Biomarkers in breast cancer

Graciela Laguens*, Silvia Coronato, Wanda Di Girolamo

Cátedra de Patología B,
Facultad de Ciencias Médicas,
Universidad nacional de La Plata, Argentina

Abstract: Breast cancer is one of the most frequently diagnosed cancers among women in the western world. Due to the aggressive behaviour of some specific types and the possibility of an early diagnosis, breast cancer has been constantly studied. Tumour size, histological type, cellular and nuclear characteristics, mitotic index, vascular invasion, hormonal receptors and axillary lymph node status are biomarkers routinely used. However, these parameters are not enough to predict the course of this disease. Molecular biology advances have made it possible to find new markers, which have already been incorporated to the clinical practice. Their ultimate goal is to reduce mortality by identifying women at risk for the development of this disease, help diagnosis, determine prognosis, detect recurrences, monitor and guide treatment, and in particular cancers they are suited for general screening. Tumour markers in breast cancer were ranked in categories reflecting their clinical utility, according to the American College of Pathologists.

This article focuses on traditional and new molecular markers stratifying them into categories and emphasizing their relevance in the routine evaluation of patients with breast cancer.

© Versita Warsaw and Springer-Verlag Berlin Heidelberg. All rights reserved.

Keywords: Tumour markers, breast cancer

1 Introduction

Breast cancer is one of the most frequently diagnosed cancers among women in the western world. Due to the aggressive behaviour of some specific types and the possibility of an early diagnosis, breast cancer has been constantly studied in relation to diagnostic and treatment methods [1].

Morphological features such as tumour size, histological type, cellular and nuclear characteristics, mitotic index, necrosis, vascular invasion, hormonal receptors and axillary

* E-mail: glaguens@atlas.med.unlp.edu.ar

tumour lymph node status are routinely used. However, these parameters are not enough to predict the course of the disease. Molecular biology advances have made it possible to discover new markers, which have already been incorporated to the clinical practice and provide key information about the tumour biological behaviour and the potential response to radiation or chemotherapy guiding to the best therapeutics. In connection with this, the use of tumour markers panel gives more accurate information than that provided by just one factor. The fact described have made the cancer prognostic more exact than twenty years ago [2, 3].

Tumour markers (TMs) are biochemical indicators of cancer. Antigens of cellular surface, cytoplasm proteins, enzymes and hormones are included among them. In clinical practice, the term is used with reference to molecules that can be detected in plasma, body fluids, solid tumours, circulating tumor cells, lymph nodes and bone marrow [4]. Some tumour markers are specific for one type of cancer, whereas others are found in several cancer types. Antigens produced by tumour cells or associated to them, are found among the specific TMs, making them antigenically different from normal cells. Any protein of tumour cells can be a potential antigen. They are specially used to help diagnosis, determine prognosis, detect recurrence, monitor and guide treatment, and in particular cancers they are adequate for general screening.

1.1 Serum Markers

When TMs are released into bloodstream and reach enough concentrations, their detection can be used for the following purposes: 1.- screening a healthy population or a high risk population for the presence of cancer; 2.- performing a diagnosis of cancer or of a specific type of cancer; 3.- determining the prognosis of disease; 4.- monitoring the therapeutic response in patients under radiation or chemotherapy. In breast cancer, there are relatively few TMs that can be measured in blood. At present, CA 15-3 mucin and carcinoembryonic antigen (CEA) are found among the most utilized ones [5, 6].

1.2 Tissue markers

To detect tissue markers in cancer tissues, immunohistochemical (IHC) and immunofluorecence (IF) techniques can be performed. Enzyme-linked immunosorbent assay (ELISA) is used to visualize and quantify those markers against which specific monoclonal antibodies are formed. These techniques are very useful to identify intracellular and membrane antigens in tissue sections or in specimens obtained by puncture. Hormonal receptor detection by IHC is a widely used technique to evaluate the susceptibility of breast cancer to anti-estrogen therapeutics [7].

1.3 Tumour markers in breast cancer

Under the auspices of the American College of Pathologists in 1999 a multidisciplinary group of clinicians, pathologists and experts on statistics considered the use of tumour markers as prognostic and predictive factors in breast cancer and classified them into categories reflecting their clinical utility.

Factors were ranked in three categories (Table 1):

Category I: markers proven to be of prognostic value and useful in clinical patient management

Category II: markers which have been extensively studied clinically and biologically, but whose importance remains to be validated in statistical studies.

Category III: any other markers not sufficiently studied to demonstrate their prognostic value.

This categorization consists of a detailed outline of the findings and recommendations of the consensus conference group and offers an invaluable help for medical doctors and technicians dedicated to the clinical evaluation of breast cancer (Table 1) [8].

Table 1 Biomarkers in breast cancer.

