REVIEW ARTICLE

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Emerging concepts regarding the molecular profile of breast carcinoma: one-year experience in a University Center

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Abstract: *Breast carcinoma represents the leading cause of oncologic mortality for women. Due to therapeutic and diagnostic advances, the mortality and morbidity in the last decade declined, but breast cancer still has a great impact on quality of life and also on medical service cost. In the light of these facts, an integrated approach, considering histopathology features, molecular profiles and corroboration with clinical and imagistic data is necessary. A descriptive retrospective one-year study analyzed the breast cancer heterogeneity in 121 cases registered in the Pathology Department of the University Emergency Hospital in Bucharest, Romania. Our purpose was to evaluate histopathological, immunohistochemically, clinical and imagistic aspects of breast cancer considering the current molecular classification (Luminal A, Luminal B, HER2 enriched and triple negative/basal like).*

Our assay revealed that most prevalent histotype was NST (no special type) followed by invasive lobular carcinoma. Considering the molecular pattern, the most common was luminal B. Triple negative basal like and HER 2-enriched were correlated with an aggressive morphologic pattern and lymph-nodes positivity. Considering the imagistic acquisitions, mammography proved to be the most accurate technique for measuring the dimension of NST.

In conclusion, breast carcinoma is a heterogeneous disease that needs an integrated approach and personalized treatment based on the histopathologic and molecular features. Considering the great number of advanced stages as diagnosis, a national screening program for breast cancer is imperiously needed.

Keywords: *breast cancer, NST, ILC, IHC, molecular subtypes, biopsy*

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INTRODUCTION

According to the most recent statistic database of WHO 2015, breast carcinoma represents the main cause of mortality for Romanian women [1]. The decline of breast cancer mortality is a trend that, according to the most recent epidemiologic estimation will continue [2]. This direction is mostly due to advancement in diagnosis and treatment [3].

A better understanding of pathogeny and a more accurate diagnostic approach of breast cancer led to several updates in guidelines for classification and treatment. Since 2003, for grading breast carcinomas, the Elston Ellis semiquantitative method modified score was used according to the 3rd edition of WHO guidelines for classification the breast tumours. The most recent WHO diagnos-

tic guideline (4th edition, 2014) considers that for prognostic and predictive factors a panel of antibodies is needed for a further classification into molecular subtypes (luminal A, luminal B, Her2 enriched and triple negative basal-like). This approach is supported by multiple studies and summarized in the most important European or American conferences or guidelines. For example, the 14th St. Gallen International Breast Cancer Conference, 2015 [4], considers breast carcinoma a heterogeneous pathology according to its intrinsic molecular types whereas the 8th edition of AJCC (American Joint Committee on Cancer) states that breast malignant tumours should be staged according to the prognostic group, based on population that had been offered and mostly treated with appropriate endocrine or systemic therapy which includes anatomic T, N and M plus grade and status of HER2, ER, PR. [5]

MATERIAL AND METHOD

The aim of this retrospective analysis was to offer an exhaustive perspective of breast cancer considering clinical and imagistic presentation, morphological and molecular profiles of the main mortality factor for women. We analyzed the breast cancer heterogeneity in 121 cases registered as mastectomies, lumpectomies, quadrantectomies or biopsies in the Pathology Department of the University Emergency Hospital in Bucharest, performed over a period of 1 year (1st January 2017-31st of December 2017). The diagnostic varied from benign lesions (fibroadenoma, fibroadenomatoid mastopathy) to the most common malignancies, NST carcinoma and even rare cases like carcinoma with medullary features, mucinous, adenosquamous, papillary carcinoma or Phyllodes tumor.

