases was observed in 42 (36.5%) patients, while multiple metastases were recorded in 27 (23.5%) patients (p=0.250). In these patients, the lymph nodes were oval in 59 (51.3%) cases, irregular in 10 (8.7%) cases, and round in 46 (40.0%) cases (p=0.276). The cortical layer of metastatic axillary lymph nodes was slightly and/or uniformly thickened (<3.0 mm) in 41 (35.7%) patients, asymmetrically and/or focally unevenly thickened (>3.0 mm) in 19 (16.5%) patients, while in 55 (47.8%) patients the bilayer structure was completely disrupted, with the hilum either poorly visualized or completely absent (p=0.053). Conglomerates in metastatic axillary lymph nodes were observed in 2 (1.7%) patients (p=0.135).

Thus, in patients with PR-positive tumors, no statistically significant differences were identified compared to PR-negative tumors in

terms of the presence and number of axillary lymph node metastases, as well as the shape and bilayer structure of metastatic lymph nodes.

Among 41 patients with HER2-positive tumors, axillary lymph node metastases were detected in 30 (73.2%) patients (p=0.129). Of these, a small number of metastases were observed in 15 (36.6%) patients, while multiple metastases were observed in another 15 (36.6%) patients (p=0.052). Among these patients, ultrasonographic examination showed that axillary lymph nodes were oval in 13 (31.7%) cases, irregular in 4 (9.8%) cases, and round in 24 (58.5%) cases (p=0.014). The cortical layer of axillary lymph nodes was slightly and/or uniformly thickened (<3.0 mm) in 9 (22.0%) patients, asymmetrically and/or focally unevenly thickened (>3.0 mm) in 5 (12.2%) patients, while in 27 (65.9%) patients the bilayer structure was completely disrupted (p=0.054). Conglomerates in metastatic axillary lymph nodes were observed in 3 (7.3%) patients (p=0.098).

Thus, in patients with HER2-positive BC, metastases to both axillary and supraclavicular lymph nodes were observed compared to HER2-negative patients. In patients with HER2-positive tumors, metastatic axillary lymph nodes were predominantly round in shape. Statistical results demonstrated that HER2-positive tumors have a high propensity for metastasis to axillary lymph nodes.

Among 137 patients with tumors characterized by Ki-67 ≥14%, metastases to the axillary lymph nodes were detected in 90 (65.7%) patients (p = 0.205). Of these, 49 (35.8%) patients had a low number of metastases, while 41 (29.9%) patients had multiple metastases (p = 0.043). In this group, the shape of the axillary lymph nodes was oval in 62 (45.3%) patients, irregular in 12 (8.8%) patients, and round in 63 (46.0%) patients (p = 0.137). Regarding cortical morphology, slight and/or uniform cortical thickening (<3.0 mm) was observed in 37 (27.0%) patients, whereas asymmetric and/or focal uneven cortical thickening (>3.0 mm) was detected in 13 (16.8%) patients. In 77 (56.2%) patients, the normal biphasic structure was completely disrupted, with an indistinct boundary between the cortex and medulla (p = 0.064).

Thus, in patients with BC and tumors characterized by Ki-67 ≥14%, no statistically significant differences were found compared to

tumors with Ki-67 <14% in terms of the shape and structure of metastatic lymph nodes or the presence of distant metastases. In this group, a statistically significant increase in the incidence of limited metastases to both axillary and supraclavicular lymph nodes was observed compared to patients with Ki-67 <14% tumors. Additionally, in patients with axillary lymph node metastases, the Ki-67 level (31.7 ± 1.9%) was higher than in those without metastases (28.8 ± 2.7%).

Changes in radio-morphological features of axillary lymph nodes in breast cancer patients according to molecular subtypes

Among the 184 patients included in the study, metastases to the axillary lymph nodes were detected in 116 (63.0%) patients. Few metastases were identified in 69 (37.5%) patients, while multiple metastases were observed in 47 (25.5%) patients (Table 5).