Category I	Category II	Category III
Tumor size	c-erb B2	DNA Ploidy
State of Lymph Nodes	p53	Angiogenesis
Micrometastasis	Vascular invasion	EGFr
Sentinel node	Ki 67	bcl-2
Histological grade	DNA synthesis	pS2
Histological type		Cathepsine D
Mitotic index		
Hormonal state		

1.4 Category I

1.4.1 Tumour size

After lymph node status, tumor size (measured at least 2 dimensions and using the single greatest one) is the most important prognostic factor for patients with breast cancer. Precise assessment of tumor size is necessary to properly stratify the patient, particularly since screening mammography has resulted in a steadily increasing proportion of early cancers.

1.4.2 Lymph nodes status

The topographic level of nodal involvement and the number of axillary lymph nodes with metastasis are highly important prognostic factors since they are correlated to the disease-free survival, overall survival, cancer recurrence and failure of treatments.

1.4.3   Micrometastasis

Micrometastasis is defined as a histologically detected tumour foci measuring less than 2.0 mm. It can be detected by routine hematoxylin and eosin staining, and its prognostic value is widely recognized. Immunohistochemistry with anti-cytokeratin antibodies to detect unapparent foci of malignant epithelial cells in lymph nodes is even more sensitive. Micrometastasis has been correlated to vascular peritumoral invasion and tumour size [9].

1.4.4   Sentinel lymph node

Sentinel node is the first lymph station receiving tumor lymph drainage and is considered to be the place where the early metastases are found. Sentinel lymph node biopsy is now widely used to evaluate the status of the axilla and is very useful when the ordinary pathological evaluation is combined with anti-cytokeratin antibodies [10, 11].

1.4.5   Histological grading

In 1957 Bloom and Richardson [12] developed a grading system based on strict histological criteria. It combines tubule formation grade, nuclear features and mitotic figures count. These histological factors were scored with numbers from 1 to 3. The obtained point score allows tumour grading according to their degree of differentiation, which is an important determinant of prognosis.

In 1989, Helpap proposed a modification of this method, including nucleolar findings such as size, number and localization [13].

At present, the Nottingham Prognostic Index (NPI) is utilized, which is based on tumour size, histological grading and lymph nodes status [14].

1.4.6   Histological type

Some breast cancer types –medullar, tubular, mucinous or colloid carcinoma- are tumours with low grade of malignancy, usually with negative lymph nodes and favourable prognosis. Poorly differentiated carcinomas such as signet rings, inflammatory carcinoma and carcinosarcomas are considered more aggressive. Lobular infiltrating carcinoma exhibits a propensity for multicentricity and bilaterality [15].

1.4.7   Mitotic index

The number of mitotic figures in a delimited area of a tumour is an accurate means of estimating tumour cell proliferation. High mitotic index is correlated to a poor prognosis. This represents a part of the Nottingham combined histological grade (NPI).

1.4.8   Estrogen (ER) and progesterone (PR) receptors

The presence of steroid hormone receptors (estrogen receptor [ER] and progesterone receptor [PR]) represents a relatively weak prognostic factor for patients with breast cancer, but these receptors are the strongest predictive factors for the response to endocrine

therapy [16]. In recent years, immunohistochemistry has replaced the ligand-binding bio-chemical assay for the assessment of ER and PR status. In fact, this method is easier to perform and has been shown to be equal to or better than the biochemical assay in predicting the response to adjuvant endocrine therapy [17]. Estrogen and progesterone are essential hormones for breast growth since both of them bind to the nuclear receptors and regulate the transcription of several genes. Estrogens stimulate proliferation of ductal epithelial cells in normal mammary glands and play a key role in the development and the progression of breast cancer. Thus life-long exposure to estrogen plays an important role in development of breast cancer. Studies that have identified risk factors for breast cancer have found that women who had menarche at an early age or menopause at a later age have a higher risk of breast cancer. This also supports the theory that the number of menstrual cycles a woman, and hence the length of exposure to estrogen during her lifetime enhance the risk of breast cancer [18].

Several cell cycle regulatory proteins have been implicated in the ER-signaling pathway involved in estrogen-mediated growth stimulation and antiestrogen-mediated growth arrest. A cyclin-dependent kinase inhibitor such as p21 is a component of this pathway and can mediate the estrogen action in ER-negative breast cancer cells [19].

Approximately one third of breast cancer are ER-positive and are characterized by slow growth, high degree of differentiation, increase of the relapse-free and overall survival and good response to anti-estrogen drugs such as Tamoxifen. The antitumour effects of Tamoxifen are thought to be caused by its antiestrogen activity, mediated by competitive inhibition of estrogen binding to their receptors. However, most ER-positive tumors can eventually become resistant to this drug [20].

About 50% of all ER-positive breast tumors are also PR-positive and these double positive tumors have a mayor benefit with hormonal therapeutics, whereas ER-positive/PR-negative ones are very unlikely to equally respond to Tamoxifen.

