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Molecular markers used in breast cancer diagnosis — current practice and future perspectives

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Breast cancer is the most frequently diagnosed cancer among women and a leading cause of cancer-related death. Despite many available diagnostic tools and techniques, tumor heterogeneity and molecular diversity of breast cancer tumors require new biomarkers in clinical practice. A potential diagnostic test can be built based on few biomarkers. Those biomarkers may not be specific and sensitive enough to be a single diagnostic tool, but might be useful as a set of biomarkers e.g. CEA, CA15-3, MammaPrint®, HER2 and BRCA1, 2. The second group of potential biomarkers are microRNAs. Changes in miRNA and altered genes expression may contribute to the development of breast cancer and metastases. Integration of proteomics, genomics, and metabolomics data is necessary to discover a new panel of biomarkers.

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Breast cancer — introduction

Breast cancer is the first malignant neoplasm in terms of incidence in women in Poland and worldwide (more than 1/5 of all cases) and the most frequent cause of death due to malignant neoplasms in women [1]. The incidence of this type of cancer increased more than twice in the last three decades — with about 16 000 new cases reported in 2010 [2]. Breast cancer is a heterogeneous cancer associated with many etiological factors [3]. Among the factors predisposing to the development of this type of cancer are: being female, age over 50 years, early menstruation and late menopause, as well as a number of environmental and genetic factors [4].

Breast cancers are divided into two main histological types: pre-invasive *in situ* cancer and invasive cancers. Pre-invasive cancers account for about 15% to 30% of all cases and are divided into: lobular carcinoma *in situ* (LCIS), characterized by the growth of the mammary glands, and ductal carcinoma *in situ* (DCIS), where cancer cell growth affects the epithelium of the mammary gland, without signs of stroma infiltration. Ductal carcinoma *in situ*, a precursor of invasive cancer, is heterogeneous in terms of biology and morphology. In 80% of cases these cancers are detected during mammography (in the form of calcifications), the remaining 20% are detected in the form of cystic tumors, Paget's disease, star-shaped lesions or nipple discharge. Lobular carcinomas *in situ* are characterized by the growth of lobular epithelium cells, which completely fills at least 50% of the follicles, these cancers do not form tumors and calcifications, and therefore they are not detected during mammography.

The most common invasive cancer is ductal cancer, which occurs in about 70–85% cases. Lobular carcinoma in the mammary glands accounts for about 15% of the cases. Both types of cancer are characterized by an increased risk of distant metastases. Rare types of invasive breast cancer are: tubular/cribriform (6%), mucous (2%), medullary (2%), papillary (1%) and metaplastic (< 1%) cancers. Due to significant differences in the biology of invasive cancers, a 3-stage histological malignancy evaluation was additionally introduced. Common features of infiltrating cancers are: infiltration in all directions, "orange peel", nipple pulling, metastases into lungs, bones, liver, adrenal glands and brain. Metastases to

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lymph nodes are the most important prognostic factor. In the absence of distant metastases, it is estimated that the 10 years overall survival reaches 80%, while the involvement of one to three lymph nodes reduces the 10 years overall survival to 35–40%.

