The Many Faces of Triple Negative Breast Cancer

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Rezumat

Multiple fețe ale carcinoamelor mamare triplu negative

Tumorile de sân triplu negative sunt caracterizate de lipsa de expresie imunohistochimică pentru receptorii hormonali (ER si PgR) și Her2/neu. Imunofenotipul este, în general, asociat cu vârstă tânără și agresivitate crescută, frecvența lor în literatură variind între 10 și 20%. În studiul nostru am realizat o clasificare retrospectivă a tumorilor mamare triplu negativ, pentru a evidenția spectrul larg de leziuni care pot îmbrățișa acest fenotip foarte special. Parametrii evaluați au inclus vârsta, mărimea tumorii și aspectul macroscopic, subtipul histologic și asocierea cu componenta in situ, gradul de diferențiere a tumorii (pleomorfism nuclear, numărul de mitoze, formarea tubulilor), prezența nodulilor limfatici sau a metastazelor la distanță. Carcinoamele de sân triplu negative reprezintă un anumit subtip de tumori, dar agresivitatea lor descrisă în literatură se aplică numai la unele subtipuri histologice. Este foarte importantă corelarea caracteristicilor histologice și a expresiei fenotipice.

Cuvinte cheie: cancer de sân, triplu negativ, clasificare

Abstract

Triple negative breast tumors are described by the lack of immunohistochemical expression for hormone receptors (ER and PgR) and Her2/neu. The immunophenotype is, in general, associated with young age and high biological aggressiveness, its frequency in the literature ranging from 10 to 20%. In our study we performed a retrospective classification of triple negative breast cancers in order to show the large spectrum of lesions that can embrace this very special phenotype. Clinicopathological parameters evaluated included age, tumor size and macroscopic appearance, histological subtype and association with in situ component, the degree of tumor differentiation (nuclear pleomorphism, mitosis count, the formation of tubules), presence of lymph nodes or distant metastases. Triple negative breast cancers represent a particular subtype of tumors but their, now famous, aggressiveness applies only to some histological subtypes. It is very important to correlate the histological features and the phenotypical expression.

Key words: triple negative, breast cancer, classification

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Introduction

Breast carcinomas, the second leading cause of cancer death in women, represent a group of malignancies receiving specific therapy both before and after surgical treatment, therapy determined by the presence of molecular targets identified by immunohistochemistry or molecular biology. So chemotherapy is modulated by the presence in the tumoral cells of steroid hormone receptors (estrogen, progesterone, androgens), of proteins encoded by some oncogenes, eg. Her-2neu/cerbB2 (with prognostic and predictive role) and on other prognostic markers.

Triple negative breast tumors are described by the lack of immunohistochemical expression for hormone receptors (ER and PgR) and Her2/neu. The immunophenotype is, in general, associated with young age and high biological aggressiveness, its frequency in the literature ranging from 10 to 20%.

In recent years, several molecular techniques have contributed to the classification of breast cancer and thus, helped determine prognosis and response to therapy. The most important contribution of these techniques is considered the classification of breast tumors based on microarray analysis technique into 4 classes: luminal, normal like, basal-like and HER2/neu by the Stanford group (1). These subtypes differ in the patterns of gene expression, clinicalmorphologic appearance, response to treatment and duration of survival. This classification has brought into attention the importance of identification of triple negative breast cancers, a very heterogeneous subtype, both from the point of view of prognosis and morphological features.

Immunohistochemical classification is based on the expression of estrogen receptor (ER), progesterone (PR) and Her2/neu protein, these three markers representing the gold standard in practice. Initially the classification was done by dividing breast tumors by identifying ER: positive and negative. The importance of identifying triple negative tumors was enhanced by the classification of Onitilo A et al (2,3) that divided breast tumors into four groups based on statistical equivalence in terms of survival, using triple negative breast cancers as a reference for the highest degree of aggressiveness: ER / PR +, Her2 +, ER / PR +, HER2-, ER/PR-, Her2 +, ER/PR-, Her2-.

In our study we performed a retrospective classification of triple negative breast cancers in order to show the large spectrum of lesions that can embrace this very special phenotype.

Material and Methods

The study group consisted of 250 cases for which we used standard stained sections of 3-5 microns (Hematoxylin and Eosin), which allowed the classification of lesions based on histology, as well as assessing additional morphological criteria.

Clinicopathological parameters evaluated included age, tumor size and macroscopic appearance, histological subtype and association with in situ component, the degree of tumor differentiation (nuclear pleomorphism, mitosis count, the formation of tubules), presence of lymph nodes or distant metastases.

Immunohistochemical reactions were performed on 4 micron sections obtained from paraffin blocks, on glass slides pre-treated with polylysine or charged electrically.

The detection and visualization system used was EnVision kit, Dako, Glostrup, Denmark, a method of immunostaining in two stages, based on the conjugation of a HRP polymer with secondary antibody.

