

## Estrogen and progesterone receptor expression in the mammary gland tumors

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### Abstract

In the primary mammary malignant tumors, including *in situ* carcinoma, it is recommended the carrying out of immunohistochemical diagnosis for the estrogen (ER) and the progesterone receptors (PR). We have studied the ER and the PR expression in malignant tumors, trying to identify the corresponding phenotypes according to the presence of these tumors. We have carried out a study on a total number of 80 carcinomas, divided into two groups: the first one constituted of 54 cases of carcinomas on which we had clinical data, and another group, constituted of 26 cases of mammary carcinoma, where no clinical data was available. We have observed that the values and the distribution of the ER and PR taken from the biopsies made in the patients with mammary carcinoma are influenced by the age and menopausal status. The combination of the ER/PR results lead to the definition of many tumoral phenotypes.

**Keywords:** mammary, malignant tumor, estrogen, progesterone, receptor.

### Introduction

The assessment of the ER and PR expression in the mammary carcinomas as a prognostic and especially predictive factors in the antiestrogenic endocrine adjuvant therapy represents the element of the traceability protocol in mammary neoplasm [1].

The immunohistochemical assessment of the ER and PR must be compulsory carried out in the detection of any primary malignant mammary tumor, including *in situ* carcinoma. The immunohistochemical testing for the ER and PR is indicated in the case of mammary tumor relapse and metastasize, due to the particular capacity of mammary carcinoma to change its hormonal status in evolution [2, 3].

The ER status conversion, from negative to positive, is advantageous for patients who could benefit of endocrine therapy. Conversely, the disappearance of the positive ER status is associated with an increased tumoral aggression and therapy resistance [4]. The receptor expression for progesterone is induced by estrogen and consequently it represents a functional marker for ER. The normal mammary tissue contains PR. In the mammary tumors, the PR expression is similar or slightly inferior in density, compared to ER, but the coloration intensity is higher. PR is a weaker predictor for the endocrine therapy response, but in exchange, it offers important information about the clinical evolution of the disease [5, 6].

There is a well-defined correlation between ER/PR expression and breast cancer initiation, progression and prognosis. Testing the breast tumor for both ER and PR

status has to take into account their double profile, as predictive and prognostic factors, which becomes significant for a correct management of breast cancer [7, 8]. Due to the importance of the ER and PR, both in the therapeutic decision and in the assessment of the disease evolution and treatment response, we have studied the estrogen and progesterone expression level in the case of malignant lesions, trying to identify some phenotypes according to the presence of these two hormonal receptors.

### Materials and Methods

We have studied the distribution of the ER and the PR, according to the histopathological type of carcinoma, as well as the correlation between the receptor presence and the differential degree, with the clinico-pathological parameters, such as the age of the patient, menopausal status, the type of axillary ganglion, the size of the tumor, the TNM staging, for the cases where the clinical data were known.

The study was carried out on a total number of 80 carcinomas, divided into two groups: the first one constituted of 54 cases of carcinomas, where we had clinical data, and another group, constituted of 26 cases of mammary carcinomas, where no clinical data were available. The study included an immunohistochemical assay of the ER and PR and an imagistic investigation.

The immunohistochemical protocol for the two types of hormonal receptors is similar. Two monoclonal antibodies, Mouse Anti-Human Estrogen Receptor, clone 1D5 (Anti-ER, 1D5) and Mouse Anti-Human

Progesterone Receptor, clone PgR 636 (Anti-PR, PgR 636), both provided from Dako Cytomation (Denmark) were used to evaluate ER and PR. The paraffin sections were dewaxed, rehydrated and incubated. Endogenous peroxidase activity was blocked by incubating with 3% hydrogen peroxide for 20 minutes, followed by LSAB2 (Labeled Streptavidin–Biotin 2 System, Horseradish Peroxidase) technique. After washing the primary antibody with phosphate buffered saline (PBS), the biotinylated link antibody and the peroxidase-labeled Streptavidin were added to be incubated. The staining is completed after the incubation with DAB. A dilution of 1:100 was used for both monoclonal antibodies. Dako Universal Negative Control +, Mouse was used for the negative control. For the ER/PR results interpretation in optical microscopy, we have taken into account only the nuclear staining pattern and we have used the Allred or Quick score, a semi-quantitative evaluation including data about the density and the intensity of the positive reaction, grades and sums up the percentage of positive nuclei cells (the percentage score) and the intensity score (Table 1).

**Table 1 – Allred or Quick score**

Percentage score (PS)	Intensity score (IS)
0 = negative	0 = negative
1 = <1% stained nuclei	1 = weakly positive
2 = 1–10% stained nuclei	2 = moderately positive
3 = 11–33% stained nuclei	3 = intensely positive
4 = 34–66% stained nuclei	
5 = 67–100% stained nuclei	
Total score (TS) = PS + IS	
TS = 2–8	

For a score of 0 to 2 (less than 10% weak stained positive nuclei), the result was considered negative; for a score of 3 and 4, we have used “+”; for a score of 5 and 6, we have used “2+”, and for a score of 7 and 9, “3+” (Table 1).

