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Analysis of breast cancer subtypes and their correlations with receptors and ultrasound

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Abstract

The study aim was to evaluate the ultrasound (US) signs of the mammary lesions classified in the Breast Imaging-Reporting and Data System (BI-RADS) score category 3, 4, and 5, corresponding to US BI-RADS. It also followed the correlation between US changes of lesions suggestive for malignancy with the histopathological results and evaluated the proper management of those lesions. There were correlations of breast cancer (BC) subtypes with the receptors [estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2)], and Ki67 index, and the signs of conventional ultrasonography and US elastography. We selected 108 female patients examined with US, mammography and fine-needle biopsy who presented suspicions for malignancy lesions. Following the immunohistochemical analysis, they were classified in one of the BC subtypes. According to *chi*-squared analysis of molecular cancer subtypes correlation to receptors and Ki67 index, we found significant associations between both luminal A and luminal B HER2-negative subtypes and hormone receptors (ER, PR). These have an inverse relationship with Ki67 index elevated values; luminal B HER2-positive subtype has a direct association with HER2 presence; HER2-enriched subtype was statistically significant associated to HER2 presence and elevated Ki67 index values but had an inverse relationship to hormone receptors (ER, PR); triple-negative subtype was strongly associated to Ki67 index values and inversely correlated to ER and PR. We found luminal A subtype as being the most common and luminal B HER2-positive subtype as having the fewer cases.

Keywords: breast cancer, ER, PR, HER2, Ki67, ultrasound.

Introduction

One of the most frequent diseases in women is breast cancer (BC), with a significant contribution to all-cause mortality [1, 2].

Several ultrasound (US) characteristics of malignant breast lesions, such as hypoechogenicity, irregular shape, vertical orientation, presence of spicules, posterior acoustic shadowing, absence of calcifications, and increased vascular signal can be associated with BC subtypes, improving both the diagnosis and case management [3].

Breast elastography is a complementary imaging technique, utilized only in association with B-mode US, which improves BC diagnosis. The Tsukuba score is used to differentiate between benign and malignant breast lesions.

Breast elastography is a rapid and simple method that can improve the sensitivity and specificity of US, especially when we have focal lesions categorized as Breast Imaging-Reporting and Data System (BI-RADS) 3 and 4. This technique improves patient management and reduces unnecessary biopsies but requires follow-up [4–7].

The need for a more accurate understanding of BC, including the need for appropriate treatment, led to research concluded in the introduction of new information regarding the molecular characterization of breast tumors. This data is meant to complete the existing histopathology and imagistic information defining a breast tumor.

Their definition is based on the presence of four different tumor cells compounds: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki67.

The therapeutic decision must be based on understanding the disease and also on the use of different types of investigation and treatment, which need to respect the ethical rules, to obtain a right informed consent [8] and analyze the different types of cancer not only in a public health context [9] but also like an individual major health problem in any stage of detection and treatment, including palliative care period [10].

Immunohistochemistry plays an important role in detecting biomarker expression concerning breast pathology and it has demonstrated a strong correlation between receptor expression and patient's response to therapy. Gene profile analysis is important but expensive and difficult to perform routinely. ER, PR, HER2, and Ki67 are the most common immunomarkers used in determining the prognostic of BC and for deciding the therapeutic strategy [11].

According to *St. Gallen Consensus* 2013 Classification, there are five molecular subtypes of BC (Table 1) [12, 13].

The grouped calcifications evident on the US exam at the level of a lesion and confirmed mammography and histopathological (HP) examination, more than five per 1 cm, raise suspicion of malignancy [14].

Table 1 – *Characterization of BC molecular subtypes*

Molecular BC subtype	Receptor			*Ki67 index*	Prognosis	Responds to therapy
	ER	*PR*	*HER2*			
Luminal A	+	+	–	Low (<14%)	Good	Hormonal therapy
Luminal B HER2-negative	+	+	–	Low (≥14%)	Worse	Hormonal therapy
Luminal B HER2-positive	+	+	+	–	Worse	HER2-targeted therapy (Trastuzumab) + hormonal therapy
HER2-enriched	–	–	+	–	Worse	HER2-targeted therapy (Trastuzumab)
Triple-negative (basal-like)	–	–	–	Possible high	Bad	Chemotherapy

BC: Breast cancer; ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; PR: Progesterone receptor.

Aim

The study analyzed the correlation of US changes of lesions suggestive of malignancy with the HP results. There were correlations of BC subtypes with the ER, PR, and HER2 receptors, and Ki67 index, and the US aspect of the mammary tumor formations.

Patients, Materials and Methods

The study was prospective for a period of six months, in 2019, as part of a screening conducted in a private Hospital in Braşov, Romania, within the Department of Radiology and Medical Imaging. Out of 2502 patients, 108 were selected who were US-framed in one of the lesions categorized as BI-RADS 3, 4, and 5 scores, and on whom biopsy was performed [immunohistochemical (IHC) detection] at the level of the suspected US lesion.

