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New Prognostic Factors in Breast Cancer

Nowe czynniki prognostyczne w raku piersi

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Abstract

For many years, the age of the patient, the condition of axillar lymph nodes, the size of the tumour, histological traits (in particular histological grade of malignancy and invasion of lymphatic vessels), condition of hormonal receptors and HER2 represented principal factors used for the stratification of breast cancer patients for the purposes of evaluating the prognosis and determining the appropriate strategy of treatment. Although the variables are useful for the prognostic evaluation of individual groups of breast cancer patients, their role in determining the individual risk level of the patient and in the selection of supplementary treatment is quite restricted. This article shows the prognostic value of additional parameters, whose expression is associated with chemoresistance (MRP2, BCRP, YB1) or individual assessment of the dynamics of tumor progression (S100P, BUBR1). In addition, it describes the role of an online database of “The Kaplan-Meier plotter” which contains the assessment of the effects of expression of various genes on the clinical outcome of patients with breast cancer (*Adv Clin Exp Med* 2013, 22, 1, 5–15).

Key words: breast cancer, prognostic factors, chemoresistance, KMplotter.

Streszczenie

Od wielu lat wiek pacjenta, stan pachowych węzłów chłonnych, wielkość guza, cechy histologiczne (zwłaszcza histologiczny stopień złośliwości i inwazja naczyń limfatycznych), stan receptorów hormonalnych oraz HER2 są głównymi czynnikami stosowanymi do stratyfikacji pacjentów z rakiem piersi w celu oceny rokowania i określenia odpowiedniego sposobu leczenia. Chociaż te dane są bardzo przydatne do oceny rokowniczej poszczególnych grup pacjentów chorych na raka piersi, to jednak ich rola w określaniu indywidualnego poziomu ryzyka progresji choroby oraz w doborze dedykowanego sposobu leczenia uzupełniającego jest ograniczona. W artykule przedstawiono wartość prognostyczną dodatkowych parametrów, których ekspresja jest wiązana z chemioopornością guza (MRP2, YB1) lub indywidualną oceną ryzyka progresji zmiany nowotworowej (S100P, BUBR1). Dodatkowo opisano rolę bazy danych „The Kaplan-Meier plotter”, zawierającej oceny wpływu ekspresji różnych genów na wyniki kliniczne chorych na raka piersi (*Adv Clin Exp Med* 2013, 22, 1, 5–15).

Słowa kluczowe: rak piersi, czynniki prognostyczne, chemiooporność, KMplotter.

The classical morphological factors considered in the selection of patients for systemic therapy include size and the extent of histological malignancy of the tumour (grade), Ki67 proliferative index and condition of axillar lymph nodes. Also, the evaluation of steroid receptor expression and of Human Epidermal Growth Factor Receptor 2 (HER2) are of principal importance [1–3]. The analysis of all the mentioned factors manifests a much higher clinical significance than a consideration of each of them in separation, and such a comprehensive analysis provides grounds for several schemes used in grouping the patients to individual risk categories,

such as Saint Gallen criteria, *National Institutes of Health (NIH)* consensus, prognostic index from Nottingham (NIP) or decisive base related to adjuvant treatment, the Adjuvant!Online (www.adjuvantonline.com) [1–4].

The evaluation of individual prognostic factors, even such as estrogen receptors (ER), progesteron receptors (PgR) or HER2, does not always provide an insight into the functional activity of the receptors: other supplementary signalling pathways may affect the function of neoplastic cells and the efficacy of the implemented therapy. The problem may be resolved by parallel evaluation of

several variables and, then, appraisal of the risk for progression and of possible advantages stemming from use of an appropriate manner of therapy. A progress in this branch was permitted by introduction of DNA microarrays. The examination involves a pangenomic evaluation of tumor cells. Until now, high costs of such studies and difficulties with the interpretation of such a pile of obtained data prevented broader application of DNA microarrays in practice. Studies of other authors evaluated expression of only selected genes, linked to cell cycle and proliferation, which resulted in the determination of molecular profiles for breast cancer [1–5]. Nevertheless, the selection of an appropriate panel of genes that would credibly fulfill expectations remains a challenge. The most frequently described two multigenic prognostic tests include Oncotype DX and MammaPrint. Until now, only the Oncotype DX has been recognized by most (84%) experts in the Evaluation Panel at the conference in St. Gallen, in 2011 as a test which in a reliable way may help in qualifying the patients for systemic treatment [2, 3, 5]. This multigenic test defines the probability of breast cancer relapse in patients with no metastases and positive status of estrogen receptor. The efficacy of the test has been confirmed in a group of patients treated hormonally with tamoxifen. In the Oncotype DX test a panel of 21 cancer cell genes is evaluated and a probability is estimated in the scale of 0 to 100 for manifestation of a relapse within 10 years from primary diagnosis (recurrence score). The usefulness of the test is illustrated by the fact that the evaluation is performed on a tissue sampled for a routine histopathological evaluation, fixed and embedded in paraffin. MammaPrint represents also a multigene test but it is aimed at the evaluation risk in developing metastases. It was designed in Holland and published for the first time in 2002. In 2007 the American Food and Drug Administration (FDA) approved the test for use in female patients below 61 years of age with breast cancer of up to 5 cm in diameter, with no metastases to lymph nodes. A significant obstacle in broad application of the test is its requirement that it requires fresh (unfixed) tissue. The material is sampled during surgery using an appropriately prepared kit and sent for evaluation to laboratories of the producer [1, 3, 5]. It should be stressed that experts of St. Gallen conference, American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) admit the potential for using the molecular profiles in defining the risk of relapse in patients with equivocal indications for adjuvant chemotherapy. Nevertheless, it is recommended that results of the tests should be interpreted with caution and that the traditional

