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The importance of selected biomarkers in the clinical practice of breast cancer patients

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Breast cancer is considered the most commonly diagnosed tumors. Biomarkers used for the diagnosis and treatment of breast cancer are: tissue biomarkers (PR, ER, HER2, Ki-67) and serum biomarkers (CA-15-3, CA-125, CA-27-29, CEA, cytokeratins). ECD HER2, metalloproteinases and leptin are emerging as promising biomarkers for breast cancer. There is a growing need for personalized diagnostics based on tumour genome characterization, relying on a liquid biopsy containing components such as CTC and ctDNA, cell-free RNA. Biomarkers can also be used use as a target for anti-breast cancer treatment (PGRN and sortilin, AR, PD-1/PD-L1). Another potential field of application of breast cancer biomarkers is monitoring treatment side effects, such us inflammatory biomarkers causing cardiotoxicity, thyroiditis biomarkers (TSH, FT4, TPOab TgAb) in IrAE, NF-L and MCP-1 in ICI-associated neurotoxity. It is expected that new prognostic and predictive biomarkers will be developed that can provide accurate and reliable information for clinical application. Through the recognition of emerging biomarkers, it is possible to identify subgroups of patients who benefit from targeted therapies and managing treatment by monitoring side effects. However, these new biomarkers need to be validated and tested for their suitability before entering clinical use.

Key words: breast cancer, biomarkers, personalized diagnostics, anti-cancer therapy, adverse events

Introduction

According to Global Cancer Statistics 2020, breast cancer is considered the most commonly diagnosed tumor with 2.3 million new cases of breast cancer reported in 2020. It is the fifth leading cause of cancer mortality globally, whereas in women it is the leading cause of cancer death [1]. The highest incidence rates of breast cancer in 2020 were reported in Belgium and the Netherlands with the highest mortality in Barbados and Fiji [2]. In Poland in 2020, the most common cancer in women was breast (23.8%), and it is the second (15%) leading cause of death after lung cancer (18%) [3]. The risk factors for breast cancer are gender, age, genetic factors, ethnicity, early menstruation, late menopause and shorter periods of breastfeeding. The increased incidence rate is associated with lifestyle such as alcohol consumption, obesity, use of hormonal therapy and contraceptives [4].

Treatment of breast cancer depends on its clinical stage, the histological type and its accompanying biomarkers. Nowadays there are many available methods for molecular profiling, hormone indications etc. The general classification of breast cancer is based on the division into sarcomas and carcinomas [5]. Carcinomas are divided into two histopathological types: pre-invasive *in situ* cancer and invasive cancer. Pre-invasive *in situ* carcinomas are further divided into ductal *in situ* carcinomas (DCIS) and lobular *in situ* carcinomas (LCIS).

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Independently from histological subtypes, breast cancers have been classified by molecular examination: luminal A and B subtypes, epidermal growth factor receptor 2 (HER2)-positive breast cancer and triple negative breast cancer (TNBC). The luminal A type of breast cancer is characterized by the presence of an estrogen-receptor (ER) and/or progesterone-receptor (PR), the absence of *HER2* and low expression of genes associated with proliferation (Ki-67). The luminal B subtype includes either HER-positive or HER--negative tumors. Progesterone and estrogen receptors are also found here. In contrast to luminal A, luminal B tumors have higher expression of proliferation-related genes assessed by the Ki-67 designation [6]. Luminal A tumors grow slowly and have a better prognosis, while luminal B tumors are higher grade and have a poorer prognosis. ER is similarly expressed in both A and B subtypes and is used to distinguish luminal from non-luminal disease. Triple-negative breast cancer (TNBC) is a type of breast cancer that lacks the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). It is characterized by an unfavorable prognosis and aggressive biology since patients with TNBC do not benefit from endocrine or anti-HER2 therapy [7].