The panel of antibodies used in our study is shown in Table 1.

Table 1. *Antibodies used in our study*

| Antigen	Clone	Developer	Dilution
ER	6F11	Novocastra	1:30
PgR	16	Novocastra	1:100
Her2-neu	poli	DAKO	1:250

Results

In our study, TN (triple negative) phenotype included all tumors that did not express hormone receptors for estrogen and progesterone, or Her2/neu. Hormone receptors scoring was done by assessing the percentage of positive tumor cells, a percentage <1% being considered negative (*Fig. 1*).

For the identification of Her2/neu genotype we used the HercepTest score for evaluating Her2/neu immunostaining. Her2/neu staining was considered positive when we observed the presence in the plasma membranes of at least 10% of tumor cells. Although in some cases there was cytoplasmic reaction of variable intensity, it did not affect the scoring.
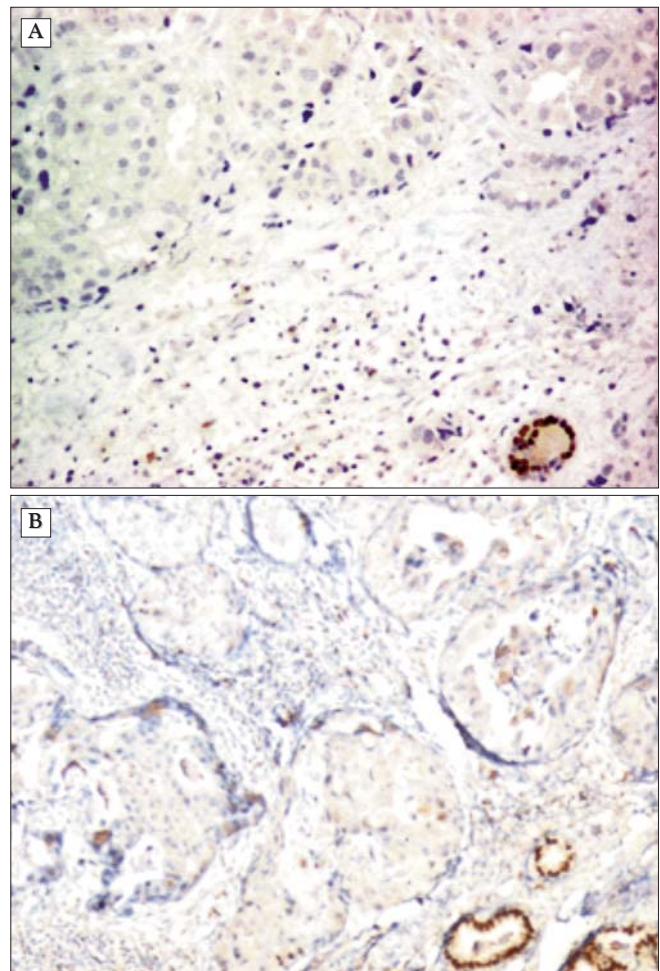
Figure 1. *(A) ER negative in tumoral cells (internal positive control– lower right), 100x; (B) PR negative in tumoral cells (internal positive control– lower right), 100x*
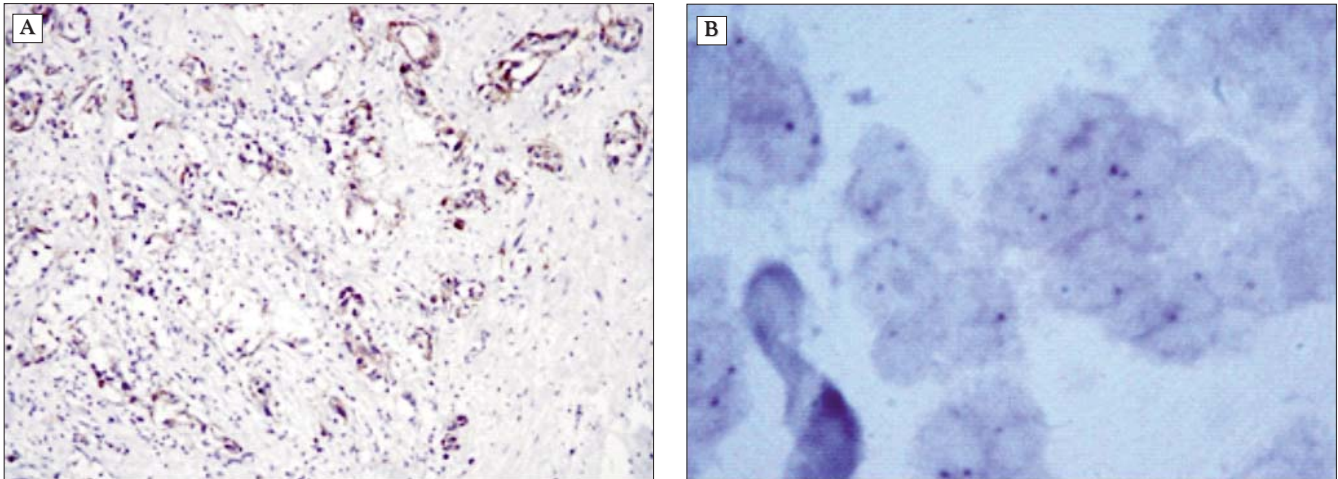
Figure 2. *(A) Her 2 /neu negative (score 1 +), 40x; (B) Her 2 negative, CISH reaction*