Table 5

Changes in the radio-morphological characteristics of axillary lymph nodes according to molecular subtypes

Parameters		Molecular subtypes					P_H
		Luminal A	Luminal B/ HER2–	Luminal B/ HER2+	HR–/ HER2 +	TNBC	
Metastases to the axillary lymph nodes	absent	17 (42.5%)	28 (37.3%)	10 (33.3%)	1 (9.1%)	12 (42.9%)	0.313
	present	23 (57.5%)	47 (62.7%)	20 (66.7%)	10 (90.9%)	16 (57.1%)	
Number of metastases in the axillary lymph nodes	few	17 (42.5%)	24 (32.0%)	9 (30.0%)	6 (54.5%)	13 (46.4%)	0.117
	numerous	6 (15.0%)	23 (30.7%)	11 (36.7%)	4 (36.4%)	3 (10.7%)	
Shape of the axillary lymph nodes	oval	22 (55.0%)	36 (48.0%)	12 (40.0%)	1 (9.1%)	17 (60.7%)	0.018
	irregular	4 (10.0%)	6 (8.0%)	3 (10.0%)	1 (9.1%)	4 (14.3%)	
	round	14 (35.0%)	33 (44.0%)	15 (50.0%)	9 (81.8%)	7 (25.0%)	

Table 5 (continuation)

Parameters		Molecular subtypes					P_H
		Luminal A	Luminal B/ HER2–	Luminal B/ HER2+	HR–/ HER2+	TNBC	
Structure of the axillary lymph nodes	slight and uniform cortical thickening	16 (40.0%)	23 (30.7%)	9 (30.0%)	0 (0.0%)	7 (25.0%)	0.104
	uneven cortical thickening	7 (17.5%)	11 (14.7%)	3 (10.0%)	2 (18.2%)	10 (35.7%)	
	complete loss of the normal biphasic structure	17 (42.5%)	41 (54.7%)	18 (60.0%)	9 (81.8%)	11 (39.3%)	

In this study, the statistical results were analyzed to predict the molecular subtypes of BC based on the radiomorphological characteristics of metastatic axillary lymph nodes. In the Luminal A subtype, axillary lymph node metastases were detected in 23 patients (57.5%), in the Luminal B/HER2− subtype, in 47 patients (62.7%), in the Luminal B/HER2+ subtype, in 20 patients (66.7%), in the TNBC subtype, in 16 patients (57.1%), and in the HR–/HER2+ subtype, in 10 patients (90.9%). As can be seen from the results, among patients with axillary lymph node metastases, particularly those with multiple metastases, Luminal B/HER2+ (36.4%) and HR−/HER2+ (36.4%) subtypes were predominant. However, this difference was not statistically significant ($p = 0.117$). Fewer axillary lymph node metastases were observed in patients with the TNBC subtype ($n = 12$; 42.9%).

As shown in Table 5, oval-shaped axillary lymph nodes were most frequently observed in the TNBC subtype (60.7%, $n = 17$), while round-shaped axillary lymph nodes were more common in the HR−/HER2+ subtype (81.8%, $n = 9$) compared with other subtypes, and this finding was statistically significant ($p = 0.018$).

Axillary lymph nodes with slight and/or uniform cortical thick-

ening (d ≤ 3 mm) were predominantly observed in the Luminal A subtype (n = 16; 40.0%). Asymmetric and/or focal cortical thickening (d > 3 mm) was mainly detected in the TNBC subtype (n = 10; 35.7%), whereas complete loss of the biphasic structure of lymph nodes was most frequently seen in the HR–/HER2+ subtype (n = 9; 81.8%). According to statistical analysis, the cortical thickness of axillary lymph nodes was not significantly associated with the molecular subtypes of BC (p = 0.104).

Tumor size and shape were also found to be associated with a risk of axillary lymph node metastasis, with multiple tumors (n=43; 38.1%; compared with n=10; 14.7%) and tumors with spiculated contours (n=93; 80.2%; p_H =0.035) being more likely to metastasize (p_H <0.001).

