IMMUNOHISTOCHEMICAL PROFILE OF THE ESTROGEN AND PROGESTERONE RECEPTORS IN MAMMARY BENIGN LESIONS

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IMMUNOHISTOCHEMICAL PROFILE OF THE ESTROGEN AND PROGESTERONE RECEPTORS IN MAMMARY BENIGN LESIONS (Abstract): The immunohistochemical diagnosis for estrogen and progesterone receptors must be carried out in the tracking of every primary tumor, benign or malignant, especially in the case of in situ carcinoma. **Material and method**: We have studied the expression of estrogen and progesterone receptors in benign lesions, identifying phenotypes depending on the presence of estrogen and progesterone receptors. **Results**: The result “positive” or “negative” in the report sent to the doctor is not sufficient, the inclusion of the total score in the case of a positive result, the clone used, the usage / non-usage of antigenic exposure and also the quality control being necessary. The benign mammary lesions occur in the context of a hormonal imbalance, which, in the long run runs the risk of developing a mammary carcinoma. **Conclusion**: The further study of the hormonal spectrum of those lesions, considered as pre-malignant, may lead to the identification of some groups of hyperplastic lesions, presenting a high risk for developing a mammary cancer. **Key words**: MAMMARY TUMORS, IMMUNOHISTOCHEMICAL DIAGNOSIS, HORMONAL PROFILE

The hormones control the development of the mammary carcinomas and thus the hormonal treatment is the preferred treatment for the vast majority of the ER positive metastatic breast cancers. At present, the evaluation of estrogen and progesterone expression in mammary carcinomas and their interpretation as prognostic and predictive factors, in the antiestrogenic endocrine therapy are elements of the traceability in female patients with mammary tumors (1). The immunohistochemical diagnosis for the estrogen and progesterone receptors must be carried out in the tracking of every primary benign and malignant tumor, especially in the case of in situ carcinoma. The immunohistochemical testing for the estrogen and progesterone receptors is highly indicated in the mammary tumors recurrence and metastases, due to the well known capacity of the mammary carcinomas of changing their hormonal status during their evolution. The conversion from a negative ER status to a positive ER status is beneficiary for the patients, benefiting of endocrine therapy. Conversely, the disappearance of the positive ER status is associated with a marked tumoral aggressiveness and therapy resistance. The expression of the progesterone receptor is induced by estrogen and consequently is a functional marker for ER. The normal mammary tissue presents receptors for progesterone. In mammary tumors the PR
expression is similar or slightly inferior in density, in comparison with ER, but the staining intensity is higher. PR is a weaker predictor for the endocrine therapy response and it offers instead important information regarding the clinical evolution of the disease (2). The positive mammary tumors for ER and PR present a high degree of histopathological heterogeneity, do not associate ganglionic metastases, present a reduced proliferation rate and have a better prognosis (3). The ER / PR mammary tumors are associated with a favorable response to endocrine therapy and only a reduced number of cases do not respond to this therapy, due to the expression of an altered form of receptors.

OBJECTIVES
We have studied the expression of the estrogen and progesterone receptors in the benign lesions identifying phenotypes depending on the presence of estrogen and progesterone receptors.

MATERIAL AND METHODS
Out of 91 cases of benign lesion evaluated (main and adjacent ones), 16 were fibroadenomas, 23 typical moderated and fluoride ductal hyperplasia, 6 atypical ductal hyperplasia, 11 adenosis and sclerosing adenosis, 17 cystic dilatations, 15 apocrine metaplasia, 1 Phyllodes tumor and 2 cases of intraductal papilloma. The immunohistochemical protocol in the case of ER and PR receptor was similar and has been applied on twin blade slides. In order to interpret the results for ER/PR using optical microscopy we have taken into account only the nuclear coloration pattern, using Allred or Quick score, which are a semi-quantitative evaluations that includes density data, the intensity of the positive reaction and also grades the positive nuclei cell percentage (percentage score), using an intensity score (tab. I).