The results were estimated as a score: 0, 1 +, 2 +, 3 +. A score of 1 + was used when a small amount of antibody was bound to the cell membrane and had a discontinuous disposition in the form of slits, and 2 + on a low or moderate circumferential membrane staining, and 3 + for a high circumferential membrane staining. 0 and 1 + scores were considered negative. For values of 2 +, we only included lesions that showed no gene amplification by CISH (*Fig. 2*).

The study group included patients with age ranging between 23 and 83 years (mean 52.64 years), the highest number of cases being present between 50-59 years (*Table 2*).

Clinical manifestations in the skin covering the breast varied, from peau d'orange (dimpling resembling an orange peel, skin retraction, nipple retraction, presence of ulceration, skin redness or eczema and erosions in the nipple and areola (*Fig. 3*). Data on duration of clinical symptoms, according to the information obtained by anamnesis, were available in 112 cases (mean 12.3 months, between 1 and 54 months).

On gross appearance, lesions varied in size from 9 to 80 mm, the most common tumor maximal diameter being between 20 and 40 mm. Gross appearance of the piece of mastectomy varied also according to the clinical appearance (*Fig. 4*) and correlated with the microscopic appearance of the tumor, especially with the distribution and appearance of the stroma.

Figure 3. *Alteration of skin overlaying breast carcinoma*

On cut section, most frequently encountered was a tumor formation, inaccurately defined, stellate or round, yellowish white or grey, showing fibrous streaks that radiated into the adjacent breast parenchyma (*Fig. 5*). We also identified areas of necrosis, haemorrhage and cyst formation.

Some cases had clear tumor extension to the skin and were classified T4 in the TNM system. (*Fig. 6*)
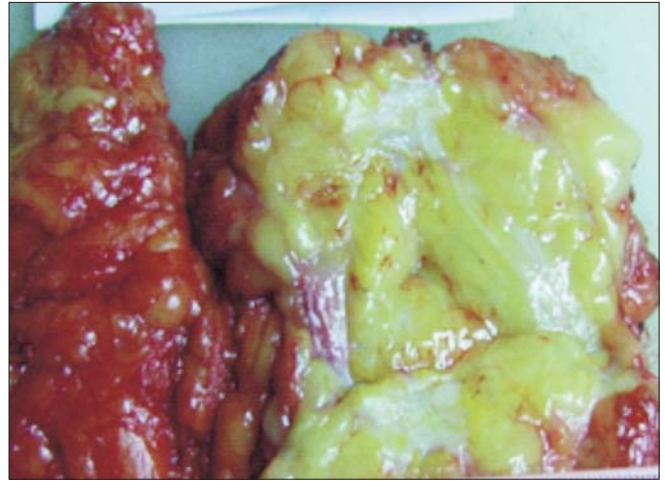
Another gross feature identified was the presence of a diffuse tumor with ill-defined borders corresponding histologically to lobular carcinoma. Papillary carcinoma was represented by the presence of a round tumor with large diameter between 15 and 25 mm, apparently well defined, white grey and the presence of clotted blood on cut section. Mucinous carcinomas presented as a well confined tumor, variable in size, gelatinous and of low consistency. Medullary carcinoma presented similar issues with fibroadenoma, being a well-defined, solid, homogenous and grey whitish tumoral mass. (*Fig. 7*)

