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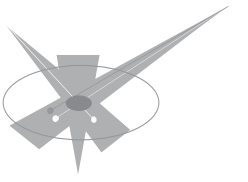

Original Paper

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Expression of selected markers in patients treated for breast cancer – based on our own data

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Background Breast cancer patients' long-term survival rate depends on many factors, such as the biological features of the tumour, stage of disease and mode of treatment. In most cases, in management of breast cancer combination therapy is used, according to indications. Qualification for adjuvant therapy is based on the assessment of several factors of established prognostic and predictive values. As a standard the patient's age, primary tumour size, axillary lymph node involvement, cancer histological type, its malignancy grade and steroid receptor expression are considered

Aim Analysis of status of selected immunohistochemical markers in a set of consecutive patients undergoing surgery for breast cancer.

Materials/Methods Samples from 623 patients were examined. Colour reaction was used for oestrogen (ER) and progesterone (PgR) receptors, P53 protein, cathepsin D and c-erbB-2. Overexpression of HER-2 protein was examined in 150 patients.

Results The presence of oestrogen receptors in cell nuclei was detected in 431 (69.2%) patients, of progesterone receptors in 504 (80.2%), and ER+, PgR+ phenotype in 382 (61%) patients. Cathepsin D expression was observed in 438 (70.3%) subjects. In 176 (27.3%) patients P53 protein accumulation was observed. Oncoprotein c-erbB-2 overexpression was observed in 99 (15.9%) and overexpression of HER-2 receptor in 29 (19.8%) patients.

Conclusions Nowadays oestrogen receptors are detected more frequently than in patients treated in the years 1980–1986. Detection rates of cathepsin D and P53 protein expression remain at comparable levels. The difference observed in detection rate of c-erbB-2 expression requires further analysis. Assessment of the correlation between expression of studied immunohistochemical markers and survival rate is necessary.

Key words **breast cancer • immunohistochemical markers**

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Background

Breast cancer patients' long-term survival rate depends on many factors, such as the biological features of the tumour, stage of disease and mode of treatment. In most cases, in management of breast cancer combination therapy is used, according to indications. Qualification for adjuvant therapy is based on the assessment of several factors of established prognostic and predictive values. As a standard the patient's age, primary tumour size, axillary lymph node involvement, cancer histological type, its malignancy grade and steroid receptor expression are considered [1]. According to the guidelines of the American Joint Committee on Cancer (AJCC), expression of HER-2 receptor was added to the group of prognostic factors in 2002 [2]. In spite of many years of studies on the usefulness of marker expression determination, the Nottingham Index parameters (based on tumour size – T, lymph node stage – T, and histological tumour grade – G) remain a reliable prognostic tool. Markers that are intensively studied among others include: c-myc, pS2, MDR; suppressor genes and their products (p53, Rb1, BRCA-1 i BRCA-2); receptors for growth factor: EGFR – epithelial growth factor, c-erbB-2, IGF-IR – insulin-like growth factor; markers of high metastasis risk, e.g. cathepsin D, plasminogen activator, nm-23; markers of tumour proliferative activity: MI – mitotic index, TLI – thymidine index, SPF – S-phase fraction, proliferation cell nuclear antigen PCNA antibody, Ki-67 antibody; DNA ploidy; tumour angiogenesis markers; heat shock proteins and others (CEA – carcinoembryonic antigen, vimentin, blood group antigens and lectins – PNA, HPL [3,4].

Detection of selected immunohistochemical markers combined with assessment of some pathoclinical features in patients treated with surgery for glandular breast cancer in the years 1980–1986 allowed us to determine the group of factors with a statistically significant influence on overall survival rate as well as to construct a prognostic index for a group of patients with no metastases to axillary lymph nodes. Routine assessment of the following markers was recommended: oestrogen receptor expression ER, P53 protein accumulation, cathepsin expression CD and c-erbB-2 protein expression [5].

Our later studies on invasive lobular cancer allowed us to formulate the prognostic index for this cancer type, in which – in spite of assessment of numerous immunohistochemical markers – the only independent prognostic factors were: size of the tumour, state of lymph nodes and expression of receptors for progesterone [6].

Aim

The aim of the present study was to analyse assessments of selected markers performed in a set of consecutive patients undergoing surgery for breast cancer in the years 1998–2003, and to compare their results with those obtained from studies from the 1980–1986 period. In the future (after the time necessary for 5-year survival assessment) an analysis of the relation between expression of selected clinical, morphological and immunohistochemical factors and overall survival rate will be performed. Thus an attempt to define a universal prognostic index, independent of histological cancer type, will be made.

