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Review article

Differentiated expression of estrogen receptors (ER) and progesterone receptors (PgR) in ductal breast cancers

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Abstract: Contents of estrogen receptors (ER) and progesterone receptors (PgR) in cells of breast cancers represent strong predictive factors. The higher is the contents of ER and PgR in breast cancer, the higher is a probability of obtaining a response to hormonal therapy and prognosis for the patient is better. In a routine manner, all tumours of mammary gland are subjected to evaluation of ER and PgR expression using immunohistochemistry. Forty ductal breast cancers (pT_2N_0) were subjected to an immunohistochemical evaluation (IHC) aimed at detection of ER and PgR expression. From every tumour three samples were taken for immunohistochemical studies: the lateral one from the side of axilla (ER-1; PgR-1); the median one (ER-2; PgR-2) and the medial one from the side of sternum (ER-3; PgR-3). The levels of both ER and PgR expression proved to be highly differentiated between the medial zone of the tumour and its periphery. The distinct expression of ER and PgR in ductal breast cancers, dependent on evaluated zone of the tumour, confirms its heterogenous character and exerts an effect on the type of applied treatment.

Key words: breast cancer, estrogen receptor, progesterone receptors

Introduction

Cancer of mammary gland represents the most frequently developing female malignant tumour in Europe and North America. In 2006 almost 300,000 new cases of the tumour were noted in the United States and it resulted in around 41,000 deaths [1].

In Poland, more than 13,000 new cases of the tumour were detected in 2005 and over 5,000 women died of the cancer [2]. In turn, in Lower Silesia the Lower Silesia Tumour Register in 2005 documented 1,117 new cases, which accounted for 21.9 % of all new cases of malignant tumours noted in this region of Poland [3]. As evident from the above data, the problem of breast cancer is extremely significant for health of the population and for financial resources devoted to combat the disease. This seems even more important when the still growing trend in the incidence and the associated mortality are taken into account [1-3].

The related risk factors, which affect incidence of breast cancer, are known to include duration of exposure to sex hormones and to estrogens in particular [4]. The elevated risk of the tumour is observed in women with early menarche (before 12th year of age) and in women with late menopause (after 55th year of age) [4,5]. Also tamoxifen, the non-steroid drug which blocks estrogen receptors, applied in women with increased risk of developing breast cancer was found to decrease probability of developing the disease by, approximately 50% [6].

Considering the above, the routine evaluation of estrogen receptors (ER) and progesterone receptors (PgR) in all diagnosed cases of breast cancer seems natural.

Ovarian granulosa cells represent the main source of natural estrogens (17-β-estradiol, estron, estriol) in the body. Aside from various functions, the hormones stimulate development of mammary gland, both by activation of growth in ductal epithelium and glandular alveoli and by development of adipose tissue and of

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connective tissue sublayer [5,6]. Estrogens and progesterone exert a mitogenic effect on the cells which, after a protracted period, may promote development of tumours in mammary gland. Estrogens act by activation of specific receptors (ER), localized mainly in cell nucleus, which play role of transcription factors [6-8]. In supplementary treatment of breast cancer also the earlier mentioned tamoxifen is employed. It represents a modifier of ER receptors, which inhibits, *i.a.*, mitotic activity of estrogens in cells of breast cancer [6,9]. Two types of ER are distinguished, including ER α and ER β, which represent two distinct proteins, coded by two separate genes [6,7]. Similarly PgR is manifested in two isoforms, PgR-A and PgR-B. The two latter proteins are coded by the same genes [10-14]. Expression of PgR depends on presence and normal function of ER, since estrogen binding to its receptor induces synthesis of PgR [10]. Presence of PgR is accepted to provide index of undisturbed function of ER and evaluation of PgR expression provides an indirect index of estrogen response mechanisms [15]. The dependence of PgR synthesis on normal function of ER is confirmed by the fact that around 50 % of breast cancers manifest expression of both ER and PgR while expression of PgR alone can be noted in only 1-12% breast cancers [16].

The variable which affect most the results of tamoxifen treatment involves levels of ER and PgR expression in cells of breast cancer. According to EORTC criteria, receptor positive cancers include the tumours in which, using immunocytochemical technique, ER expression is manifested by at least 10% cells [16]. Expression of ER and PgR is noted in, approximately 60 % patients with breast cancer while a single receptor only is expressed in around 20% patients [6].