The most common histological type was invasive ductal carcinoma (202 cases). These cases were reported in patients aged between 23 and 83 years.
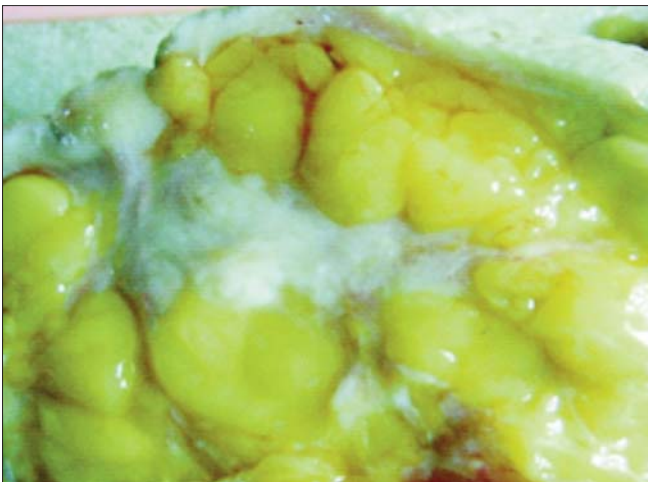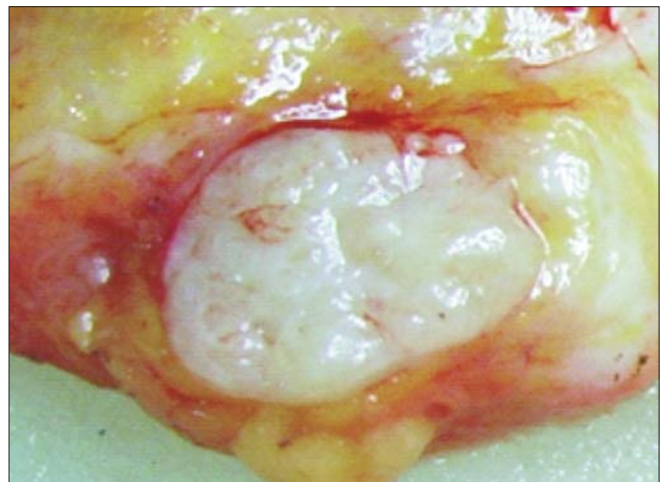
Table 2. *Age distribution of the patients in the study group*

Age	No.
20-29	11
30-39	23
40-49	57
50-59	96
60-69	40
70-79	18
80-89	5

Figure 4. *Gross appearance of the external surface of the tumors*

Figure 5. *Gross appearance of breast cancer*

Figure 6. *Direct extension of the tumor to the skin*

**Figure 7.** *Solid grey-white tumor, with well-defined borders*

Invasive ductal carcinoma (*Fig.* 8) was identified histologically by the presence of pleomorphic round or polygonal tumor cells, with nuclei and prominent nucleoli and mitoses in variable number, arranged in cords, nests, and syncytial tubules, and isolated stroma.

Mixed forms that have not been considered as separate entities were identified in 6 cases.

The tumors showed invasive ductal carcinoma and areas of mucinous carcinoma (*Fig.* 9), but since this component did not exceed 50%, they were not classified as mucinous carcinomas.

The second category as frequency in our study was that of medullary carcinomas (16 cases), subclassified into typical (7 cases) and atypical (9 cases) identified in patients aged between 35 and 74 years.

In terms of histology, typical medullary carcinoma was characterized by the presence of epithelial syncytial sheets occupying over 75% of the tumor, with round tumor cells with abundant eosinophilic cytoplasm (*Fig.* 10). Nuclei were round,
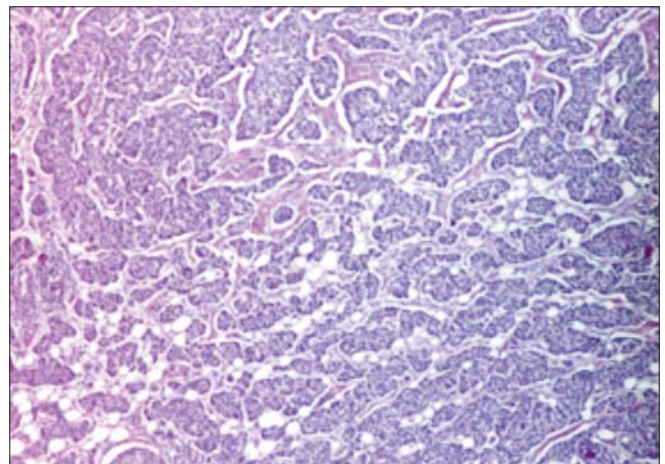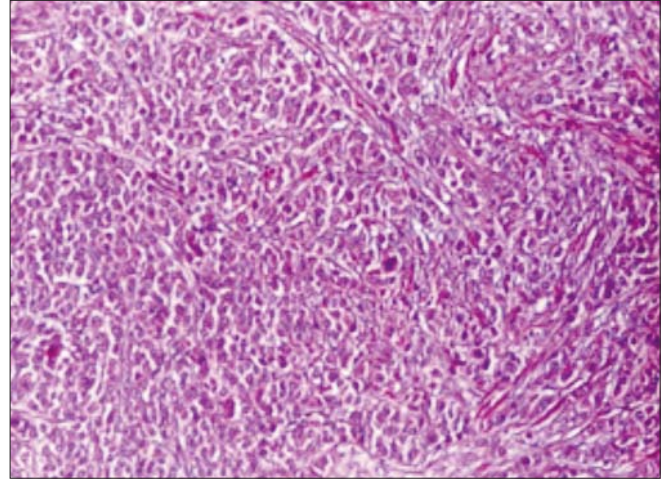