TABLE I

<table>
<thead>
<tr>
<th>Allred quantification score for estrogen and progesterone receptors</th>
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</thead>
<tbody>
<tr>
<td>Percentage score (PS)</td>
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<tr>
<td>----------------------</td>
</tr>
<tr>
<td>0 = negative</td>
</tr>
<tr>
<td>1 = &lt; 1% coloured nuclei</td>
</tr>
<tr>
<td>2 = 1-10% coloured nuclei</td>
</tr>
<tr>
<td>3 = 11-33% coloured nuclei</td>
</tr>
<tr>
<td>4 = 34-66% coloured nuclei</td>
</tr>
<tr>
<td>5 = 67-100% coloured nuclei</td>
</tr>
<tr>
<td>Total score (TS) = PS + IS</td>
</tr>
<tr>
<td>TS = 2 – 8</td>
</tr>
</tbody>
</table>

A score of 0, 1 or 2 (less than 10% weak colored positive nuclei) has been considered negative (0), a score of 3 and 4 was considered “1+”, a score of 5 and 6 was considered “2+”, and a score of 7 and 8 was considered “3+”.

The result “positive” or “negative” in the report sent to the doctor is not sufficient, the inclusion of the total score in the case of a positive result, the clone used, the usage / non-usage of antigenic exposure and also the quality control being necessary. The correlation between the immunohistochemical score and the response rate to hormonal therapy will be attached:

Score 0 hormonal therapy is inefficient.
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Score 2-3 – 20% chance of therapy response to anti-estrogen medication.
Score 4-6 – 50% response rate.
Score 7-8 – 75% positive response to therapy.

RESULTS
In the case of negative controlled sections, where the incubation phase with the primary antibody was omitted, no coloration has been noticed. The normal adjacent mammary tissue, used as internal positive control presented a positive reaction for estrogen and progesterone receptors. The positive cellular immunoreaction has been noticed only at nuclear level. The myoepithelial, myofibroblasts and fibroblasts from the stroma of each case did not present any positive reaction. The staining for progesterone receptors has been more intense than in the case of the estrogen receptors, but the percentage of positive nuclei was higher than in the case of the estrogen receptors.

The unmodified mammary epithelium presented a non-uniform, diffuse expression of the estrogen and progesterone receptors, with a more obvious expression at the level of ductal epithelium, compared with the lobular epithelium. The estrogen receptors have been stated in 25.3% of the benign lesion cases, and the progesterone receptors have been stated in 33% of the main benign lesions and associated lesions. The distribution according to benign lesion type of the estrogen and progesterone receptors can be seen in table 2. We have encountered 13 cases of intra and pericanalicular fibroadenomas and also 2 cases in which the fibroadenoma has been associated with the phyllodes tumor. Out of the cases of fibroadenomas studied, we have encountered a number of 5/16 (31.25%) positive cases for ER and 9/16 (56.25%) positive cases for PR. The immunoreaction of hormonal receptors was present at the level of epithelial cells nuclei. The fusiforme proliferated stromal cells (fibrocyte / fibroblasts) present as “spindle cells” were negative for hormonal receptors. From table II we can note the fact that most fibroadenomas were positive for progesterone receptors.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Total number of lesions (n=91)</th>
<th>ER</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ (n=23)</td>
<td>- (n=68)</td>
</tr>
<tr>
<td>Fibroadenomas (FA)</td>
<td>16</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Typical ductal hyperplasia (HDT)</td>
<td>23</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Atypical ductal hyperplasia (HDA)</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Adenosis and typical sclerosing adenosis (Ad)</td>
<td>11</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Intraductal florid papiloma (Pp)</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Apocrine metaplasia (MA)</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Cystic dilatation (DC)</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Phyllodes Tumor (TP)</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

TABLE II
Estrogen and progesterone receptor distribution in the case of benign tumors encountered (including the associated lesions)
Regarding the quantification of estrogen and progesterone receptor expression, the vast majority of the cases we have evaluated were slightly positive (1+) and only a small amount of cases were moderately positive (2+). Out of the two fibroadenomas cases associated with the invasive ductal carcinoma, one was positive both for ER and PR, and the carcinoma was well differentiated (G1); the case associated with weak differentiated carcinoma (G3) was negative for estrogen and progesterone receptors. The fibroadenomas associated with the phyllodes tumor was positive only for the progesterone receptors.

The typical ductal hyperplasia (fig. 1) was encountered in 23 cases, out of which 6 cases (26.08%) were ER positive and 8 cases (37.7%) were PR positive. The great majority of the cases were slightly positive for ER and PR (+1). The moderate or florid ductal hyperplasia is associated with a 1.5-2 times higher risk of developing a carcinoma.