Estrogen and progesterone receptors are recommended to be measured in both primary breast cancer and metastasis. In pre and postmenopausal patients, steroid hormone receptor status may be useful to identify patients who can benefit from endocrine forms of adjuvant therapy. Tamoxifen has been the mainstay of hormonal therapy in not only early but also advanced breast for approximately three decades. The availability of novel compounds such as aromatase inhibitors and fulvestrant, with different mechanisms of action, is changing the scenario of endocrine treatment of postmenopausal patients [21].

1.5   Category II

1.5.1   Her-2/neu (c-erbB-2)

c-erbB-2 is a proto-oncogene encoding a transmembrane glycoprotein with tyrosine-kinase activity. This receptor has homology to other family members (EGFR, erbB-3 and erbB-4). Immunochemical staining for detecting c-erbB-2 overexpression is more likely to be positive than fluorescence in situ hybridization (FISH) for quantifying c-erbB-2 gene amplification [22]. It is considered as a key prognostic factor in early stages of breast can-

cer and several studies have shown that they can be used as predictive markers related to chemotherapy and antiestrogen therapy responses. It is related to a poor prognosis, lymph node metastasis, p53 overexpression and an increase of cell proliferation. C-erbB-2 amplification is one of the most common genetic mutations associated to breast cancer, making them resistant to chemotherapeutic drugs [23].

High levels of c-erbB-2 expression or c-erbB-2 amplification can be used to identify patients for whom Trastuzumab (anti– c-erbB-2 antibody) may be of benefit for the treatment of metastasis, recurrent, and/or treatment-refractory, locally unresectable advanced breast cancer [24].

1.5.2   Protein 53 (p53)

The role of p53 is to maintain the integrity of genome. The activation of normal p53 leads to cell cycle arrest in G1 and induction of DNA repair. If DNA repair fails, p53 promotes apoptosis. In cells with loss or mutation of p53, DNA damage remains unrepaired, mutations become fixed in dividing cells and cells turn into a malignant transformation. p53 gene is located at chromosome 17 p13.1. Nearly over 50 % of all human tumours posses mutations of this gene. Nearly one third of breast cancers have mutations of p53, and this is associated with more aggressive and therapeutically refractory tumors [25]. Recent immunohistochemical studies suggest that p53 protein accumulation is associated with several other adverse prognostic factors such as high tumour grade, high proliferation rate, and ER and PR negativity [26].

1.5.3   Vascular invasion vascular and angiogenesis

Angiogenesis is a process consisting in the growth of new blood vessels from a preexisting vascular net. Neovascularization has a dual effect on tumor growth: perfusion supplies nutrients and oxygen and newly formed endothelial cells stimulate the growth of tumour cells by secreting growth factors. Angiogenesis is a requisite for not only tumour growth but also metastasis. A significant correlation between the extent of angiogenesis – measured through microvessel density- and the probability of metastases was found.

In breast cancer, vessel density has proven to be a significant prognostic indicator not only in patients with positive nodes but also in those with negative lymph nodes. Patients with a high grade of vascularization have a poor prognosis at early stages of the disease [27].

The most widely counting method was developed by Weidner and consists in the use of markers to stain endothelial cells. There are different staining methods: (H-E) and inmunohistochemistry with anti factor von-Willebrand, anti CD31 (vascular adhesion molecule) and anti CD34 (adhesion molecule) [28].

The clinical utility of these surrogate markers of angiogenesis in predicting clinical course has become a prognostic help especially with the latest refinements; the computerized morphometric analysis [29, 30]. Experimental and clinical data showed that breast carcinoma is an angiogenesis-dependent tumour and its hormonal receptor status is not correlated to angiogenesis [31–33].

1.5.4   Ki 67 (mib 1 antibody)

Proliferation rate in breast cancer can be measured by immunohistochemistry with the antibody mib 1, Ki 67. It is a non-histone nuclear protein found in all phases of cell cycle, except in G0. Its prognostic value is independent of age, nodal status and hormonal status. Nevertheless, it was found that it is inversely correlated with estrogen receptors status and indicates a poor prognosis. There may be a direct relationship between cell fraction in S-Phase measured by flow cytometry and the IHC tissue detection with Ki 67 [34].

1.5.5   DNA Synthesis

DNA content can be quantifiable by flow cytometry analysis or image analysis of tissue sections with IHC using antibody PCNA (cell nuclear antigen). PCNA is a non-histone nuclear protein of 36 Kda functioning as accessory of DNA polymerase δ and is related to DNA synthesis and cellular proliferation. This protein is detected in the late G1-phase of cell cycle, immediately before the onset of S-phase, where its maximum value is found, declining during G2 and M. PCNA has shown to be an independent prognostic indicator in predicting disease-free and overall survival in breast carcinoma patients [35].