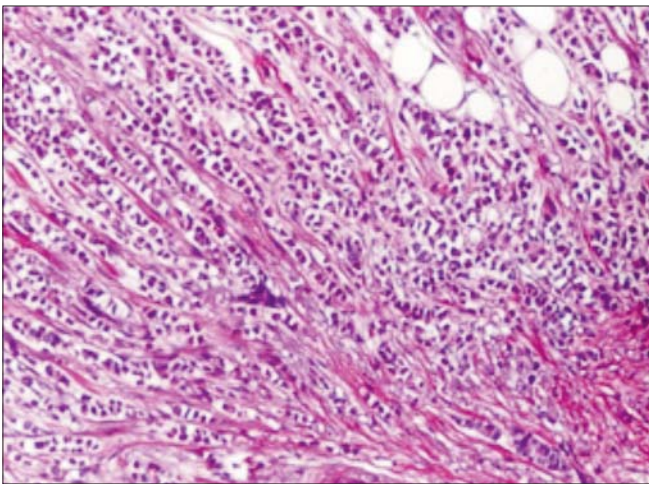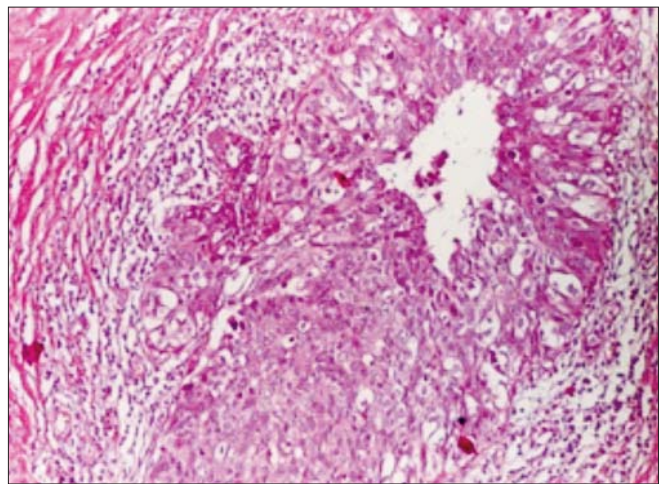**Figure 8.** *Invasive ductal carcinoma, H&E 40x*
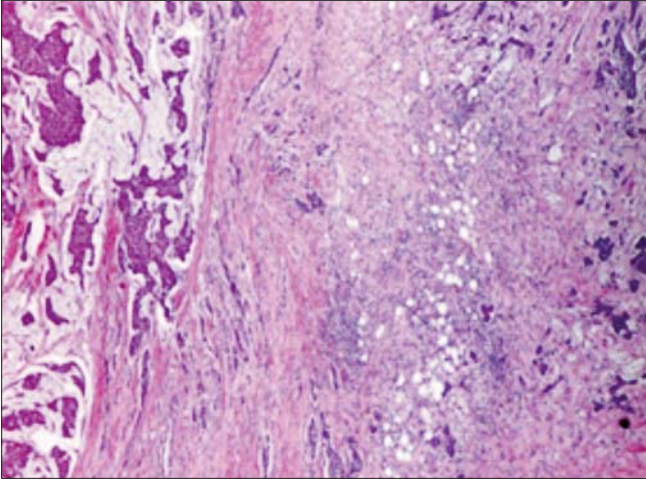
Figure 9. *Mixed mucinous and ductal breast carcinoma. H&E, 100x*

Figure 10. *Medullary carcinoma H&E, 200x*

Figure 11. *Lobular carcinoma – Indian file pattern, H&E 40x*

Figure 12. *Squamous differentiation H&E 40x*

presenting one or more nucleoli, marked pleomorphism and mitoses.

Nine cases did not show all these changes, but tumor cells occupied over 75% of the tumor, including these cases in the category of atypical medullary carcinomas.

Lobular carcinoma was identified in 9 cases: classic pattern (5 cases), solid variant (1 case), alveolar (1 case) and in 2 cases: pleomorphic lobular carcinoma.

Classic lobular carcinoma was characterized by the presence of small tumor cell proliferation, non-cohesive, uniform, round oval with rounded nuclei with variable pleomorphism and rare mitotic figures and moderate cytoplasm, usually arranged linearly - pattern of "Indian file" (*Fig. 11*).

In 7 cases metaplastic epithelial and mesenchymal lesions were identified: 4 cases with squamous metaplasia with varying degrees of differentiation (*Fig. 12*), 2 cases of metaplastic carcinoma with chondroid differentiation and a case with osteoclast-type giant cells.

The 4 cases of papillary carcinoma were characterized by

the presence of infiltrative type papillary proliferations with delicate conjunctive-vascular axis, lined by one or more layers of atypical cells, with eosinophilic cytoplasm, hyperchrome nucleus with N / C ratio greater than one and frequent mitoses.

Mucinous carcinoma (5 cases) was diagnosed based on the presence of tumor cell proliferation with uniform, round cells, with reduced eosinophilic cytoplasm, and nuclei with minimal pleomorphism, arranged in nests or trabecular in lakes of extracellular mucin (*Fig. 13*).