Atypical ductal hyperplasia is associated with a 5 times higher risk of developing a carcinoma. We have encountered this type of lesion in 6 cases, which also presented malign lesions and DCIS (ductal carcinoma in situ), out of which 2 were ER positive (33.33%) and 3 cases were PR positive (50%).

We have encountered 12 cases of adenosis, as well as cases of sclerosing adenosis, out of which 8 cases were ER positive (72.7%) and 7 cases were PR positive (63.6%).

Intraductal florid papiloma was identified in 2 cases, positive for both ER and for PR.

In the case of apocrine metaplasia we have not noticed a positive reaction for estrogen or progesterone receptors. The ductal dilatation was negative for estrogen and progesterone receptors.

From table I one can notice that the majority of lesions (17) were quantified as being slightly positive (1+), regarding ER expression, 6 cases were moderate positive, most of them in the case of adenosis. As for the progesterone receptors expression, 22 cases were slightly positive, 7 were moderate positive and one case (adenosis) was intensely positive (fig. 2).

Depending on the estrogen and progesterone receptors, we have established the prevalence of four phenotypes ER/PR in the case of the benign tumors (fig. 3).

Consequently, in case of fibroadenomas, we have encountered 4 cases (25%) for both estrogen and progesterone receptors (ER+/PR+), 1 case (6.25%) ER+/PR-, 5 cases (31.25%) ER-/PR+ and 6 cases (37.5%) ER-/PR-.

In the case of typical hyperplasia, 1 case (4.4%) was ER+/PR+, 5 cases (21.8%) were positive only for ER (ER+/PR-) , 7

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**Fig. 1.** The progesterone receptors expression in a typical ductal hyperplasia. One can notice heterogeneous distributed, positive nuclei, with an intensity score of 1+ and rare 2+ nuclei, with a percentage score of 3+ (around 30% positive nuclei).
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(30.4%) were ER-/P+ and 10(43.47%) cases were negative (ER/- PR-). Atypical hyperplasia presented the following phenotypes: 1 case (16.6%) ER+/PR+, 1 case (16.6%) ER+/PR- and 2 cases (33.3%) ER-/PR+ and ER-/PR-.

![Graph showing ER and PR quantification of benign lesions.](image1)

**Fig. 2.** ER and PR quantification of benign lesions.

![Graph showing ER / PR phenotype prevalence in the case of benign tumors.](image2)

**Fig. 3.** ER / PR phenotype prevalence in the case of benign tumors

In the case of adenosis, 6 (54.54%) cases were ER+/PR+, 2 cases (18.18%) were ER+/PR-, 1 case (9%) was ER-/PR+ and 2 cases (18.18%) were ER-/PR-. The Phyllodes tumor encountered presented an ER-/PR+ phenotype. The florid intraductal papilomas were positive both for estrogen and progesterone receptors (ER+/PR+). The non-proliferative lesions, represented by cystic dilatations and apocrine metaplasia were not positive for ER/PR. Out of the positive cases, the most frequently encountered phenotype was ER-/PR+, followed by ER+/PR+.
DISCUSSION
The benign mammary lesions occur in the context of a hormonal imbalance, which, in the long run runs the risk of developing a mammary carcinoma. The activation of α estrogen receptor (ER-α) raises the sensitiveness of the target tissue for the action of circulatory estrogens, stimulating the DNA synthesis, cell division, active biological protein production, including pS2, TGF-α (transforming growth factor), EGF (epidermal growth factor), which influences the cellular growth and differentiation (4). The cumulative exposure to estrogens contributes to the mammary carcinogenesis by means of stimulating a premalignant cells clone or the augmentation of spontaneous mutation percentage. The estrogens may also decrease the transition time in the cellular cycle, so that the spontaneous mutations become defined before the reparatory mechanism intervention. Another mechanism could be the toxic genetic effect of estrogens. It can be explained by the loss of the suppressing gene effect, whose function is to prevent the excessive proliferation of ER positive cells in the rich estrogens environment, especially in premenopausal women (5). The allelic disequilibrium was proven in some intraductal and intralobular proliferation and is significantly increased into in situ carcinomas and atypical hyperplasia, compared with usual hyperplasia (6). Increased hormonal contents, as those observed in the case of premenopausal women or those breastfeeding are associated with a reduced number of ER+ epithelial cells, while the reduced circulatory concentrations of estrogens, as those observed in the case of postmenopausal women, are associated with an increased number of positive ER cells. In normal mammary tissue, the percentage of positive ER cells grows with the age, correlated with the diminished plasmatic concentration of the circulatory steroid hormones. In the case of premalignant proliferative lesions, as well as in the case of the in situ carcinomas, this negative association is lost, being a display of the early molecular changes that appear in the neoplastic transformation (7). The relatively elevated risk of developing a carcinoma was associated with the sclerosing adenosis lesions, intraductal florid papiloma, fibroadenoma, phyllodes tumor, fibrocystic disease with proliferative lesions adenosis-type and ductal hyperplasia. Some histopathological changes, such as apocrine metaplasia and ductal ectasia were not associated with an increased risk of developing mammary cancer. The evaluation of hormonal receptors presence in benign lesions is necessary in order to evaluate the opportunity of chemotherapy, with a positive ratio cost – benefit.