The distribution of patients by age groups was as follows: five patients in the 21–30 years group, nine patients in the 31–40 years group, 20 in the 41–50 years group, 27 patients in the 51–60 years group, 31 in the 61–70 years group, 10 patients in the 71–80 age group and six patients over 81 years (Figure 1).

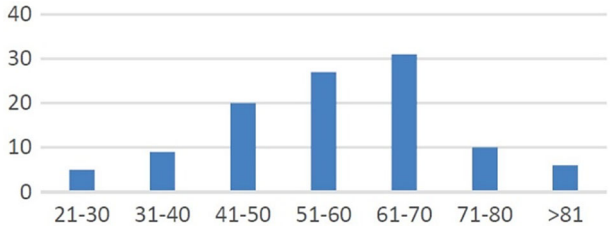

Figure 1 – *Distribution of cases by age categories.*

The devices used in the study were:

▪ for mammograms: Senographe Crystal Nova (General Electric), Mammomat 3000 Nova (Siemens);

▪ for ultrasonography: Logiq S7 (General Electric) US machine, RS80 with Prestige (Samsung) US machine.

Statistical Package for the Social Sciences (SPSS) 20.0 software was used to analyze all the patients selected. Count data were expressed and compared using χ^2 (*chi*-squared) test, $p<0.05$ (considered statistically significant), and Cramér's V test interpretation (>0.25 – very strong; >0.15 – strong; >0.10 – moderate; >0.05 – weak; >0 – no or very weak). Cramér's V statistic is frequent used as measure of association between two categorical variables. Cramér's V test is the most widely used of the nominal-based measures of *chi*-squared test, offering a good standardization from 0 to 1, regardless of the size of the table [15].

Results

Out of 2502 patients evaluated in the screening, we selected 108 patients examined by US, mammography, and fine-needle biopsy, who presented suspicions for malignancy lesions, having BI-RADS score of 4 or 5. Following the IHC analysis, they were classified in one of the BC subtypes.

The highest incidence of US lesions was more frequent between 51 and 70 years old, with an average of 27.9%, and the lowest incidence was observed under the age of 30, 4.9% respectively.

Lesions to the right breast were more common, with a difference of 4% as compared to the left breast. Unique breast lesions were observed in 57.8% of cases; multiple lesions either in one breast or bilaterally were noted in 42.2% of cases. In 60.78% of the cases, the lesion was present in the external upper quadrant, followed by 18.62% in the internal upper quadrant, and with a lower percentage in the lower quadrants (11.76% inferior external and 8.82% in the lower medial quadrant).

Over half (51%) of the mammary tumors suspected of malignancy found on US examination had dimensions over 2 cm and have been easily emphasized; in 29% of the cases, dimensions situated between 1–2 cm and 20% lesions measure less than 1 cm and were more present in younger patients.

We considered the following characteristics as being malignant lesions on the US examination: hypoechoic, inhomogeneous structure, microlobulations, including microcalcifications vascular signal, and hypervascularization inside the tumor. Eighty-three (76.85%) cases were confirmed for BC, 23 (21.3%) cases were classified as benign lesions and two (1.85%) cases were inconclusive and were due to repeat the examination.

Analyzing the distribution of BC patients by subtype (immunohistopathologically), we found the highest incidence of the luminal A subtype – 53.57% of the cases, most frequently evidenced in the 51–60 years age group. The luminal B subtype was present in 22.61% of the cases, most frequently evidenced in the 41–50 years age group. The triple-negative subtype was present in 10.71% of the cases, most frequently evidenced in the 61–70 years age group. Less than 10% were HER2-positive enriched-subtype – 7.14%, and luminal B HER2-positive subtype – 5.95%. We found luminal A subtype to be the most common and luminal B HER2-positive subtype as having fewer cases (Figure 2).

The US characteristics of the mammary lesions were analyzed and correlated to all molecular receptors (ER, PR, HER2) significant for this type of cancer, and Ki67

index, and with HP subtypes according to *St. Gallen Consensus* 2013 Classification (Table 2).

The following aspects were noted:

▪ ER and PR were positively correlated with luminal A (p<0.001) and luminal B HER2-negative subtypes (p<0.001 for ER and p=0.007 for PR), and inversely correlated with HER2-enriched (p=0.001 for ER and p=0.004 for PR) and triple-negative subtypes (p<0.001);

▪ HER2 was correlated with luminal B HER2-positive (p=0.04) and HER2-enriched (score 3) (p<0.001) subtypes;

▪ 14% of Ki67 was positively correlated with HER2-enriched (p=0.003) and triple-negative (p<0.001) subtypes, and inversely correlated with luminal A (p<0.001) and luminal B HER2-negative (p<0.001) subtypes.