The presence of proteins or other substances in the serum, body fluids and tissues allow for an early diagnosis of cancer and recurrence of the disease. A biomarker is a substance (nucleic acids, proteins, carbohydrates, lipids) which is either qualitatively or quantitatively abnormally expressed by the tumor tissue or released after cell death by apoptosis, necrosis or destruction by immune cells in biological fluids such as blood serum, urine, saliva or the cerebrospinal fluid. A biomarker can be measured as an indicator of normal biological or pathogenic processes. Some of these biomarkers can be used by physicians to identify the type of cancer and stages of progression, as well as determining a specific treatment and further monitoring response to treatment. However, a lack of specificity is observed for some biomarkers, which is a barrier for their use in cancer screening. As a non-specific tool they complement imaging tests.

Based on their clinical use, two major types of biomarkers can be distinguished: prognostic and predictive. Prognostic markers predict the natural course of a cancer and differentiate good-outcome tumors from poor-outcome tumors. However, no prognostic marker can exactly predict an outcome for a particular patient. It informs about the outcome for a heterogeneous patient population. A predictive marker delivers in advance information on whether the patient is or is not likely to benefit from a particular therapy. The absence of a given marker or a decrease in its concentration during therapy is also of prognostic importance. Therefore, the use of predictive markers enables reducing the overtreatment of patients with benign malignancy and avoiding undertreatment of patients with aggressive tumors [8, 9].

This review covers information about biomarkers currently available for breast cancer management, as well as new promising biomarkers and their potential use in the future.

Biomarkers for the diagnosis and treatment of breast cancer

Biomarkers used in clinical practice are helpful in:

- risk assessment for patients who are unaffected and considering preventive strategies,
- screening for detection of early-stage cancer,
- diagnosis in staging, grading and choice of therapy,
- for prognostic purposes, predicting and monitoring treatment response,
- detecting recurrence after therapy.

Some biomarkers are only used for specific purposes, whereas another can serve in more than one type of application.

Tissue biomarkers

Biomarkers in the biopsy material play an important role in the diagnosis of breast cancer and the choice of treatment.

Determination of various subtypes of breast cancer based on diagnostic evaluation of hormone receptors (ER and PR), HER2 is recommended to be assessed by American Society of Clinical Oncology (ASCO) guidelines due to their prognostic and predictive relevance [10]. These markers are highly specific, and nowadays are routinely used for the diagnostic of breast cancer. Additionally, Ki-67 proliferation index is helpful in differentiating luminal A and luminal B molecular subtypes. The detection of ER, PR, HER2 and Ki-67 affects decisions on the type of undertaken therapy. They are tissue biomarkers, their disadvantage is that an invasive surgical biopsy is required.

At present, the most important predictive biomarker for breast cancer is the estrogen receptor (ER). The measurement of ER is mandatory in all newly diagnosed cases of breast cancer. Its main application is as a predictive marker for endocrine therapy, since ER levels may be correlated with the beneficial effects of antiestrogen therapy. The occurrence of ER helps to identify patients with early breast cancer for adjuvant treatment with drugs such as estrogen receptor modulators (tamoxifen) or aromatase inhibitors (AI), preventing the stimulation of breast cancer proliferation [11]. Two izoforms of estrogen receptor have been identified ER-α, and -β [12]. They have different effects on cancer cells. ER-α stimulates transcription while ER-β inhibits it. The proportions of ER-α and ER-β in the cell determine cell division or inhibition and resistance to hormonal treatment. ER-β is a negative regulator of ER-α [13]. ER-α plays a crucial role in the progression and proliferation of breast cancer. There are some inconsistencies about the role of the ER-β, since there are studies indicating its anticancer and carcinogenic role in breast cancer [14]. The progesterone receptor (PR) is routinely examined together with ER in breast cancer as an important biomarker. PR is involved in molecular

subtyping and plays a substantial role in treatment decisions. It is thought that the absence of PR reflected a nonfunctional ER pathway and was less responsive to tamoxifen [15]. ER+/PR+ breast cancers respond better to hormone therapy than ER+/PR– breast cancers and have a better breast cancer-specific survival rate [16, 17]. The tumors that are ER– and PR+ demonstrate an intermediate response to endocrine therapy [18]. The expression of ER and PR receptors is not permanent and may change spontaneously during the course of the disease or as a result of therapy. The complete loss of ER during endocrine therapy is rare, whereas about half of tumors lose PR completely becoming resistant to therapy. Metastatic tumors have a much more aggressive course after the loss of PR in comparison with tumors with PR expression [19].