The results of the US examination showed that in BC patients who did not receive chemotherapy, the shape of metastatic axillary lymph nodes was oval in 13 (17.1%) patients, irregular in 5 (6.6%) patients, and round in 58 (76.3%) patients (p_H < 0.001) (Figure 1).

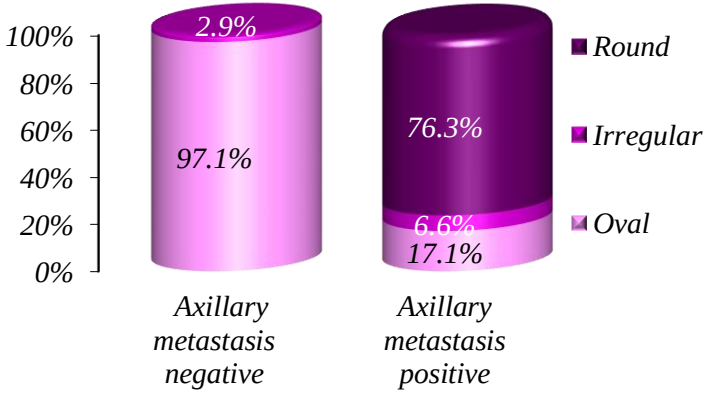


Figure 1. Shape of metastatic axillary lymph nodes

In BC patients who did not receive CT, metastatic axillary lymph nodes showed slight and/or uniform cortical thickening (d ≤ 3.0 mm) in 1.3% (n = 1) of cases, asymmetric and/or focal cortical thickening (d > 3.0 mm) in 15.8% (n = 12), and complete loss of the biphasic structure in 82.9% (n = 63) (p_H < 0.001) (Figure 2).

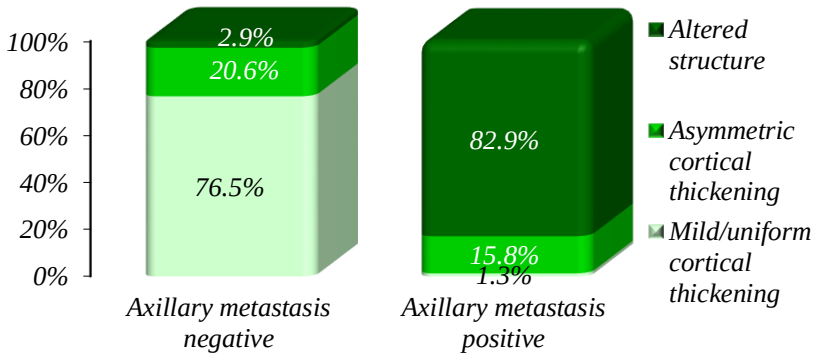


Figure 2. Structure of metastatic axillary lymph nodes

Thus, the main predictors of metastasis to the axillary lymph nodes include tumor size, number, and shape. As can be seen from the obtained results, metastatic axillary lymph nodes are predominantly round in shape (whereas normal lymph nodes are typically oval), with cortical thickening (>3 mm) and complete loss of the biphasic structure (i.e., loss of differentiation between the cortex and medulla).

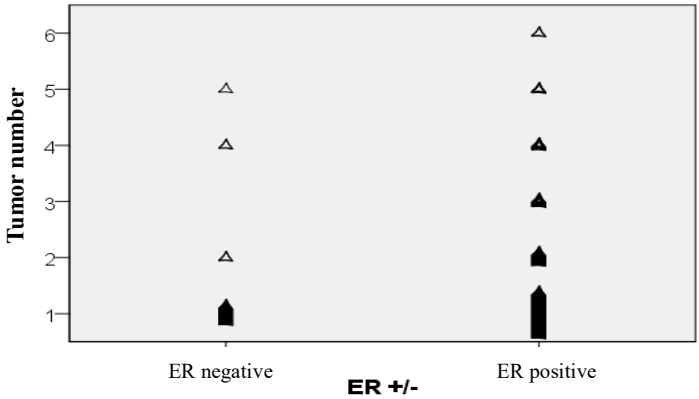
Correlation between US findings and hormonal receptors, molecular subtypes, and their prognostic value in BC patients

In order to determine the correlation between radiomorphological features of the tumor and axillary lymph nodes and hormonal receptors and molecular subtypes in BC patients, Spearman correlation analysis was used.