Three cases showed the presence of well-differentiated, round or oval, tubular structures, with clear lumina and bordered by a single layer of tumor cells and were diagnosed as tubular carcinoma.

The presence of islands of poorly differentiated tumor cells with reduced cytoplasm, large, hyperchrome nuclei, the presence of mitoses and prominent basaloid aspects was found in one case and diagnosed as adenoid cystic carcinoma of the breast, solid version.
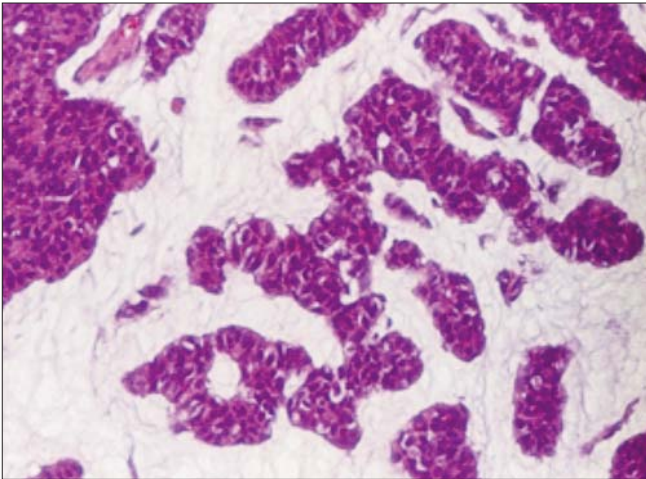
Figure 13. *Mucinous carcinoma of the breast H&E, 100x*
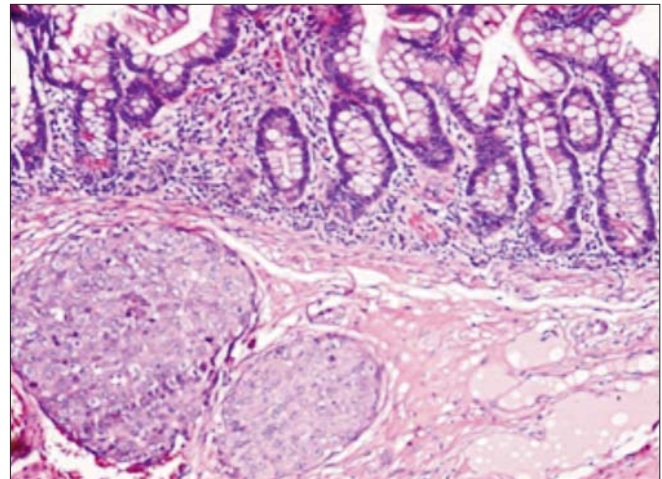
Figure 14. *Intestinal metastasis of breast cancer*

For the diagnosis of apocrine carcinoma (1 case) we used the following criteria: presence of proliferation of tumor cells with abundant eosinophilic, granular cytoplasm, with large nuclei, pleomorphic and small nucleoli, arranged in trabecular structures and occupying more than 90% of the tumor surface.

Tumor staging using the TNM classification system (2002) presented the following results: 80 lesions with a maximum diameter ≤ 2 cm (T1), 122 lesions with a diameter > 2 cm and ≤ 5 cm (T2), 20 lesions with a diameter > 5 cm (T3) and 28 lesions of any size, but with the direct extension of the tumor to the skin of the thoracic wall (T4) (*Table 3*).

After assessing regional lymph nodes the following data were recorded: N0 - 97 cases, N1 - 105 cases, N2 - 40 cases and N3 - 8 cases.

Distant metastases (M1) were found in 12 cases, presenting the following locations: brain metastases - 3 cases, bone metastases - 2 cases, bowel metastasis - 1 case (*Fig. 14*), liver metastasis - 1 case, pulmonary metastasis - 1 case, pleural metastasis - 1 case, gingival metastasis - 1 case, ovarian metastases - 2 cases.

The differentiation grade of the tumor was identified using the Nottingham grading system. Most cases were classified as low differentiated G3 (142 cases) and moderately differentiated G2 (103 cases), only 5 cases were classified as

well differentiated G1.

Other morphological features investigated included the presence and quantity of necrosis, tumoral borders' appearance and presence of lymphovascular invasion.

The presence of necrosis, in any amount, was registered in 190 cases. Quantification varied between focal necrosis, with only a small percentage of tumor cells involved, and extended, the tumor having been replaced almost entirely by the area of necrosis.

Tumor borders were considered infiltrating when tumor cells were identified beyond the tumor-stroma interface (98 cases) and pushing when the periphery of the tumor had a roundish appearance (152 cases) (*Fig. 15*). Lymphovascular invasion was present in 93 cases.

Discussions

From these results we considered triple negative phenotype as a heterogeneous entity, including various tumor subtypes.

