The role of oestrogen and progesterone receptors in breast cancer – immunohistochemical evaluation of oestrogen and progesterone receptor expression in invasive breast cancer in women

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Introduction

Expression of oestrogen receptors (ER) and progesterone receptors (PR) is a very powerful and useful predictor. Because the response rate to hormonal treatment in breast cancer is associated with the presence of oestrogen and progesterone receptors, assessment of the receptor expression profile allows for prediction of breast cancer response to hormonal treatment. The aim of this study was to assess whether the expression of receptors for oestrogen (ER) and progesterone (PR) in the tumour tissue of patients with invasive breast cancer correlated with tumour histological type, histological grade of malignancy, tumour size, and lymph node status.

A similar relationship was described for the progesterone receptor [8, 9], but not all authors confirm this relationship [10]. Expression of oestrogen receptors is also associated with age and menopausal status. Oestrogen receptor is more frequently detected in breast cancers in postmenopausal women than in premenopausal women [11, 12] and more frequently in older women than younger ones [13]. Expression of ER and PR is not constant and changes with disease progression [14]. Typically, the number of cells expressing ER and/or PR progressively decreases with disease progression [15]; an example of this is the inverse relationship between expression of ER and the size of the primary tumour. Many authors agree that the prognosis is better in the case of patients whose tumours exhibit ER and/or PR expression than in patients whose cancers do not show such expression [16]. But opinions on the value of oestrogen and progesterone receptors as prognostic factors are divided. In light of the above information, it seemed like an interesting topic.

The aim of the study: Expression of oestrogen and progesterone receptors is a very powerful and useful predictor. Because the response rate to hormonal treatment in breast cancer is associated with the presence of oestrogen and progesterone receptors, assessment of the receptor expression profile allows for prediction of breast cancer response to hormonal treatment. The aim of this study was to assess whether the expression of receptors for oestrogen (ER) and progesterone (PR) in the tumour tissue of patients with invasive breast cancer correlated with tumour histological type, histological grade of malignancy, tumour size, and lymph node status.
Material and methods

The materials consisted of histological preparations derived from patients treated for invasive breast cancer. Histological and immunohistochemical studies were performed at the Department of Pathology of the Military Medical Institute in Warsaw. Samples of tumours were fixed in 10% buffered formalin phosphate. Paraffin blocks were cut into sections with a thickness of 4 μm. The resulting sections were stained with different methods for diagnostic purposes. Preparations stained with haematoxylin and eosin were used to identify tumour type (WHO classification) and histological grade of malignancy.

Immunohistochemistry was performed using the EnVision TM + complex HRP DakoCytomation (DAKO) (EnVision™ Dual Link System-HRP DAB+, Code: K4065). In order to determine the expression of steroid receptors, monoclonal antibodies against receptors for oestrogen (Monoclonal Mouse Anti-Human Oestrogen Receptor alpha, 1 : 50 dilution, Clone: 1D5, Code: IR654, DAKO) and progesterone (Monoclonal Mouse Anti-Human Progesterone Receptor, 1 : 400 dilution, Clone: PgR636, Code: IR608, DAKO) were used. The study was conducted as follows: sections were incubated in an incubator at 60°C overnight and then dewaxed. The next step was to reveal the epitope by heating slides in a buffer for 40 minutes. Subsequently, preparations were left at room temperature for 20 minutes. Preparations were rinsed in buffer, and then endogenous peroxidase was blocked in 3% H2O2. Subsequently, preparations were left at room temperature for 20 minutes. Preparations were rinsed in buffer, and then endogenous peroxidase was blocked in 3% H2O2. Subsequently, preparations were left at room temperature for 20 minutes. Preparations were rinsed in buffer, and then endogenous peroxidase was blocked in 3% H2O2.

In immunohistochemical studies, expression of ER was observed in the nuclei of cancer cells. Expression of these receptors was demonstrated in 78% of patients in the age group younger than 50 years, and in 22% of patients in this age group there was no expression of ER. In the same age group, in 80% of patients, expression of PR was found, and 20% of patients showed no expression of PR. In women over 50 years old (postmenopausal) ER was detected in 73% of patients, while PR expression was observed in 64%, but there was no expression of PR in 36% of cases. Taking into account the histological type of tumour, a positive reaction for oestrogen receptor was observed in 74.2% of IDC and 77.8% of ILC, and the positive response to PR was observed in 67.1% of IDC and 61.1% of ILC (Table 1, Fig. 1).