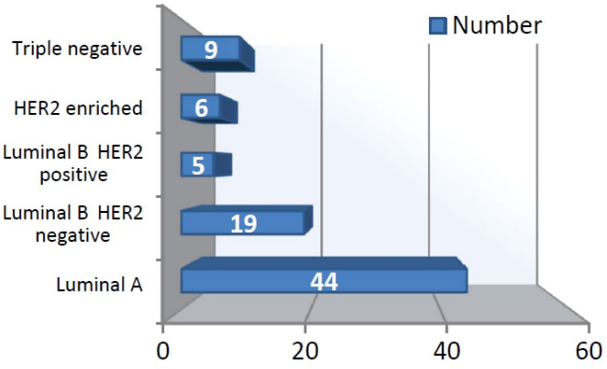

Figure 2 – ***Distribution of breast cancer patients by immunohistopathological subtype.***

Table 2 – ***Correlation between breast cancer molecular subtypes and ER, PR, HER2 molecular receptors, and Ki67 index***

Molecular receptors	p / OR	Subtype				
		Luminal A	*Luminal B HER2-negative*	*Luminal B HER2-positive*	*HER2-enriched*	*Triple-negative*
ER-positive	*p*	<0.0001	0.0002	0.0790	0.0010	<0.0001
	OR	>1	>1	>1	<1	<1
PR-positive	*p*	<0.0001	0.0072	0.2774	0.0040	0.0003
	OR	>1	>1	>1	<1	<1
HER2-positive	*p*	0.0910	0.3463	0.0481	<0.0001	0.5388
	OR	<1	<1	>1	>1	>1
Ki67 index >14%	*p*	<0.0001	<0.0001	0.0938	0.0033	<0.0001
	OR	>1	>1	>1	>1	>1

ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; OR: Odds ratio; PR: Progesterone receptor.

Multiple US findings were correlated with ER presence (p=0.045) and HER2-positive (p=0.012) immunoexpression.

According to the data analysis using Cramér's V test, the following HP subtypes were correlated with the presence of vascularization at the level of US-examined lesions: the luminal B HER2-negative and triple-negative subtypes were moderately correlated (values >0.10), and the luminal B HER2-positive and luminal B HER2-positive subtypes were poorly correlated (values >0.05) (Table 3). The increased vascularization present in US was correlated with the absence of PR (p=0.014).

Table 3 – ***Correlations between breast cancer subtypes and ultrasound signs – lesion vascularization and the presence of adenopathy (Phi / Cramér's V test)***

Subtype	Phi / Cramér's V test values	
	Vascularization	*Adenopathy*
Luminal A	0.004359	0.116868
Luminal B HER2-negative	0.108289	0.123667
Luminal B HER2-positive	0.073902	0.038259
HER2-enriched	0.098183	0.150414
Triple-negative	0.127273	0.112194

HER2: Human epidermal growth factor receptor 2.

In our study, 70.58% of the evaluated patients have adenopathy; the majority being axillary and less parasternal in the case of lesions in the internal quadrants, adenopathy was anatomopathologically confirmed, but sometimes without describing their HP characteristics by the examining doctor, respectively partially or totally increase of the thickness of the cortex, reduction, or disappearance of the sinus. 29.41% of the patients did not have axillary pathological adenopathy at the first evaluation; the observed cases had lesions with dimensions less than 1 cm compared to those who had adenopathy, in which the tumor formation was frequently over 2 cm.

According to the data analysis performed using Cramér's V test, the following HP subtypes were correlated with the presence of adenopathy in the US-examined lesions: the luminal A subtype, the luminal B HER2-negative subtype, the non-luminal HER2-positive subtype, and the triple-negative subtype correlated moderately (values >0.10) with US appearance of adenopathy (Table 4).

Table 4 – ***Correlations between breast cancer subtypes and ultrasound signs – contour (Phi / Cramér's V test)***

Subtype	Phi / Cramér's V test values	
	Well-defined contour	*Inaccurate contour delimited / speculated*
Luminal A	0.203144	0.201165
Luminal B HER2-negative	0.066156	0.121548
Luminal B HER2-positive	0.095229	0.114610
HER2-enriched	0.117161	0.071309
Triple-negative	0.130319	0.075011

HER2: Human epidermal growth factor receptor 2.

The US presence of adenopathy was associated with the absence of PR (p=0.007), with equivocal score (score 2) of HER2 (p=0.038), and with increased score (>14%) of Ki67 index (p=0.005). In addition, adenopathy correlated with the onset of invasive ductal carcinoma (p=0.018) of mucinous carcinoma (p=0.013) and with grade 3 malignancy of BC (p=0.007).