1.6   Category III

1.6.1   DNA Ploidy

In addition to determine S-fraction, DNA analysis is useful for identifying tumours with abnormal DNA profiles (aneuploidy). The degree of DNA abnormality is established by an index which is the ratio between G0-G1 peak locations of tumour cells and normal cells. Until recently this parameter has not been considered as an independent prognostic marker and remains in research phase.

Common criteria for classifyng abnormalities of DNA content seem to be inadequate for the dynamics of genome instability characterizing human tumors [36].

1.6.2   Vascular endothelial growth factor (VEGF)

VEGF is an important regulator of angiogenesis and vascular permeability and it is considered the most powerful mitogen for endothelial cells. It is secreted by tumour cells and by stromal monocytes and macrophages, responding to stimuli such as hypoxia or cytokines (IL 1, 3, 6 and 10). The level of circulating VEGF provides a less subjective analysis than IHC. In breast cancer VEGF is used as a marker of unfavourable prognosis since it is associated to recurrence. Because angiogenesis is essential for growth and tumour progression, the use of its inhibitors is becoming of interest as co-adjuvant of other therapeutics. It has been observed that patients with metastasis have higher levels of serum VEGF and could benefit from anti-VEGF treatment [37].

1.6.3 Epidermal growth factor receptor (EGFR)

EGFR, also known as erbB1 receptor, has a tyrosine–kinase activity and it is involved in the division of epithelial cells and fibroblasts induced by EGF. It is overexpressed in 30 % of breast cancer. EGFR expression in primary breast cancer has been extensively investigated in its prognostic and predictive value [38]. It is associated with a shorter disease-free period and a decrease of overall survival, showing an inversed relationship with the highly significant expression of ER. Consequently, the response to the hormonal therapeutics seems to be impaired. Several anti-EGFR agents are being clinically tested on patients with breast cancer. A better understanding of the EGFR pathways will facilitate the identification of patients prone to respond to these agents [39].

1.6.4 Cathepsin D

One of the most reliable features of malignant cells is their invasiveness. In order to accomplish this, malignant cell sub-clones separate from the rest of the tumour mass, penetrate the basement membrane, adhere to the matrix components to which they degrade, and then they migrate through the vascular basement membrane and reach circulation. Several protease families are implied in the degradation of extracellular matrix: plasminogen activator urokinase, cathepsins B, D and L and several metalloproteinases. In breast cancer, there is a close relationship between high levels of proteases with an unfavorable prognosis of the disease.

Cathepsin D (CathD) is a lysosomal aspartylprotease induced by estrogen and growth factors that can be expressed not only in ER-positive breast carcinoma but also in ER-negative ones [40]. CathD overexpression in breast cancer is associated with recurrence, metastasis and a shorter disease-free and overall survival [41].

1.6.5 Bcl-2

Bcl-2 is a member of a gene family whose function is to regulate apoptosis.

It is frequently expressed in breast cancer and is related to low rate of proliferation, high degree of differentiation, low expression of stromal cathepsin D, DNA diploidy and ER expression.

Bcl-2 expression is more frequent in breast carcinoma of lobular than of ductal type. The reason for this difference is unknown, but it could reflect the different histological origin of both types of tumours. It is considered that breast cancer expressing this protein has more favorable outcome and good response to Tamoxifen [42].

1.7 Tumour markers not included in categorization

1.7.1 Mucins

Mucins are large glycoproteins with high carbohydrate content (50 to 90 % by weight). They are expressed by a diversity of normal and malignant epithelial cells. One of the most studied ones is MUC1. Cancer-associated MUC1 is incompletely glycosilated. It

exposes internal sugar units and non exposed peptide sequences, being essential for the normal molecule [5].

Mucins have diverse functions: they mediate in epithelium morphogenesis, cytosketal remodeling and downregulate other adhesion molecules. The increase of MUC1 expression by tumour cells might facilitate detachment from original tumoral mass and from cellular matrix. During the process of bloodborne metastasis, MUC1 can protect tumour cells from destruction by natural killer cells or other immune cells [43].

MUC1, is commonly detected in serum such as CA 15.3 or CA 27.29 and can be utilized to monitor endocrine or chemotherapy and predict metastasis in advanced disease. CA 15.3 is not breast cancer-specific since a proportion of patients with prostate, ovary and pancreas cancer also show high serum levels of this mucin. 50% of the patients with breast cancer in stage IV and between 10 and 20% in stage II show high levels [44].

CA27.29 is similar to CA15.3 but is more specific. Both mucins can help clinically for the follow-up and handling of patients with advanced breast cancer. They are also used in asymptomatic population screening. High levels of mucins are associated with a poor prognosis and progression in some types of cancers.

Recently another mucin, MUC5B, is being investigated and may be considered as a potential marker of tumour cell dissemination to bone marrow [45].

