Androgen receptors as a prognostic and predictive factor in breast cancer

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Abstract: Many theoretical and experimental models indicate that androgen receptors can play an important role as prognostic factors in breast cancer. The aim of this study was to evaluate the correlations between the presence of androgen receptors on cancer cells and other selected prognostic and predictive factors with established clinical significance in women with breast cancer after radical surgical treatment. 488 adult females were included in the study, who underwent primary radical surgery for breast cancer. 428 patients (87.7%) had Patey's conservative radical mastectomy and 60 (12.3%) Halsted's radical mastectomy. The mean age at operation was 54.3, ranging from 32 to 79. The mean length of hospitalization was 7.2 days for the patients after Patey's mastectomy and 9.8 days for those after Halsted's mastectomy. The androgen receptor is the most frequently detected steroid receptor on breast cancer cells. Therapeutic efficacy of adjuvant hormone therapy was higher in the group of androgen receptor-positive patients than in androgen receptor-negative ones. The prognosis for androgen receptor-positive patients who underwent adjuvant hormone therapy was better than for those androgen receptor-positive patients who did not receive hormone therapy after primary radical surgery for breast cancer. Assessment of androgen receptor levels on cancer cells should become a routine procedure with patients undergoing primary radical surgery for breast cancer, as it seems to be an important predictive factor.

Key words: Breast cancer - Androgen receptors - Prognostic factors - Predictive factors

Introduction

Prognostic factors in breast cancer were stratified into 3 categories by a multidisciplinary group of experts of the College of American Pathologists (CAP) in 1999 [1,2]. Category I included factors proven to be of prognostic value in numerous studies, as tumor size, axillary lymph node metastases, histological grade (G), histological type, and estrogen and progesterone receptor status. Category II comprised factors that had been studied, but whose importance remains to be validated: mitotic index, HER-2 overexpression, p53 mutation, expression of Ki-67, and peritumoral lymphatic or vascular channel invasion. Finally, category III were other factors, not sufficiently studied and demonstrated to be of prognostic factors only by some investigators: DNA ploidy, tumor angiogenesis, expression of EGFR, TGF-alpha, Bcl-2, pS2, and cathepsin D. The fact that steroid receptors were included in category I emphasizes their role in predicting the prognosis and choice of a particular algorithm of management in breast cancer patients.

Recently, there is a tendency in English literature on the subject to distinguish between prognostic factors, which are useful for determining the expected further course of the disease, and predictive factors, which determine the response to treatment (steroid receptors and HER-2 overexpression) [1,2].

Normal function of the mammary glandular tissue is regulated by various hormones, like steroid hormones (i.e., estrogens, prostaglandins, and androgens), peptide hormones (insulin, growth hormone, prolactin), and amino acid hormones (thyroid hormones). Estrogens and prostaglandins regulate the epithelial growth and morphogenesis of the ducts and alveoli of the mammary gland. Changing activity of these hor-
Mones leads to the physiological changes in the breast gland's density [3].

Apart from normal proliferation, morphogenesis and physiological activity of mammary gland cells, the steroid hormones also influence the development and progression of breast cancer. The hormones' action on the tissues starts after binding to specific receptor proteins: steroid receptors. The steroid hormone receptors: estrogen (ER), progesterone (PR), and androgen (AR) belong to the superfamily of nuclear receptors [4].

Nowadays, great hope is linked with finding out the role that androgen receptors play in breast cancer, as they belong to the same family of steroid hormone receptors, and thus show high structural, functional, and topographic similarity to the aforementioned estrogen and progesterone receptors.

The purpose of our study was to evaluate the prognostic role of androgen receptor status of breast cancer cells in patients who underwent primary radical surgical treatment. Special consideration was given to the following aspects:

1. determination of incidence of androgen receptor expression on breast cancer cells in the analyzed group of patients
2. evaluation of the response to different methods of treatment depending on the androgen receptor status of breast cancer cells
3. uni- and multivariate analysis of the prognostic and predictive value of androgen receptor expression on breast cancer cells considering different methods of adjuvant treatment (hormone therapy, chemotherapy, and radiotherapy), based on the course of the disease within 5 years after surgery.