Molecular markers currently used to diagnose breast cancer

A molecular marker may be a gene, transcript or protein (or sets of such molecules) whose condition and quantity is related to the risk, occurrence or advancement of the disease. Cancer markers by origin can be divided into two types. The first are markers produced by cancer cells and these are specific antigens of tumors, i.e. TSA (tumor specific antigens). The second type of cancer markers are antigens accompanying cancer produced by normal cells as a result of their response to pathological changes in the environment, also referred to as TAA (tumor associated antigens) [5]. The markers can be nucleic acids, proteins, lipids and other metabolites, but also whole tumor cells, which can be found in blood (CTC — circulating tumor cells) [6]. Molecular markers, including cancer markers, can be detected in the patient's blood, urine or tissue. Depending on the method of material collecting, markers can be divided into invasive and non-invasive [7]. Invasive markers are characterized by the need for surgical intervention in order to collect the material for testing. This group includes immunohistochemical markers such as ER, PR, HER2, Ki-67, p53, whose analysis requires biopsy or tissue removal during surgery. The advantage of this type of markers is their high specificity, which makes them commonly used for accurate diagnosis and prognosis of the disease. Non-invasive markers are proteins, enzymes, hormones or circulating tumor cells present in body fluids, such as serum, plasma, nipple secretion, tears, urine and saliva [8]. Collection of material for testing is not problematic and burdensome for the patient, although markers are evaluated in non-cancer material, which means that parameters such as sensitivity and specificity are debatable. Immunological methods, mass spectrometry, flow cytometry [9] and qRT-PCR [10] are used to determine these markers. In the analysis of molecular markers, the most important parameters are sensitivity and specificity as well as their predictive value. The sensitivity of the marker reflects the number of correctly identified samples from ill patients and it is calculated as the ratio of true positive results to the sum of true positive and false negative results (value expressed as a percentage). The specificity of the marker reflects the number of healthy people misclassified as a group of sick persons and it is calculated as the ratio of true negative results and the sum of false positive and false negative results. An ideal marker should have 100% sensitivity and specificity. A positive predictive value (PPV) indicates a high probability of disease occurrence when a marker is detected and it is calculated as the ratio of the number of true positive results to the sum of true and false positive results. Similarly, a negative predictive value (NPV) indicates a low probability of disease presence in the absence of a detected marker. The following are the markers currently used in clinical practice to diagnose breast cancer.

Status of hormonal receptors

The hormonal status of patients is important both in the etiology of the disease and in response to treatment, so the choice of treatment depends on the expression of individual receptors. Estrogen receptor (ER) expression is found in 70% of breast cancers. It has two isoforms: α and β, in clinical practice ERα expression is mainly determined. The presence of β form in the tumor is associated with better prognosis and a longer disease free survival [11]. Patients with ER+ status have significantly better response to anti-estrogenic treatment (e.g. tamoxifen) than patients with ER- [12]. Moreover, in the work of Guo et al. it has been shown that high ERβ concentration in patients impair the efficiency of endocrine therapy, while low ERβ concentrations in patients treated with hormones were correlated with prolonged disease free survival in relation to the group without hormonal treatment [13]. Estrogens, by binding to their receptors, induce the synthesis of the progesterone receptor [14]. Clinical trials have shown that patients classified as ER-/PR- have a higher mortality rate than ER-/PR+ patients, which was associated with a better response to hormone therapy in the latter group [15]. The expression of ER and PR receptors is not constant and changes with the progression of the disease [16].

HER2 receptor status

Important prognostic factors in breast cancer include HER2 receptor status (human epidermal growth factor receptor 2). HER2 is a glycoprotein acting as a membrane receptor, belonging to the family of receptors for epidermal growth factors. Protooncogen HER2/neu/eRBb-2 is detected in multiplied form in 10–35% of breast cancer patients, which is an important adverse prognostic factor in both early and advanced breast cancer [17]. The first overexpression of this receptor was confirmed in breast cancer patients by the Van de Vijver group in 1988. In the same year the Berger group showed a correlation between HER2 receptor overexpression and lymph node status (N) and tumor size [18, 19]. In the following years, overexpression of this receptor was associated with a more aggressive cancer phenotype and worse prognoses [20]. The basic test for assessing the HER2 receptor status is an immunohistochemical procedure supplemented by FISH gene amplification test (fluorescent hybridization *in situ*), recommended each time when the result of protein expression determination is ambiguous. The antibody directed against the extracellular domain of HER2 receptor is trastuzumab. This antibody is used in the

adjuvant treatment of HER2 patients, reducing the risk of relapse as well as mortality in early stage cancer patients [21]. HER2 overexpression has been shown to be associated with an increased risk of cerebral metastases in patients with advanced cancer [21, 22]. Moreover, quantitative measurements of HER2, HER3 and p95HER2 protein levels allow for detailed analysis of their potential prognostic value. This assessment showed that the relationship between HER2 expression and overall survival of patients receiving lapatinib after trastuzumab progression is U-shaped with the best response among patients with moderate HER2 overexpression and high p95HER2 expression [23]. Moreover, quantitative assessment of p95HER2 and HER2 expression may be useful in assessing the risk of cerebral metastases, which requires further research [24].