Table 1. Frequency of the surgical procedures used for the histopathological diagnoses in the analyzed population

Type of lesion	Mastectomy + axillary in dissection	Lumpectomy	Core needle biopsy	Total
NST	40	19	19	78
NST with papillary features		1		1
Invasive lobular carcinoma	4	1	3	8
Medullary carcinoma	1			1
Adenosquamous carcinoma	1			1
Phyllodes tumour	1			1
Adenomioepithelioma		1		1
Metastasis of B cell lymphoma		1		1
Benign lesions, non-inflammatory	5	5	1	11
Fibroadenomas	1	8	2	11
Benign lesions, inflammatory	1	3		4
Metastasis of breast carcinoma			3	3
Total	54	39	28	121

For clinical data and imagistic interpretation, we used the hospital electronic database. The histologic specimens were studied using both conventional histopathological examination with HE stains and also immunohistochemically methods, classifying them further in the main 4 surrogate-molecular subtypes: luminal A, luminal B, HER2-neu non-luminal and basal-like/triple-negative. For diagnosing the main histologic subtypes, the last WHO guideline criteria were applied. For data collection and processing Microsoft Office Excel 2010 was used.

RESULTS

This one-year retrospective study analyzed 121 cases female patients registered in Pathology Department of the University Emergency Hospital in Bucharest with diagnostic of benign lesions and also with malignant

pathologies. Those included carcinomas, with NST histotype being the most common (78 cases), ILC (8 cases) and also rare types. Detailed information is found in Table 1.

We focused our attention on the most common types of breast carcinoma: NST and ILC. In our study the most common histotype of breast carcinoma was NST, with a predominance of G2 grade of differentiation (59%), followed by an equal percentage of G1 and G3 (8% for each one).

In the absence of precise data about menstrual status, we considering the climax age at 50 years old. With this presumption, most women were postmenopausal (79%), and the average age at presentation was 61.48 years, varying from 35 to 86 years old. Distribution of the analyzed patients according to their age, histotype and molecular profile of their tumor is detailed in Table 2.

Table 2. Age distribution according to the main histotype, grade of differentiation and molecular profile of the most important histotypes of breast carcinoma

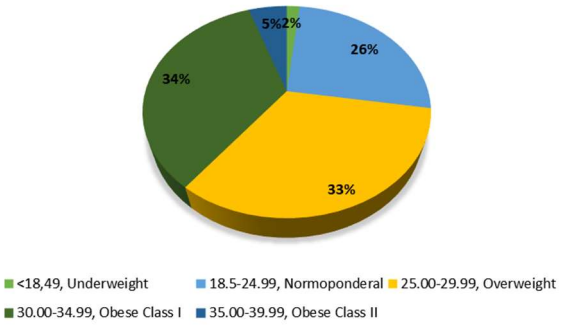
Molecular Profile	Diagnostic					Age					
	0 NST G1	NST G2	NST G3	CLI G2	CLI G3	31-40	41-50	51-60	61-70	71-80	81-90
Luminal A	9%	11%	0%	2%	0%	1%	5%	5%	8%	3%	1%
Luminal B	5%	32%	3%	1%	2%	2%	5%	10%	14%	13%	3%
HER 2 Enriched	0%	3%	1%	0%	0%	0%	1%	0%	1%	2%	0%
Triple Negative Basal Like	0%	6%	5%	0%	0%	0%	1%	1%	7%	2%	0%
Missing Data	1%	9%	0%	3%	0%	1%	3%	1%	6%	2%	0%

Most patients addressed to surgery or Obstetrics-Gynecology Departments of the University Emergency Hospital in Bucharest for painless tumor masses. We also noticed sporadic cases of ulcerated bleeding tumors. A single patient presented for asymptomatic tumor incidental discovered with mammographic screening.

A demographic brief analysis revealed that most patient came from urban areas, with a percentage of 56% for the latter and 44% for rural areas; taking a closer look, we observed that the majority of advanced stages (III and IV) were more frequent in women with urban provenience (10.7% for stage III respective 4.7% for stage IV) in comparison to rural population that addressed to our hospital (1.2% stage III and 0.6% for stage IV).

Regarding clinical data obtained from hospital database, we noticed that the metabolic profile of a majority of analyzed women was impaired, most of them being overweight and obese, only a quarter bearing a normal weight, as the Figure 1 reveals. More than half of studied patients suffered dyslipidemia and experienced cardiovascular complications (essential hypertension and atherosclerosis were most noted). A subgroup also registered a history of gynecologic or endocrine pathology but not enough for a trustable correlation.