**Materials and methods**

**Patients.** 488 adult females were included in the study, who underwent primary radical surgery for breast cancer at the 2nd Department of General and Oncological Surgery, Wroclaw Medical University, from 1996 to 2002. 428 patients (87.7%) had Patey's conservative radical mastectomy and 60 (12.3%) Halsted's radical mastectomy. The mean age at operation was 54.3, ranging from 32 to 79. The mean length of hospitalization was 7.2 days for the patients after Patey's mastectomy and 9.8 days for those after Halsted's mastectomy.

Hormone therapy was based on tamoxifen administered 20 mg daily p.o. for 5 years. Adjuvant chemotherapy and radiotherapy were given in accordance with the current standards recommended by the Polish Union of Oncology.

Postoperative hormone therapy was used in the largest number of patients, either as a single measure (in 216 patients, 44.3%) or combined with other methods: radiotherapy (40 patients, 8.2%), chemotherapy (24, 4.9%), or both chemotherapy and radiotherapy (48, 9.8%). Postoperative chemotherapy and radiotherapy was also a frequent combination (72 patients, 14.8%) (Table 1).

On the other hand, patients after radical mastectomy infrequently underwent chemotherapy (16 patients - 3.3%) or radiotherapy (4 patients - 0.8%) as a sole adjuvant treatment. In 68 patients (13.9%) surgery was the only therapeutic option used.

488 adult females underwent primary radical operations for breast cancer at the 2nd Department of General and Oncological Surgery in Wroclaw from 1993 to 2002.

**Immunohistochemistry.** The expression of the androgen receptor on cancer cells was detected immunohistochemically. 4 μm paraffin sections were used, mounted on silanized slides (DAKO, cat. No. S 3003). The specimens were then deparaffined with xylene and passed through a graded ethanol series of decreasing concentrations, down to pure water. The antigen of tissues fixed with formalin was retrieved with Target Retrieval Solution (DAKO, cat. No. S 1700) by warming in water bath at 96°C for 20 min. Endogenous peroxidase was blocked by 3% H2O2 for 10 min. Afterwards the slides were treated with primary antibodies: Monoclonal Mouse Anti Human Androgen Receptor Clone, AR441 (DAKO, cat. No. M3562), diluted 1/50, incubated for 30 min. at ambient temperature. Thus prepared slides were rinsed with TBS and then biotinylated antibody (LSAB+ kit, DAKO) was added for 15 min. After rinse with TBS, the slides were incubated with streptavidin-peroxidase complex (LSAB+ kit, DAKO, cat. No. K 0675) for 15 min. The immunocytochemical reaction was triggered by 3,3-diaminobenzidine tetrahydrochloride (DAB+ Liquid, DAKO, cat. No. K 3486). The slides were then rinsed in running water and dehydrated in graded ethanol series. Finally, the slides were treated with xylene and embedded in balsam.

To assess the value of the androgen receptor on cancer cells as an independent prognostic factor, a correlation was sought between its expression and selected well-documented prognostic factors (5-year survival rate after surgery, incidence of local recurrences, and symptom-free survival time).
The course of disease within 5 years of the operation was also included in the analysis, considering the survival time, local recurrences, symptom-free survival time, in relation to the adjuvant treatment used and the expression of the androgen receptor. This allowed determination of predictive value of the androgen receptor expression on cancer cells.

The information was obtained either personally during routine follow-up visits, or by phone or mail from the patients and their families.

Ethical issues. The permission to carry out this work was given by Polish Bioethical committee, Wroclaw Medical University, Nr. KB - 478/2006.

Statistical analysis. All the aforementioned data were organized with MS Excel. The relationship between different patient-related factors or the course of disease after surgery and the expression of androgen receptors on cancer cells was tested by Pearson's Chi-square test. Significance was set at the level of p<0.05.

Results

From 1993 to 2002, primary radical operations for breast cancer were performed in 488 female patients. They underwent either Halsted's mastectomy or Patey's mastectomy and these operations accounted for 3.7% of all surgical procedures made in that time at the 2nd Department of General and Oncological Surgery, Wroclaw Medical University.