Clinical studies revealed a relationship between some histological types of benign mammary lesions and the risk of developing breast cancer, in accordance with the histological type of lesion and the hormonal status (8, 9, 10). The associated risk for the histological factors is the same for both breasts, suggesting that those markers must be regarded into a general context rather than as simple predecessor factors, depending on the histological type of lesion. There is also an interaction between the histological risk factors and the estrogen hormones in order to determine the level of associated risk. According to some studies, tamoxifen has diminished by over 86% the risk of mammary cancer in the case of a subset of female patients, diagnosed with atypical
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Ductal hyperplasia, suggesting the utility of the antiestrogenic therapy, especially in the case of a high risk group of female patients (11). Although there are disagreements, it is well known that small dimension tumors, in the early stage, cannot be detected using mammography (12, 13, 14).

Within the benign lesions studied, ER are expressed in 25.3% and PR in 33% of the cases. Non-malignant proliferative lesions with an increased risk of developing mammary cancer presented a positive reaction for the estrogen and progesterone receptors. Thus, atypical ductal hyperplasia, with a 5 times higher risk, presented positive ER in 33% of the cases and PR positive in 50% of the cases, with the most frequent phenotype being ER-/PR+. The sclerosing adenosis lesions, characterized by the proliferation of an epithelial and mioepithelial group of cells distorted by abundant fibrous tissue, with a 3 times higher risk of developing mammary cancer presented the highest number of positive cases for estrogen receptors (72%) and progesterone (64%), with most of the cases (45%) being ER+/PR+. In the case of fibroadenomas, estrogen receptors were present in 31% of the cases, PR in 56% of the cases, with ER-/PR+ as the most frequent phenotype (31%). The Phyllodes tumors with malignant area changes, which we have encountered accompanying a fibroadenoma, preserved the progesterone receptors positive status for ER and PR and presented a reduced or moderate level of expression (2+), especially for PR. Non-proliferative lesions which were not a diagnose on their own, but were encountered in the case of fibrocystic disease, with a lower risk of developing a carcinoma, did not present a positive reaction for both estrogen and progesterone receptors (15, 16, 17). All cystic dilatations, either small or large, with a flattened epithelium were negative for the investigated markers (ER-/PR-). In the same manner the apocrine metaplasia lesions were ER/PR negative in all cases.

**CONCLUSIONS**

Regarding the benign lesions in our study, ER are expressed in 25.3% and PR in 33% of the cases. The proliferative lesions, especially sclerosing adenosis, atypical hyperplasia, florid hyperplasia and papiloma were expressed as ER and PR. The sclerosing adenosis presented most frequent ER (73%) and PR (64%). With the exception of adenosis, which expressed more frequently estrogenic receptors, in the other cases, PR presented a higher expression level. Fibroadenomas revealed PR in 56% cases and ER in 31% of the cases. The Phyllodes tumors expressed only PR. Cystic dilatations and apocrine metaplasia did not express ER/PR. The progesterone receptor prevalence in most of these cases proves a progesterone insufficiency which supports the hormonal disequilibrium theory in tumoral transformation and explains the hormonal therapy success in fibrocystic disease with proliferative lesions. The further study of the hormonal spectrum of those lesions, considered as pre-malignant, may lead to the identification of some groups of hyperplastic lesions, presenting a high risk for developing a mammary cancer and may contribute to the selection of a female patients group requiring a careful observation who might benefit from an of antiproliferative and antiestrogenic treatment.
REFERENCES