In treatment of breast cancer, tamoxifen can be used only after a precise determination of ER and PgR expression. In tumours of more than 20 mm in diameter, determination of ER expression in a single random biopsy may lead to erroneous evaluation. This reflects, *i.a.*, frequent heterogeneity of texture in breast tumours.

This study aimed at examination of correlation between ER and PgR immunohistochemical expression in ductal breast cancer of >20 mm in diameter, in which biopsies of the tumour were sampled from three distinct locations.

Materials and methods

Tissue samples. The material included ductal breast cancers (pT_2N_0) manifesting G2 grade of malignancy and diameter of over 20 mm, sampled from 40 women of 56 to 75 years in age, subjected to surgery in the post-menopausal period in 2002-2003 in Lower Silesia Centre of Oncology in Wroclaw (DCO).

Immunohistochemistry. Following surgical dissection of the lesion the entire tumour with the surrounding tissues was transferred for intra-operative examination in Department of Pathomorphology, DCO. In the course of the examination, three independent samples were taken for immunohistochemical tests from each tumour, including the lateral one from the side of the axilla (ER-1; PgR-1); the median one (ER-2; PgR-2) and the medial one, from the side of the sternum (ER-3; PgR-3). From every tumour a representative sample was also taken for the intraoperative examination. All the samples were fixed in 5% solution of buffered formaldehyde and, then, embedded in paraffin blocks. Formalin-fixed, paraffin-embedded tissue was cut (4 μm). The sections were mounted on Superfrost-Plus slides (Menzel Gläser, Germany), dewaxed with xylene and gradually rehydrated. Activity of endogenous peroxidase was blocked by 5 min exposure to 3% H_20_2. Detection of ER and PgR expression was preceded by 15 min exposure of the sections in a microwave oven to boiling Antigen Retrieval Solution (DakoCytomation, Denmark) at 250 W. For demonstration of ER and PgR expression in the paraffin sections, were used mouse monoclonal antibodies: clone 1D5 and clone PgR636 in the following concentrations: 1:50 and 1:100 (DakoCytomation, Denmark). The antibodies were diluted in the Antibody Diluent, Background Reducing (DakoCytomation, Denmark). The sections were incubated with an antibody for 1h at room temperature. Subsequently, incubations were preformed with biotinylated antibodies (15min, room temperature) with streptavidin-biotinylated peroxidise complex (15min, room temperature) (LSAB2, HRP, DakoCytomation, Denmark). DAB (DakoCytomation, Denmark) was used as a chromogen (7 min, room temperature). All the sections were counterstained with Meyer's hematoxylin. In every case, controls were included in which specific antibody was substituted by the Primary Negative Control (DakoCytomation, Denmark).

Light microscopy. Intensity of ER and PgR expression was studied using OLYMPUS BX-41 light microscope coupled to a vision pathway and computer-assisted image analysis system, AnalySIS 3.2 (Olympus; Japan). In each evaluated section (examined at the magnification of ×200) three hot spots were selected in which expression of ER and PgR was most pronounced. Subsequently, at the magnification of ×400, the computer-assisted image analysis in the regions permitted to automatically score cancer cell nuclei with positive reaction as related to all cell nuclei in the tumour. The result represented percentage of positive cancer cell nuclei as related to all tumour cell nuclei in evaluated regions.

Statistical analysis. The obtained results were subjected to statistical analysis using Pearson's correlation and taking advantage of STATISTICA 7.1 software. Values corresponding to the level of $p<0.05$ were accepted as statistically significant.

Results

In 21 out of 40 examined cases of breast cancer nuclear expression of both ER and PgR was detected in all isolated samples (Fig. 1). Distribution of ER and PgR expression intensity in individual regions of the tumour is presented in Table 1. Using the results of proportional expression of ER and PgR analysis of Pearson's correlation was conducted in individual regions of the tumours.

Comparing results related to ER-1 and ER-2 expression in the examined breast cancers their moderately positive correlation was disclosed ($r=0.43$; $p<0.05$) (Fig. 2A); ER-1 and ER-3 manifested a strongly positive correlation ($r=0.62$; $p<0.05$) (Fig. 2B) while ER-2 and ER-3 showed a weak positive correlation ($r=0.28$; $p<0.05$) (Fig. 2C).