The androgen receptor was determined to be expressed on cancer cells of the excised mammary glands in 212 patients, i.e., 43.4% of all the patients operated on for breast cancer (Group 1, androgen receptor positive, AR (+) (Fig. 1). In 276 patients (56.6%) the androgen receptor was not found to be expressed on cancer cells (Group 2, androgen receptor negative, AR (-).

The estrogen receptor was slightly less common, found in 192 patients (39.3%). The progesterone receptor was detected least frequently, in 120 patients (24.6%) (Table 2).

The prognostic value of androgen receptor expression was evaluated on the basis of the analysis of 5-year survival rate after surgery, incidence of local recurrences, and symptom-free survival time.

The mean rate of 5-year survival for the total number of mastectomized female patients was 59.8%. For the group of breast cancer patients with expressed androgen receptor this 5-year survival rate was higher, 71.7%, whereas for the androgen receptor-negative patients it was lower, 50.7%. However, the difference was not statistically significant (p=0.19).

The analysis also comprised the incidence of local recurrences and symptom-free survival time in the two different groups within 5 years of the operation. Local recurrences were found much more frequently in the androgen receptor-negative patients (144 patients, 52.2% of Group 2). In Group 1 local recurrences were present in 48 androgen receptor-positive patients, 22.6%. Among the patients who actually had recurrence of breast cancer, the rate of women with expressed androgen receptor was lower than in the whole study group of patients after radical surgery for breast cancer (25%). The difference was statistically significant (p=0.023) (Table 3).

420 of 488 mastectomized patients qualified for adjuvant therapy (86.1%). 160 patients (32.8%) underwent adjuvant chemotherapy, 164 (33.6%) postoperative radiotherapy, and 328 (67.2%) hormone therapy (Table 4).

Also the symptom-free survival time was statistically significantly shorter for Group 2 (3.02 years) compared to Group 1, androgen receptor-positive (4.08 years) (p=0.0001).
Most of the patients who underwent mastectomy (420, 86.1%) had also adjuvant therapy: chemotherapy, radiotherapy, and/or hormone therapy.

Different modes of adjuvant therapy were used in a similar number of patients from both groups.

The relation between androgen receptor expression on breast cancer cells and the patients' positive response to adjuvant treatment was evaluated by the analysis of 5-year survival rate after surgery, incidence of local recurrences, and symptom-free survival time.

The influence of androgen receptor expression on the beneficial therapeutic effect of hormone therapy was found to be statistically significant. For the androgen receptor-positive patients who were given adjuvant hormone therapy the 5-year survival rate was higher, symptom-free survival time longer, and the incidence of local recurrences lower as compared with both the androgen receptor-positive patients who did not receive hormone therapy, and with those androgen receptor-negative patients who were given hormone therapy postoperatively. All the above differences were statistically significant (p<0.05) (Table 5). On the other hand, the 5-year survival rate was lower, symptom-free survival time shorter, and the incidence of local recurrences higher among those women who were administered adjuvant hormone therapy and/or radiotherapy after radical surgery compared with the patients who had none of the above adjuvant therapy, both AR (+) and AR (-) (p<0.05). The androgen receptor-positive patients developed better response to chemotherapy and radiotherapy than the androgen receptor-negative patients, though the differences were not significant statistically (p>0.05) (Tables 6 and 7).

### Table 4. Methods of adjuvant treatment used in female patients with no undergone radical mastectomy in the years 1993-2002.

<table>
<thead>
<tr>
<th>Group 1 AR (+) patients</th>
<th>Hormone therapy</th>
<th>Chemotherapy</th>
<th>Radiotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>148 (69.8%)</td>
<td>72 (34%)</td>
<td>72 (34%)</td>
</tr>
<tr>
<td>No</td>
<td>64 (30.2%)</td>
<td>88 (31.9%)</td>
<td>92 (33.3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2 AR (-) patients</th>
<th>Hormone therapy</th>
<th>Chemotherapy</th>
<th>Radiotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>180 (65.2%)</td>
<td>88 (31.9%)</td>
<td>92 (33.3%)</td>
</tr>
<tr>
<td>No</td>
<td>96 (34.8%)</td>
<td>160</td>
<td>164</td>
</tr>
</tbody>
</table>

### Table 5. Therapeutic efficacy of hormone therapy in female patients who underwent radical mastectomy in the years 1993-2002.