Figure 1. BMI of analyzed cases

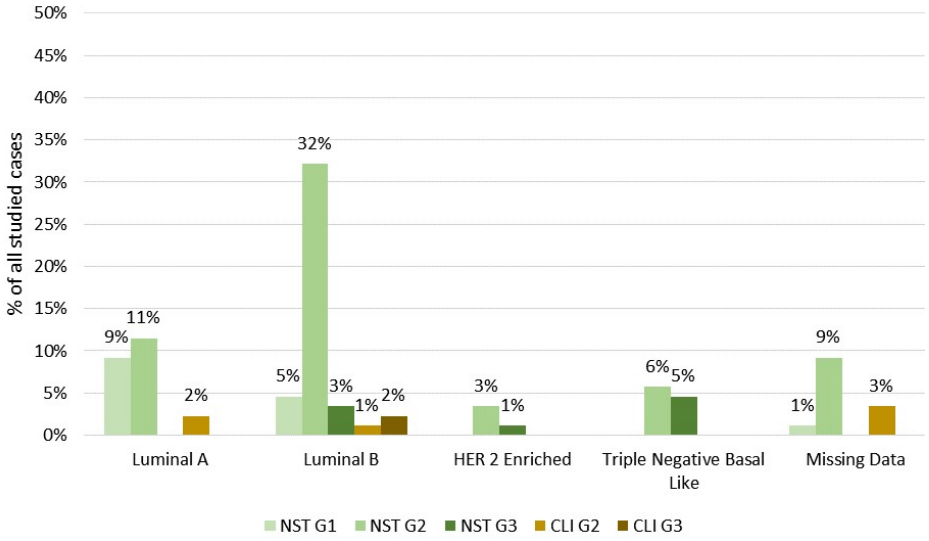


When we put side by side the matric data of different types of investigations (ultrasonography, mammography at CT) with histopathological measurement (macroscopically and microscopically evaluation) we noticed that the highest accuracy in appreciating the dimension of breast lesions is radiologic examination (mammography and CT), followed by ultrasonography which tend to underestimate most of lesions. Considering the main histotypes, the most difficult breast tumor to be precisely measured in conventional imagistic methods is invasive lobular carcinoma, in accordance with its manner of infiltrating the mammary tissue.

With the available data, we observed that invasive breast carcinomas were all scored with BIRADS 5 or 6, a single case of NST G1, luminal A, T1c being

underappreciated as 3.

Figure 2. Molecular profile of breast carcinoma according to histotype (%)



In relation to histologic examination of breast cancer, formalin fixed-paraffin-embedded tissue were studied using both conventional histopathological examination including HE stains and also immunohistochemically methods, classifying them further in the 4 main surrogate-molecular subtypes: luminal A, luminal B, HER2-neu non-luminal and basal-like/triple-negative. For diagnosing the main histologic subtypes, the last WHO guideline criteria were used.

When we evaluated breast cancer histotypes and their molecular pattern, we observed that the most prevalent is luminal B with a pick incidence in NST grade 2, followed by luminal A, also most prevalent for NST G2.

The oncologic stage according to WHO 2014 for HER 2 enriched and triple negative varied between IIB and IV. We did not find any single case in stage I. We also observed that for luminal A category the majority of cancer were in stage I and II. An interesting aspect is registered for luminal B, the most frequent molecular type of our statistic. We can easily observe that it has the highest frequency in most of cases, but not in stage I of disease. A more explicative illustration of the preponderance of tumor stages according to the 7th edition of UICC for TNM classification of malignant tumors, in relation to their molecular profile is highlighted in figure 3.