1.7.2   Breast cancer 1 and 2 (BRCA-1 and BRCA-2)

Nearly 5 to 10% of breast cancers are associated with a hereditary predisposition and 80 % are related to two suppressor genes mutations: BRCA-1 and BRCA-2. These genes encode nuclear phosphoproteins interacting with multiple biological processes such as damaged DNA repair, regulation of the transcription, centrosome duplication and negative regulation of cell cycle. In spontaneous breast cancer gene, mutations are not common. The gene encoding BCRA-1 was isolated in chromosome 17 and BRCA-2 gen was isolated in chromosome 13 [46]. Not every woman expressing these mutated suppressor genes will develop breast cancer. BRCA-1 mutation is associated to a poorly differentiated, RE negative and Her- 2/neu positive breast carcinoma. This mutation also increases the risk of ovarian cancer. On the other hand, mutation of both genes enhances the risk of more aggressive bilateral breast cancer and also predisposes to colon, prostate and pancreas cancer [47].

1.7.3   Transforming growth factor α (TGFα)

TGFα is a polypeptide closely related to EGF, which has been shown to have a stimulatory effect on the growth of some types of breast cancers. The receptor binds both EGF and TGF. It can stimulate angiogenesis and cellular proliferation. In breast cancer, TGFα overexpression is associated with the presence of positive lymph nodes, absence of ER and poor response to Tamoxifen [48].

1.7.4 Protease: uPA

Uroquinase-type plasminogen activator (uPA) is a 53Kd serine kinase converting plasminogen into plasmine. It is involved in cancer invasion and metastasis and it has been shown to be a prognostic marker in breast cancer. This serine kinase produces degradation of extracellular matrix, stimulates mitotic rate and cellular migration, induces cell adhesion molecules expression and promotes angiogenesis. It has been recently demonstrated that uPA prevents apoptosis, increasing survival of the malignant cells during metastasis and was the first tumor marker that had been clinically evaluated. It is routinely utilized to determine the prognosis in patients with breast cancer, especially in advanced stage or in indolent course when lymph nodes are negative. Node-negative patients with high levels of uPA in breast tumor tissue might benefit with adjuvant therapy, whereas those uPA-negative patients can prevent side effects and the cost of the treatment [49].

1.8 Oncogenes and tumour suppressor genes

1.8.1 c-myc gene

c-myc is a gene encoding a nuclear phosphoprotein functioning as a cell cycle regulator. In normal cells it rises during phase G1, but in transformed cells it may be continuously expressed during the whole cycle. Amplification of c-myc has been found in breast cancer. It is related to a short survival and early recurrence of the disease. Genetic alterations of c-myc oncogen play a key role in induction and progression of breast cancer. Its amplification is related to a poor prognosis not only in lobular but also in ductal carcinoma. It does not seem to be associated to other prognostic factors. When c-myc gene is not altered, the presence of the protein is related to a low incidence of metastasis in axillary lymph nodes [50].

1.8.2 Rb gene

Gene family of retinoblastoma , one of the best studied tumor suppressor genes, consists of three members: gene product (pRb) and two related proteins: pRb2/130 and p107, which are structural and functionally similar to pRb. All of them show properties of inhibiting growth cell. pRb2/130 is a possible target to be used in gene therapy [51].

1.8.3 E2F-1

E2F1 protein is a nuclear transcription factor whose activity is regulated by Rb protein. Its increase is correlated to other prognostic factors such as tumour grade, metastasis, ER, PgR and p53. Therefore, it could be used as a prognostic marker [52].

1.8.4 Heat shock proteins (Hsps)

Some of the highly conserved heat shock proteins also called stress response proteins, such as Hsp27 is constitutively expressed in normal tissue breast. In malignant cells, overexpression of this molecule is frequently found. Most authors find correlation between

Hsp 27 overexpression, ER, metastatic lymph nodes and vascular invasion. According to these parameters, the protein could be a marker of tumour aggressiveness [53, 54].

It has been observed that Hsp27 induces resistance to chemotherapy. The modulation of expression levels of Hsp could be used in clinical application in order to be in detriment of the resistance to drugs and control tumoral growth [55].

In experiments performed in our laboratory [56], we demonstrated that there is a significant correlation between the absence of Hsp 27 expression by tumour cells of breast cancer and the presence of metastasis in axillary nodes. Its highest expression is correlated with early stages of breast cancer.

1.8.5 Cytokeratins

Cytokeratin-19: the molecular study with PCR of mRNA positive cells for CK-19 in peripheral blood of patients with breast cancer is the most sensitive method and allows the detection of occult tumour cells. Several studies have shown that this protein can be detected in patients before or after the treatment with chemotherapy drugs [57, 58].

1.9 Adhesion molecules

1.9.1 Ep-CAM

Epithelial cell adhesion molecule (Ep-CAM) is a glycoprotein expressed in most epithelia and in tumours derived from this tissue. Ep-cam overexpression is an indicator of a poor prognosis, independent of other parameters like tumour size, histological grade, expression of hormonal receptors and c-erbB-2 [59, 60].