According to correlation analysis, in BC patients a significant positive correlation was identified between tumor size and number and the risk of axillary lymph node metastasis ($\rho = 0.399$; $p < 0.001$ and $\rho = 0.265$; $p < 0.001$), the number of axillary lymph node metastases ($\rho = 0.391$; $p < 0.001$ and $\rho = 0.352$; $p < 0.001$), lymph node shape ($\rho = 0.282$; $p < 0.001$ and $\rho = 0.323$; $p < 0.001$), and lymph node structure ($\rho = 0.408$; $p < 0.001$ and $\rho = 0.268$; $p < 0.001$). A positive correlation was also found between tumor shape and the risk of axillary lymph node metastasis ($\rho = 0.156$; $p = 0.034$), lymph node shape ($\rho = 0.207$; $p = 0.005$), and lymph node structure ($\rho = 0.160$; $p = 0.030$). These findings indicate that larger tumors, tumors with spiculated margins, and multiple tumors are associated with a higher risk of axillary lymph node metastasis.

Metastatic involvement of axillary lymph nodes is associated with a change in their shape ($\rho = 0.731$; $p < 0.001$), narrowing of the medulla, and loss of the normal biphasic structure ($\rho = 0.854$; $p < 0.001$). In BC patients with axillary lymph node metastases, the risk of multiple metastases is also higher in the subclavicular ($\rho = 0.394$; $p < 0.001$) and supraclavicular ($\rho = 0.265$; $p < 0.001$) lymph nodes.

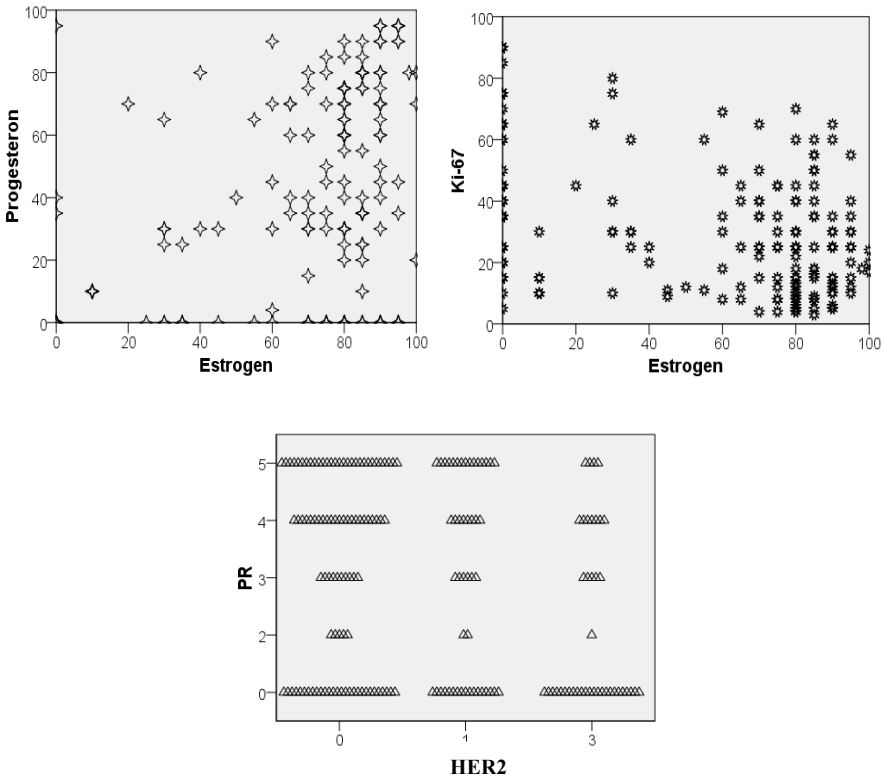
It was found that high expression of the ER receptor showed a positive correlation with tumor number ($\rho = 0.218$; $p < 0.001$), bone metastasis ($\rho = 0.194$; $p = 0.008$), and PR receptor expression ($\rho = 0.622$; $p < 0.001$). In contrast, it showed a negative correlation with tumor differentiation grade ($\rho = -0.204$; $p = 0.006$) and Ki-67 ≥ 14% ($\rho = -0.361$; $p < 0.001$) (Graph 1).