Materials and Methods

Immunohistochemical assays were performed in the Immunohistochemical Laboratory of the Department of Tumour Pathology in the Department of Oncology, University of Medical Sciences, Poznań. Selected markers' expression (ER, PgR, P53 protein, CD, c-erbB-2 and HER-2) was assessed in paraffin wax embedded breast tissue from 623 patients undergoing surgery for breast cancer in the Oncological Surgery Department of the Oncology Department in the years 1998–2003 (group A). Receptors for oestrogens and progesterone, as well as P53 protein, were detected in the nuclei of cancer cells using the EnVision+™/HRP (Mouse) method, cathepsin D in cytoplasm using complex EnVision+™/HRP (Rabbit), and c-erbB-2 membrane staining was performed according to the ABC technique. Moreover, for the past two years HER-2 protein overexpression has been routinely assessed using the DAKO Hercept Test, with positive results in 150 patients. Assessments of control reactions and colour reactions have been carried out according to unified criteria accepted in the Immunohistochemical Laboratory of the Department of Tumour Pathology in the Oncology Department, and for the Hercept Test observing the manufacturer's guidelines. Examples of colour reactions are shown in Figures 1 and 2.

The results were compared with those obtained from a group of 617 patients treated in the years 1980–1986, from which immunohistochemical assays were performed in the years 1996–1997 (group B). ER and P53 expression was determined using the ABC method (Autoclave), CD

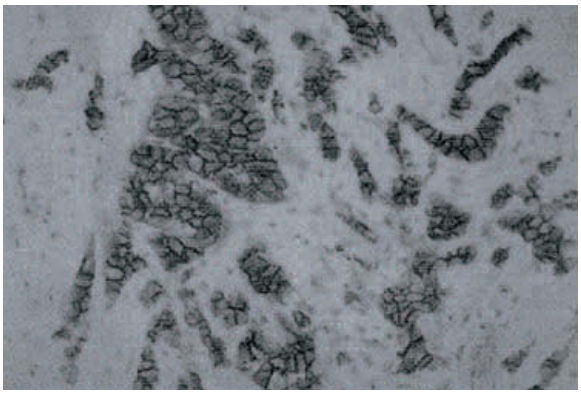

Figure 1. Reakcja barwna nadekspresji białka HER2.

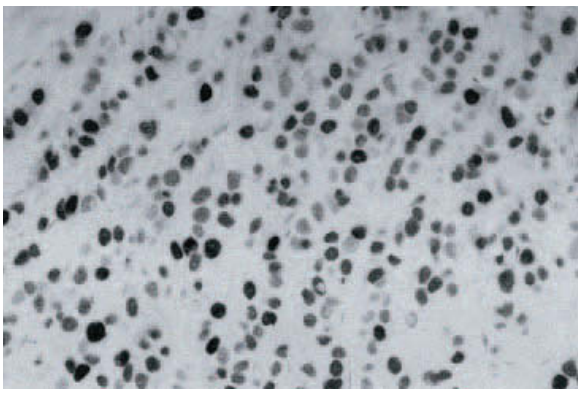

Figure 2. Reakcja barwna obecności receptorów progesteronu.

Table 1. Expression of selected immunohistochemical markers in a group of 623 patients treated surgically in the years 1998–2003 (group A).

Group A	n=623 (100%)		pN0 n=354 (56.8%)		pN1 n=269 (43.2%)	
ER+	431	(69.2%)	226	(52.4%)	205	(47.6%)
PgR+	504	(80.2%)	246	(48.8%)	258	(51.2%)
ER+ i PgR+	382	(61%)	189	(49.5%)	193	(50.5%)
katepsyna D+	438	(70.3%)	209	(47.7%)	229	(52.3%)
P53+	176	(27.3%)	93	(52.8%)	83	(47.2%)
c-erbB-2	99	(15.9%)	42	(42.4%)	57	(57.8%)

with modified PAP method, and c-erbB-2 with three-step ABC method.

Results

Presence of receptors for oestrogens ER and progesterone PgR, as well as P53 and cathepsin expression, was examined in all 623 patients, and HER-2 overexpression in 150 patients treated in the years 1998–2003. In patients presenting for diagnostic work-up only markers considered useful for prognosis based on our own results were tested, as well as receptors for progesterone and HER-2 – which have not been examined previously.

The presence of oestrogen receptors in cell nuclei was detected in 431 (69.2%) patients, of progesterone receptors in 504 (80.2%), and ER+, PgR+ phenotype in 382 (61%) patients. In 176 (27.3%) patients P53 protein accumulation was observed.

In our group overexpression of HER-2 receptor was detected in 29 (19.8%) patients, and among those a high degree of overexpression (+3) was determined in 19 (12.7%) and a moderate degree (+2) requiring confirmation with FISH test in 10 (6.7%).

Oncoprotein c-erbB-2 overexpression was observed in 99 (15.9%).

In 176 (27.3%) patients P53 protein accumulation was observed – a result similar to those found in the literature.

Cathepsin D expression in our group was observed in 438 (70.3%) subjects.

Results are presented in Table 1.

In Table 2 status of HER-2 expression in a group of 150 patients treated in the years 1998-2003 is shown.

In Table 3 results of tests for selected markers in paraffin wax embedded breast tissue from 617 breast cancer patients from the years 1980–1986 (group B) are presented.

Discussion

The usefulness of oestrogen and progesterone receptor expression evaluation is unquestionable and as such it remains a standard procedure in breast cancer management. Presence of these

Table 2. HER-2 expression in a group of 150 patients.