1.9.2 Alcam

Activated leucocyte cell adhesion molecule (ALCAM) is a molecule intervening in cellular migration. It belongs to the superfamily of immunoglobulines and is expressed in the normal breast. Its decreased expression in breast cancer would be associated to a more aggressive phenotype and poor prognosis [61].

2 Conclusions

The identification of new biological and molecular indicators of clinical outcome and the response to therapy in patients with breast cancer has been an area of active investigation during the last two decades. Although numerous biological and molecular markers have been identified during this period, traditional factors such as lymph node status, tumour size, histological type, histological grade and hormone receptor status remain as the most useful indicators of prognosis and therapeutic response. Recent advances in the understanding of breast cancer biology have made it possible to develop new molecular markers, with potential utility in identification, screening, prognosis, detection and monitoring. Nevertheless, it is difficult to translate research advances into prognostic and predictive markers that are useful in clinical management.

In 1999, a multidisciplinary group of pathologists, clinicians and statisticians reviewed prognostic and predictive factors of breast cancer and categorized them into three groups reflecting their clinical utility.

Category I: Well supported by the literature. They are generally used in patient management (tumour size, lymph node status, histological type, histological grade, mitotic index, and hormone receptor status). Sentinel node biopsy is added.

Category II: Markers have been extensively studied biologically, clinically or both and tested in clinical trials, but more studies are necessary to statistically validate, c-erbB-2, p53, vascular invasion and proliferation markers like Ki-67 or PCNA. C-erbB-2 is mainly utilized in patients with advanced breast cancer in order to evaluate the potential response to Trastuzumab [62].

Category III: Any other factors not sufficiently studied to demonstrate their prognostic value (ploidy, cathepsin D, intratumoral angiogenesis, EGFR, Bcl-2).

The determination of tumour angiogenesis expressed as density of microvessels by microscopic field is considered of great importance since there are papers supporting this correlation with the grade of neoplastic proliferation in breast cancer and also with the prediction of micrometastasis in bone marrow in clinically metastasis-free patients [63].

Recommendations are presented for the routine clinical use of serum and tissue-based markers in the diagnosis and management of patients with breast cancer. Their low sensitivity and specificity preclude the use of serum markers such as the MUC-1 mucin glycoproteins (CA 15.3, BR 27.29) and CEA in the diagnosis of early breast cancer, but their serial measurement can be useful in early detection of recurrences.

Since breast cancer is a hormone-dependent cancer, estrogen and progesterone receptor status is important to predict the likelihood of response to hormonal therapies.

Her-2 detection may support selection of optimal therapy for breast cancer patients with antibody directed against this protein or adjuvant chemotherapy in advanced breast cancer. Mucin CA 15.3 is used to monitor the efficacy of treatment and detect recurrences. CA 27.29 may predict disease relapses and can be used to detect when the treatment fails [64].

Urokinase plasminogen activator (uPA) has been recently validated as prognostic markers in patients with breast cancer and metastasis- free lymph nodes and thus may be of value in selecting node-negative patients that do not require adjuvant chemotherapy [65].

Although high Ki-67 is a sign of poor prognosis, it is associated with a good chance of clinical response to chemotherapy. Its independent significance is modest and does not merit measurement in clinical routine. However, this molecule is becoming a useful tool for evaluation of the effectiveness of medical therapy and rapid evaluation of new drugs [66]. Based on data of preclinical models, several antiangiogenic compounds have been shown to modify activated tumour endothelium, suggesting that these compounds can improve cytotoxic drug delivery [67].

This brief and concise review describes both traditional and new molecular bio-

markers with great potential to become useful tools to determine prognosis, detect recurrences, design therapeutic strategies and monitor treatment in breast cancer.

References

[1]   K. Dhingra and G.N. Hortobagyi: "Critical evaluation of prognostic factors", *Sem. Oncol.*, Vol. 23, (1996), pp. 436-445.

[2]   J. Sjostrom, C. Blomqvist, P. Heikkila, K.V. Bogulawski, A. Raiseanen-Sokolowski, N.O. Bengtsson, I. Mjaaland, P. Malmstron, B. Ostenstadt, J. Berg, E. Wist, V. Valvere and E. Saksela: "Predictive value of p53, mdm-2, p21, and mib-1 for chemotherapy response in advanced breast cancer", *Clin. Cancer Res.*, Vol. 6(8), (2000), pp. 3103–3110.

[3]   K.L. Cheung, C.R. Graves and J.F. Robertson: "Tumour marker measurements in the diagnosis and monitoring of breast cancer", *Cancer Treat. Res.*, Vol. 26, (2000), pp. 91–102.

[4]   V. Stearns, H. Yamauchi and D.F. Hayes: "Circulating tumour markers in breast cancer: accepted utilities a novel prospect", *Breast Cancer Res. Treat.*, Vol. 52, (1998), pp. 239–259.