Graph 1. Correlation between tumor number and ER+ expression

The same tendency was observed in cases of low ER expression. Low ER expression showed a positive correlation with tumor number ($\rho = 0.151$; $p = 0.042$), bone metastasis ($\rho = 0.227$; $p = 0.002$), and PR expression ($\rho = 0.550$; $p < 0.001$). In contrast, it showed a negative correlation with tumor differentiation grade ($\rho = -0.261$; $p < 0.001$) and Ki-67 ≥ 14% ($\rho = -0.350$; $p < 0.001$). As can be seen from the results, tumors with high ER expression are also characterized by high PR expression, whereas Ki-67 expression is lower. ER+ tumors are mainly associated with bone metastasis and a lower degree of differentiation.

High PR expression showed a negative correlation with Ki-67 ≥ 14% (ρ = −0.353; p < 0.001) and HER2 receptor expression (ρ = −0.215; p = 0.003). At the same time, an increase in PR expression was identified as one of the indicators of bone metastasis (ρ = 0.201; p = 0.006). In tumors with high PR levels, both Ki-67 (ρ = −0.395; p < 0.001) and HER2 expression (ρ = −0.221; p = 0.003) were lower (Graph 2).



Graph 2. Correlation between estrogen receptors and progesterone receptors, as well as Ki-67 ≥14%, and between progesterone receptors and the HER2 receptor in BC patients.

A positive correlation was identified between high Ki-67 expression and tumor size (ρ = 0.155; p = 0.038), the number of metastases to axillary lymph nodes (ρ = 0.165; p = 0.025), and the T

descriptor ($\rho = 0.196$; $p = 0.008$). These findings indicate that in BC patients with high Ki-67 expression (≥14%), larger tumors with spiculated contours are more frequently observed, the risk of multiple metastases to axillary lymph nodes is higher, and HER2 expression increased.

A positive correlation between high HER2 concentration and changes in the shape of axillary lymph nodes ($\rho = 0.182$; $p = 0.013$), as well as the risk of lung metastasis ($\rho = 0.167$; $p = 0.024$), indicates that in breast cancer (BC) patients with HER2-positive tumors, axillary lymph nodes are predominantly spherical in shape, and these patients have a higher likelihood of developing lung metastases.

A positive correlation between high HER2 concentration and changes in the shape of axillary lymph nodes ($\rho = 0.182$; $p = 0.013$), as well as the risk of lung metastasis ($\rho = 0.167$; $p = 0.024$), demonstrates that in BC patients with HER2-positive tumors, axillary lymph nodes are predominantly spherical in shape, and these patients have a higher probability of developing lung metastases.

A comparative analysis of US parameters in BC patients ($n = 110$) who did not receive chemotherapy allowed for obtaining more accurate and informative data in studying the correlation between radiomorphological characteristics of axillary lymph nodes and hormonal receptors. Among these patients, metastases to axillary lymph nodes were detected in 76 cases. The obtained results demonstrated that the risk of metastasis to axillary lymph nodes ($\rho = 0.450$; $p < 0.001$), the number of metastases ($\rho = 0.441$; $p < 0.001$), the shape ($\rho = 0.386$; $p < 0.001$) and structure ($\rho = 0.529$; $p < 0.001$) of axillary lymph nodes, as well as the number of metastases to subclavicular lymph nodes ($\rho = 0.210$; $p = 0.030$), are directly dependent on tumor size (34.5 ± 1.6 mm). A positive correlation was also identified between tumor shape and the risk of metastasis to axillary lymph nodes ($\rho = 0.209$; $p = 0.029$), as well as the shape ($\rho = 0.215$; $p = 0.024$) and structure ($\rho = 0.208$; $p = 0.029$) of axillary lymph nodes.