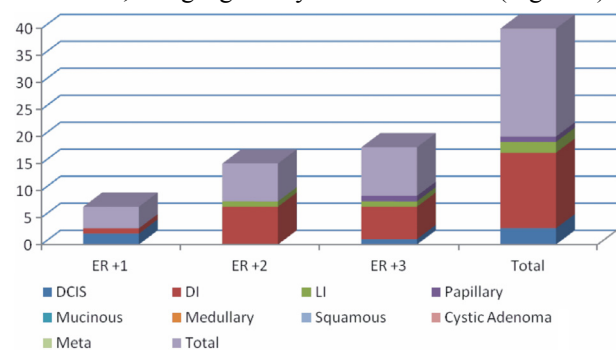
The clinical diagnose of the attending physician is not sufficient in the case of a positive result, for that reason it is necessary the inclusion of a total score, of the used clone and the usage of the antigenic exposure as well as the control quality. There must be also attached the correlation between the immunohistochemical score and the response rate of the hormonal therapy, thus:

- 0 score: the hormonal therapy is not sufficient;

- 2–3 score: 20% chance of therapy response with antiestrogenic preparation;
- 4–6 score: 50% response rates;
- 7–8 score: 75% positive response to therapy.

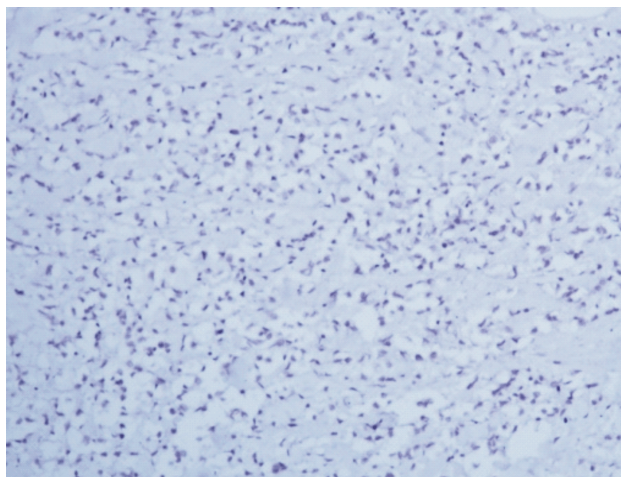
## Results

We have studied both the ER as well as the PR prevalence in the malignant mammary tumors. In the study of the ER prevalence, according to the type of carcinoma, we have found the following diversity: five cases of DCIS (three solid type, one cribriform and one apocrine), 48 invasive ductal carcinoma, 10 lobular carcinoma, four papillary, five metaplastic, two mucinous, two medullary, one cystic adenoid, one cutaneous metastasis, one ganglionic and one cerebral (Figure 1).

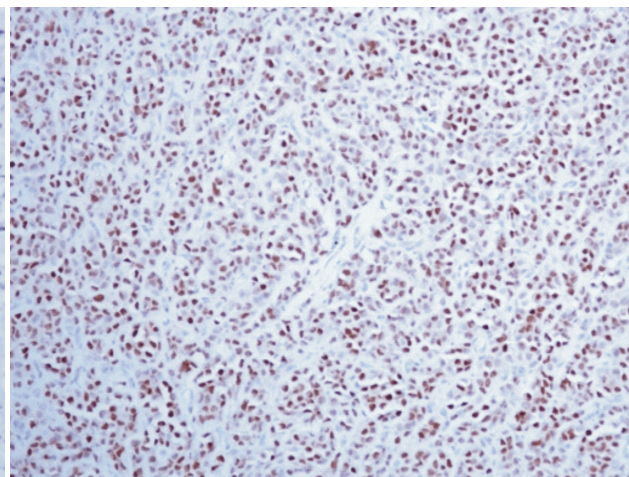


**Figure 1 – ER prevalence depending on histopathological type of carcinoma.**

Ductal carcinomas *in situ* were positive for the ER in a percentage of 60% (three cases), one cribriform DCIS high positive (ER3+) and two solid DCIS – one of them weak positive (ER1+) and the other high positive (ER3+). DCIS with apocrine differentiation and one of the solid DCIS were negative. Out of the 48 invasive ductal carcinomas, a number of 14 (29%) cases were positive estrogen receptor, the rest being negative (Figure 2). Regarding the expression level of the ER expression, one carcinoma was weak positive (ER1+), seven cases were moderate positive (ER2+) and six carcinomas were intense positive (ER3+). Out of the estrogen positive invasive carcinoma cases, the ductal invasive type presented the most cases with intense positive ER (ER3+) (Figure 3).