Hormonal resistance of breast cancer can be primary or acquired. Primary resistance occurs from the beginning of treatment. It may result from an inappropriate proportion between the level of ER-α and ER-β receptors. This results in the transcription of estrogen-dependent genes and the synthesis of proteins leading to breast progression of tamoxifen resistance. Primary resistance to tamoxifen occurs in breast cancers with high overexpression of the HER2 receptor. Acquired resistance develops as a response to a long-term block or impairment in DNA transcription and protein synthesis responsible for tumor progression. The cancer cell bypasses the tamoxifen-induced blockade and becomes hypersensitive to estrogens and tamoxifen. This causes even small doses of estrogen or tamoxifen to lead to transcription and tumor progression [13].

HER2 is a glycoprotein tyrosine kinase receptor belonging to the EGFR family. HER2 consists of three parts: an intracellular tyrosine kinase domain, a transmembrane lipophilic segment and an extracellular domain (ECD). According to the ASCO testing guideline, breast cancer is considered HER2 positive if the presence of transmembrane HER2 overexpression in the tumor tissue is confirmed by an immunohistochemistry assay or fluorescence in situ hybridization (FISH) [10]. HER2 is overexpressed in approximately 20% of breast cancers and it correlates with a poor clinical prognosis [17, 20, 21]. HER2 is important in choosing the right management in breast cancer patients. An overexpression of HER2 in breast cancer is a strong predictor of benefitting from treatment with trastuzumab (Herceptin) [22]. Trastuzumab is a monoclonal antibody against the extracellular domain of *HER2*, which, when used in adjuvant therapy, significantly extends overall survival in early breast cancer patients [23]. Except for breast cancer, *HER2* overexpression has been recognized in several different solid tumors such as lung, head and neck.

KI-67 is an index providing the information about the proliferation of malignant tumors. High levels of Ki-67 are associated with poorer outcomes. According to St. Gallen's recommendation from 2015, a cut-off point of Ki-67 ≥ 20% could be used to differentiate between low and high values [24]. Ki-67 has been shown to be prognostic of clinical outcomes in breast cancer as well as a predictor of response to neoadjuvant chemotherapy or endocrine treatment. Expression of Ki-67 is often used to identify patients with a high risk of relapse.

Serum biomarkers

Serum biomarkers (so-called "wet biomarkers") are easily accessible at any time and through any blood collection. They are minimally invasive, and therefore can be detected more often than tissue indicators [25]. Biomarkers can quickly provide additional information on patient prognosis and response to treatment. For breast cancer patients' prognosis and response to treatment, serum biomarkers are more convenient and cost-effective compared to mammograms and frequent tissue biopsies.

Among the standard serum tumor markers, CA-15-3 is dedicated to breast cancer. Due to the low diagnostic sensitivity, it is not used in the diagnosis of cancer, but may be important in monitoring treatment. Other recognized serum markers, such as CEA, CA-125 or CA-27-29, may be elevated in metastatic disease.

Increased expression of CA-15-3 in breast tumors is related to invasiveness and metastatic potential [26]. The main utility of CA-15-3 as a biomarker is monitoring therapy in patients with advanced breast cancer, because a relationship between in CA-15-3 levels and the response to chemotherapy has been observed [27]. CA-27-29 is clinically comparable to CA-15-3 due to lack of specificity. Higher serum levels of CA-27-29 may reflect an increased tumor burden [28, 29]. Persistently elevated CA-27-29 levels may indicate treatment failure or the progression of disease [30]. Increased levels of CA-125 have been observed in the majority of metastatic breast cancer patients [31]. The limitations of serum biomarkers such as CA-15-3 and CA-125 are that their temporary elevated levels in serum may occur after starting therapy, due to tumor lysis caused by chemotherapy. High levels of CEA in the blood are usually related to metastasis of breast cancer [32]. A combination of CA-15-3 and CEA is used as a diagnostic tool for relapse of breast cancer [33]. High levels of CA-15-3 together with CEA are associated with worse clinical outcomes since they indicate high tumor burden [34, 35].