Currently, immunohistochemistry is an indisputable indication for early diagnosis of breast cancer. Estrogen and progesterone receptors were among the first prognostic factors identified by immunohistochemistry tissue. They both have a role in assessing progress, cases with high percentage of positivity having a better outcome in the same anatomical - clinical group.

Endocrine treatment of breast tumors is indicated in most patients with hormone - positive tumors due to increased efficacy and acceptable side effects. (4)

Chemotherapy is used in most hormone negative tumors, which is however accompanied by numerous side effects and long-term risk of leukemia or heart disease. (5)

The genotype of triple negative tumors is characterized by the absence of gene amplification for Her2/Neu and as a result they cannot be assigned to targeted therapy. This, combined with the lack of response to hormone receptors, so the absence of an indication for endocrine therapy, enter this category of tumors in a class apart due to the lack of a

Table 3. *TNM staging of tumors in the study group*

Stage	No.
0	0
I	51
IIA	69
IIB	46
IIIA	42
IIIB	28
IIIC	8
IV	6
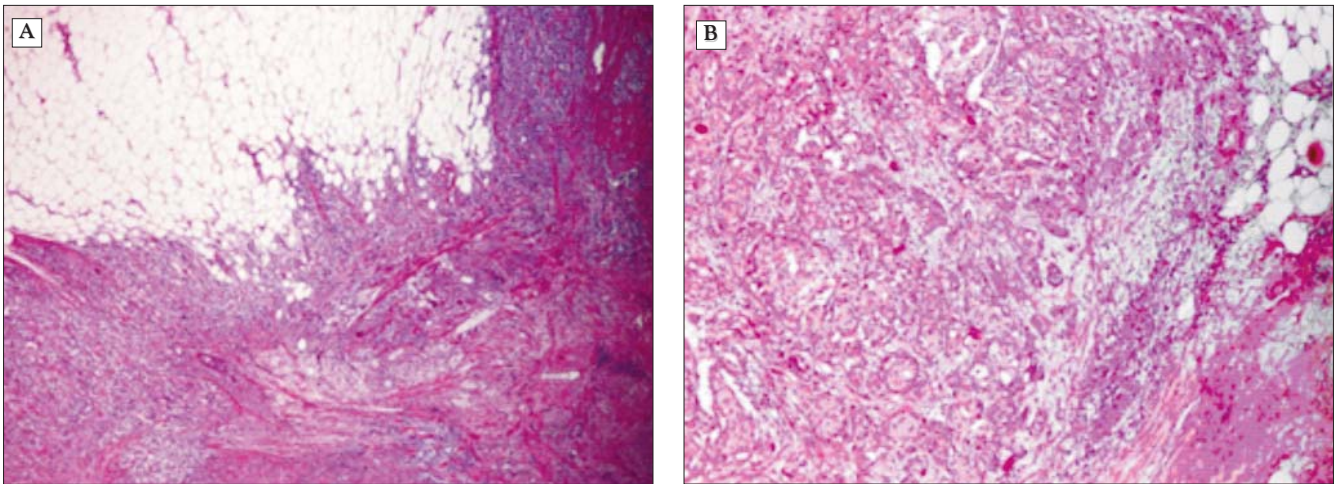
Figure 15. *Infiltrative and pushing borders in breast cancer. H&E40x*

targeted treatment, representing still an open field of research.

Although the information brought by molecular techniques have led to a better understanding of breast tumors in general, the heterogeneity of triple negative breast tumors requires attention in assessing the histological subtype.

A non-genetic risk factor is the age, breast tumors being very rare under the age of 20 and rare in those under 30 years. Incidence rates increase during premenopausal period (under 50 years). About 80% of breast tumors are diagnosed in women over 50 years old, the likelihood of developing a breast tumor within 10 years being less than 1.5% at 40 years, 3% at 50 years and more than 4% at 70 years. The lifetime risk of developing a breast tumor is 1 in 8, or 13.2%, being 9 times more common in women over 50 years (6).

Although the majority of breast tumors are found in post-menopause, about 2 to 5% are found in women under 35 years. The literature is contradictory in terms of age as an independent prognostic factor for survival: some studies show a poor prognosis of young women compared to older ones, while others argue the lack of impact of age on prognosis (7).

In our study, although the mean age was over 50 years (52.64 years), an increased number of cases were identified in the range 20-50 years (91 cases) representing 36.4% of the study group. The literature describes triple negative breast cancer as more common in women under 40 years. (8)

In our cases only 13.6% of patients were aged under 40. We did not consider this percentage as having statistical significance because in many cases, patients presented to the doctor in advanced stages of the tumor.

In our study, the 250 invasive breast tumors investigated showed variations in the histopathologic subtype, tumor grading and TNM classification.