**Figure 2 – Invasive ductal carcinoma. Negative ER expression, ob. x400.**



**Figure 3 – ER3+ expression: invasive ductal carcinoma, ob. x400.**

*In situ* ductal carcinomas were most frequently associated with invasive ductal carcinoma. Mammographically, *in situ* ductal carcinoma appears as a radiopaque lesion, which is relatively homogeneous, poorly defined for the most part BI-RADS 4 type, but the most characteristic element was the presence of the microcalcifications. There have been identified focal, segmentary and diffuse microcalcifications, round, regular, either pleomorphic or “broken glass” type (Figure 4). Highly suggestive for DCIS “comedo” type were the branching microcalcification that seemed to follow the trajectory of a duct (Figure 5).

Out of the 10 lobular carcinomas, only two (20%) were ER positive, on high positive, the majority of the tumoral cell nuclei being intensely colored in brown (ER3+) and one weak positive (ER1+). Out of the four papillary carcinomas, only one was ER high positive (ER3+) (Figure 6).

Medullary carcinoma, metaplastic, cystic adenoid as well as cerebral, ganglionic and cutaneous metastasis were ER negative. Out of the total number of carcinomas, 20 (25%) were ER positive, and out of the ER positive cases, four (20%) were weak positive, seven (35%) were moderate positive (ER2+) and nine (45%) were high positive (ER3+).

PR were positive in a percentage of 30% (24 carcinomas) and out of the positive cases, four (16.6%) were weak positive (PR1+), 13 (54.16%) moderate positive (PR2+) and seven (29.16%) intense positive (PR3+) (Figure 7).

Out of the *in situ* ductal carcinomas, only two (40%) have been moderately positive (PR2+), namely the cribriform type and one of the solid type (Figure 4); we have encountered these cases in the premenopause female patients, as in the case of positive DCIS for the estrogen receptors. DCIS with apocrine differentiation have been PR negative.

Invasive ductal carcinoma have constituted the most frequent histological type and accordingly presented the highest number of positive cases. Thus, 14 out of 48 (29%) were PR positive. According to the preset quantification with an intensity and a percentage score, three cases have been quantified as being weak positive (PR1+) (Figure 8), six moderate positive (PR2+) (Figure 9), five intense positive (PR3+) (Figures 10 and 11).

Mammographically, the typical image for invasive ductal carcinoma is that of a radiopaque, relatively homogeneous, poorly defined (Figure 12), and the calcifications identified in four cases have been pleomorphic, granular (Figure 13). The ultrasound examination in the case of invasive ductal carcinoma showed poorly defined hypoechoic masses (Figure 14); the Doppler colored echography showed diffuse vascularization of the tumor (Figure 15). The best way to investigate the invasive ductal carcinoma is represented by the MRI, especially for the small, irregular lesions, which can easily be a source of confusion, due to their overlapping on the normal tissues (Figure 16).

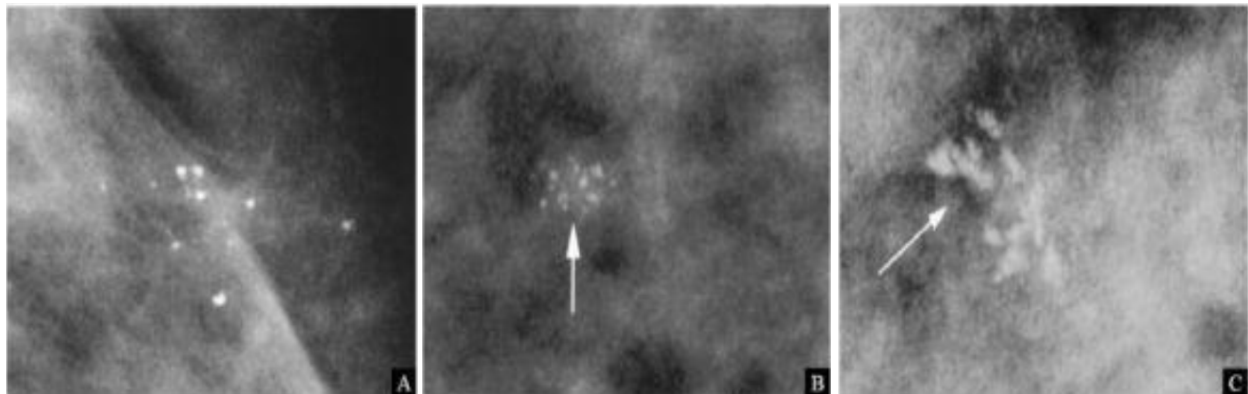


Figure 4 – Mammography: *in situ* ductal carcinomas – microcalcifications. (A) Round, regular; (B) Diffuse; (C) “Broken glass” aspect.