Regarding the correlations between BC subtype and US signs (contour, lobulation), we found the following: (*i*) US-examined malignant lesions showed an irregular contour in 83.33% of cases, compared to the lesions

highlighted mammographically in the same cases; (*ii*) US was able to correctly describe the contour, especially if the breast tissue was dense, compared to mammography, where the inaccurate contour was described in 53.16% of the lesions.

A percentage of 16.67% of the US-detected tumors presented well-defined contour; these patients were mostly less than 50 years of age.

Concerning lobulation, the most frequent US characteristic was microlobulation in 44% cases, followed by macrolobulation in 19% cases, and absence of lobulation in 37% of cases. This statement demonstrates the necessity of lesions revaluation, especially when first they were considered benign and if the age of the patient is young. The US revaluation of these lesions after six months showed their evolution towards a suspected US malignant lesion, which leads to biopsy and determination of the BC subtype.

According to the data analysis using Cramér's V test, the following HP subtypes correlated with well-defined margins of the US-examined lesions: luminal A subtype was strongly correlated (values >0.15), luminal B HER2-negative and luminal B HER2-positive subtypes were weakly correlated (values >0.05), and non-luminal HER2-positive and triple-negative subtypes were moderately correlated (values >0.10).

The following HP subtypes were correlated with indefinitely delimited margins of the US-examined lesions: luminal A subtype strongly correlated (values >0.15), luminal B HER2-negative and luminal B HER2-positive subtypes moderately correlated (values >0.10), and HER2-enriched and triple-negative subtypes poorly correlated (values >0.05) (Table 5). The malignant characters of the breast tumor formation are highlighted in Figure 3.

Table 5 – ***Correlations between breast cancer subtypes and ultrasound signs – lobulation (Phi / Cramér's V test)***

Subtype	Phi / Cramér's V test values		
	No lobulation	*Macro-lobulation*	*Micro-lobulation*
Luminal A	0.097921	0.062323	0.063706
Luminal B HER2-negative	0.187659	0.021881	0.250740
Luminal B HER2-positive	0.114488	0.101800	0.007448
HER2-enriched	0.075241	0.005898	0.040996
Triple-negative	0.011692	0.036661	0.050965

HER2: Human epidermal growth factor receptor 2.

Inaccurate or spiked margins were correlated with the presence of ER (p<0.001), the presence of PR (p=0.001), HER2-negative (p<0.001), and low values (<14%) of Ki67 index (p=0.018). In addition, luminal A, invasive ductal carcinoma, grade 2 malignancy HP subtype was correlated with the US character of imprecise or spiculate margins.

The data analyzed by means of the Cramér's V test revealed that the following HP subtypes were correlated with the presence of microlobulations in the US-examined lesions: the luminal A subtype and the triple-negative subtype were weakly correlated (values >0.05), and the luminal B HER2-negative subtype was strongly correlated (values >0.15).

Microlobulations were correlated with the presence of ER (p=0.007), the presence of PR (p=0.003), the absence of HER2 (p=0.008), and the luminal B HER2-negative subtype (p=0.009).

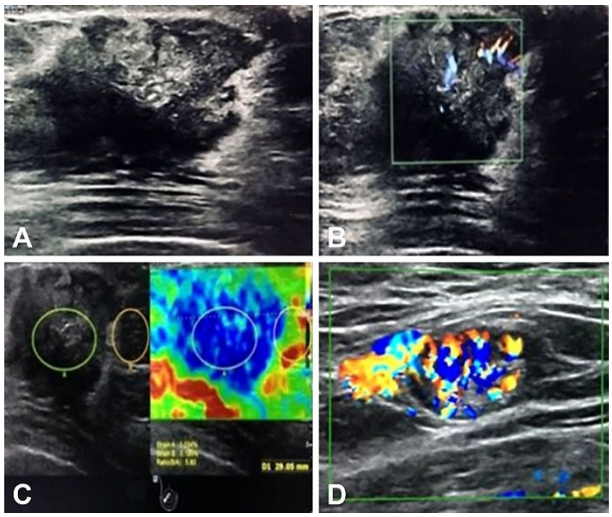

Figure 3 – ***Ultrasound examination of a 78-year-old patient, right breast, upper and external quadrant: hypoechoic lesion, irregular, with microlobulations (A), and increased vascular signal (B), increased elastographic value – strain ratio 5.8 (C), axillary pathological adenopathy (D). Anatomopathological characteristics: invasive breast carcinoma; ER-positive 80%; PR-positive 60–70%; HER2-negative (score 0); Ki67 index 10%; luminal A subtype. ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; PR: Progesterone receptor.***

The following HP subtypes were correlated with the presence of macrolobulation in the US-examined lesions: the luminal A subtype weakly correlated (values >0.05) and the luminal B HER2 subtype was moderately positively correlated (values >0.10) (Table 6).