In breast cancer, cytokeratins are applicable as serum biomarkers. The complex of cytokeratin fragments 8, 18 and 19 constitute a circulating polypeptide TPA (tissue polypeptide antigen). TPA indicates ongoing cell death and lysis [36]. TPS (tissue polypeptide specific antigen), an antigenic determinant associated with human cytokeratin 18, is released from proliferating cells during tumor development, when intensive multiplication-and disintegration of cells may take place. The rate of concentration increase of TPS is correlated with the rate of progression of the neoplastic process. This increase provides information about the growth of the tumor before the clinical manifestations of the cancer. The level of TPS indicates the proliferative activity of neoplastic tissue regardless

of tumor size, and it is an independent prognostic factor for disease-free survival and overall survival [37, 38].

The use of the biomarkers listed above has some limitations in their use in diagnostic tests for breast cancer. Their main disadvantage is the lack of sensitivity and specificity, which makes them useless for screening purposes. At low stages of cancer, serum biomarkers have low diagnostic sensitivity [39]. The conventional serum biomarker testing is recommended but not mandatory. Their application plays an auxiliary role in the clinical management of breast cancer. Therefore, it is important to continue to search for new factors involved in tumor progression which can help to identify the risk groups, detect the disease at early stages and assess the risk of future relapse.

New biomarkers with a potential application in breast cancer

An example of a promising biomarker in breast cancer can be the extracellular domain (ECD) of *HER2*. The ECD HER2 is released into the blood by means of proteolytic enzymes (shedding). The remaining shortened peptide in the cell membrane is more oncogenic than the full length receptor. The release of the extracellular domain into the serum is increased in metastases compared to primary breast cancer [40]. HER2 is a risk factor of relapse, high-grade malignancy index and metastasis. Some studies suggest that the soluble HER2 ECD is a better prognostic tool than tissue HER2 and its prognostic value is independent of the *HER2* status of the tumor [41, 42].

Metalloproteinases are proteolytic enzymes that digest basement membrane and extracellular matrix (ECM) components, enabling metastasis and angiogenesis in breast cancer [43]. Some studies suggest the potential to use MMP-9 as a predictor of breast cancer progression, since there is a relationship between high MMP-9 expression and the occurrence of distant metastases in breast cancer patients and poor prognosis [44].

Another potential biomarker for breast cancer risk is leptin. Leptin is produced mainly by fat cells and is overexpressed in obese individuals. It is known as the "obesity hormone", the blood level of which increases in proportion to the amount of body fat. In physiological conditions, leptin plays a crucial role in the regulation of energy balance by reducing appetite and increasing metabolism. Leptin has also been shown to promote cell proliferation and the development of breast cancer. Leptin and its receptors regulate progression, angiogenesis, metastasis and immunosuppression. Elevated serum leptin levels are associated with poor cancer prognosis, therefore it may be a potential biomarker of breast cancer risk, especially in overweight women or postmenopausal women [44, 45].

Biomarkers in liquid biopsy

Tumor biopsy is still the gold standard for diagnosis and classification of breast cancer, however, there is a growing need for personalized diagnostics based on tumor genome characterization, relying on blood samples known as liquid biopsy. The liquid biopsy, similar to serum biomarkers, is non-invasive, enables frequent sampling and following patients over time. It can deliver information for understanding tumor characteristics and cell dissemination. Various components of tumor cells are released into the bloodstream: circulating tumor cells (CTC) and circulating DNA (ctDNA), cell-free RNA and exosomes. These elements can be used as potential biomarkers personalizing cancer treatment based on these real-time results.