Molecular subtypes evaluated on the basis of gene expressions

Based on the characteristics of gene expression profile in tumour tissue, four breast cancer subtypes can be distinguished as luminal type A, luminal type B, basal cell type and HER2-positive, also called non-luminal [25, 26]. Luminal type A is characterized by high expression of genes associated with estrogen receptor activity and low expression of genes associated with proliferation and genes associated with HER2 receptor expression. Luminal type B is characterized by positive ER status combined with low expression of genes associated with this receptor and higher expression of proliferation-related genes than in type A assessed by the Ki-67 designation. A team of panellists from St. Gallen considered the degree of malignancy and Ki-67 expression to be factors that can be used to distinguish between luminal type A and subtype B-like tumours. This is important in the prognostic assessment, which is better in type A [27]. The third type is basal-like breast carcinoma, also called triple negative due to the absence of estrogen and progesterone receptors and the lack of expression of the HER2 receptor — as a consequence, there is no expression of genes associated with these receptors. A group of patients with this type of cancer with cerebral metastases is particularly interesting; in their case the use of biological markers (CK 5/6, HER1, c-KIT) may help to differentiate the basal subtype of a like and no-like one, but their clinical usefulness is ambiguous [28]. The last molecular subtype of breast cancer is characterized by HER2 overexpression combined with absence of ER and PR [22].

Molecular breast cancer subtype can be assessed by performing one of several available genomic tests. The Oncotype DX® test allows for analysing the expression of a panel of 21 genes to estimate the individual risk of relapse in patients diagnosed with early stage breast cancer. This test also allows for individualizing the therapy because of the information about the chemotherapy benefits. A risk assessment of relapses based on a 21-gen test result for breast cancer predicts the benefits of chemotherapy if it is high, while a low risk of relapses in the absence of chemotherapy if it is low. In addition, complementary hormone therapy and chemotherapy combined with hormone therapy showed similar efficacy in women with hormone positive, HER2-negative, N0 breast cancer, who obtained an indirect test result, although the benefits of chemotherapy were reported in some women aged 50 years and younger [29, 30].

Another multi-gene diagnostic assay is MammaPrint®. This test is based on an analysis of 70 gene signatures. It is used by clinicians to choose a therapy that minimizes the risk of relapse. The Breast Cancer Index test analyses the expression of genes associated with two types of signal pathways — estrogen-related and cell proliferation, with its use it is possible to estimate the benefits of endocrine therapy as well as the risk of relapse [31]. In addition, recent results of a randomized EORTC 10041/BIG 3-04 MINDACT study showed the usefulness of molecular evaluation (BluePrint and MammaPrint) of breast cancer subtype compared to immunohistochemical evaluation. On the basis of molecular evaluation 54% of patients with luminal B subtype could be qualified to luminal A subtype with similar treatment results. Therefore, molecular classification may help to identify a larger group of patients with a low risk of relapse compared to the more modern classification methodology, including the high quality assessment of the Ki-67 [32].

Multi-gene panels are better than traditional predictive factors in predicting clinical response and identifying women who can safely skip chemotherapy. The available evidence confirms the clinical validation of multi-gene panels, of which Oncotype DX® and MammaPrint® have the strongest evidence to support their clinical usefulness and decision making effectiveness in luminal breast cancer [33, 34].