Table 1. Clinicopathological characteristics of studied groups

| Type of tumour | Tumour grade | Tumour size | Lymph-node |
|----------------|--------------|-------------|------------|
| IDC (%)        | ILC (%)      | p ≤ 0.05    | p ≤ 0.05   |
tions were found between oestrogen receptors and second- (G2) \((p = 0.023)\) and third- (G3) \((p = 0.0027)\) grade cancers. In the case of progesterone receptor, statistically significant differences were found in G2 \((p < 0.001)\) and G3 carcinomas \((p = 0.002)\) (Table 3).

Analysis of the pre-operative staging of cancers studied and expression of steroid receptors showed that the largest group expressing ER were cancers in T2 stage \((46\%)\). PR-positive response was most frequently found in cancers in stages T2 \((44\%)\) and T1c \((40\%)\). In the case of invasive cancers assessed as T1b, positive response to the ER was seen in 9\% of tumours, and PR expression was found in 10\% (Table 3). Statistically significant differences were found for both steroid receptors in the case of tumours in T4 stage \((for \text{ER}, \ p = 0.0191 \text{ for PR}, \ p = 0.01228)\) (Table 3).

We also assessed the lymph node status and its relationship to the expression of steroid receptors. In patients without metastasis to regional lymph nodes \((pN0)\), 63\% of invasive carcinomas showed positive reaction for ER, and 62\% showed PR-positive reactions. In patients with metastases to deep inguinal lymph nodes \((pN3)\) a positive response to ER was seen in 5\% of cancers, as was in the case for PR (Table 3).

Statistically significant differences were shown between ER and pN0 tumours \((p = 0.0443)\) (Table 3). The results were compared for the expression of oestrogen and progesterone receptors. Analysis of data shows that the highest percentage of patients with invasive breast cancer showed a positive response to both steroid receptors.

### Table 2. The percentage of tumours exhibiting positive and negative response to oestrogen and progesterone receptors in tumour cells according to histological type of tumour

| Type of tumour | ER (%) | PR (%) |
|---------------|-------|--------|
| Expression of steroid receptors      | negative | positive | \(p \leq 0.05\) | negative | positive | \(p \leq 0.05\) |
| IDC           | 55    | 158    | 0.7367 | 70    | 143    | 0.6026 |
| ILC           | 4     | 14     |        | 7     | 11     |        |

### Table 3. The percentage of tumours positive or negative for the presence of oestrogen and progesterone receptors depending on the histological grade of tumour malignancy, the tumour size and the status of regional lymph-node involvement

| Tumour grade | Expression of steroid receptors | \(p \leq 0.05\) |
|--------------|---------------------------------|----------------|
| G1           | 3                               | 5              | 0.9427 |
| G2           | 53                              | 72             | 0.023  |
| G3           | 44                              | 23             | 0.0027 |

| Tumour size  | Expression of steroid receptors | \(p \leq 0.05\) |
|--------------|---------------------------------|----------------|
| T1           | 2                               | 1              | 0.9748 |
| T1a          | 2                               | 4              | 0.7806 |
| T1b          | 3                               | 9              | 0.2710 |
| T1c          | 37                              | 37             | 0.9807 |
| T2           | 41                              | 46             | 0.4457 |
| T3           | 5                               | 1              | 0.7072 |
| T4           | 10                              | 2              | 0.0191 |

| Lymph-node   | Expression of steroid receptors | \(p \leq 0.05\) |
|--------------|---------------------------------|----------------|
| Nx           | 5                               | 6              | 0.7291 |
| N0           | 49                              | 63             | 0.0443 |
| N1           | 22                              | 23             | 0.7845 |
| N2           | 12                              | 6              | 0.1932 |
| N3           | 12                              | 5              | 0.0588 |

Fig. 1. Showing positive nuclear staining of progesterone receptor in invasive ductal carcinoma (IDC) (original magnification 20×)
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(ER(+)/PR(+)) (63.6%). The smallest group consisted of patients who had a positive reaction to PR and negative reaction to ER (PR(+)/ER(–)) (3.5%). As many as 22.1% of women with invasive breast cancer had no reaction to steroid receptors.

Discussion

Oestrogen receptor expression is a recognised marker of breast cancer in women. In normal epithelium of female mammary gland ER is detected in 7–17% of cells. It is estimated that approximately 70–80% of breast tumours in women express ER. These tumours are characterised by slower growth, differentiation, and better prognosis with a suitable treatment regimen, which correlates with the length of survival after surgical removal [17].

In our study, in women younger than 50 years, the expression of oestrogen receptors was found in 78% of cases. In postmenopausal women positive nuclear reaction for ER was observed in 73% of patients. Women in whom positive reaction for ER occurs in more than 10% of tumour cells are classified as suitable for hormonal therapy, as in this group of people this kind of treatment is effective [18, 19]. If the reaction is observed in 1–10% of cells, it means a fragile sensitivity to hormonal treatment. In routine pathological diagnosis not only the percentage of tumour cells showing nuclear colour reaction is taken into consideration, but also the intensity of the reaction [20].