Circulating tumor cells (CTCs) are malignant cells that following apoptosis, necrosis or active release are shed into the lymphatic or vascular system. CTCs in the bloodstream could be responsible for metastatic progression of breast cancer. The presence of CTCs indicates residual disease, increased risk of metastasis and poorer results for CTC-positive patients. Tracking the presence of ctDNA in serial postoperative serum samples may be used as a predictor of early relapse in ctDNA-positive patients [46]. Some researchers observed that breast cancer patients with levels of CTCs lower than 5 per 7.5 ml had a higher progression-free survival and overall survival in comparison with patients with higher levels of CTCs [47]. The strong correlation between CTCs results and radiographically confirmed progression of metastatic breast cancer indicates that CTCs numeration is useful in assessing the effectiveness of therapy [48]. Although it can be difficult to isolate CTCs from blood due to their short half-life, they have proven to be beneficial as a prognostic tool for cancer patients. CTCs circulating in the bloodstream can also be analyzed for their contents such as protein, DNA, messenger/matrix RNA (mRNA), mitochondrial RNA (miRNA). One of the protein biomarkers contained in CTCs is for example CA-15-3 [49]. CTCs markers often reflect the genetic profile of tumors because they represent a part of the patient's tumor that could be assessed for target antigens. However, some difficulties have been observed in differentiating between primary and metastatic tumors with CTC origin. Researchers found that CTCs represented metastatic tumors rather than primary tumors. There is some evidence that primary ER+/PR+ breast tumors have spread CTCs that are ER–/ PR–, which have a significant importance in decisions regarding the choice of treatment [50]. Additionally, discrepancies between HER2 level of expression in ductal breast tumors and plasma CTCs have been observed, confirming the difference in expression profiling between CTCs and primary tumors [51].

Circulating tumor DNA (ctDNA) is fragmented DNA derived directly from tumor cells or circulating tumor cells (CTC). Cell-free DNA can be detected in free form in sera or plasma [52]. In healthy individuals, ctDNA is present at low levels, whereas higher levels of ctDNA in cancer patients reflect progressive tumor sizes, nodal involvement and metastasis.

Determination of circulating tumor DNA may serve as a marker for the presence of disease and a tool for molecular tumor assessment at different time points in the disease.

It has been proven that analyses of mutations in ctDNA could detect tumors at early stages [53]. CtDNA compared with DNA isolated from primary tumors shows the presence of identical genetic changes that are specific to the tumor type. At present, the diagnosis and selection of breast cancer treatment is based on the analysis of tumor biopsy, but the information from the biopsy is not permanent due to changes in the tumor and its resistance to treatment. Examination of ctDNA overcomes tumor heterogeneity. Some researchers report on ctDNA's platform detecting genomic changes in breast cancer patients, showing its clinical utility for monitoring of disease [54]. In breast cancer, ctDNA enables monitoring the response to treatment and clinical prognosis. In tumors responding to treatment, a sharp decrease in ctDNA levels is observed [55, 56]. The levels of ctDNA are very high in advanced cancer, therefore it is possible to perform a liquid biopsy for molecular testing of ctDNA which may serve as a non-invasive tool for real-time monitoring of disease development [57].

Tumorgenesis is accompanied by high gene expression which leads to synthesis of large amounts of RNA shed from the tumor cells into the blood. The released RNA particles are called cell-free mRNA (cfRNA), and consist of mRNA and miRNA. In cancer patients the amount and composition of miRNA is modified. CfRNA analysis is useful due to its higher concentration in the blood compared to ctDNA in patients at an early stage of cancer. Analysis of cfRNA provides valuable information about tumor gene expression that could be used to monitor treatment and drug resistance of the tumor. For instance, miRNA was used to predict resistance to trastuzumab in HER2+ metastatic breast cancer patients. A several type of miRNA with distinct expression of HER2+ metastatic breast cancer patients with different sensitivities to trastuzumab have been found [58]. The prognostic and predictive value of a real-time PCR assay for cytokeratin-19 (CK-19) mRNA isolated from CTCs has been evaluated. The study suggested that detection of CK-19 mRNA expression may have a clinical impact on overall survival in patients with breast cancer, since they showed poor overall survival [59].