clinical and pathomorphological indices are also taken into account [1, 3, 4].

Molecular Subtypes of Breast Cancer

An analysis of gene expression profiles in microarray platforms resulted in distinguishing a few molecular subtypes of breast cancer. The first study which initiated the development of the concept of defining molecular subtypes of breast cancer involved the study of Perou et al., employing DNA microarray technology, with analysis of expression involving 8102 genes, obtained from 65 breast tumors (originating from 42 patients) [6].

Considering the gene expression, five molecular subtypes of breast cancer were distinguished. However, it was most important that the evaluation of breast cancer subtypes carried not only a prognostic value but also provided an indication as to the group of systemic agents which in the group of patients resulted in the best clinical effect. In 2011, a group of experts from St. Gallen demonstrated that for a correct categorization of the patients it was sufficient at the time to determine parameters accessible by classical histopathological approach, enriched by additional immunohistochemical data, including definition of histological type of breast cancer, status of steroid receptors and of HER2 and Ki67 proliferative index (MiB1) [2–4].

Depending on the results of the mentioned morphological studies, all infiltrative breast cancers were divided into five principal groups, including: luminal cancers A, luminal cancers B (HER2-negative), luminal cancers B (HER2-positive), HER2-positive cancers (nonluminal ones) and triple negative cancers (nonluminal ones), cancers of basal type. In addition, two other special types of breast cancers were distinguished: Somatotrophic hormone (STH)-hormone-dependent cancers (cribriform, ductal, mucinous cancers) and STH-hormone-independent cancers (apocrine, medullary cancers, adenoid cystic carcinoma, metaplastic cancer).

Cancers of the luminal A type used to be highly differentiated and linked to a favorable prognosis. They manifested the expression of ER and/or PgR and a low proliferative activity (Ki67 index < 14%). In this group of cancers amplification of HER2 gene can be detected only in exceptional cases. As a rule, such patients are sufficiently managed using hormonal therapy. The application of chemotherapy is justified only in some patients with metastases more numerous than three lymph nodes [3, 7, 8].

The luminal type B cancers include also tumors with expression of steroid receptors but, in comparison with type A, tumors of type B manifest high proliferative index or co-expression of HER2 receptor. In cases of tumors manifesting no over-expression of HER2 and showing high proliferative index ($Ki67 \geq 14\%$) hormonal therapy and/or chemotherapy is used. On the other hand, in patients with luminal type B tumors with over-expression and/or amplification of HER2 it is recommended to apply combined hormonal treatment and treatment with trastuzumab. Patients with this type of cancer may also take advantage of chemotherapy [3, 7, 8].

Cancers of HER2 type comprise around 15% of all breast cancers and manifest over-expression or amplification of HER2 with absence of expression of steroid receptors (ER and PgR). The tumors used to show low differentiation, accompanied by metastases to lymph nodes. The prognosis is unfavorable. In patients with this type of cancer treatment with trastuzumab and anthracyclin-based chemotherapy is recommended [3, 7].

Cancers of basal type comprise 10–20% of all breast cancers. In this group reactions aimed to detect the presence of ER, PgR and HER2 are negative. Proliferative activity of basal cancer cells is high. Cancers of basal type used to metastasize with blood, mainly to the brain and lungs, less frequently to lymph nodes and bones. The group of basal type cancers is not homogenous, containing cancers with BRCA-1 (breast cancer gene 1) mutation, medullary carcinomas and adenoid cystic carcinomas, with a low risk of distant metastases. In contrast to luminal cancers, most basal type cancers are sensitive to chemotherapy: in 76% of cases traits of a complete clinical and pathomorphological response to chemotherapy is noted. Nevertheless, due to the high rate of relapses, the clinical course in patients with this type of cancer is unfavourable [3, 7].