HER-2	n=150 (100%)		pN0 n=72 (48%)		pN1 n=78 (52%)	
HER-2 (+3)	19	(12.7%)	6	(4%)	13	(8.7%)
HER-2 (+2)	10	(6.7%)	4	(2.7%)	6	(4%)

Table 3. Expression of selected immunohistochemical markers in a group of 617 patients treated surgically in the years 1980–1986 (group B).

Group B	n=617 (100%)		pN0 n=302 (48.9%)		pN1 n=315 (51.1%)	
ER+	310	(50.2%)	158	(50.9%)	152	(49.1%)
cathepsin D +	413	(66.9%)	194	(47%)	219	(53%)
P53 +	132	(21.4%)	63	(47.7%)	69	(52.3%)
c-erbB-2 +	217	(35.2%)	87	(40.1%)	130	(59.9%)

receptors is a classical favourable prognostic and predictive factor. In approximately 60% of patients both receptor types are found, in 20% one type, and in the remaining 20% no receptors are present [7–10].

ER expression frequency of nearly 70% and PgR expression of 80% of our group A patients, as well as phenotype ER+ PgR+ in 61% are similar to the literature data [4,5,9,12]. For oestrogen receptors the results are significantly higher in group A than in B (69.2% vs 50.2%). One possible explanation of this observation is a difference in sample conservation technique in compared time periods. Another may be a possible loss of a certain part of archived material. Even higher ER+ (72.6%) and PgR+ (82.4%) frequency were observed in our studies on lobular cancer, which is a known feature of this histological type of tumour [6]. HER-2 overexpression in 19.8% also matches the results reported in the literature.

Overexpression of HER-2 protein (acting as a membrane receptor stimulating cellular growth) correlates with lymph node metastases, higher histological malignancy, higher mitotic activity, lack of oestrogen receptors (meaning worse response to hormonal therapy), early disease relapse and with overall shorter survival period. At the same time, detecting HER-2 expression enables the selection of patients who may benefit from monoclonal antibody therapy [9,10,12–14] (Trastuzumab), which block the extracellular part of the receptor. Remission rate in therapy with trastuzumab is estimated to reach nearly 40%. Qualification for adjuvant therapy of HER-2–2+ patients may be problematic, since in 75% of examined subjects fluorescent in situ hybridization does not detect gene amplification and in those patients no clinical benefits from trastuzumab therapy are observed [15].

For the samples from 1999–2003 there were no significant differences observed between the positive reaction for c-erbB-2 receptor overexpression rate using three-step ABC technique and Herceptest (15.9% vs 12.7% HER-2–3+). In archival material c-erbB-2 overexpression was present in nearly 35% of the patients. This discrepancy may result from different criteria of colour reaction evaluation, since in its final interpretation the 1+, 2+, 3+ groups were not differentiated. In fact, in no more than 16% of the c-erbB-2+ group were the reactions evaluated as 2+ and 3+. Another explanation may be heterogeneity of the compared groups. In recent years earlier stages of the cancer are more often diagnosed, and 56.8% of the subjects were metastasis-free (in this group cerbB-2 expression is significantly lower: 42% vs 58%). In comparison, in the group of previously examined patients approximately 48% had pN0 status.

The suppressor gene product P53 protein is responsible for stopping replication and for activating DNA repair in the case of DNA damage detection; it may also initiate apoptosis. P53 gene mutation is connected with later disease stages and thus with a shorter disease-free period and shorter overall survival time [1,4,9,15–19].

The higher number of P53+ patients in our lymph node metastasis-free subgroup is unclear. This

observation requires an analysis of the correlation with survival period and disease-free period. Especially interesting may be the evaluation of survival time of patients with accumulation of unfavourable prognostic factors: HER-2 receptor overexpression and P53 protein accumulation. According to Yamashita et al., a combination of excess of these two proteins may be a remarkably bad prognostic factor [16].

Excessive cathepsin D (CD) expression in breast cancer results from induction by oestrogens. CD overexpression correlates with shorter disease-free and survival periods [3,4].

In both our groups CD overexpression was observed in nearly 70% of patients, and in both groups less frequently in pN0 than in pN1 patients (47.7% vs. 53%). So far, observations indicate that in a group of patients without node metastases, cathepsin overexpression has an independent, significant negative influence on overall survival period [5]. No CD overexpression influence on survival rate in lobular cancer was observed [6]. Determination of the clinical significance of present results requires further studies. Correlating present results with survival rate of glandular breast cancer patients will enable an attempt to define a universal prognostic index using immunohistochemical markers, independent of histological cancer type.

Conclusions

1. Nowadays oestrogen receptors are detected more frequently than in patients treated in the years 1980–1986.

2. Detection rates of cathepsin D and P53 protein expression remain at comparable levels.

3. The difference observed in detection rate of c-erbB-2 expression requires further analysis.

4. Assessment of the correlation between the expression of studied immunohistochemical markers and survival rate is necessary.

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