Despite numerous reports on the benefits of liquid biopsy, it has not yet been standardized as a routine diagnostic method in clinical settings of breast cancer. It is expected that the sequencing of the genetic material contained in ctDNA and cfRNA obtained from liquid biopsy will lead to the implementation of this diagnostic tool for routine diagnosis, early detection and follow-up of breast cancer patients.

Biomarkers as a target for anticancer therapy

An important potential application of biomarkers in breast cancer management is their use as a target for anticancer treatment.

Progranulin (PGRN) promotes tumorigenesis as a growth factor since it stimulates the proliferation and survival of several cancer cell types [60]. Progranulin and its receptor sortilin are highly expressed in breast cancer and are associated with various clinical properties. PGRN is considered a poor prognostic factor because it inhibits tamoxifen-induced apoptosis [61]. The expression of progranulin in tumor and serum samples correlates with pathological grading, lymph node metastasis and angiogenesis [62]. Sortilin is linked to breast cancer progression and recurrence in advanced diseases [63]. High co-expression of progranulin and sortilin is associated with decreased breast cancer specific survival [64].

PGRN and sortilin targeting has potentials of application in novel targeted therapy of breast cancer consisting of blocking their tumor-promoting interplay. This offers a unique cancer treatment principle based on selectively targeting the microenvironment of the communication system. In vitro studies indicate that the use of PGRN-neutralizing antibodies and their receptors cause decreased expression of tyrosine-protein kinase and the tyrosine-protein kinase receptor involved in the metastasis of breast cancer [65]. Another in vitro study showed that inhibiting progranulin with the anti-progranulin antibody caused an inhibition of survival and a reduction in migration of TNBC cell lines. The decrease in Ki-67 expression and reduction in the expression of angiogenic proteins VEGF and HIF-1α was also observed [66]. Blocking PGRN with antibody treatment may provide novel-targeted solutions in TNBC treatment resulting in the inhibition of breast cell tumor proliferation. An *in vivo* study proved that sortilin inhibition decreases progranulin-dependent breast cancer progression and the expansion of cancer stem cells [67]. These results suggest that targeting PGRN may be involved in optimizing treatment protocols for breast cancer patients, however further *in vivo* studies regarding serum PGRN should be conducted.

Another emerging potential therapeutic target for breast cancer treatment is the androgen receptor (AR). AR been detected in around 70–90% of breast cancers [68]. AR is considered as a good prognostic factor in ER-α positive breast cancer, since it interferes with the function of ER-α and suppresses tumor growth. However, in the case of ER-α negative breast cancer patients such as HER2+ and TNBC, the AR exhibits oncogenic properties contributing to cancer development. Androgen receptor–targeted therapies have demonstrated promising results in clinical trials in patients with breast cancer. A potential treatment for breast cancer cells is a selective AR modulator such as enobosarm. In vitro studies in the cell line of TMBC indicate that enobosarm inhibits the metastasis promoting factors (IL-6, MPO-13) and therefore blocks migration and invasion. Several AR antagonists have been examined as well. Bicalutamide interrupts the DNA-binding domain binding to the androgen related element. The outcome of the application of bicalutamide has achieved a 19% clinical benefit rate at 6 months and 12 weeks median progression-free survival (PFS) in patients with AR-negative and AR-positive advanced breast cancer. Other biomarkers of response to AR inhibitors should be established in the future [69].

Immune checkpoints play a very important role in the regulation of immune responses involved in cancer elimination. One of them is the programmed cell death-1 receptor (PD-1). PD-1 is expressed in immune effector system cells such as T cells, B cells, natural killer cells and dendritic cells. It is activated by PD-L1, expressed by the majority of human cells. The PD-1/PD-L1 pathway is crucial in maintaining immune tolerance, thus creating a mechanism of immune escape in response to cancer. Cancer cells are capable of activating PD-1 on T cells specific for the cancer antigen by abnormally expressing programmed death-ligand 1 (PD-L1) on their surface. The PD-1/PD-L1 inhibitory pathway is used by solid tumors to silence the immune system [70]. PD-L1 expression is correlated with large tumor size, high grade and high proliferation rate, as well as being inversely related to the survival of breast cancer patients [71]. It has been proven that the blockade of immune checkpoints anti-PD-1/PD-L1 using appropriate monoclonal antibodies triggers effective anticancer responses in many types of solid tumors, such as breast cancers. The inhibitors against PD-1/PD-L1 prevent the suppression of anti-cancer immune responses, allowing the immune system to attack and eliminate tumor cells by modulation T-cell activation and suppressing tumor growth. Immune checkpoint inhibitors (ICIs) are new immunotherapeutic agents that interrupt the interaction between PD-1 and PD-L1.