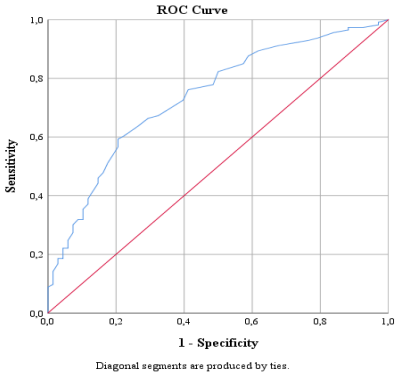
In patients who did not receive chemotherapy (CT), a high Ki-67 concentration (Ki-67 ≥14%) showed a positive correlation with tumor size ($\rho = 0.257$; $p = 0.007$) and shape ($\rho = 0.199$; $p = 0.037$), multiple metastases to axillary ($\rho = 0.246$; $p = 0.010$) and supraclavicular ($\rho = 0.208$; $p = 0.029$) lymph nodes, as well as the shape

(ρ = 0.206; p = 0.031) and structure (ρ = 0.200; p = 0.036) of axillary lymph nodes. Specifically, tumors with high Ki-67 proliferative index were predominantly characterized by spiculated contours (ρ = 0.199; p = 0.037), an increased risk of multiple metastases to axillary lymph nodes (ρ = 0.246; p = 0.010), spherical morphology of axillary lymph nodes (ρ = 0.206; p = 0.031), and disruption of their normal bilayer structure (cortex–medulla differentiation) (ρ = 0.200; p = 0.036). Metastases to supraclavicular lymph nodes (ρ = 0.208; p = 0.029) were also observed.

In BC patients, the main biochemical indicator of metastases to both axillary lymph nodes and other organs is the tumor marker CA 15-3. Correlation analysis revealed a positive association between the level of this tumor marker and the risk of metastasis to axillary lymph nodes (ρ = 0.168; p = 0.031) and subclavicular lymph nodes (ρ = 0.179; p = 0.021), tumor size (ρ = 0.187; p = 0.017), T descriptor (ρ = 0.362; p < 0.001), as well as the shape of axillary lymph nodes (ρ = 0.211; p = 0.006) and disruption of their normal bilayer structure (ρ = 0.226; p = 0.003). An increase in CA 15-3 concentration in larger tumors indicates intensive tumor breakdown and a higher likelihood of metastasis.

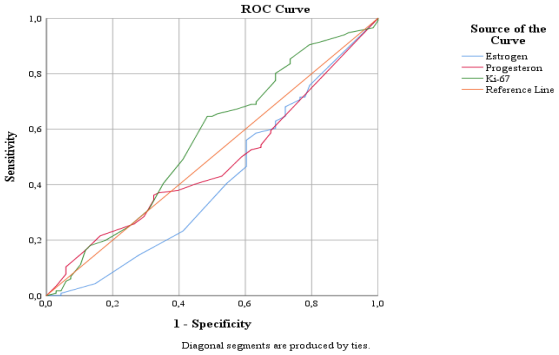
The results of our study also demonstrated that in breast cancer (BC) patients with metastases to axillary lymph nodes, tumor size is significantly larger compared to patients without metastases (p < 0.001). This finding was also confirmed by ROC analysis. Specifically, according to ROC statistical analysis (AUC = 0.737, 95% CI: 0.664–0.811; p < 0.001), tumor size has both high specificity and sensitivity in predicting metastasis to axillary lymph nodes (Graph 3).