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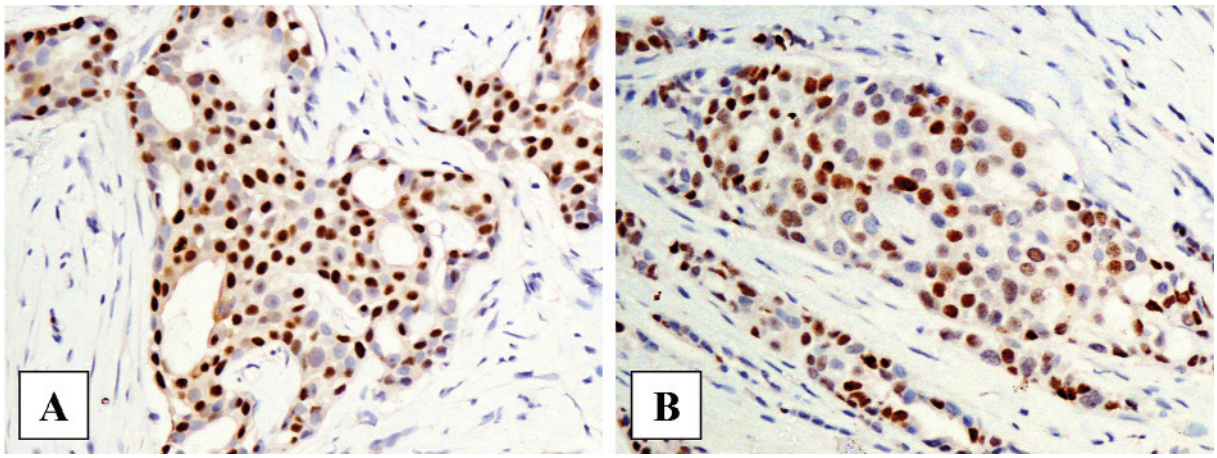

Fig. 1. Nuclear expression (brown nuclei) of estrogen receptors (ER)-(A) and progesterone receptors (PgR)-(B) in ductal breast cancers. Immunohistochemical technique, counterstained with hematoxylin (original magnification x200).

Table 1. Distribution of ER and PgR expression intensity in individual tumour zones.

Variable pattern of ER and PGR expression in all isolated samples	Breast cancer G2 – number of cases
Expression of ER and PgR in all samples	21
No ER and PGR expression in all samples	8
No ER-2 expression; expression of ER-1 and/or ER-3	3
No ER-1 and/or ER-3 expression; expression of ER-2	1
No PgR-2 expression; expression of PgR-1 and/or PgR-3	5
No PgR-1 and/or PgR-3 expression; expression of PgR-2	2

Studies on relationships between PgR expressions detected a weak positive correlation between expressions of PgR-1 and PgR-2 ($r= 0.25$; $p<0.05$) (Fig. 3A); a relatively strong positive correlation between PgR-1 and PgR-3 ($r=0.49$; $p<0.05$) (Fig. 3B) and a very weak positive correlation between PgR-2 and PgR-3 ($r=0.12$; $p<0.05$) (Fig. 3C). In addition we conducted an analysis of correlation between expression of ER with that of PgR in the same regions of studied tumours and we demonstrated a strong positive relationship between ER-1 and PgR-1 expressions ($r=0.64$; $p<0.05$) (Fig. 4A) and between ER-2 and PgR -2 expressions ($r=0.60$; $p<0.05$) (Fig. 4B) while in the case of ER-3 and PgR-3 expression we observed a moderately positive relationship ($r=0.39$; $p<0.05$) (Fig. 4C).

Discussion

Role of ER and PgR in biology of breast cancer has been widely documented in literature of the subject. Contents of the receptors in tumour cells represent a strong predictive factor, which plays a significant role in prognosis and in selection of therapeutic decision, including start of hormonal therapy [17].

Almost 75% of primary breast cancers demonstrate positive expression of ER and more than half of them co-express PgR [6]. The higher is ER and PgR content in breast cancer, the higher is probability of obtaining response to hormonal therapy [18-20].

Evaluation of ER and PgR contents in breast cancer cells used to take advantage of three principal techniques, including the ligand binding test, immunoenzymatic test (EIA) and immunohistochemical tests (IHC) [16].