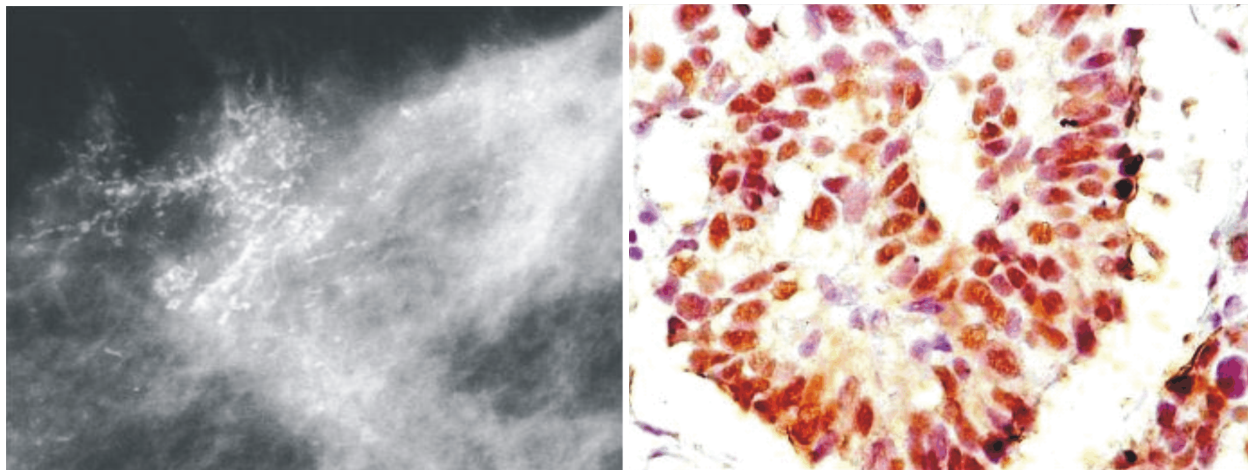
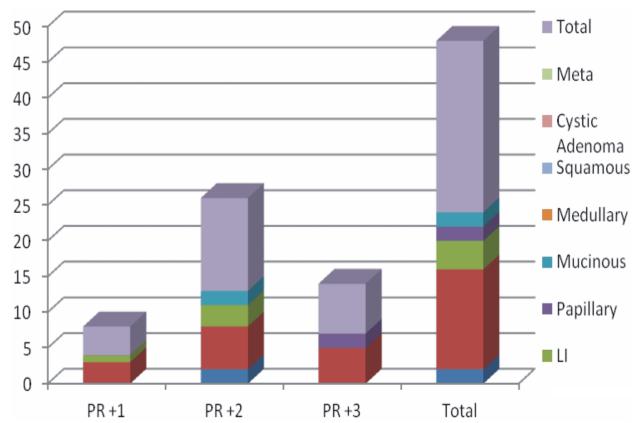


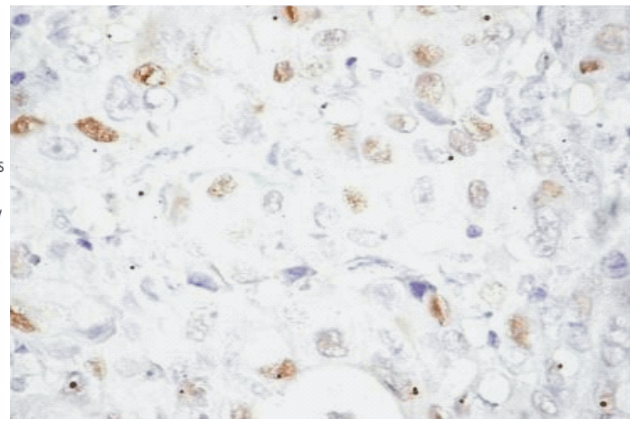
Figure 5 – Mammography: *in situ* ductal carcinoma. Branching microcalcifications.

Figure 6 – ER3+ expression in papillary carcinoma, ob.  $\times 400$ .

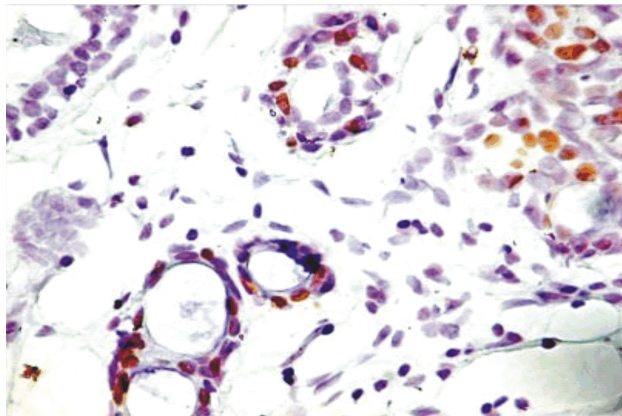




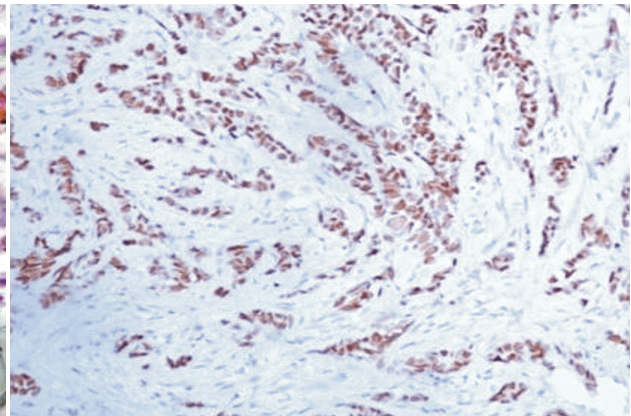
**Figure 7 – PR prevalence depending on histopathological type of carcinoma.**