However, categorization of a patient to a specific group provides only a very general indication. Reports of a variable sensitivity to cytostatic agents in patients who seemed to represent the same type of the tumor remain valid. The clear differences in efficacy of treatment in various patients with the same diagnosis prove that oncological treatment cannot be universal and it remains indispensable to identify additional markers which would allow a more individualized selection of an appropriate therapy.

New Markers

Intense development of molecular biology allowed the detection of several mechanisms, which

condition the development of neoplastic disease. Membrane receptors were identified, signaling pathways and transcription factors were detected, the pathological activity of which defines malignant phenotype of a neoplastic cell. In this way the information was gained on a number of proteins which could be used as potential targets of new therapies [7].

Most clinical studies conducted in oncology pay significant attention to the identification of biomarkers that would promote optimization, individualization and an increased efficacy of novel therapeutic strategies. Following the identification of pathologically activated signaling pathways, molecular mechanisms conditioning drug resistance and an analysis of general condition manifested by the patient, it will be possible to suggest to the patient an individualized therapy, manifesting high clinical efficacy with very restricted toxicity [7, 8].

Multidrug Resistance to Cytostatic Agents

Resistance to drugs poses a serious challenge to chemotherapy of tumors. Mechanisms in which the resistance develops are reflected first of all in the changes in transport of the drug between extracellular space and cell interior and between cell organelles (resistance dependent on transport), changes in activation of the drug (resistance dependent on activation), in the activity of targeted enzymes/proteins reflecting their augmented expression in tumor cells or a decreased affinity to the drug (resistance dependent on targeted substance), altered processes of DNA repair and an altered ability of neoplastic cells to inhibit apoptotic mechanisms [9, 10].

Neoplastic cells may acquire resistance to a single drug or to an entire group of cytotoxic agents. Nevertheless, the most frequently encountered mechanism of resistance involves active export of the drug from the cells, mediated by proteins acting as transmembrane transporters. The transport results in a decreased effective intracellular concentration of the drug, reflecting its reduced inflow to the neoplastic cells or an intensified elimination from the cell. The mechanism may also affect the transport of the drug from cytoplasm to cell nucleus or to other intracellular compartments/organelles [9]. The first identified transporter of the type, glycoprotein P (ABCB1) belongs to the extensive superfamily of transport ABC (ATP Binding Cassette transporters) proteins transporting endo- and exogenous substances through the cell membrane using energy resulting from ATP hydrolysis. In the human body around 50 genes were identified which code ABC proteins. They form

seven subfamilies, ascribed with the sequential alphabet letters of A to G. Most of ABC proteins represent energy-dependent transporters but the superfamily contains also transport proteins gated by binding and hydrolysis of ATP (Adenosine Triphosphate), e.g. CFTR/ABCC7 (Cystic Fibrosis Transmembrane conductance Regulator) and ATP-dependent controllers of potassium channel, e.g. receptors of sulphonylurea SUR1/ABCC8 (Sulphonylurea Receptor 1) and SUR2/ABCC9 [9]

The first to be identified and the most frequently described protein of the group involves P-glycoprotein (Pgp/ABCB1), composed of 1280 amino acids and coded by MDR1 (Multidrug Resistance Protein 1) gene, located on the long arm of chromosome 7 (7q21). Pgp is thought to participate in the transport of endogenous products out of a cell. The presence of Pgp on the surface of capillary endothelial cells in various organs may constitute one of the functional components not only of the blood-brain barrier but also of intestinal barrier or renal barrier. The role of Pgp in normal lymphocytes remains to be recognized. It is suggested that Pgp present in lymphocytes CD8+ (Cluster of Differentiation 8) and NK (*Natural killer*) cells may be important for their cytotoxic function. Relatively high amounts of Pgp are present in haemopoietic stem cells and its concentration decreases in the course of development and differentiation of the stem cells to mature terminal blood cells. Probably, Pgp affects proliferation and differentiation of haemopoietic stem cells through controlling factors of the processes [11]. Tumors in which the expression of Pgp is detected form two groups. The first group comprises tumours stemming from tissues which originally demonstrate expression of Pgp (among other tumours of liver, kidneys, pancreas, intestines and adrenal cortex), thought to be primarily resistant. The other group contains tumours originating from tissues originally manifesting low concentrations of Pgp and in the course of chemotherapy developing resistance, which persists following termination of chemotherapy. The group includes, among other, breast cancer, small-cell pulmonary carcinoma, acute and chronic myeloid leukaemia [11–15].

In studies conducted on breast cancer patients (metaanalysis) the expression of Pgp was demonstrated in 41% patients before the start of treatment and the proportion increased following treatment [16]. Other authors demonstrated the expression of Pgp in as many as 80% patients [13]. Burger et al. confirmed as significant the correlation between the expression level of Pgp and the response to treatment. The reaction to treatment was markedly lower in patients manifesting high

expression of Pgp (2/12; 17%) than in those with low expression of Pgp 32/47 (68%) [13, 17].