Even if biochemical studies and immunohistochemical tests yield consistent data and the obtained results corroborate each other in 60% to almost 90% cases [16,21-23], at present IHC represents the recommended technique [24-26]. In comparison to EIA the technique is more sensitive and more specific [24]. Moreover, it allows for analysis of neoplastic cells only, excluding the stromal cells and the normal glandular epithelium [24]. IHC yields positive results both for ER and PgR, even if EIA test is negative, particularly in poorly differentiated tumours [25]. IHC represents also the only option in cases of tumours of small dimensions (of few mm in diameter) [24].

Results of our study unequivocally point to relatively extensive variability in the level of receptor expression between median zone of the tumours and tumour margins. The relationship pertain both, ER and PgR. Moreover, a pronounced significant positive correlation has been disclosed between expressions of the two types of receptors in a given tumour zone. The results may be explained by the variable levels of ER

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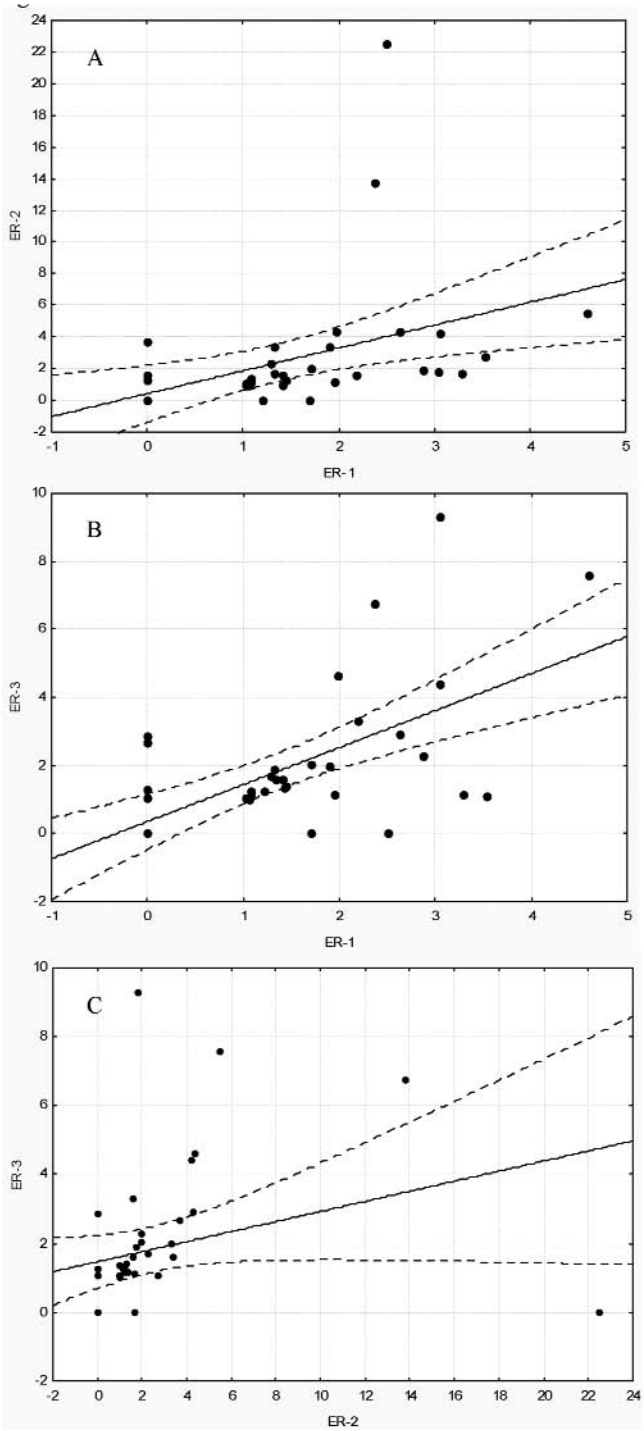

Fig. 2. Correlation ($p<0.05$) of estrogen receptors (ER) and progesterone receptors (PgR) expression as related to localization within the tumour: 1 – margin on axillary side; 2 – median zone; 3 – margin on sternal side. A: ER-1 vs ER-2; r=0.43. B: ER-1 vs ER-3; r=0.62. C: ER-2 vs ER-3; r=0.28.