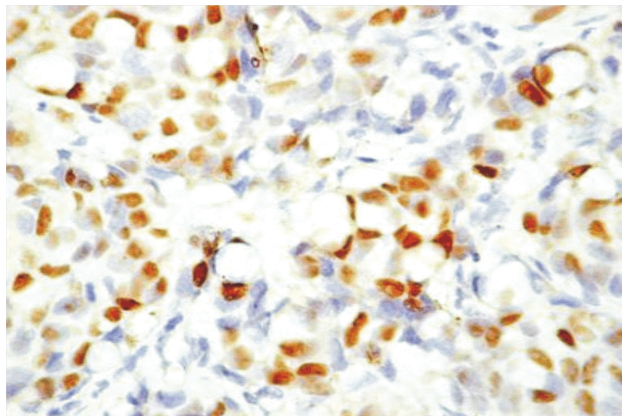
**Figure 8 – PR1+ expression in poorly differentiated ductal carcinoma, ob.  $\times 400$ .**



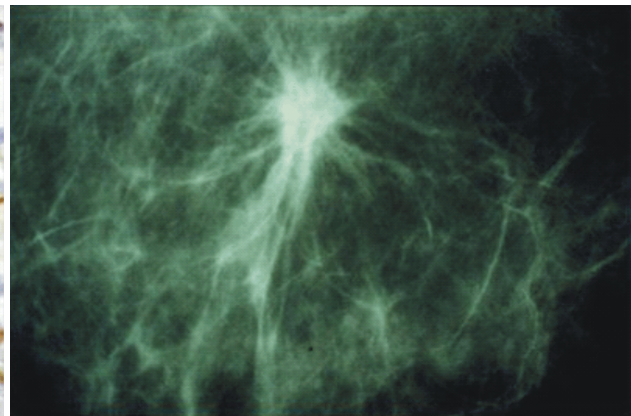
**Figure 9 – PR2+ expression in invasive ductal carcinoma, ob.  $\times 400$ .**



**Figure 10 – PR3+ expression in invasive ductal carcinoma, ob.  $\times 400$ .**



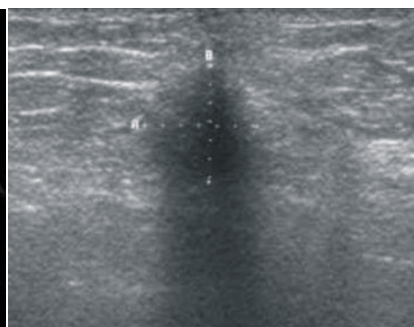
**Figure 11 – PR3+ nuclear heterogeneity in invasive ductal carcinoma, ob.  $\times 200$ .**