The application of ICIs against PD-1/PD-L1 is emerging as a new treatment option in breast cancer [72]. The expression of PD-L1 is higher in TNBC than in other molecular subtypes of breast cancer. There are 2 monoclonal antibodies approved by the FDA to treat breast cancer: pembrolizumab and atezolizumab [73]. It was shown *in vivo* that responses to antibody therapy were greater in tumors with high PD-L1 expression. The presence of PD-1 and PD-L1 have been proposed as biomarkers predictive of a response to PD-1/PD-L1 inhibition. The antagonists of the PD-1/PD-L1 pathway induce clinical responses in some patients with metastatic TNBC [74]. However, there are some patients positive for PD-L1 who do not respond to the treatment, while some patients negative for PD-L1 may respond [75]. This makes PD-L1 an imperfect predictive biomarker. Tumor responses with anti-PD-1 and PD-L1 antibodies are mediated by tumor antigen-specific T cells that were previously blocked by the PD-1/PD-L1 pathway.

Awareness of the presence or absence of T cells in breast cancer is crucial in understanding the mechanisms of cancer escape from immune surveillance and for response to anti-PD-1 and PD-L1 antibody therapy. Decisions on the use of anti-PD-1/L1 antibody therapy should be based on the assessment of the presence or absence of T cells specific for the tumor antigen, which are inhibited by PD-L1 expression by tumor cells [76]. Tumor infiltrating lymphocytes (TILs) are an important biomarker in immunotherapy of breast cancer. The presence of tumor-infiltrating lymphocytes (TILs) is a favorable prognostic factor in breast cancer, since they interact with ICI therapy to improve the clinical response. A higher density of TILs has been associated with favorable clinical outcomes in breast cancer: a significantly lower risk of relapse or death, metastasis and overall mortality. To date, the strongest relationship between TILs and treatment outcomes has been demonstrated for the TNBC type of breast cancer [77]. Another study in HER2+ breast cancer patients treated with adjuvant trastuzumab found that increased levels of TILs were correlated with decreased distant recurrence [78].

Biomarkers in adverse events in anti-cancer therapy

Another potential field of application of breast cancer biomarkers is their application in monitoring the side effects of treatment.

The most serious toxic effect of chemotherapy in breast cancer treatment is heart muscle failure, known as so-called "cardiotoxicity". The role of anti-breast cancer drugs such as trastuzumab and anthracyclines in determining cardiotoxicity has been demonstrated in numerous studies [79, 80]. It has been proven that tumor-related inflammation is an important factor in the development and progression of heart failure. Many studies point to biomarkers of inflammation for the risk assessment of breast cancer patients treated for cancer in early detection of cardiotoxicity.

These inflammatory biomarkers are high-sensitivity C-reactive protein (hsCRP), myeloperoxidase (MPO), soluble growth stimulation expressed gene 2 (sST2), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), growth differentiation factor-15 (GDF-15), endothelin-1 (ET-1) and galectin-3. Two of these factors, sST2 and galectin-3, were recommended in the latest ACC/AHA HF (American College of Cardiology and American Heart Association, guideline for management of heart failure) guidelines to be used as useful in risk of heart failure stratification in clinical settings, since they are able to track treatment responses [81]. Additionally, troponins and creatinine kinase have been identified as the serum cardiac biomarkers of choice for assessing myocardial injury. Various studies have evaluated the role of natriuretic peptides (NPs) in the diagnosis and prediction of anticancer drug-induced cardiotoxicity [82].