The morphologic spectrum of triple negative breast cancer includes not only invasive ductal carcinoma and medullary carcinomas, but also at the lower end of the spectrum, adenoid cystic carcinomas, and at the upper end of

the spectrum, high-grade and metaplastic breast carcinomas (9). In our study, the histopathological aspects of selected cases were heterogenous.

The most common histological type was invasive ductal carcinoma (80.8%), lobular carcinoma being rarely associated with this phenotype (10). Medullary breast carcinoma and apocrine are frequently described in the literature in association with triple negative phenotype (11) and in our study were found in 6.4% and 0.4% of cases.

Although the triple negative phenotype is generally associated with decreased prognosis, it seems that in metaplastic carcinomas it represents actually a favorable prognosis, but studies are still needed to explore the mechanisms of action. (12)

Although mucinous and tubular breast carcinomas are rarely triple negative, being generally ER + / PR + / Her2/neu- (13), in our study we met these two categories in equal proportions of 1.2%.

The morphological pattern most commonly described in the literature in association with triple negative phenotype is increased histological grade, translated by poor tumor differentiation, nuclear pleomorphism and increased mitotic index, all these being aspects correlated with a decreased prognosis.

Analysis of clinicopathological variables in our study showed that the triple negative phenotype has aggressive features: large tumor size (4.45 cm), increased frequency of metastases in regional lymph nodes (61.2%), high histologic grade, increased frequency of classification tumors in advanced stages (III and IV - 31.2%). Thus, our data are consistent with the literature.

Prognostic assessment of the likelihood of response to systemic therapy in malignant tumors is based on a series of clinical and morphological parameters underlying the TNM classification and pTNM (tumor size, relationship with surrounding tissue, lymph nodes or distant metastases, the type and degree histology of the tumor, etc.).

Data from the literature indicate a link between impor-

tant prognostic factors in breast cancer and tumor size, which is inversely proportional to survival. (14)

Numerous studies have analysed the importance of histologic grade as a prognostic factor.

Breast cancer patients with a high degree of malignancy most commonly present involvement of the regional lymph nodes, and death can occur as a result of metastatic disease. (15) However, there are data in the literature that state that lymph node status is not a major prognostic factor in breast tumors TN. (16) In our study, the majority of cases have been G3 (56.8%) and G2 (41.2%), and only 2% of the cases G1.

Several studies have demonstrated the presence of a large number of visceral metastases compared with bone metastases in breast tumors. Also, these patients had an increased risk of central nervous system metastasis. (17) In our cases, visceral metastases were 41.66% of all metastases and bone metastases 16.66%. Gaedcke et al. (18) have shown that the majority of breast cancer brain metastases derived from a triple negative breast tumor. In our study, brain metastases were 25% of all metastases. Fulford et al. (19) did not find any difference between the number of lung and pleural metastases in triple negative breast tumors, although some studies describe a higher frequency of lung metastases. In our study, lung and pleural metastases were in equal number.

In defining the clinicopathological profiling of TN breast tumors, in addition to the aggressive characteristics described above, we identified additional morphological features such as the presence of necrosis (76%) and of the "pushing" borders (60.8%). These data are consistent with the literature. (20)

Tumour necrosis occurs due to the rapid proliferation of tumor cells beyond the capacity of blood supply to those areas, thus the appearance of poorly vascularized tumor areas that become ischemic.

The increased aggressiveness of TN phenotype might explain the high percentage of presence of tumor necrosis. Morphological studies of triple negative breast cancers have shown a higher prevalence of the presence of necrosis. (21) Use of tumor necrosis as a prognostic factor is still debated due to the lack of criteria for defining significant necrosis.

Borders at the tumor invasion front in our study were infiltrative (39.2%) or pushing type (60.8%).

The major cause of death in breast tumors is the formation of metastases by dissemination. Lymphovascular invasion is considered an important independent prognostic factor, although there are conflicting data in the literature in this regard. A possible explanation of these discrepancies could be the inter-observer variation in reporting the frequency of lymphovascular invasion and the difficulty in differentiating it from tissue retraction artefacts. In our study we considered as retraction artefacts the groups of tumor cells that mimicked the shape of spaces they were in.

The prognostic importance of lymphovascular invasion has been highlighted by studies in which the survival rate in a group of patients with T1N0 breast tumors was reduced in those who showed lymphovascular invasion than those in which it was absent. (22) Furthermore, Rosen et al. (23) showed a decrease of prognosis in patients with lymphovascular invasion without lymph nodes metastasis.

Conclusion

Triple negative breast cancers represent a particular subtype of tumors but their, now famous, aggressiveness applies only to some histological subtypes. It is very important to correlate the histological features and the phenotypical expression.

A multitude of factors contribute to the complexity of the lesions occurring in breast cancer. These factors depend on the biology and natural history of cancer, variability in diagnostic criteria, the importance of management and therapy procedures for screening and therapy. It is important to draw attention to the detection and treatment of malignant breast lesions through biomedical research, education and treatment module.

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