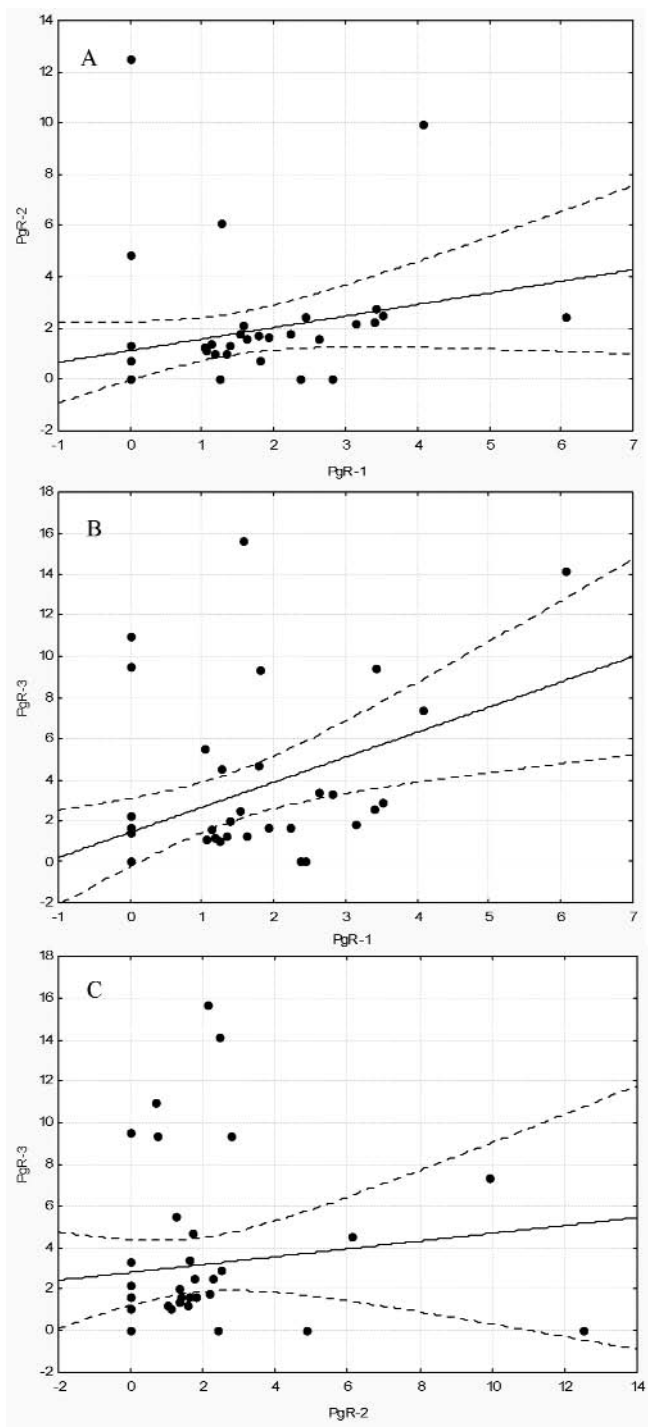

Fig. 3. Correlation ($p<0.05$) between expression of estrogen receptors (ER) and of progesterone receptors (PgR) expression as related to localization within the tumour: 1 – margin on axillary side; 2 – median zone; 3 – margin on sternal side. A: PgR-1 vs PgR-2; r=0.25. B: PgR-1 vs PgR-3; r=0.49. C: PgR-2 vs PgR-3; r=0.12.

and PgR expressions depending on proliferative activity of tumour cells, commented in the literature [27-29]. ER have been shown to manifest a relatively short half-life and, therefore, they are most abundant in mitotically active cells [27-29]. Comparing our results related to distribution of ER and PgR expression intensity with the data obtained by other authors it could be suggested that our results reflect an enhanced mitotic activity of breast cancer cells at margins of the tumour [27-29]. Median zone of a tumour used to manifest