Research by Potemski et al. in 2007 shows that there is a correlation between the level of steroid receptor expression and survival in patients treated with hormonal therapy for breast cancer [21]. Research shows that the best prognosis is seen in patients with the highest expression of oestrogen and progesterone receptors, as well as patients in whom both receptors were present at the same time [21]. In our study, ER and PR expression occurred simultaneously in most patients (63.6%). Potemski et al. (2007) obtained similar results and also showed that the greater the level of receptor expression [21], the lesser the mortality. Such data may indicate the role of the progesterone receptor as a factor increasing the predictive value of ER.

Elledge et al. (2000) [22] observed the relationship between the percentage of cells showing positive nuclear staining with anti-ER and response to tamoxifen, and survival [22] in women with metastatic breast cancer.

Bardou et al. (2003) [23] found, in women undergoing hormonal adjuvant therapy, that patients with positive response to nuclear oestrogen and progesterone receptors had a lower risk of death compared with patients in whom there was no positive response to both receptors [23]. It was proven that pharmacological chemophrophylaxis with tamoxifen is effective in cases of tumours positive for nuclear oestrogen receptors, and it reduces the risk to 69% [24–26]. However, there are scientific reports showing that tamoxifen has undesirable effects, such as osteoporosis, enhanced blood clotting, and embolisms, as well as increased risk of endometrial and liver cancer [27]. In our study, statistically significant differences were found for the expression of oestrogen and progesterone receptors and tumour size. This relationship was demonstrated in the case of tumours in stage T4 (for ER, \( p = 0.0191 \) for PR, \( p = 0.01228 \)). From the results of our work we can conclude that the expression of oestrogen and progesterone receptors may be important in assessing the malignancy of cancers. There were significant associations between both steroid receptors and malignancy in G2 and G3 tumours (ER/G2 \( p = 0.023 \), ER/G3 \( p = 0.0027 \), PR/G2 \( p < 0.001 \), PR/G3 \( p = 0.002 \)) (Table 3). The role of oestrogens in the process of carcinogenesis is well known. The effects of oestrogens in this process are linked to their direct impact on the target cell and interaction with other exogenous factors: physical, chemical, and viral.

Oestrogens alter the metabolism of carcinogenic substances and impair the immune system [28, 29]. They stimulate cell proliferation and they induce receptor protein and DNA synthesis both in glandular and stromal organs, which stimulates the development and growth of tumours [30]. These hormones act on target cells by binding the steroid-receptor complex to DNA, altering the transcription of genes [31]. Expression of oestrogen receptors in breast cancers in women is an important prognostic factor and predictor. In the treatment of breast cancer, drugs are applied that inhibit the synthesis of oestrogens (aromatase inhibitors) [32], drugs that lower blood oestrogen levels (luteinising hormone-releasing hormone – LH-RH), and drugs that act on oestrogen receptors themselves [33]. Approximately 60% of patients with breast cancer exhibit the presence of both oestrogen and progesterone receptors. In approximately 20% of patients, the presence of only one of the receptors has been found, in the rest, receptors are not expressed. Expression of receptors is associated with age, and it is often found in older patients [34]. In the case of adjuvant therapy for breast cancer, patients with confirmed presence of oestrogen receptors obtain greater benefit from adjuvant tamoxifen treatment than patients who do not express any of these receptors [35].

Studies Skotnicki et al. (2012) demonstrated that invasive lobular carcinomas were characterised by a lack of E-cadherin expression, high rate of steroid receptor expression, low rate of PS3 and c-erb-B2 expressing tumours, low MIB-1 labelling index, and low S-phase fraction, as well as high rate of diploid lesions [36].

Zowczak-Drabarczyk et al. (2013) in their study evaluated the plasma total antioxidant capacity (TAS) in breast cancer patients in relation to ER\( \beta \) expression. Based on their studies, the authors concluded that the plasma TAS was significantly decreased in breast cancer patients in comparison to controls, independently of hormonal and lymph node status. The TAS level was not significantly different between breast cancer subgroups either in relation to the ER\( \beta \) expression or considering the steroid receptor status, even in the selected lymph node-negative subgroup. The authors observed a tendency towards higher TAS level in all ER\( \beta \)-negative breast cancer subgroups. A study conducted by Zowczak-Drabarczyk et al. (2013) confirmed enhanced consumption of plasma antioxidants in breast cancer patients. These studies can be considered as an attempt to determine ER\( \beta \) isoforms along with pa-
The authors declare no conflict of interest.

Study was carried out at the Department of Pathology, Military Medical Institute in Warsaw, Szaserów 128, 04-141 Warsaw, Poland.

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