Figure 3. Molecular profiles of tumor stages related to the molecular profile

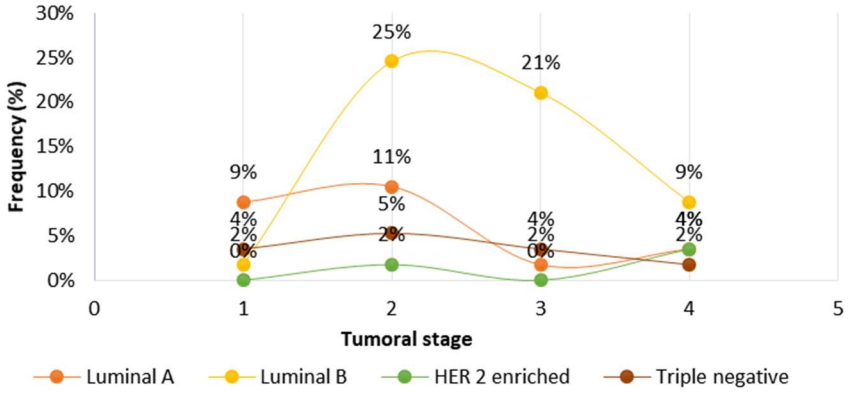
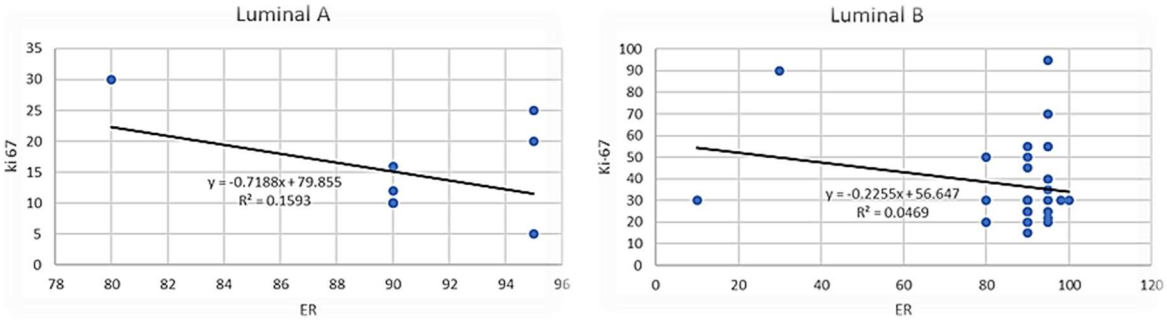


Figure 4. Regression between ER expression and KI 67 in luminal A and luminal B breast carcinomas



Considering the hormonal expression in the luminal type carcinomas and their proliferation index, we observed the regression between ER expression and the mitotic index evaluated by Ki 67 in luminal molecular profiles. For luminal A there is a better correlation between a higher expression of ER and a lower Ki 67 (R2=0.159) comparing to luminal B (R2=0.046), as in the Figure 4 can be easily seen. The juxtapose of the two types was not only useful for understanding the variation of hormonal variation regarding the proliferation rate, but also necessary considering the limited number of analyzed cases.

In our study, no invasive lobular breast carcinoma was identified among non-luminal carcinomas. We registered more triple negative than HER 2 enriched molecular subtype, and their prevalence were highest in climax women. As expected, their histologic features were more aggressive, with large areas of necrosis, infiltrative pattern of growth, and large numbers of mitoses, none of those being graded as well differentiated.