[5]   J. Martinez-Trufero, A.R. de Lobera, J. Lao, T. Puertolas, A. Artal-Cortes, M. Zorrilla, V. Alonso, R. Pazo, M.I. Valero, M.J. Rios-Mitchell, V. Calderero, A. Herrero and A. Anton: "Serum markers and prognosis in locally advanced breast cancer", *Tumori*, Vol. 91, (2005), pp. 522–530.

[6]   D. Haidopoulos, M.M. Konstadoulakis, P.T. Antonakis, D.G. Alexiou, A.M. Manouras, S.M. Katsaragakis and G.F. Androulakis: "Circulating anti-CEA antibodies in the sera of patients with breast cancer", *Eur. J. Surg. Oncol.*, Vol. 26, (2000), pp. 742–746.

[7]   D. Allred, J. Harvey, M. Berardo and G. Clark: "Prognostic and predictive factors in breast cancer by immunohistochemical analysis", *Mod. Pathol.*, Vol. 11, (1998), pp. 155–168.

[8]   P.L. Fitzgibbons, D.L. Page, D. Weaver, A.D. Thor, D.C. Allred, G.M.. Clark, S.G. Ruby, F.O'Malley, J.F. Simpson, J.L. Conolly, D.F. Hayes, S.B. Edge, A.Lichter and S.J. Schnitt: "Prognostic factors in breast cancer", *Arch. Pathol. Lab. Med.*, Vol. 124, (2000), pp. 966–978.

[9]   R. Heimann and S. Hellman: "Individual characterisation of the metastatic capacity of human breast carcinoma", *Eur. J. Cancer*, Vol. 36, (2000), pp. 1631–1639.

[10] E.L. Dupont, M.A. Kuhn, C. McCann, C. Salud, J.L. Spanton and C.E. Cox : "The role of sentinel lymph node biopsy in women undergoing prophylactic mastectomy", *Am. J. Surg.*, Vol. 180, (2000), pp. 274–277.

[11] P.J. Borgstein, S. Meijer, R.J. Pijpers and P.J. van Diest: " Functional lymphatic anatomy for sentinel node biopsy in breast cancer: echoes from the past and the periareolar blue method", *Ann. Surg.*, Vol. 232, (2000), pp. 81–89.

[12] H.J.G. Bloom and W.W. Richardson: "Histological grading and prognosis in breast cancer: A study of 1709 cases of which 359 have been followed for 15 years", *Br. J. Cancer*, Vol. 2, (1957), pp. 353–377.

[13] B. Helpap: "Nucleolar grading of breast cancer", *Virchows Archiv. A Pathol. Anat.* Vol. 415 (1989), pp. 501–508.

[14] I.O. Elis, C.W. Elston and R.W. Blamey: "The Nottingham prognosis index (NPI) a combination of multiple prognostic factors derived from the Nottingham Tenovus primary cancer study", *Mod. Pathol.*, Vol. 4, (1991), p. 11.

[15] E.R. Fisher and B. Fisher: " Lobular carcinoma of the breast: an overview", *Ann. Surg.,* Vol. 185, (1977), pp. 377-385.

[16] X. Cui, R. Schiff, G. Arpino, C.K. Osborne and A.V. Lee: "Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy", *J. Clin. Oncol.,* Vol. 23, (2005), pp. 7721–7735.

[17] B. Jasani , A Douglas-Jones , A. Rhodes, S. Wozniak, P.J. Barrett-Lee, J. Gee and R. Nicholson: "Measurement of estrogen receptor status by immunocytochemistry in paraffin wax sections", *Methods Mol. Med.*, Vol. 120, (2006), pp. 127–146.

[18] J.D.Yager and N.E. Davidson: "Estrogen carcinogenesis in breast cancer", *N. Engl. J. Med.,* Vol. 354, (2006), pp. 270–282.

[19] X.Chen , C. Danes, M. Lowe, T.W. Herliczek and K. Keyomarsi: "Activation of the Estrogen-Signaling Pathway by p21 (WAF1/CIP1) in Estrogen Receptor-negative breast cancer cells", *J. Natl. Cancer Inst.,* Vol. 92, (2000), pp. 1403–1413.

[20] R. Schiff, S.A. Massarweh, J. Shou, L. Bharwani, G. Arpino, M. Rimawi and C.K. Osborne: "Advanced concepts in estrogen receptor biology and breast cancer endocrine resistance: implicated role of growth factor signaling and estrogen receptor coregulators", *Cancer Chemother. Pharmacol.*, Vol. 56, (2005), pp. 10–20.

[21] N. Normanno, M. Di Maio, E. Di Maio, A. De Luca, A. de Matteis, A. Giordano and F. Perrone: "Mechanisms of endocrine resistance and novel therapeutic strategies in breast cancer", *Endocr. Relat. Cancer,* Vol. 12, (2005), pp. 721–747.