Table 6 – ***Correlations between breast cancer subtypes and ultrasound signs – structure (Phi / Cramér's V test)***

Subtype	Phi / Cramér's V test values	
	Homogeneous	*Non-homogeneous ± halo*
Luminal A	0.134840	0.133598
Luminal B HER2-negative	0.020197	0.055777
Luminal B HER2-positive	0.070381	0.091882
HER2-enriched	0.061980	0.012643
Triple-negative	0.019263	0.062869

HER2: Human epidermal growth factor receptor 2.

According to the data analysis using Cramér's V test, the following HP subtypes were correlated with the homogeneous structure of the US-examined lesions: the luminal A subtype was moderately correlated (values >0.10), and the luminal B HER2-positive and HER2-enriched subtypes were weakly correlated (values >0.05).

The following HP subtypes were correlated with the non-homogeneous structure of the US-examined lesions: the luminal A subtype was moderately correlated (values >0.10), and the luminal B HER2-negative, luminal B HER2-positive, and triple-negative subtypes were weakly correlated (values >0.05).

The non-homogeneous structure or presence of the peripheral wall was correlated with the presence of ER (p=0.022) and the presence of PR (p=0.006). In addition, invasive ductal breast carcinoma was correlated with the presence of the non-homogeneous character of the tumor (p=0.045) (Table 7).

Table 7 – ***Correlations between breast cancer subtypes and ultrasound signs – calcifications (Phi / Cramér's V test)***

Subtype	Phi / Cramér's V test values			
	Without calcifications	*Macrocalcification*	*Isolated microcalcifications*	*Focal microcalcifications*
Luminal A	0.057085	0.025885	0.039479	0.053300
Luminal B HER2-negative	0.078145	0.116912	0.038880	0.037830
Luminal B HER2-positive	0.139798	0.030264	0.085029	0.062318
HER2-enriched	0.024821	0.033315	0.026743	0.085749
Triple-negative	0.030857	0.041416	0.016623	0.042640

HER2: Human epidermal growth factor receptor 2.

It is important to differentiate benign (with increase with age) from suspected malignant microcalcifications, as more than half of non-palpable cancers are ductal carcinoma *in situ*.

According to the data analysis based on the Cramér's V test, the following HP subtypes were correlated with the absence of calcifications in the US-examined lesions: the luminal A and luminal B HER2-negative subtypes weakly correlated (values >0.05), and luminal B HER2-positive subtype moderately correlated (values >0.10).

The luminal B HER2 subtype was positively correlated (values >0.05) with the presence of isolated microcalcifications in the US-examined lesions.

The analysis of the data performed by means of the Cramér's V test revealed that the HP subtypes were correlated with the presence of focal microcalcifications in the US-examined lesions: luminal A, luminal B HER2-positive, and HER2-enriched subtypes poorly correlated (values >0.05).

The luminal B HER2-negative subtype was moderately correlated (values >0.10) with the presence of macrocalcifications in the US-examined lesions (Table 8).

Table 8 – ***Correlations between breast cancer subtypes and ultrasound signs – posterior acoustic changes (Phi / Cramér's V test)***

Subtype	Phi / Cramér's V test values				
	Posterior amplification	*Without attenuation*	*Total attenuation*	*Total bilateral symmetrical marginal shadow*	*Bilateral asymmetric marginal shadow*
Luminal A	0.080158	0.222727	0.075011	0.159091	0.093275
Luminal B HER2-negative	0.044667	0.010265	0.056649	0.051325	0.034391
Luminal B HER2-positive	0.021300	0.050487	0.100657	0.066431	0.031159
HER2-enriched	0.023447	0.190131	0.027427	0.073127	0.085749
Triple-negative	0.029148	0.018182	0.075011	0.030303	0.000000

HER2: Human epidermal growth factor receptor 2.

US analysis of the attenuation created by the suspicious mammary lesion revealed that most lesions presented asymmetric bilateral shadow (35.29% of cases); in 32.35% of cases, no posterior acoustic changes were noted. The total attenuation was found in 22.54% of cases and the bilateral symmetrical marginal shadow in 8.82% of cases. In less than 1% of cases, the lesions were described as presenting acoustic amplification. The malignant characters of the breast tumor formation, especially details of the contour and structure, were highlighted in Figure 4.

The Cramér's V test-based data analysis showed that the following HP subtypes were correlated with the presence of US-examined lesions without posterior acoustic changes: the non-luminal positive, luminal A and HER2 subtypes strongly correlated (values >0.15), and the luminal B HER2-positive subtype poorly correlated (values >0.05). The luminal A subtype was weakly correlated (values >0.05) with the US character of the posterior acoustic amplification of the examined lesions.