**Figure 12 – Mammography. Invasive ductal carcinoma: irregular radiopacity extending into parenchyma.**



**Figure 13 – Invasive ductal carcinoma: granular, pleomorphic microcalcifications.**

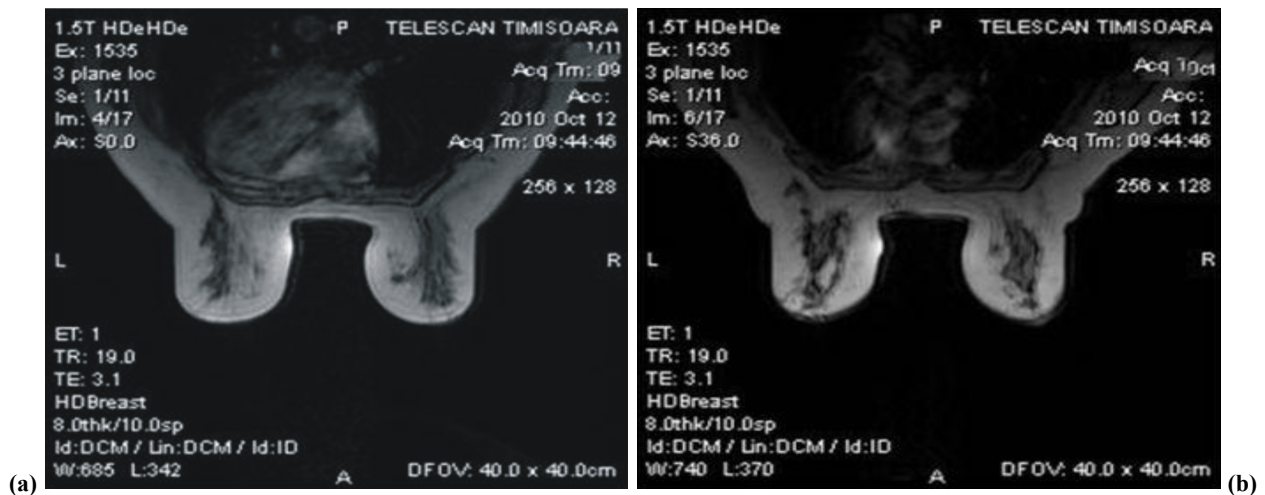


**Figure 14 – Echographic image of an invasive carcinoma.**



**Figure 15 – Doppler echography: invasive ductal carcinoma.**



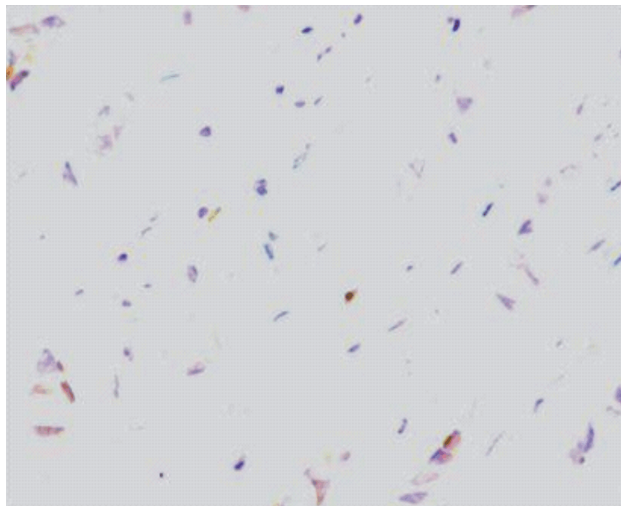


**Figure 16 – (a and b) MRI: invasive ductal carcinoma.**

In the case of lobular carcinoma, four out of 10 cases (40%) have been positive, one of them being weak positive (PR1+) and three cases have been moderately positive. In the case of associated LCIS, it has presented a similar behavior to the main lesion, as for the other markers studied. We can point out the positive reaction from the nuclei of the tumoral cells, disposed in “Indian row” (Figure 17). Invasive lobular carcinoma is difficult to be mammographically visualized, unlike the ductal invasive carcinoma. For its identification, a local compression spot mammography was required. Even so, the lesion was very subtle, identified by a light asymmetry of the mammographical density. Mammary echography was very useful in order to identify a structure resembling to invasive ductal carcinoma, with more irregular margins (Figure 18).

Two out of four (50%) papillary carcinomas presented an intense positive reaction for the progesterone receptors (PR3+) only at the nuclear level, the stroma of the fine conjunctive axis being negative (Figure 19).

An intense positive reaction is observed in the majority of the characteristically tumoral cells of papillary carcinoma, disposed perpendicular on the ductal axis. Poor stromal elements are negative.



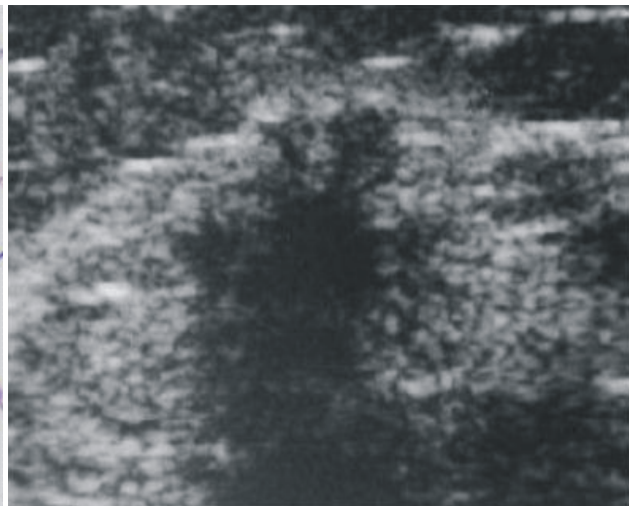
**Figure 17 – PR1+ expression in lobular carcinoma, ob. ×400.**

The invasive papillary carcinoma has a similar mammographic aspect to that of the papiloma, a tumoral mass partially circumscribed, contained in a cystic dilatation (Figure 20, a and b). The galactography can lead to confusion with the papiloma (Figure 21).

Mucinous carcinomas have been positive for PR, with a moderate intensity, established using the percentage and intensity score of the nuclear immunoreactions in the tumoral cells. The intra- and inter-lobular stroma, as well as the extracellular mucin were negative. The medullar, metaplastic with squamous differentiation, cystic adenoid, as well as the three mammary carcinoma metastases have been PR and ER negative. In some of those cases, only a weak cytoplasmic correlation has been observed, which has been ignored (Figure 22).

The mucinous carcinomas were found in four female patients, appearing partially circumscribed, relatively homogeneous on mammography (Figure 23); the echography has a higher diagnostic relevance by showing the heterogeneity of the lesion (Figure 24).