<table>
<thead>
<tr>
<th>Group 1 AR (+) patients</th>
<th>Hormone therapy</th>
<th>No. of patients</th>
<th>5-year survival rate</th>
<th>Local recurrences (% of the group)</th>
<th>Symptom-free survival time (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>148</td>
<td>78.4%</td>
<td>28 (18.9%)</td>
<td>4.31</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>64</td>
<td>56.3%</td>
<td>20 (31.3%)</td>
<td>3.44</td>
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</table>

### Table 6. Therapeutic efficacy of chemotherapy in female patients who underwent radical mastectomy in the years 1993-2002.

<table>
<thead>
<tr>
<th>Group 1 AR (+) patients</th>
<th>Chemotherapy</th>
<th>No. of patients</th>
<th>5-year survival rate</th>
<th>Local recurrences (% of the group)</th>
<th>Symptom-free survival time (years)</th>
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<tr>
<td>Yes</td>
<td>Yes</td>
<td>72</td>
<td>44.4%</td>
<td>36 (50%)</td>
<td>3.25</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>140</td>
<td>85.7%</td>
<td>12 (8.6%)</td>
<td>4.51</td>
</tr>
</tbody>
</table>

### Table 7. Therapeutic efficacy of radiotherapy in female patients who underwent radical mastectomy in the years 1993-2002.

<table>
<thead>
<tr>
<th>Group 1 AR (+) patients</th>
<th>Radiotherapy</th>
<th>No. of patients</th>
<th>5-year survival rate</th>
<th>Local recurrences (% of the group)</th>
<th>Symptom-free survival time (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>72</td>
<td>44.4%</td>
<td>32 (44.4%)</td>
<td>3.19</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>140</td>
<td>85.7%</td>
<td>16 (11.4%)</td>
<td>4.54</td>
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</table>

<table>
<thead>
<tr>
<th>Group 2 AR (-) patients</th>
<th>Hormone therapy</th>
<th>No. of patients</th>
<th>5-year survival rate</th>
<th>Local recurrences (% of the group)</th>
<th>Symptom-free survival time (years)</th>
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</thead>
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<tr>
<td>Yes</td>
<td>Yes</td>
<td>92</td>
<td>52.2%</td>
<td>72 (39.1%)</td>
<td>2.56</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>184</td>
<td>52.2%</td>
<td>72 (39.1%)</td>
<td>3.32</td>
</tr>
</tbody>
</table>

Tables 5-7: AR (+) - androgen receptor positive, AR (-) - androgen receptor negative
Discussion

The results of treatment of breast cancer are not satisfactory yet. Every year 4000 women die in Poland for this malignancy, which gives 40% mortality. Many randomized clinical trials, retrospective analyses and metaanalyses indicate that the greatest chance for cure is associated with adequate adjuvant treatment: chemotherapy, hormone therapy, and radiotherapy.

Prognostic factors, factors responsible for positive reaction to treatment, and various grading systems of this malignant disease facilitate making therapeutic decisions optimal for the patient. The value of some of these factors has already been well proven (e.g. G feature, status of axillary lymph node metastasis, estrogen and progesterone receptor, BRCA gene mutation), and the usefulness of others is being verified (HER2 overexpressionmutations of genes involved in regulation of the cell cycle, angioinvasion and neuroinvasion, or micrometastasis).

Understanding the role of estrogen and progesterone receptor in pathogenesis of breast cancer made it possible to use this knowledge in practice in the 50's of the past century for determining adjuvant treatment of women with this disease.

Recently, prognostic value in breast cancer has also been attributed to the androgen receptor (AR). Studies on induced breast cancer cells in rats have demonstrated that AR is the steroid receptor most commonly expressed on the cells of primary and metastatic breast tumors [5]. According to different investigators, the incidence of AR expression in breast cancer patients is from 35% [6] to 90% [5].