The following HP subtypes were correlated with the total posterior acoustic attenuation of the US-examined lesions: luminal A, luminal B HER2-negative and triple-negative subtypes were poorly correlated (values >0.05), and luminal B HER2-positive subtype was moderately correlated (values >0.10).

Regarding the correlation of the BC subtypes with the bilateral symmetric marginal shadow character of the US-examined lesions, it was found that luminal A subtype was strongly correlated (values >0.15), and luminal B HER2-negative, luminal B HER2-positive and HER2-enriched subtypes were weakly correlated (values >0.05).

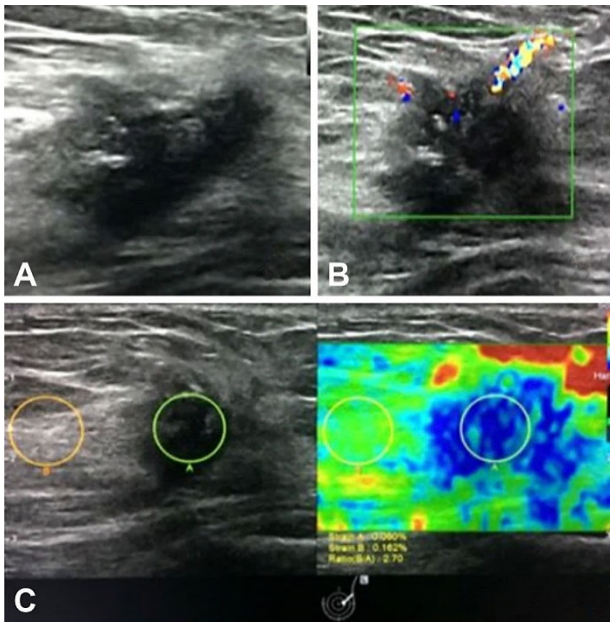

Figure 4 – ***Ultrasound examination of a 65-year-old patient, left breast, upper and outer quadrant: hypoechoic lesion, irregular mass, echogenic rim (A), increased vascular signal, focal microcalcifications (B), increased elastography value – strain ratio 2.70 (C). Anatomopathological characteristics: ER-negative; PR-negative; HER2-positive (score 2+); Ki67 index 60–70%; HER2-enriched subtype. ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; PR: Progesterone receptor.***

The luminal A and HER2-enriched subtypes were weakly correlated (values >0.05) with the bilateral asymmetric marginal shadow character of the US-examined lesions.

Asymmetrical bilateral acoustic attenuation on US examination was correlated with the presence of PR (p=0.02) and grade 2 malignant BC (p=0.04).

The statistics obtained by means of the Cramér's V test showed the following results:

- Tumors' vascularization has a direct relationship with luminal B HER2-negative and triple-negative subtypes with moderate correlation (values >0.10), and with the absence of PR (p=0.014);
- Pathological lymph nodes were positively correlated with luminal A, luminal B HER2-negative, HER2-enriched, and triple-negative subtypes, and with the absence of PR (p=0.007), with HER2 equivocal score (score 2) (p=0.038), with increased score (>14%) of Ki67 index (p=0.005), with the onset of invasive ductal carcinoma (p=0.018), mucinous carcinoma (p=0.013), and with grade 3 malignancy of BC (p=0.007);
- Ill-defined margins had a positive relationship with luminal A, luminal B HER2-negative, luminal B HER2-positive subtypes, and with the presence of ER (p<0.001), the presence of PR (p=0.001), HER2-negative (score 1) (p<0.001), low values (<14%) of Ki67 index (p=0.018), invasive ductal carcinoma, and grade 2 malignancy;
- The luminal B HER2-negative subtype was correlated with the presence of microlobulations in the US-examined lesions; microlobulations were correlated with ER-positive (p=0.007), PR-positive (p=0.003), the absence (score 1) of HER2 (p=0.008), and the luminal B HER2-negative subtype (p=0.009), as well;
- The luminal B HER2-positive subtype was positively correlated with the presence of macrolobulation in the US-examined lesions;
- The luminal A subtype was positively correlated with the inhomogeneous internal structure of the US-examined lesions; this ultrasonography characteristic was correlated with ER-positive (p=0.022), PR-positive (p=0.006), and invasive ductal breast carcinoma (p=0.045), as well;
- The luminal B HER2-negative subtype was moderately correlated (values >0.10) with the presence of macrocalcifications in the US-examined lesions;
- The luminal B HER2-positive subtype was correlated with the total posterior acoustic attenuation of the US-examined lesions;
- The luminal A subtype was correlated with the bilateral symmetrical marginal shadowing character of the US-examined lesions; asymmetrical bilateral acoustic attenuation on US was correlated with the presence of PR (p=0.02) and grade 2 malignant BC (p=0.04).