The ER/PR phenotypes have been distributed as follows: ER+/PR+ in 19 (23.75%) cases, ER+/PR- in one (1.25%) case, ER-/PR+ in five (6.25%) cases and ER-/PR- in 55 (68.75%) cases (Figure 25).



**Figure 18 – Echographic hypoechogenic irregular image of invasive carcinoma.**

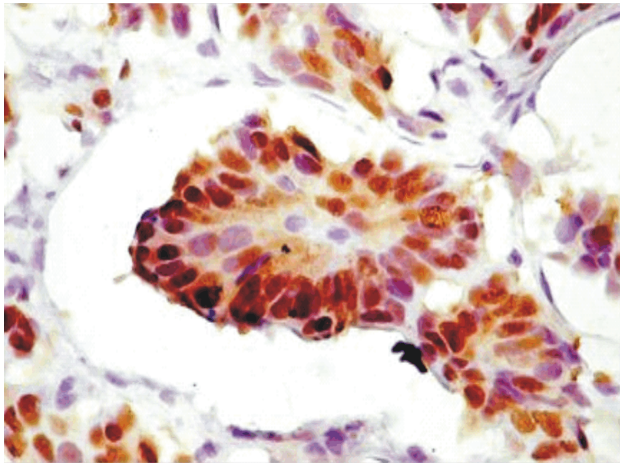


Figure 19 – PR3+ expression in a papillary carcinoma, ob.  $\times 400$ .

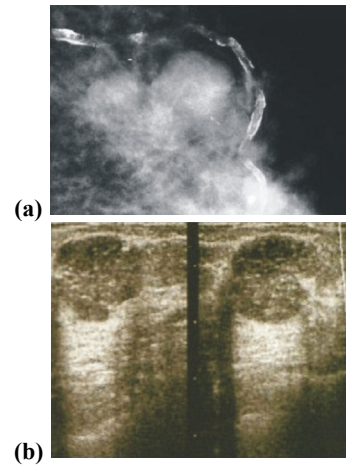


Figure 20 – Mammography – papillary carcinoma: (a) Incompletely circumscribed cystic structure; (b) Well-defined structure in a dilated cystic duct.

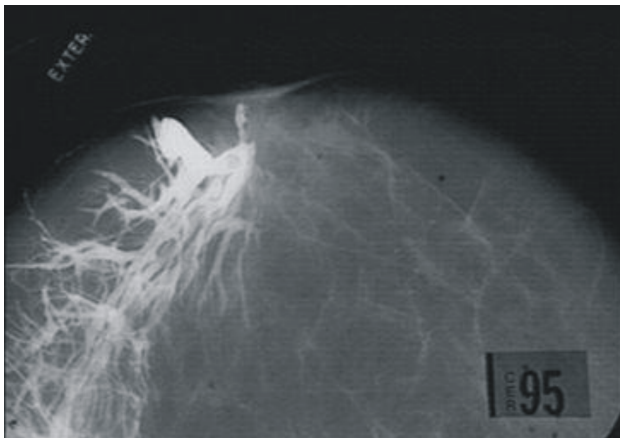


Figure 21 – Galactography: invasive papillary carcinoma.

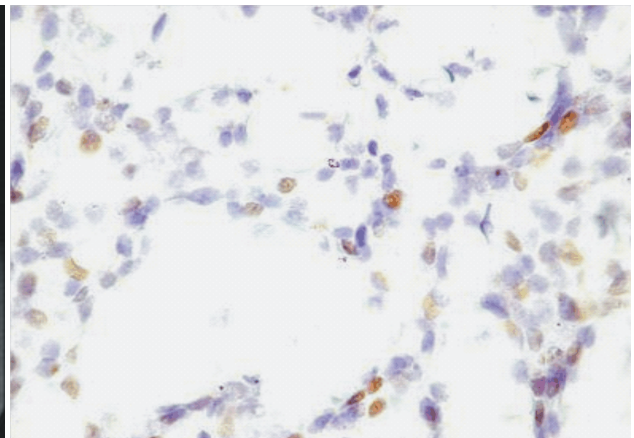


Figure 22 – PR expression in a mucinous carcinoma, ob.  $\times 400$ .

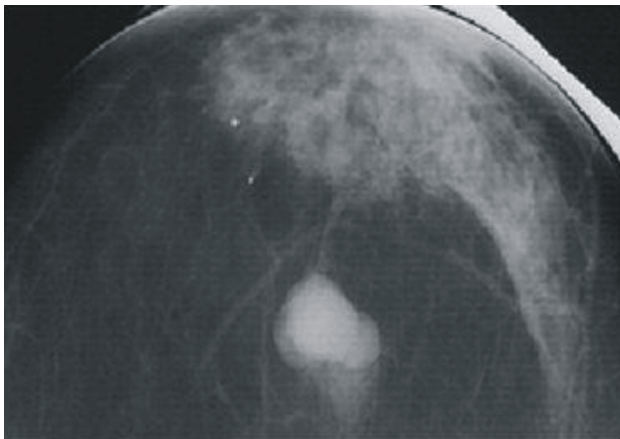


Figure 23 – Mammography: mucinous carcinoma. Homogeneous radiopacity.