Such common expression of AR on breast cancer cells brings about the question of the role of androgens in the pathogenesis of human breast cancer. The role of AR in breast cancer was already noticed 30 years ago. Persijn, in a short communication, observed that expression of AR in 51 patients was a determinant of better response to treatment with ethinyl estradiol [7]. Experiments on animals prove that androgens may induce tumor growth. Also proliferation of the tumor in human mammary gland seems to be modified by androgens. Androgens may have stimulating or inhibitory effect on tumor growth. These apparently paradoxal contradictory effects of steroid hormones are probably due to the mediation of ER and/or PR. Androgens show two main effects on cancer cells of the mammary gland. When estrogens are lacking, they stimulate tumor growth by direct binding to ER alpha; this effect is blocked by antiestrogens. When estrogens are present, however, androgens act as their antagonists and inhibit the growth of the tumor; this is probably mediated by AR and can be blocked by antiandrogens [8]. The clinical consequence of such influence of androgens on cell receptors is poor prognosis of those female patients receiving hormone therapy, whose cancer cells do not show AR expression. Many authors emphasize that expression of AR in breast tumors is a favorable prognostic factor, while the lack of AR correlates with a higher grade of malignancy [9-12].

AR (-) patients show worse response to hormone therapy that AR (+) women. Teulings has observed that regression of tumor in 34 patients who received megestrol acetate was associated with high concentrations of AR [13]. Therefore testing AR may be important for the choice of therapy, especially for patients with high proliferative activity of the tumor. In 1984 Bryan observed in a group of 1371 breast cancer patients that AR (+) had better response to treatment and higher 5-year survival than AR (-) ones [14]. On the other hand, Allegra stated that AR expression in patients with advanced tumors was not associated with better response to hormone therapy [15]. Thus the expression of AR and ER on cancer cells may have additional therapeutic consequences, particularly for tumors showing high proliferative activity and AR expression as the sole steroid receptor. The absence of AR correlates with higher malignancy and poorer response to hormone therapy in AR (-) women.

Research made in numerous centers all over the world have clearly proven that estrogen and progesterone receptors cannot be considered as independent prognostic factors of survival, but that their expression determines positive response to hormone therapy, thus enhancing the probability of complete cure of the operated woman. Having this in mind, demonstrating an association between androgen receptor and the expression of the other types of steroid receptors could make indications for hormone therapy stronger or weaker, especially in correlation with the analysis of response to adjuvant treatment within particular groups of patients.

It has been demonstrated that the expression of androgen receptor on cancer cells is often accompanied by the expression of estrogen receptor, and even more frequently progesterone receptor [16-19]. Therefore many authors maintain that knowing the status of all the three types of steroid receptors will facilitate predicting more accurately the response of patients to hormone therapy [16-21].

The role of androgens and their receptor in the progression of breast cancer remains unexplained [22-25]. Many studies showed that elevated level of androgens is directly proportional to the risk of developing breast cancer in postmenopausal women [26-30]. Clinical trials have demonstrated that taking antiandrogens and blocking the androgen receptor correlates with the increased incidence of breast cancer [31-33]. In our study, lower 5-year survival rate was demonstrated in women with breast cancer without androgen receptor expression (50.7% patients of Group 2). The prognosis
for patients with positive androgen receptor expression was better: 5-year survival rate of 71.7%. The above difference in survival was not statistically significant, however. Therefore androgen receptor expression cannot be considered as an independent prognostic factor for breast cancer.

The analysis of incidence of local recurrences and symptom-free survival time in separate study groups has shown that the number of local recurrences was lower in Group 1 (22.6% in Group 1 vs. 52.2% in Group 2), and symptom-free survival time in this group was longer (4.08 years in Group 1 vs. 3.06 in Group 2).

Our results are consistent with those presented by Shippinger, who found the mean survival time after recurrence to be significantly longer in AR (+) patients (21.89 months vs. 11.99 months) [34]. Correno did not observe any statistically significant association between AR expression and local recurrences [35], which, however, has not been confirmed in our study.