Discussions

According to *St. Gallen Consensus* 2013, breast tumors were classified into five categories based on their molecular characteristics [12]: three non-basal subtypes (luminal A, luminal B HER2-negative and luminal B HER2-positive) and two basal-like subtypes (HER2-enriched and triple-negative) [16]. Luminal A and luminal B subtypes were considered those which were positive for ER and PR, and which form the luminal-like group [17–19].

By analyzing the distribution of BC patients depending on IHC subtype, we found the highest incidence of the luminal A subtype in 53.57% of the cases, which aligns with Naeem *et al.* findings, most frequently evidenced in the 51–60 years old age group [20]. Patients diagnosed with BC luminal A subtype were among the youngest ones, while patients presenting HER2 immunoexpression were found to be older of age (p=0.01), as Wen *et al.* postulate in their study [3].

The luminal B HER2-negative subtype was present in 22.61% of the cases, which is different from the study conducted by Su *et al.*, most frequently evidenced in the 41–50 years age groups [21].

The triple-negative subtype was present in 10.71% of the cases, as resulted in other studies, most frequently evidenced in the 61–70 years age groups [22].

Less than 10% of BCs were HER2-enhanced (7.14%) and luminal B HER2-positive (5.95%) subtypes.

Kondov *et al.* determined the incidence of different BC subtypes, as follows: luminal A was the most frequent one, followed by luminal B HER2-negative, luminal B HER2-positive, HER2-enriched, and the least frequent one was triple-negative; in the present study, we found HER2-enriched patients to be more numerous than the luminal B HER2-positive ones [23].

Determining BC subtype has a major impact on therapeutic decisions, as well as on disease prognosis [23]. The necessity for BC molecular subtyping is emphasized by the need for individualized, more targeted therapy, as several studies have shown [24–27].

The luminal A subtype was associated in our study with posterior acoustic attenuation, as mentioned by Irshad *et al.* [28], with inhomogeneous internal structure and presence of pathological lymph nodes. Wen *et al.* study suggested that the basal-like subtype had no posterior features (p=0.041), compared to the present study where HER2 and basal-like subtypes presented acoustic shadowing (p=0.03) [3].

The luminal B HER2-negative subtype was especially correlated with microlobulations, macrocalcifications, pathological lymph nodes, and increased vascular signal. Zhang *et al.* found that luminal B subtypes could be associated with increased vascularity [29].

According to Wen *et al.*, US characteristics, such as microlobulated margins, are more frequently found in BC HER2 subtypes (p=0.002), while the spiculated contour seems to be characteristic of the luminal (A and B) subtypes [3].

The luminal B HER2-positive subtype had a positive correlation with ill-defined margins, macrolobulation, and posterior acoustic attenuation [30, 31].

Çelebi *et al.* mentioned the association of ill-defined margins and posterior acoustic shadowing with luminal A and luminal B subtypes [32].

The HER2-enriched subtype had a strong correlation with the presence of pathological lymph nodes and well-defined margins, whiles the triple-negative subtype was associated with pathological lymph nodes, enhanced

vascularization, and well-defined margins [30, 33, 34]. Imagistic investigations, such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) or US, represent important tools for detecting lymph node metastases.

Imaging investigation is a mandatory step in determining the staging of tumor, node, and metastasis (TNM) and focuses on the management of the patient's disease. Furthermore, the prognosis is influenced by the presence or absence of metastases [35].

US and mammography were the two imagistic methods used in our study to assess breast tumors. US is considered a tool that can be used regardless of age, especially on women less than 40, both as a screening method and as a complementary investigation when a woman presents a palpable tumor [36, 37]. It is recommended to be performed as a routine for breast control, as it helps to evaluate false positive results [38–40].

In our study, we used US-guided biopsy, as referred in studies underlying its utility [37].

An attempt in identifying US features correlated to the risk of malignancy was conducted by Nam *et al.* They did not manage to draw a consistent conclusion regarding this topic because of the insignificant number of patients diagnosed by US investigation with a BI-RADS 3 formation; these patients developed histopathologically confirmed BC during the follow-up. Both Nam *et al.* study and ours revealed a high incidence of bilateral implication on patients receiving a BI-RADS 3 score, while BC detected on these patients was infrequent. Based on these remarks, we recommend routine breast US evaluation for patients on average risk for malignancy diagnosed with a BI-RADS 3 lesion [41].

Ultrasonography brought important information and adjusted the diagnosis when the dense breast was examined on mammography, as in other studies of Houssami *et al.* [42] and Tagliafico *et al.* [43].

An additional useful US imaging method for breast examination is elastography, a non-invasive technique based on determining tissue stiffness. Small dimension tumors, cystic formations, or tumors possessing a corpuscular core represents the lesions most frequently evaluated using this tool. It has been concluded that breast elastography can both reduce the number of unnecessary biopsies and postpone reappraisal for BI-RADS 3 US lesions [44].