According to ROC statistical analysis, in patients with BC, the level of ER (AUC = 0.412, 95% CI: 0.324–0.500; p = 0.046) may be considered a marker with relatively high specificity and sensitivity for the detection of axillary lymph node metastases. As shown in the ROC curves, the levels of PR (AUC = 0.474, 95% CI: 0.389–0.559; p = 0.556) and Ki-67 (AUC = 0.557, 95% CI: 0.469–0.646; p = 0.195) cannot be considered specific or sensitive indicators for the prediction of axillary lymph node metastasis (Graph 4)



Area under the curve					
Test	Area (AUC)	Standard error	P-value	95%CI	
				Lower bound	Upper bound
Tumor size	0.737	0.038	0.000	0.664	0.811

Graph 3. ROC analysis of the relationship between tumor size and axillary lymph node metastasis



Area under the curve					
Tests	Area	Standard error	P-value	95% CI	
				Lower bound	Upper bound
Estrogen	0.412	0.045	0.046	0.324	0.500
Progesterone	0.474	0.043	0.556	0.389	0.559
Ki-67	0.557	0.045	0.195	0.469	0.646

Graph 4. ROC analysis of the relationship between axillary lymph node metastasis and hormonal receptors

Thus, in our study, axillary lymph node metastases were detected in 63% of BC patients, particularly with multiple metastatic involvement. The results demonstrated that the risk of axillary lymph node metastasis in BC patients depends on tumor size, shape, and number. In other words, larger tumors with spiculated margins have a higher metastatic potential. Axillary lymph node metastases were mainly observed in T4 locally advanced and N3 stage tumors. In patients with axillary lymph node metastasis, the risk of distant metastasis to the lungs and bones is also increased. A high level of the CA 15-3 tumor marker in BC patients was confirmed as one of the main indicators of axillary lymph node metastasis.

Results showed that among BC patients, the luminal B/HER2− subtype was predominant, whereas the HR−/HER2+ subtype was rarely observed. This subtype is more aggressive and demonstrates a higher risk of axillary lymph node metastasis compared with other subtypes. These findings are consistent with the results of our study. The obtained results indicated that TNBC and luminal A subtypes have a lower risk of lymph node metastasis compared with luminal B and HR–/HER2+ subtypes. The radiomorphological characteristics of axillary lymph nodes differ according to the histological subtypes of BC. In the TNBC subtype, oval lymph nodes with cortical thickening (<3.0 mm) are more common, whereas in the HR−/HER2+ subtype, spherical lymph nodes with disrupted normal structure predominate. A single, well-defined tumor contour and absence of calcifications are considered typical features of the TNBC subtype. Although this subtype has a high metastatic potential, an aggressive clinical course, and a poor prognosis, it causes less damage to the axillary lymph nodes compared to other subtypes.

Correlation relationships between US features of metastatic axillary lymph nodes and the molecular-biological characteristics of the tumor serve as important predictors for the future determination of BC subtypes. In patients with BC, when it is not possible to identify molecular subtypes based on the radiomorphological characteristics of the axillary lymph nodes and the tumor, the assessment of hormonal receptors may have significant practical value in making early treatment decisions.

CONCLUSIONS

1. Intratumoral microcalcifications were most frequently observed in the luminal B/HER2+ subtype (60.0%) and least frequently in the TNBC subtype (17.9%) ($p = 0.017$). In the luminal A subtype (37.5%), tumors with well-defined and irregular contours were more commonly observed compared to other subtypes, whereas in luminal B subtypes (luminal B/HER2− (80.0%) and luminal B/HER2+ (86.7%)), spiculated tumor contours were more frequently detected. In the HR−/HER2+ subtype, larger tumor sizes were recorded ($p = 0.123$) [11, 15].
2. Changes in the shape of axillary lymph nodes ($\rho = 0.731$; $p < 0.001$) and the loss of corticomedullary differentiation, i.e., disruption of the biphasic structure ($\rho = 0.854$; $p < 0.001$), are among the significant indicators of metastasis. In the HR−/HER2+ subtype, spherical lymph nodes (81.8%; $p = 0.018$) and disrupted biphasic structure (81.8%; $p = 0.104$) were more frequently observed, whereas in the TNBC subtype, oval-shaped axillary lymph nodes (60.7%; $p = 0.018$) were statistically more common compared to other subtypes [12, 14, 23].
3. A significant positive correlation was found between high Ki-67 expression in tumor cells (Ki-67 ≥14%) and multiple axillary lymph node metastases ($\rho = 0.246$; $p = 0.010$), as well as lymph node shape ($\rho = 0.206$; $p = 0.031$) and structure ($\rho = 0.200$; $p = 0.036$). Additionally, a positive correlation was observed with HER2 receptor expression ($\rho = 0.164$; $p = 0.025$) [13, 15].
4. A higher frequency of axillary lymph node metastasis was observed in the HR−/HER2+ subtype (90.9%). In contrast, patients with the TNBC subtype had a lower risk of axillary lymph node metastasis (57.1%) ($p = 0.313$). Spiculated tumor contours (80.2%; $p = 0.035$), tumor multiplicity (38.1%; $p < 0.001$), and tumor size (37.5 ± 1.6 mm vs. 25.6 ± 1.3 mm; $p < 0.001$) were identified as predictors of axillary lymph node metastasis [11, 15].
5. According to ROC statistical analysis, tumor size (AUC = 0.737, $p < 0.001$) and ER level (AUC = 0.412, $p = 0.046$) demonstrated both high specificity and sensitivity in predicting axillary lymph node