Even if Pgp protein represents one of the principal causes of drug resistance in tumours, also other transport proteins may be linked to this phenomenon. In the group of transport protein an important role is played by proteins of MRP group (Multidrug Resistance related Proteins). They constitute one of the largest groups in the ABC superfamily, in humans composed of 13 proteins [9, 18, 19].

MRP are broadly expressed in human body, in tissues responsible for elimination of toxic metabolites while protection of strategic compartments in human body is linked to their function.

Until now, MRP1 represents the best-recognized protein of ABCC family. Expression of MRP1 was detected in cells of solid tumors in lungs, breasts, prostate. Some authors regard the expression of MRP1 to represent an unfavourable prognostic index in locally advanced breast cancer, resulting in abbreviated relapse-free survival and total survival [9, 20]. It is also indicated that an augmented MRP1 expression is manifested in relapsing breast cancers following neoadjuvant treatment and in metastatic tumours although it has no predictive significance for application of a systemic treatment [9, 17, 20, 21].

MRP2

MRP2 (Multidrug Resistance Protein 2), termed also ABCC2, belongs to the group of transport proteins responsible for resistance to chemotherapy. In physiological conditions it represents a transporter of organic ions and undergoes expression in several tissue types in which it transports anionic substances. Activity of MRP2 was shown to be linked to the resistance to methotrexate, etoposide, vincristine, vinblastine, cisplatin, doxorubicin and epirubicin [12–14, 22, 23].

Present studies suggested that immunohistochemical examination represents an appropriate technique to evaluate the expression of ABCC2 and, in addition, it allows us to evaluate the differences related to the subcellular localization of the protein (cell membrane or nuclear envelope). The expression of MRP2 in nuclear envelope is typical for cells of low differentiation while the expression in cell membrane is typical of higher differentiated cells. Cases manifesting nuclear expression of ABCC2 demonstrated a more aggressive clinical course, which reflected lower differentiation of tumour cells and an increased resistance to some drugs. The observation confirmed the hypothesis of the relationship between localization of ABCC2 on one hand and extent of differentiation and cell polarity on the other [23, 24].

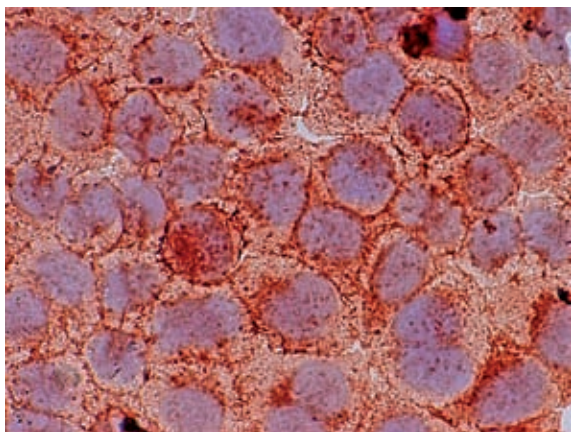


Fig. 1. Immunohistochemical localization of P-glycoprotein expression in a cytostatic drug resistant cell line of EPG 257/85 RDB

Ryc. 1. Immunohistochemiczna lokalizacja ekspresji P-glikoproteiny w komórkach linii odpornej na cytostatyki EPG 257/85 RDB

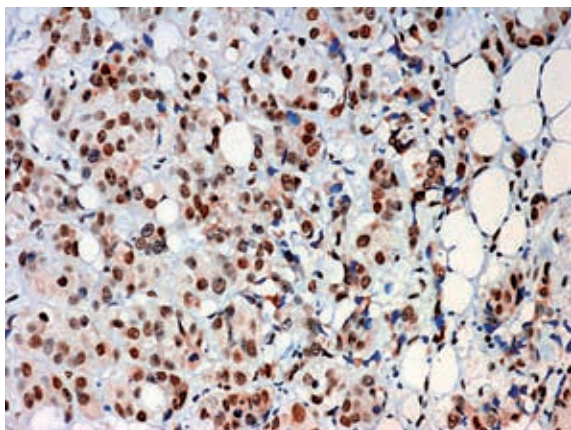


Fig. 2. Immunohistochemical localization of MRP2 in a sample of breast cancer: evident prevalence of nuclear localization

Ryc. 2. Immunohistochemiczna lokalizacja MRP2 w wycinku z raka piersi – wyraźna przewaga lokalizacji jądrowej

Tumors as heterogenous structures manifest a significant structural differentiation. Most probably, the resistance mechanism linked to the activity of MRP2 is particularly significant in the resistance to chemotherapy manifested by all cells of low differentiation, such as tumor stem cells. Located in nuclear envelope, ABCC2 decreases the sensitivity of tumor stem cells to chemotherapy, the cells representing the principal target of adjuvant treatment which, as a result, leads to both local progression and to generalized disease [24].