PAM-50 is a qPCR-based assay. It enables the analysis of the expression of 50 genes based on biopsy material allowing to classify cancers into subtypes and predicts the risk of relapse (ROR) after 10 years. The additional inclusion of known clinical and pathological factors significantly increases its predictive value [25, 35, 36]. The PAM-50 test result may help to identify a group of patients who may benefit from the additional use of taxans in complementary treatment [37]. Further clinical studies should assess the ability of the PAM-50 test and other gene analysis to divide patients and provide individual treatment depending upon the predicted risk of relapse and metastases. Especially, many problems remain to be solved before multi-gene panels have a greater impact on breast cancer treatment, such as accurate prediction of late relapse in ER-positive breast cancer or greater access to multi-gene panels [34].

Patient's genetic profile

The most important genetic factors that determine predisposition to breast cancer are the *BRCA1* and *BRCA2*

genes. These genes are classified as so-called suppressor genes, and their protein products are involved in the regulation of transcription, repair of damaged DNA and in the differentiation process. Germinal mutations within the *BRCA* genes are associated with an increased risk of disease (up to 70% for people without mutations). The frequency of different types of mutations within these genes depends on the ethnic group and geographical region [38, 39]. The assessment of the occurrence of mutations in *BRCA* genes is important not only as a risk factor for cancer, but also plays an important role in the choice of systemic treatment. It has been shown that in advanced breast cancer patients carrying mutations benefit more from the use of carboplatin compared to docetaxel [40]. In addition to mutations in the *BRCA* genes, other genetic changes are associated with an increased risk of breast cancer. These genes include: *TP53* (in patients with Li-Fraumeni syndrome), *STK11* (in patients with Peutz-Jeghers syndrome) and *PTEN*. In addition, mutations in genes such as *CHEK2*, *ATM*, *PALB2* and *BRIP1* were also found in the studied families with breast cancer [41].

Proliferation potential

Ki-67 protein is the protein that enables the assessment of the proliferation rate in the malignant tissue. Patients with more than half of the tumour cells showing expression of this factor are at higher risk of recurrence. Furthermore, high expression of this factor is associated with worse prognosis, but with a better response to chemotherapy [42, 43]. The Ki-67 protein expression may be an independent prognostic factor determining the disease free survival, however, it requires standardization of parameter evaluation [44]. A significant factor in the assessment of Ki-67 expression is a properly performed analysis by qualified professionals. Although the data concerning the standardization of scores are encouraging, there are still discrepancies between departments of pathomorphology. Moreover, the information on Ki-67 expressions has a significant influence on the decision making process regarding the use of systemic treatment. In the case of high expression of Ki-67, chemotherapy may be considered even for the early stage breast cancer patients and ER positive tumours [27].

Cancer markers are also detected in serum or plasma. The most commonly used cancer markers are glycoproteins present in the membrane of cancer cells. These include mucins such as CA15-3 (cancer antigen 15-3), which is a product of the *MUC1* gene. Other members of this marker group are: PEM, MCA, MSA and CA125 (cancer antigen 125). Their level may be elevated in the course of several types of cancer (apart from breast cancer these are ovarian cancer, endometrial cancer, fallopian tube cancer or lung cancer). Another cancer marker used in diagnostics of many types of cancers, including breast cancer (especially in ductal cancers), is CEA (carcinoembryonic antigen), which is a glycoprotein of blood serum [45]. CEA together with CA15-3 are considered to be a markers for relapse. CA15-3 is overexpressed in 90% of breast cancer cases, but it is not (similarly as CEA) a marker specific for this type of cancer. The concentration of these markers increases with the size of the tumour, and an increase in their concentration was also observed in hepatitis, benign breast and ovarian lesions, cancer of the uterus, ovary or lung. CA15-3 is characterized by low diagnostic sensitivity at the early stages of the disease (I, II), because it varies between 20% and 30%, therefore it is not suitable for screening, but this marker is used in the diagnosis of metastases [46]. In advanced stages of the disease (stage III and IV) the sensitivity of CA15-3 increases to 70%. CEA synthesis is intensified in breast cancer cells as well as in cells derived from colorectal, pancreas and stomach cancer. For this reason, CEA is characterized by limited sensitivity and diagnostic specificity. Like CA15-3, this marker is not suitable for screening, but it is used for the detection of relapses and distant metastases. The positive predictive value of CEA concentration increase for progression confirmation is over 90%, so it is considered a universal marker of cancer metastases [47].