In most our patients who underwent surgery adjuvant treatment was given: chemotherapy, radiotherapy, and/or hormone therapy. The distribution of corresponding methods of adjuvant treatment among steroid receptor-positive and negative patients was similar in both groups. It was thus the method of adjuvant treatment did not affect the differences in long-term results in the two groups, AR (+) and AR (-). The 5-year survival rate was proven to be higher, local recurrence rate lower, and symptom-free survival time longer for the androgen receptor-positive patients who received adjuvant hormone therapy compared with the androgen receptor-negative patients with the same adjuvant therapy. The androgen receptor-positive patients who received adjuvant hormone therapy had also more favorable prognosis than those androgen receptor-positive patients in whom hormone therapy was not used. Our results are in agreement with those presented by Bryan, who demonstrated that the response to treatment of androgen receptor-negative patients is worse than that of androgen receptor-positive patients. AR (-) patients had shorter survival time than the AR (+) patients [36].

The relatively more aggressive course of the malignant disease and in consequence worse prognosis noted in patients who received radiotherapy or chemotherapy in comparison to those who did not receive these methods of adjuvant treatment was not related to the expression of androgen receptor. These results were most probably the consequence of the fact, that according to the recommendations of Polish Union of Oncology patients with more advanced cancer were qualified for adjuvant chemotherapy and radiotherapy.

Also the inhibitory effect of androgens on breast cancer cell cultures with expressed androgen receptor has been proven [37]. Zhou investigated the influence of estrogens, either alone or in combination with progesterone and testosterone, administered for 3 days. Estrogens alone caused a 6-fold increase of proliferation of mammary gland epithelium and increase of estrogen receptor expression by about 50%. Estrogens combined with testosterone led to a decrease of epithelial proliferation by 40% and the expression of estrogen receptor [38]. These observations prove that androgens can inhibit the estrogen-induced proliferation of mammary gland epithelium, and their addition to substitution hormone therapy may reduce the risk of breast cancer.

Hardin demonstrated anti-cancer efficacy of dehydroepiandrosterone sulfate (DHEAS) in the treatment of tumors that lacked estrogen and progesterone receptors and had androgen receptor expressed. In his opinion DHEAS may be an effective therapeutic option for the patients with positive androgen receptors not receiving standard hormone therapy. This was supported by experiments in vitro, in which introduction of the gene for androgen receptor to cells primarily deprived of all the three types of steroid receptors led to their destruction by DHEAS and aromatase inhibitors [43].

The clinical role of androgen receptor (AR) in breast cancer is much less documented than that of estrogen and progesterone receptors.

Though the expression of androgen receptor in male breast cancer has been investigated by some authors, the articles on this subject considering female breast cancer are very scarce. There are several theoretical arguments supporting such considerations. First, androgen receptor belongs to the same nuclear receptor superfamily as estrogen and progesterone receptor. Therefore their mechanisms of action are similar. Nowadays it is also known that apart from the effector mechanism of their stimulation, which consists in direct binding with DNA and regulating the processes of transcription and translation, their action can also be mediated by a cascade of second transmitters like membrane receptors. This opens a possibility of mutual modulation of the transmission path in the cell, thanks to the activation of different signal transmission paths inside the cell. Moreover, steroid hor-
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...mones, agonists of estrogen, progesterone, and androgen receptors, due to their similar chemical structure can react not only with the specific receptor but also cross-react with the other two types of steroid receptors. Obviously, in the latter instance the reaction will be much weaker than with the main receptor, but when the concentration of the receptors is high this other way of signal transmission may play an important role in pathogenesis, too. One should also have in mind that in normal physiology steroid hormones can be converted into each other, i.e., androgens may undergo peripheral conversion to estrogens in the fatty tissue.

Possible associations between the expression of androgen receptor in cancer cells and other prognostic factors and those determining the response to treatment need further analysis, which might elucidate the indications for choosing one of the available therapeutic options or facilitate prognosing the further course of disease. This might be particularly beneficial to the patients who are androgen receptor-positive and both estrogen and progesterone-negative. In about 10% of all female patients the androgen receptor is the only steroid receptor detected on breast cancer cells. According to the present recommendations, these patients are not qualified for hormone therapy. Therefore clearing the prognostic and therapeutic role of androgen receptor in women with breast cancer may turn out to be another milestone in our fight with this disease.

References


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