A number of reports have appeared which point to justified attempts to evaluate the clinical role of other proteins linked to multidrug resistance and breast cancer resistance protein (BCRP) in particular.

BCRP

BCRP, also termed ABCG2, belongs to the group of proteins linked to multidrug resistance. An analysis of BCRP properties represents one of the developing directions in studies aimed at recognising mechanisms in which resistance to treatment develops. In physiological conditions BCRP is mainly localized in epithelia of natural organ barriers, such as blood-brain barrier, blood-testis barrier and, in particular, within placenta [12–14, 25]. It plays a protective role, assuring the active export of xenobiotics and other chemical substances, toxins out of the cell. Similarly to P-glycoprotein, BCRP is also present on the surface of haemopoietic stem cells. Over-expression of BCRP on stem cells was observed to take place in conditions of anoxia, linked to the signalling pathway of HIF-1 (Hypoxia-inducible Factor 1). Such a mechanism of action manifested by the protein suggests a significant role played by BCRP over-expression in the development of radioresistance [12, 25]. Within a tumor, the principal mechanism of BCRP activity involves active transport of xenobiotics, including drugs, out of the cell. Over-expression of BCRP in cells of human tumors is linked to the resistance of the cells to several cytostatic agents. The anti-neoplastic drugs actively removed from the cells by BCRP include mitoxantrone, doxorubicin, daunorubicin, methotrexate, SN-38 (active metabolite of irinotecan) and topotecan [12–14]. In this paper the authors have shown that, in the describe group of patients (with low advancement of the disease, upon a long monitoring of treatment results, with a mean duration of observation of around 14 years) BCRP proved to provide a very useful prognostic index. Its value was confirmed by unifactorial and multifactorial analysis; it should be stressed that the only equally significant prognostic factor involved the widely used and the most important clinical prognostic parameter: a condition of regional lymph nodes [25]. In the future, the evaluation of BCRP activity may be helpful in the stratification of breast cancer patients which are supposed to be subjected to chemotherapy.

YB1

YB-1 (Y-Box-Binding 1) is a multifunctional protein, participating in the control of oxidation and reduction pathways, cell growth and, first of all, controlling responses to toxic agents [12–14]. Nuclear localization of YB-1 is linked to its function of a transcription factor for several genes associated with the replication of DNA, proliferation process and resistance to cytostatic agents. YB-1 induces expression of ER and HER2, im-

The screenshot shows the 'Kaplan-Meier Plotter' web interface for Breast Cancer. The top navigation bar includes 'KM plotter', 'Background', 'Multiple Testing', 'Updates', and 'Contact'. The main interface has a header with 'Kaplan-Meier Plotter' and 'Breast Cancer' (with a link to 'Switch to Ovarian Cancer'). Below the header, there are input fields for 'Affy id/Gene symbol' (209993_at), 'Survival' (RFS (n=2898)), 'Split patients by' (median), 'Follow up threshold' (all), and 'Compute median over entire dataset' (unchecked). A 'Settings' button is in the top right. The 'Probe set options' section has radio buttons for 'user selected probe set' (selected), 'all probe sets per gene', and 'only JetSet best probe set'. A 'Plot beeswarm graph of probe distribution' checkbox is also present. The 'Restrict analysis to subtypes...' section includes dropdowns for ER status (n=1942), PR status (n=565), Lymph node status (n=2190), Grade (n=1556), and Molecular subtype (n=2978). The 'Restrict analysis to selected cohorts...' section has radio buttons for 'systemically untreated patients' (include n=1056), 'systemically treated patients' (endocrine therapy: any n=2310, chemotherapy: any n=2299), and 'patient cohort similar to SEER prevalences'. A 'Use earlier release of the database' dropdown is set to '2012 version (n=2976)'. A green box displays '2898 patients'. A note at the bottom states: 'Please note: the generated n value does not include correction for multiple hypothesis testing by default.'

Fig. 3. Pattern of KMplot screen: beginning of analysis of the relationship between expression of ABCB1 gene and relapse-free time. The studied group included 2898 female patients with breast cancer. Effects of ER, PR expression, involvement of lymph nodes, grade of malignancy, molecular subtype or of the applied therapy were not analyzed

Ryc. 3. Zrzut ekranu z KMplot – początek analizy związku między ekspresją genu – ABCB1 a czasem wolnym od wznów. Badana grupa liczy 2898 pacjentek chorych na raka piersi, nie uwzględniono stanu ekspresji ER, PR, zajęcia węzłów chłonnych, stopnia złośliwości, ani subtypu molekularnego. Nie uwzględniono rodzaju stosowanej terapii

portant for treatment of breast cancer. Due to its nuclear activity, the nuclear form of YB-1 seems to provide a potential target for modulating signals transmitted by the receptors. The nuclear form of YB-1 participates also in signalling pathways linked to the protection of DNA and cellular stability from genotoxic effects of cisplatin, mitomycin and ionizing radiation [26].