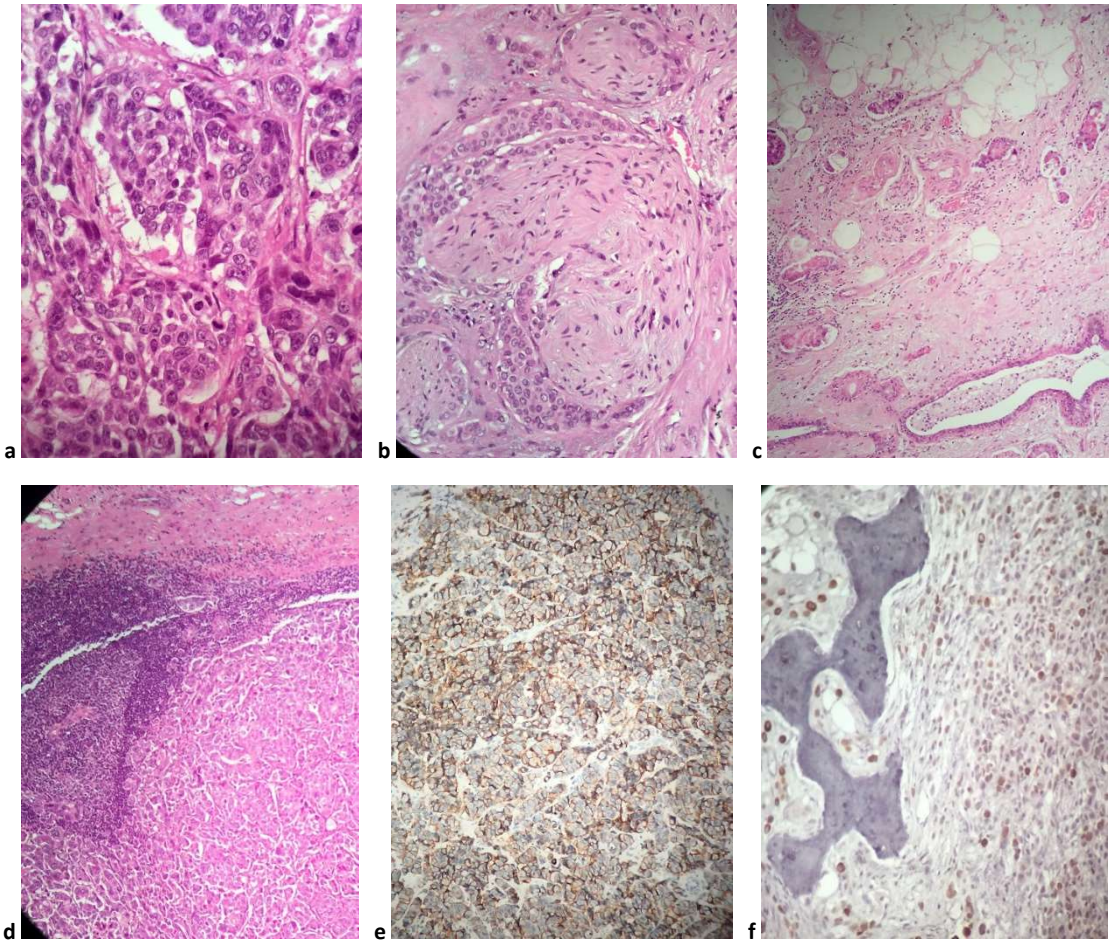
DISCUSSION

Breast carcinoma is not a single disease, it has a large panel of shapes, clinical manifestations, imagistic and histologic representations. The current WHO guidelines for breast carcinoma counts 37 different coded histotypes, but this classification it is far from being adequate for proper treatment [6]. Tumors with identical clinical and pathological presentations may have different behaviors. Beside the TNM classification and grading method (today the Nottingham histologic score system is universally accepted), the interest for treating in the most efficient and precise way each particular biological form of disease gave birth to the molecular subtype classification. This procedure evaluates the hormonal expression of tumor tissue (ER, PR), presence or absence of HER2 protein and levels of Ki-67.

We started our analysis by looking on the preferred diagnostic procedure, and in some cases, it was similar with the surgical treatment. The method of choice for the majority of invasive breast carcinoma clinically diagnosed is represented by mastectomies with axillary lymph node dissections were. Most of these procedures started with a frozen section for certifying the malignant nature of the lesion. After the frozen section, surgical interventions ended with mastectomy and lymph node dissection. Less than half of analyzed women received a neoadjuvant chemo-radio-therapy after core needle biopsy and came back for completing the surgical treatment.

The concordance of core needle biopsy and surgical excision specimen for diagnosing the histotype and hormonal profile of the tumor was proofed to be safe in large studies [7, 8]. According to ESMO, assessment of primary tumor includes: physical examination, mammography, breast ultrasound for lymph node determination, breast MRI and core biopsy with pathology determination of histotype, grade, ER, PR, HER-2 and Ki67 status. If preoperative systemic therapy is planned, a core needle biopsy is mandatory to ensure a diagnosis of invasive disease and assess biomarkers (recommendation IIIA) [9].