Another group of markers used in the diagnosis of breast cancer are cytokeratins, which are insoluble structural proteins of epithelial cells, the presence of which in the blood indicates progressive cell death processes in tissues. Cytokeratin 8, 18 and 19 fragments present in serum are potential markers for the diagnosis of early stages of breast cancer [48, 49]. TPA (tissue polypeptide-specific antigen) is a peptide complex associated with cytokeratin 18. This marker is related to the proliferative abilities of tumour cells, the presence of this complex in blood is an unfavourable factor, as it indicates a rapid relapse of the disease [50, 51].

New molecular factors with potential use in breast cancer diagnostics

The "classic" breast cancer biomarkers applied so far are not used in screening. The main reasons are their low concentration in non-advanced cancers (*in situ*) and low sensitivity and specificity at the early stages of the disease [52]. These markers are usually used to monitor a possible relapse during the treatment, as well as after the treatment [53]. Moreover, there are no data on the possibility of using these markers in the risk assessment of metastases in patients with non-advanced breast cancer, which are the main cause of treatment failure in this group of patients. For this reason, an intensive research is underway to identify markers with potential use in early diagnosis and risk assessment of metastases, especially in patients with early detection of cancer. Several groups of potential biomarkers tested in this context are presented below.

Metalloproteinases

Among the new cancer markers, also tested for their usefulness in the diagnosis of breast cancer, there are

metalloproteinases, which as proteolytic enzymes enable digestion of extracellular matrix components and numerous molecules on the cell surface, and thus participate in the formation of metastases and angiogenesis. Metalloproteinases of potential diagnostic significance include MMP-9. It is believed that the concentration ratio of MMP-9/TIMP-1 (a specific MMP-9 inhibitor) may be prognostic in breast cancer [54].

Nuclear proteins related to proliferation activities

PCNA (proliferating cell nuclear antigen) is a non-histone nuclear protein that participates in both DNA synthesis and in the response to genetic material damage. In clinical practice it is considered as a marker of mitotic activity, just like Ki-67 in breast cancer patients. Phosphorylation of this protein in tyrosine at position 211 is positively correlated with the increase in proliferation of cancer cells, and thus it is a prognostic disadvantage [55, 56].

Chemokines and their membrane receptors

The CCR2 chemokine receptor is a membrane protein, specifically binding with CCL2 chemokines. These chemokines are secreted by monocytes and macrophages. The overexpression of this chemokine and its receptor in breast cancers metastases to the lungs and bones was demonstrated [57, 58]. Moreover, CCL2 correlates with the advancement of the breast cancer and is a prognostic factor for the estimation of metastases free survival/relapse free survival [58]. Under normal conditions CCL2 together with CCL5 chemokine stimulate the migration of monocytes and T cells to damaged or infected sites. These chemokines show higher expression in tumor tissues than in normal tissues, and CCL5 is a characteristic chemokine for patients with triple negative molecular subtype [58–60]. CXCR4 (C-X-C chemokine receptor type 4) is a transmembrane protein receptor responsible for the migration of cells from the primary tumor to the lungs, bones and lymph nodes. Its mechanism of action is based on chemotaxis, as these organs release CXCL12 chemokine, which is a ligand of this receptor. In patients with triple negative breast cancer, high expression of this receptor may indicate a more aggressive tumor phenotype than in patients with low CXCR4 levels [61].