In present work the authors detected a link between the expression of nuclear YB-1 form and less favourable prognosis in breast cancer patients. It is particularly significant that in our studies we have confirmed the prognostic value of the protein in a group of patients with a relatively good prognosis. Thus, evaluation of the nuclear form of YB-1 may manifest a potentially high clinical value in the selection of patients requiring a more intense adjuvant treatment. Similar observations were published by other authors. An augmented expression of YB-1 was detected in patients with a less favourable prognosis [26]. Gluz et al. dem-

onstrated that high activity of YB-1 was associated with other unfavourable prognostic factors, such as the absence of hormonal receptors, positive status of HER-2 and p53, intense proliferation and malignancy grade 3 [27]. In this analysis the authors have observed a correlation between an increased expression of YB-1 nuclear form and the presence of metastases to lymph nodes. Such a relationship, noted in a group of patients with a good prognosis (II stage of advancement), seems to be particularly interesting both in respect to prognostic and predictive value of the biomarker [26].

CD46 and Other Inhibitors of the Complement

The system of complement encompasses a set of a few tens of proteins present in plasma and other body fluids, together with functionally numerous receptors linked to them and controlling proteins. The complement system plays an

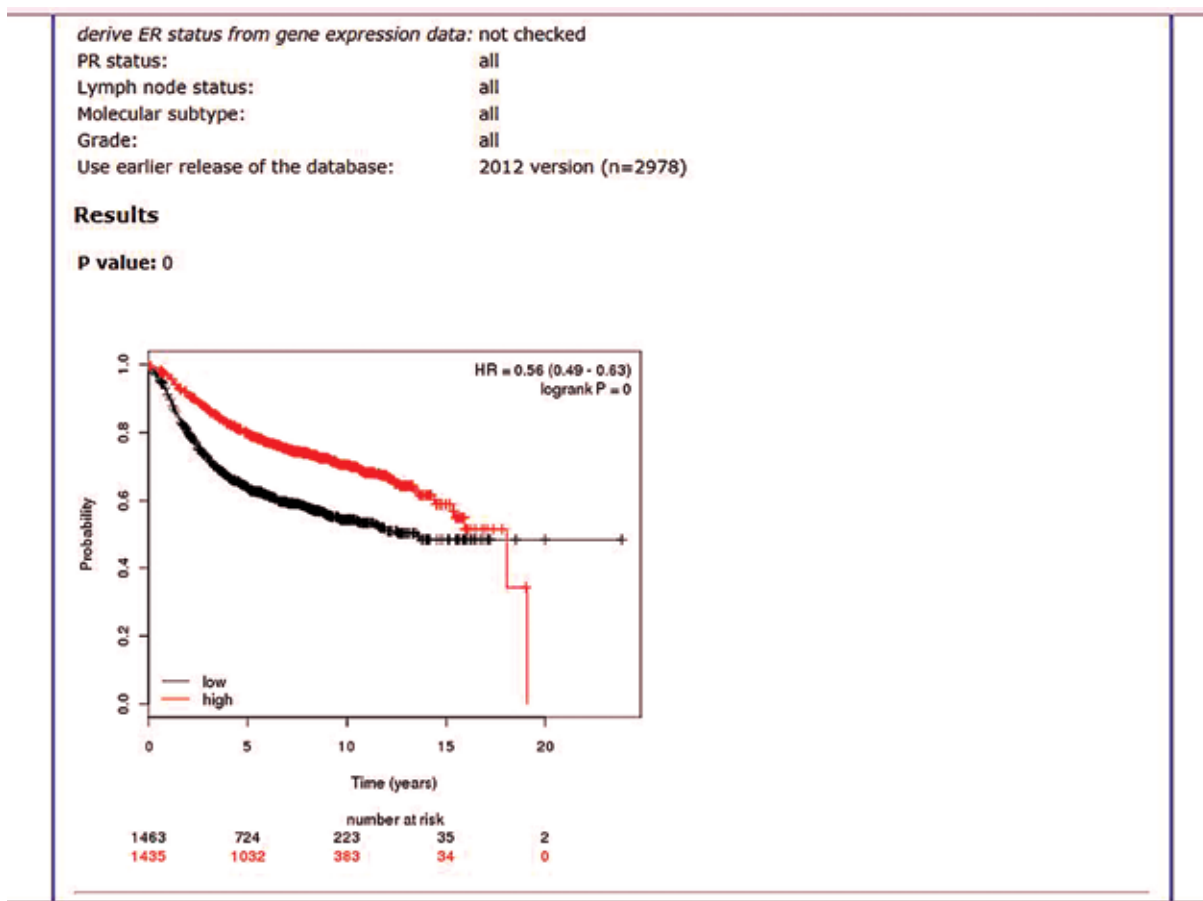


Fig. 4. Pattern of KMplot screen: relationship between expression of ABCB1 and relapse-free time in the group described in Fig. 3. Patients with higher ABCB1 expression demonstrated shorter relapse free survival