Immunotherapy has significantly improved the prognosis for many breast cancer patients, but it can also generate a wide range of serious immune-related adverse events (irAEs) which can be serious and even fatal. IrAEs are autoimmune conditions that can affect any organ. The most common are dermatitis, diarrhea/colitis and endocrinopathies such as thyroid disorders. IrAEs appear later and have a longer duration compared to chemotherapy-related adverse events. Since IrAEs can interfere with treatment management, it would be helpful to determine IrAE-related biomarkers.

In monitoring the ICI treatment of breast cancer thyroiditis, biomarker levels are useful. Thyroiditis following ICIs in breast cancer patients should be detected by routine blood tests

of TSH and FT4 and morning cortisol levels for concurrent adrenal insufficiency. Baseline TSH levels were observed to be significantly higher in patients who developed hypothyroidism as the initial thyroid irAE. The association of hypothyroidism with baseline TSH levels may suggest progression of pre-existing Hashimoto's subclinical thyroiditis accelerated by ICI treatment rather than ICI-induced thyroiditis [83]. ICI treatment may be continued if patients with asymptomatic and subclinical hypothyroidism have elevated TSH but normal T4 levels [84]. Moreover, additional testing for thyroid peroxidase antibodies (TPOab) and thyroglobulin antibody (TgAb) is recommended. Some studies show an association between TPOAb and TgAb positivity at baseline and the incidence of thyroid irAE associated with ICI. The presence of TPOAb and TgAb was evident in patients who developed thyroid dysfunction. The titers of these antibodies were higher in patients with overt thyroid irAEs than in patients with or without subclinical thyroid irAEs. These results suggest that pre-existing thyroid autoimmunity may be a strong risk factor for the future development of ICI-associated thyroid toxicity [83].

Neurological adverse events associated with ICI and chemotherapy are of particular interest. One of them is chemotherapy-induced peripheral neuropathy (CIPN). The occurrence of CIPN often forces clinicians to change the course of therapy which is associated with a decrease in anti-cancer effectiveness. Therefore, it is necessary to determine the biomarkers of neurotoxicity.

In the blood serum of patients with severe CIPN, researchers observed significantly higher concentrations of neurofilament light chains (NF-L). NF-Ls are part of the cytoskeleton of peripheral and central nervous system neurons. Due to the damage to the peripheral nervous system in chemotherapy-induced peripheral neuropathy, NF-L is released to the cerebrospinal fluid. Very low concentration of NF-Ls are also detected in serum of treated patients. Previous studies have confirmed the relationship between the degree of CIPN and the increase in NF-L concentration, underlining NF-L's potential as a translational biomarker [84, 85].

A potential biomarker for ICI-associated neurotoxicity is the monocyte chemotactic protein 1 (MCP-1). MCP-1 is a chemoattractant and activator of monocytes, promoting their infiltration into the tumor, it also causes the production of angiogenesis factors that promote angiogenesis and stimulate cell proliferation. MCP-1 is one of the chemokines with the highest expression during inflammation. There are studies indicating that patients with higher-grade neurotoxicity had significantly elevated serum MCP-1 levels at baseline compared to patients without neurological adverse events [86].

Conclusions

Since breast cancer is one of the most prevalent diagnosed cancers among women, there is an expectation for developing new prognostic and predictive biomarkers that would provide accurate and reliable information for clinical applications. In recent years, particular emphasis has been placed on the development of personalized breast cancer diagnosis with the use of the liquid biopsy, enabling accurate characterization of the tumor. Through the recognition of emerging biomarkers, it is possible to identify subgroups of patients who benefit from targeted therapies and manage treatment by monitoring side effects. There is still a huge clinical need for new objective prognostic biomarkers for adverse events in breast cancer therapies**.** However, these new biomarkers need to be validated and tested for their suitability before entering clinical use.

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Agata Makówka – literature review and manuscript preparation. Beata Kotowicz – conception and critical revision of the article.

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