It is recommended that breast elastography only be used as a complementary method to the traditional US technique, when assessing suspected malignancy breast lesions. It would adjust the US BI-RADS score according to tissue stiffness [45].

Mammography was used both for asymptomatic and symptomatic patients, as it has a higher sensitivity than US for non-invasive BCs, correlated with breast calcifications presence detected only by mammography [46]. The dense breast tissue examined on women under 50 represented a difficulty in detecting mammographic lesions but the US examination completed the diagnosis due to the hypoechogenic appearance of the tumors, which brought a significant contribution [47]. In women over 50 years, the occurrence of a predominantly adipose tissue allowed the correct highlighting of the mammary lesion [48]. Multiple suspicious mammographic lesions required complementary US examination for an appropriate characterization. Therefore, we must mention that both mammography and US brought an important contribution in detecting and characterizing breast lesions.

Pathological lymph nodes were present in 70.58% of the examined patients, a finding similar to that in a study conducted by Zhang *et al.* [49]. Most of them had an axillary localization and fewer parasternal (in case of internal quadrants lesions). Pathological lymph nodes were also histopathologically confirmed but on US examination, some of them were not characterized as presenting increased thickness of the cortex (partially or totally) and reduction or disappearance of the sinus. These false-negative results on an US could be a consequence of small dimensions of the lymph nodes [50]. 29.41% of the patients did not have axillary pathological lymph nodes on the first evaluation. Those without pathological lymph nodes most frequently had tumors less than 1 cm in diameter; in comparison, those who had pathological lymph nodes had tumors over 2 cm.

On US evaluation, suspicious mammary tumors were described as presenting an ill-defined contour or spiculate margins on 83.33% of cases, compared to only 53.16% on mammography examination of the same patients, emphasizing the fact that ultrasonography has a higher sensitivity, especially when describing fibro-glandular tissue [51]. The rest of them (16.67%) presented well-defined margins and this characteristic was most often present in patients under 50.

Regarding lobulation of mammary tumors, micro-lobulations were present in 44% of cases, macrolobulations in 19% cases, and 37% of cases were without lobulations; this highlights the necessity for follow-up when describing benign lesions on the first examination, especially if the patient is very young [52]. The six months US reevaluation of these lesions demonstrated their evolution towards a suspicion for malignant tumor, which implied biopsy and histopathological and IHC analysis.

US examination of the posterior acoustic attenuation character related to suspicious mammary lesion emphasized the fact that most lesions presented asymmetric bilateral shadowing (35.29% of the cases); in 32.35% of the cases, no posterior acoustic changes were noticed. The total attenuation was highlighted in 22.54% of cases and the bilateral symmetrical marginal shadowing in 8.82% of cases. In less than 1% of the cases, the lesions were described as presenting posterior acoustic amplification. The posterior acoustic attenuation was rather associated with two subtypes (luminal A and B), a finding similar to other studies [31, 34, 28].

According to *chi*-squared analysis of molecular cancer subtypes correlation to receptors and Ki67, we found a significant association between both luminal A and luminal B HER2-negative subtypes and hormone receptors (ER, PR); these have an inverse relationship with Ki67 index elevated values; the luminal B HER2-positive subtype has a direct association with HER2 presence; the HER2-enriched subtype was statistically significant associated with HER2

presence and elevated Ki67 values but had an inverse relationship to hormone receptors (ER, PR); the triple-negative subtype was strongly associated to Ki67 values and inversely correlated to ER and PR. Stolnicu *et al.* stated that the ER-/PR+ BC phenotype can be associated with small-size tumors, the HER2 presence, the absence of lymph node metastases, and younger patients; although it was found to be the most infrequent cancer subtype in their study, it had the best long-term prognosis probably correlated to PR targeted therapy [53].

Conclusions

In our study, luminal A subtype has the highest incidence, and luminal B HER2-positive subtype was the least common. Posterior acoustic shading, inhomogeneous internal structure, and poorly defined edges appear to be associated with both luminal A and luminal B subtypes, while the well-defined contour was characteristic of triple-negative BCs. On the other hand, the presence of pathological lymph nodes was accentuated on all subtypes: luminal A, luminal B, HER2-enriched and triple-negative subtypes. Improved vascularization was rather associated with luminal B and triple-negative subtypes, according to our findings. The study showed that the expression of the value of IHC receptors influences the therapeutic decision, and immunological therapy has an essential role in the patient's evolution and in the prognosis of the disease. Moreover, different associations between the IHC subtypes of BC and molecular receptors could predict a certain genetic predisposition to develop specific BC and can be used in medical practice.

Conflict of interests

The authors declare that they have no conflict of interests.

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