Ryc. 4. Zrzut ekranu z KMplot – wynik analizy zależności między ekspresją ABCB1 a czasem wolnym od wznów w grupie opisanej w rycinie 3. Pacjentki o wyższej ekspresji ABCB1 mają krótszy czas wolny od wznów

important role in the mechanisms of immune responses; its action involves the activation of an enzymatic cascade leading to a number of phenomena significant for the course of immune and inflammatory reactions. The important functions played by the complement in the body include lysis of bacterial, parasitic or neoplastic cells, cell opsonization, which facilitates phagocytosis, activation of phagocytic cells and clearance of immune complexes. Even if the complement system encompasses around 30 proteins, in recent years the highest attention of investigators was focused on the appraisal of properties manifested by the expression of membrane proteins which control complement system, mCRPs, the elevated expression of which is frequently detected on neoplastic cells [28, 29]. The most important of them include three proteins: CD46 (Cluster of Differentiation 46) (the protein cofactor binding C3b and C4b components), CD55 (a factor which accelerates degradation of C3 and C5 convertases) and CD59 (which blocks membrane attacking complex). An increased expression of CD46 seems to be linked

to active protection of neoplastic cells from effects of cytotoxic agents. The role of the system is universal: it was detected in various types of tumors and it is particularly significant in cases of tumors with higher malignancy [28].

In present studies the authors have detected elevated expression of CD46 protein on ovarian cancer cells in a group of patients with abbreviated survival. Also, analysis of breast cancer patients in this respect has detected elevated expression of CD46 in patients with a shorter total and free of progression survival [29]. Also clinical and experimental data confirm the potential role of complement system in indirect control of cancer cell proliferation through the effects of STAT3 (Signal Transducer and Activator of Transcription 3) transcription factor [30]. Buettner et al. drew attention of investigators to the link between CD46 gene and the mechanism of action manifested by activated STAT3, which accelerates tumor cell growth and to the fact that a stable activation of STAT3 promotes tumor angiogenesis and increases dynamics of metastatic spread [31]. Summing up the above,

it should be stressed that CD46 is a particularly interesting protein of a complex manner of action, the increased expression of which clearly manifests a prognostic value. Further studies on the marker will permit not only to additionally document its prognostic value but, perhaps, to find new approaches to the therapy.

Markers of Tumour Progression

Progression is a multistage process, in which the tumour invades the surrounding tissues and spreads metastases to distant organs. At present, a number of molecular markers can be evaluated which illustrate the risk for progression in breast cancer.

CA 15.3, CA 27.29, uPA and PAI-1

The process of invasion is accompanied by alterations in the expression of cytokeratins and proteins levels of MUC1 (Mucins1) family, pointing to the destabilization of cytoskeleton and the process can be monitored using the markers of CA 15.3 (Carcinoma Antigen 15-3) and CA 27.29. A number of proteins take part in tumor cell invasion and adhesion: adhesins, integrins, cathepsins, collagenases, metalloproteases and proteins of urokinase-type plasminogen activator. Disturbances in the normal expression of adhesins, including CD44 antigen and E-cadherin, cause detachment of tumor cells from the tumor mass and their re-adhesion in a distant organ. The two processes can take place due to a local proteolysis of extracellular matrix proteins, executed by matrix metalloproteases, first of all by MMP-1 (Matrix metalloproteinase-1) and MMP-9. The enzymes co-operate with components of urokinase-type plasminogen activator, including uPA (urokinase-type Plasminogen Activator), its receptor, uPAR (urokinase-type Plasminogen Activator Receptor) and its inhibitors, PAI-1 (Plasminogen Activator Inhibitor-1) and PAI-2. uPA represents the key protein involved in the decomposition of extracellular matrix. It is secreted in an inactive precursor, pro-uPA and subsequently converted to its active form following its binding to its receptor, uPAR. In cells of breast cancer uPA promotes infiltration of surrounding tissues and development of metastases. Its mechanism of action involves the degradation of the extracellular matrix, stimulation of angiogenesis, control of cell migration and their adhesion and inhibition of apoptotic processes. Neoplastic cells and, in particular, those in peripheral portions of the tumour manifest higher levels of uPAR expression, which is linked to

greater amounts of active proteolytic enzymes on the tumor surface. This allows for the stimulation of digestion and the penetration of surrounding tissues by tumor cells, in effect leading to the infiltration by the tumor and its progression. In peripheral regions of the tumor, apart from increased expression of uPAR, an elevated expression of uPA itself is also noted. The elevated expression of uPA correlates with higher invasiveness of the tumor and abbreviated survival of breast cancer patients. Many investigators stress that uPA protein is a tumor marker of particular importance in patients with the expression of estrogen receptors. Components of plasminogen activation system represent useful markers pointing to the progression of breast cancer, while uPA and uPAR may prove particularly useful in designing new types of therapy. At present, CA15-3, CA 27.29, uPA and PAI-1 belong to the group of tests recommended by ASCO (American Society of Clinical Oncology) [7, 8].