Figure 5. NST HER2 enriched carcinoma a. Numerous atypical mitosis HE 40X; b. perineural invasion, HE 40X; c. Intravascular tumor emboli; d. Lymph node invasion, HE 10X; e. HER2 intensely positive, scored 3+, 20X; HER2 negative, score 0, 20X.; f. Bone metastases with GATA 3 positivity suggesting breast origin, 20X.



Since it is not yet a standardized method, CNB does not represent a IA indication for non-operative diagnostic. Even if the Royal College of Pathology [10], the WHO guidelines for breast cancer and AJCC 8th edition [5] enclose certain recommendation for reporting the results of a CNB, this practice is controversial regarding the rate of metastasis [11], the reliability of histological grade considering the dimensions of collected fragments [12], the limitation in diagnosing certain subtypes of carcinoma (e.g. mucinous carcinoma) [13], and even rise the problem of "vanishing carcinoma", when tumor cells are present on CNB but absent in the surgical specimen [14];

We focused our attention on the most frequent histotypes of breast tumors, NST and ILC. The rare types of malignancies like papillary carcinoma, medullary carcinoma, Phyllodes tumor or metastasis to the breast are not well represented in this one-year study and for the sake of statistic relevance, we choose not to include them in our calculation. Also, the benign lesions (fibroadenoma, adenomyoepithelioma, abscesses or fibrocystic disease) were not relevant for the heterogeneity of breast carcinoma.

The interesting demographic results that we obtained underlines that the majority of severe stages of disease (stage III and IV) were addressed from urban population. This trend is not observed in other studies made in Australia [15], Africa [16] or even in multiethnic and multiracial population [17], but in our opinion, these unexpected results may appear because of many reasons: probably the most important one is the limited number of cases that

were included in our analysis. Other reasons may consider the higher addressability of urban women to private health system, the migration of urban population to the country side or a better surveillance of patients from rural area by their family doctor. On a deeper assessment, it is easily noticed that most of our luminal B cases had a high Ki 67 index (above 20%), only a third of them being HER2 positive or equivoque on IHC. A possible explanation could be the absence of a -ISH method in our department for a precise assessment of HER 2 mutation. Anyway, considering the limited period and number of analyzed cases, for a more accurate distribution of luminal subtypes more studies are needed.

Taking into account hormonal expression, using regressions, we observed that for luminal A molecular subtype the higher the estrogen receptor expression is, the lower the proliferation index is (quantified by Ki 67); in comparison, for luminal B subtype p-value was only 0.0469.

Regarding the age, the highest amount of breast cancers appeared in the 6th decade of life, with a considerable delimitation between pre- and post-menopausal age. Even so, in the 3rd decade we registered only luminal subtypes of cancer (considered to have a better prognosis) but in the 4th decade we registered all the molecular subtypes.

Regarding clinical correlation, we became aware of the impaired weight of our studied group, most patients being overweight, with a medium BMI of 27.73. Also, we noticed that a great proportion of studied women who underwent a diagnostic biopsy and returned after approximately 3 months for surgical treatment gained extra kg, their BMI being augmented. This observation could be an alarm signal for a better nutritional surveillance and psychologic management of oncologic patients, considering that obesity it is an important risk factor for malignancy, comorbidities and mortality [18 - 20].

Unfortunately, our clinical correlation was limited because we did not had access to variables like family and history of menstruation, oral contraceptive use, age of first full-term pregnancy, number of children, duration of lactation or hormonal replacement therapy.

CONCLUSIONS

In our study, primary malignant epithelial tumors of the breast were NST with a moderate grade of differentiation with an age of incidence ranging from 3rd to 8th decade, being most prevalent in the 6th decade. Regarding molecular classification, luminal B was more common than others, with a wide clinical and histologic appearance; it was found in all analyzed histotypes and in all pathologic stages of disease. Because of its predominance, most of severe cases we registered (metastatic breast disease, ulcerated tumors) were also luminal B subtype. We admit the limitations of our study (few cases in a single year in a single hospital with emergency profile) but we are convinced that for a better breast cancer management, keeping in mind the most important aim, a lower mortality and morbidity, a proper national screening program is of an imperative necessity.

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