Figure 24 – Echographic hypoechogenic irregular image of a mucinous carcinoma.

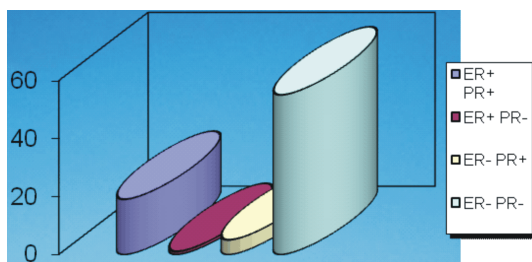


Figure 25 – The prevalence of ER/PR phenotypes.

The DCIS cases we have encountered, associated with invasive carcinoma, presented the same phenotypes with the base lesions. The five metaplastic carcinoma cases with squamocellular differentiation have been negative (ER-/PR-). Medullary carcinomas have been negative for ER and PR, and in the case of mucinous carcinoma, the phenotype was ER-/PR+.

In the case of papillary carcinomas, we found one positive case for ER/PR, one positive case only for PR, and two negative cases for ER/PR. Cystic adenoid



carcinoma was negative for ER and PR, as well as cutaneous, ganglionic and cerebral metastases of mammary carcinoma, which are negative for hormonal receptors. We have studied the correlations between the ER and PR expression and the clinico-pathological data with impact on the prognosis of the breast cancer: menopausal status, presence of axillary ganglion, size of tumor, tumor staging according to TNM classification, the histopathological type of tumor, the degree of tumoral differentiation, as well as the correlation between the estrogen and progesterone receptors.

## Discussion

In the identified malignant lesions, we have noticed the prevalence and the expression level of the ER and PR were correlated with clinico-pathological parameters recognized as prognostic factors in mammary cancer: menopausal status, stage of tumor, size of tumor, nodal status, differentiation degree, histological type [9, 10].

We have also identified the ER/PR phenotypes recognized as having an impact on the response of the antiestrogenic treatment. There are numerous studies which sustain our results, pointing on the particular relationship between estrogen, progesterone and therapeutic management of breast tumors [11, 12]. In total, 25% of malignant tumor expressed ER, and 30% expressed PR. Regarding the ER/PR receptor distribution according to carcinoma type, we have obtained the following results: DCIS expressed ER/PR in 6/40 cases, invasive ductal carcinoma in 29/29, lobular in 20/20 papillary in 25/50, mucinous 0/50.

Hormonal background of mammary gland tumors development and progression is firmly connected with the estrogen-progestative profile [13]. The expression of the ER and PR was revealed by immunohistochemical assay; the relationship between the expression and the prognostic significance of ER/PR regarding the mammary carcinomas seems to be the premise not only for an accurate diagnosis but also for a therapeutic decision [14–16].

Medullary carcinoma, metaplastic, cystic adenoid, as well as the three metastasized tumors did not express ER/PR. The response at treatment depends also on the expression level of the hormonal receptors [17, 18]. As long as the breast cancer is still a significant cause of death in women, the search for the proper therapy has led to a complex investigation of the hormonal involvement; both ER and PR are valid biomarkers, even the biological and clinical PR significance is more or less controversial [19, 20].

Thus, most of the positive ER carcinomas (45%) presented a high intensity nuclear expression level, in the majority of the tumoral nuclei cells of epithelial nature (ER3+).

Regarding the PR expression, the majority of positive PR cases presented a moderate level expression (2+). The phenotypes identified have been distributed so: ER+/PR+ in 23.75% cases, ER+/PR1 in 1.25% cases, ER-/PR+ in 6.25%, and ER-/PR- in 68.75% cases. We have obtained a lower ER- percentage, and a higher PR+ cases.

At the statistical analysis, we have noticed a statistical significance as expected between the two receptor type

expression, as well as the differential histological degree ( $p=0.02$ ). We did not observed a statistical significant difference between the ER/PR expression and the tumor stage, lymphatic invasion, size of the tumor, histological type and menopausal status.

Those data support the hypothesis that the estrogen and progesterone hormonal status has a higher impact on the antiestrogenic treatment response and less on the prognosis.

## Conclusions

The values and the estrogen/progesterone receptor distribution from the biopsies of the mammary carcinoma are influenced by age and menopausal status; thus, the biopsies taken from the premenopausal patients have smaller values of those receptors compared to those of postmenopausal patients, reason for which it will be taken into account the age and the menopausal status in the interpretation of the immunohistochemical reactions. Combining the result for ER/PR led to the definition of more tumoral phenotypes with different response rate at hormonal therapy, especially in advanced mammary neoplasm or metastasis.

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