Membrane proteins

Integral cell membrane proteins that form membrane invaginations (so-called caveoles) are caveolins, which take part in the transmission of cellular signals and in alveolar transport. The expression of CAV1 and CAV2 caveolins is often associated with triple negative cancers and a high histological advancement of the disease [62]. Caveolin 1 (CAV1) can be a marker of oxidative stress, and its level seems to be related with response to chemo- and radiotherapy [63].

Microtubule-associated proteins

One of the methods of breast cancer treatment is the administration of drugs which affect the structure of microtubules. Decomposition or stabilization of these cellular structures reduces cell proliferation. One of the proteins associated with microtubules is the ATIP3 protein, which prolongs the time of cell division, thus reducing the number of dividing cells. Patients with invasive breast cancer and metastases showed significantly decreased levels of ATIP3 protein and its encoding gene (*MTUS1*) by 74.7% and 62.4% respectively in the group with metastases. These data indicate ATIP3 protein as a therapeutic target and as a potential biomarker for development of metastases [64, 65].

Transcription factors

GATA4 is a transcription factor, which plays an important role in cancer progression (expression of genes regulated by this factor is correlated with metastases and HER2 status). This marker is particularly useful for patients with invasive ductal carcinoma and may be a prognostic factor for this group of patients [66]. Transcription factor HIF2α activates, among other things, the expression of gene encoding MMP-9 metalloproteinase. In immunohistochemical studies based on material from breast cancer patients, the expression of these proteins was found in 60% and 66% respectively. Both HIF2α and MMP expression correlate with stage of the breast cancer, while high HIF2α protein expression correlates with short overall survival [67].

Growth factors

Growth factors such as EGF, HGF, IGF, VEGF and TGF-β are examined both for cancer risk and tumor progression. TGF-β as a biomarker is useful as a prognostic information for breast cancer patients in stages I to III. It is believed that elevated TGF-β levels may characterize patients at risk of relapse [68].

Protein panels detected by proteomics methods

Proteomics studies allow us to get to know the full set of proteins present in a given tissue, their structure, modification and mutual relations. Information on the qualitative and quantitative composition of cellular proteins can be generated using a number of techniques (mainly mass spectrometry), and for the final result of such studies, correct bioinformatic data processing and their integration from various fields is crucial. Clinical proteomics makes it possible to learn about changes in body tissues and fluids during the development of the disease and during therapy [69, 70]. Proteomics methods are also used for the "unsupervised" search for proteins of potential importance for the diagnosis of breast cancer. Table I summarizes a series of studies aimed at detecting potential biomarkers of breast cancer in both tumor tissue and body fluids. It should be

noted that potential breast cancer markers detected with proteomics methods in serum and plasma are dominated by inflammatory proteins. It is estimated that the higher level of amyloid (SAA) and S100A4 protein increases the risk of metastases [71–73]. Moreover, the risk of breast cancer seems to be correlated with the level and modification of apolipoproteins [74, 75].

Circulating tumor cells

The presence of circulating tumor cells (CTC) in the blood is an important diagnostic and prognostic factor in many types of cancer [84]. It is believed that the CTC level may be a useful prognostic marker at early stages of breast cancer and may correlate with the level of invasiveness and aggressiveness of the tumor [85]. CTC can also be used as research material for other biomarkers. It was found that TFF1 protein present in CTC in breast cancer patients correlates strongly with bone metastases [86]. Moreover, patients with high ERβ expression in circulating tumor cells showed good response to hormonal treatment [87].