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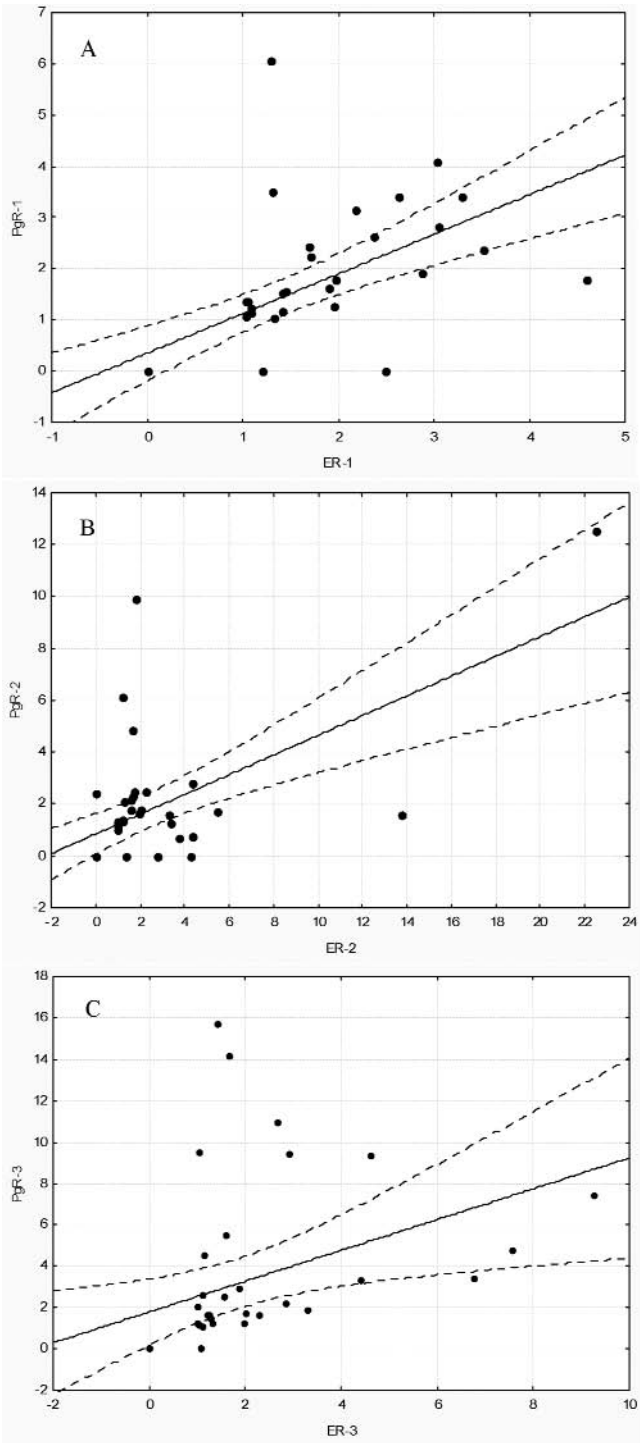

Fig. 4. Correlation ($p<0.05$) between expression of estrogen receptors (ER) and progesterone receptors (PgR) as related to localization within the tumour: 1 – margin on axillary side; 2 – median zone; 3 – margin on sternal side. A: ER-1 vs PgR-1; r=0.64. B: ER-2 vs PgR-2; r=0.60. C: ER-3 vs PgR-3; r=0.39.

lower proliferative activity and, therefore, lower expression of ER [27-29]. The relation may also be responsible for results of studies conducted by Jakesz *et al.* [27] who demonstrated higher expression of receptors in metastatic cells (in lymph node metastases) of a higher proliferative activity as compared to cells in the primary lesion.

Our observations have also been confirmed by the study performed by Davis *et al.* [30]. The authors analysed tumours of lower dimensions (diameter <20 mm), detecting differential levels of ER and PgR expression depending on localization of the studied material within the tumour mass. A markedly higher ER and PgR expression was noted at the periphery of small tumours as compared to their centre [30]. Thus, it seems that distribution of ER and PgR expression does not change with increase in tumour volume, remaining most pronounced at margins of the tumour. Considering clinico-therapeutic implications of ER and PgR expression it seems necessary to perform detailed IHC studies on margins of large tumours. In such situations, in contrast to tumours of small dimensions, a significantly higher risk exists for subjecting to analysis of exclusively their central zone, which in contrast to their peripheral zone may prove receptor-negative or receptor poorly positive. This in turn may result in an erroneous therapeutic decision: the patient my be disqualified from hormonal therapy. This in turn may negatively affect the prognosis.

Conclusion

In our studies we have demonstrated a significant variability in ER and PgR expression, related to evaluated zone of the tumour. This confirms the heterogenous character of histological texture of ductal breast cancers and it may carry diagnostic and therapeutic implications in the context of administration of hormonal therapy.

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