S100P

The gene family of S100 codes for low molecular weight proteins linked to cancer progression. The proteins of S100 family exhibit a high affinity to calcium ions and act as transducers of calcium signal. An appropriate level of calcium ions acts as a universal signal involved in the control of several processes, including gene expression, apoptosis and cell differentiation. Alterations in the expression of S100 family proteins are reported with increasing frequency in various tumours, including breast cancer [32]. McKiernan et al. found that various S100 genes (S100A1, S100A2, S100A4, S100A6, S100A8, S100A9, S100A10, S100A11 and S100A14) manifest an increased activity in basal type of breast cancer and they may provide potential targets for therapy of the cancer type [33]. Relationships between members of S000 family and tumors can be explained in various ways, but it is significant that in humans the chromosomal region of 1q21, which accumulates most of S100 genes, is particularly susceptible to genomic rearrangements [33, 34].

S100P involves a low molecular weight protein which influences the processes of proliferation and angiogenesis [32–34]. In breast cancer, the expression of S100P correlates with a particularly aggressive disease course. In the present study, both in unifactorial and multifactorial analysis, higher expression of nuclear form of S100P proved to be an unfavourable prognostic factor, noted in the group of patients with abbreviated total and relapse-free survival [35]. The protein may provide a target for therapy and it requires further observations of its prognostic and potentially predictive value.

BUBR

An interesting sector of currently developing studies includes the prognostic evaluation of markers, linked functionally to control points in the process of cell division. In the group of compounds, the mechanism can be distinguished which controls chromosomal segregation process, termed also the spindle assembly checkpoint (SAC). The group of compounds controls an appropriate course of mitosis at the moment when abnormalities are detected in chromosomal division (BUBR – budding uninhibited by benzimidazole-related). If the karyokinetic spindle checkpoint fails to act properly, abnormalities result in structure of cellular progeny, most frequently involving aneuploidy. Within methods in which the anomalies can be monitored a particularly interesting aspect seems to be the evaluation of the activity manifested by BUBR1, the protein representing the main component of the complex controlling mitotic process. BUBR1 expression in breast cancer was reported in several papers but till now its prognostic value was not examined. In the present study, the authors have described the prognostic role of BUBR1 expression in a group of breast cancer patients, detecting a pronounced correlation between the high activity of BUBR1 and the unfavourable course of the disease. An interesting idea seems to involve the examination of BUBR1 role as a potential therapeutic target for drugs belonging to microtubule inhibitors. This opens a new investigative path aimed at confirming predictive value of BUBR1 protein.

Other

KM Plotter

A selection of an appropriate in a predictive and prognostic respect gene group represents the highest challenge. One of the more interesting so-

lutions involves the formation of an on line tool which could be used for the evaluation of effects exerted by the expression levels manifested by various genes on clinical results in patients with breast cancer. The “The Kaplan-Meier plotter” (KM plotter) database was formed using data on expression manifested by 22,277 genes in a group of 1,809 patients [36, 37]. The software took advantage of the data obtained from Gene Expression Omnibus (GEO – www.ncbi.nlm.nih.gov/geo/) database, in which the expression level manifested by individual genes was estimated using Affymetrix HGU133A and HG-U133 Plus 2.0 microarrays. For the purposes of analysis involving prognostic value of individual genes the data were supplemented with clinical results related to relapse-free survival and total survival of the patients. Using the database the results can be demonstrated using survival curves. The database is generally accessible under address of www.kmplot.com. The tool manifests high value for preliminary analysis of planned to use biomarkers. However, the most important trait of the software represents the fact that the data stored in the database continue to be supplemented and, therefore, in the future it can be used to assure even higher accuracy in ascribing prognostic value for individual markers and in the qualification of patients to individual prognostic groups [34–37].

Summing up the above, the importance of clinical and scientific efforts should be stressed in designing a personalized strategy for treatment. The therapeutic efforts to an increasing extent will be based on classical and the developing new prognostic and predictive markers. It should be hoped that personalized oncology will allow to treat most of the tumors as a chronic disease while the individualized therapy will provide effective and well tolerated procedures, permitting the patients to preserve life quality comparable to that before the diagnosis of neoplastic disease was established.

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