MicroRNA

In the last decade, intensive research on microRNA has been carried out in terms of its diagnostic and prognostic usefulness. MicroRNA are short (21–24 nucleotides), non-coding RNA molecules that typically bind to the 3'UTR regions present in mRNA transcripts and regulate the expression level of many genes. Molecules of miRNA can be detected in both tissues and body fluids. Probably cancer-related miRNA molecules enter the bloodstream when tumor cells die. Another possibility is the active secretion of miRNA molecules through exosomes [88]. In the work of Wu et al. [89] more than 800 different miRNA molecules were detected in breast cancer patients. Two of them, mi-R357 and mir-122, showed a strong correlation with the outcome of treatment and thus with the choice of treatment. Their analysis showed that myrrh-497 expression correlates negatively with the advancement of the disease, lymph node metastases and tumor size. However, there was no correlation with classical markers such as ER, PR or p53 status. In turn, the Huang group showed, both *in vitro* and *in vivo*, that mir-373 and mir-520c molecules stimulate the migration of cancer cells and their invasiveness [90–92]. The loss of suppressor miRNA molecules such as: miR-206, miR-17-5p, miR-205, miR-125b, miR-200, miR-34a, miR-27b, miR-126, miR-101, miR-145, miR-205 and miR-31 and/or oncogen overexpression of miRNA molecules (miR-21, miR-155, miR-10b, miR-373, miR-520c, miR-27a, miR-221/222) were observed in breast cancer patients [93]. In 2012, the Schrauder group carried out micromatrix analyses of miRNA molecules in peripheral blood of 48 patients at early stages of the disease and 57 healthy people. There were 59 miRNA molecules differentiating these two groups of women, 13 of which were characterized by overexpression and 46 by decrease in

Table I. Potential biomarkers for breast cancer identified by different methods of proteomics

Potential biomarker	Method used	Material used for testing	Bibliography
CBP1, PDZ, LIM, PDLIM2, RNF25	iTRAQ-2D LC MS/MS immunohistochemistry	24 samples from metastatic lymph nodes, 24 samples from non-metastatic lymph nodes, 48 samples taken from patient tumors	Bouchal et al. [76]
TCEAL4, AZGP1, S100A10, CAPS ALDH6A1, AHNAK, FBP1, S100A4, MX1, HSP90AB1, PDXK, GFPT1, RAB21,	iTRAQ	12 samples taken from tumors from patients with recurrence 12 samples taken from tumors from patients without symptoms of the disease > 7 years	Johansson et al. [77]
ECM1, MAST4, Filaggrins	Label-Free LCMS/ MS	20 urine samples taken from breast cancer patients 20 urine samples taken from healthy women	Beretov et al. [78]
15SFAA	UPLC–MS	27 saliva samples from breast cancer patients 28 saliva samples from healthy women	Cheng et al. [79]
Apolipoprotein C1, carbonic anhydrase 1, L1CAM	MRM- MS	80 plasma samples from breast cancer patients 80 plasma samples from healthy women	Lee et al. [80]
Apolipoprotein AI, POTEE, HPX	Filaggrins MS	20 tissue samples taken from breast cancer patients	Cine et al. [81]
Apolipoprotein H, ApoCL, apolipoprotein AI, C3a, TTR	SELDI-TOF MS Western blott MALDI-TOF/TOF MS	99 blood samples from breast cancer patients 51 blood samples from healthy women	Chung et al. [70]
Parathyroid hormone-related protein	SELDI-TOF MS	111 plasma samples from breast cancer patients	Washam et al. [82]
Serum amyloid Haptoglobin	ELISA	118 blood samples from breast cancer patients 51 blood samples from healthy women	Zhang et al. [83]

relation to the control group [94]. These reports indicate the enormous information potential of miRNA research, both as factors regulating many processes in the cell, as well as their diagnostic and prognostic potential.

Summary

The results of advanced breast cancer treatment are still unsatisfactory, therefore, it is necessary to develop new diagnostic tests for the early detection of the disease. Moreover, a panel of prognostic and predictive biomarkers is awaited, which would allow for individualization of patients treatment. Personalized medicine assumes a systemic approach to the disease. The key to success may be the integration of molecular data: genomic, proteomics and metabolic together with clinical parameters.

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