metastasis. In contrast, PR (AUC = 0.474, p = 0.556) and Ki-67 (AUC = 0.557, p = 0.195) cannot be considered reliable specific or sensitive indicators. Furthermore, in patients with metastatic axillary lymph nodes, an increased serum concentration of the CA 15-3 tumor marker was identified as an important indicator and showed a positive correlation with lymph node shape ($\rho = 0.211$; $p = 0.006$) and disruption of the biphasic structure ($\rho = 0.226$; $p = 0.003$) [7, 16, 17].

PRACTICAL RECOMMENDATIONS

1. In the initial evaluation of axillary lymph nodes, the assessment of US findings in relation to the molecular subtypes of the tumor enables more accurate prediction of metastatic risk and supports evidence-based decision-making in selecting either sentinel (signal) lymph node biopsy or complete dissection in surgical management.
2. During US examination, metastatic axillary lymph nodes characterized by a spherical shape, loss of corticomedullary differentiation, and a narrowed or absent hilum may predict high HER2 receptor expression in BC. The HR−/HER2+ subtype is considered a more aggressive subtype in terms of axillary lymph node metastasis. Therefore, in BC patients with this subtype, removal of both the primary tumor and axillary lymph nodes during surgery is recommended.
3. In patients with BC, the high risk of axillary lymph node metastasis associated with large tumors with spiculated margins should be taken into consideration in clinical practice.

List of publications related to the dissertation topic

1. Akhundova, J.N. Radiological imaging methods in the diagnosis of breast cancer / J.N. Akhundova, M.J. Sultanova, N.V. Gasimov // Saghlamlig, – Baku: – 2021. Vol. 27, No. 2, – pp. 35–39 (in Azerbaijani).
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13. Akhundova, J.N. The role of hormonal receptors in the development of metastases to axillary lymph nodes in patients with breast cancer / J.N. Akhundova, N.V. Gasimov, M.J. Sultanova // Journal of Medicine and Science, – Baku: – 2024. No. 1, – pp. 22–28 (in Azerbaijani).
14. Akhundova, J.N. The relationship between molecular subtypes of metastatic axillary lymph nodes and radiomorphological parameters in patients with breast cancer who did not receive neoadjuvant-chemotherapy//–Moscow: Effective Pharmacotherapy, — 2024. Vol. 20, No 36, – pp. 16–23 (in Russian).
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AZƏRBAYCAN RESPUBLİKASI SƏHİYYƏ NAZİRLİYİ
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List of abbreviations

ER – Estrogen receptor
PR – Progesterone receptor
HER2 – Human Epidermal Growth Factor Receptor 2
HR – Hormone receptors
CT – Chemotherapy
Ki-67 – Ki-67 antigen or Ki-67 nuclear antigen
CT– Computed tomography
LN – Lymph nodes
MRI – Magnetic resonance imaging
BC – Breast cancer
TNBC – Triple-negative breast cancer
US – Ultrasound examination

The defense will be held on 22 may at 14⁰⁰ at the meeting of the Dissertation Council FD 1.02 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the National Oncology Center of the Ministry of Health of the Republic of Azerbaijan.

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