[22] A. Starr, K. Bloom, D. Oleske and M. Cobleigh: "Comparison of fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) in determining HER-2/neu status in breast cancer patients", *Proc. Am. Soc. Clin. Oncol.,* 2000 ASCO Annual Meeting, abstract No. 2610.

[23] A.A. Sahin: "Biologic and clinical significance of HER-2/neu (c-erbB-2) in breast cancer", *Adv. Anat. Pathol.,* Vol. 7, (2000), pp. 158–166.

[24] F.J. Esteva, C.D. Cheli, H. Fritsche, M.D. Slamon, R.P. Thiel, D. Luftner and G. Farooq: "Clinical utility of serum HER2/neu in monitoring and prediction of progression-free survival in metastatic breast cancer patients treated with trastuzumab-based therapies", *Breast Cancer Res.*, Vol. 7, (2005), pp. 436–443.

[25] L.D. Miller, J. Smeds, J. George, V.B. Vega, L. Vergara, A. Ploner, Y. Pawitan, P. Hall, S. Klaar, E.T. Liu and J. Bergh: "An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival", *Proc. Natl. Acad. Sci. USA.*, Vol. 102, (2005), pp. 13550–13555.

[26] S.J. Schnitt: "Traditional and Newer Pathologic Factors", *J. Nat. Cancer Ins. Mon.*, Vol. 30, (2001), pp. 22–26.

[27] H. Takei, Y. Iino, J. Horiguchi, M. Maemura, H. Nagaoka, Y. Koibuchi, T. Yokoe, T. Oyama and Y. Moreshita : "Highest microvessel count as a long-term prognostic factor in Japanase breast cancer patients", *Cancer Lett.*, Vol. 156, (2000), pp. 109–116.

[28] G. Gasparini: "Clinical significance of determination of surrogate markers of angiogenesis in breast cancer", *Crit. Rev.Oncol. Hematol.*, Vol. 37, (2001), pp. 97–114

[29] J. Boyle: "Histological measurement of tumour angiogenesis", *Eur. J. Cancer,* Vol. 39, (2003), pp. 859–860.

[30] D. Cruz, C. Valenti, A. Dias, M. Seixas and F. Schmitt: "Microvessel density counting in breast cancer. Slides vs. digital images", *Anal. Quant. Cytol. Histo.,* Vol. 23, (2001), pp. 15–20.

[31] G. Gasparini: "Prognostic value of vascular endothelial growth factor in breast cancer", *Oncologist,* Vol. 5, (2000), pp. 37–44.

[32] G. Callagy, E. Dimitriadis, J. Harmey, D. Bouchier-Hayes, M. Leader and E. Kay: "Immunohistochemical measurement of tumor vascular endothelial growth factor in breast cancer. A more reliable predictor of tumor stage than microvessel density or serum vascular endothelial growth factor". *Appl. Immunohistochem. Molecul. Morphol.,* Vol. 8, (2000), pp. 104–109.

[33] N. Boudreau and C. Myers: "Breast cancer-induced angiogenesis: multiple mechanisms and the role of the microenvironment", *Breast Cancer Res.*, Vol. 5, (2003), pp. 140–146.

[34] P.H. Tan, B.H. Bay, G.Yip, S. Selvarrajan, P. Tan, J. Wu, C.H. Lee and K. B. Lee: "Immunohistochemical detection of Ki 67 in breast cancer correlates with transcriptional regulation of genes related to apoptosis and cell death", *Mod. Pathol.,* Vol. 18, (2005), pp. 374–381.

[35] S.A. Aziz, S. Pervez, S.M. Khan, N. Kayani and M. I. Nasir: "Prognostic value of proliferating cell nuclear antigen (PCNA) in infiltrating ductal carcinoma breast", *J. Coll. Physicians Surg. Pak.*, Vol. 15, (2005), pp. 225–229.

[36] A. Sidoni , G.A. Cavalieri, G. D'Amico, G. Brachelente and E. Bucchiarelli: "Biopathological significance of single cell DNA aneuploidy measured by static cytometry in breast cancer", *The Breast*, Vol. 10, (2001), pp. 325–329.

[37] J. Adams, P.J. Carder, S. Downey, M.A. Forbes, K. MacLennan, V. Allgar, S. Kaufman, S. Hallam, R. Bicknell, J.J. Walker, F. Cairnduff, P.J. Selby, T.J. Perren, M. Lansdown and R.E. Banks: "Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum and tissue VEGF and microvessel density and effects of tamoxifen", *Cancer Res.*, Vol. 60, (2000), pp. 2898–2905.

[38] R.S. Rampaul, S.E. Pinder, R.I. Nicholson, W.J. Gullick, J.F. Robertson and I.O. Ellis: "Clinical value of epidermal growth factor receptor expression in primary breast cancer", *Adv. Anat. Pathol.*, Vol. 12, (2005), pp. 271–273.

[39] H.W. Lo, S.C. Hsu and M.C. Hung: "EGFR signaling pathway in breast cancers: from tradicional signal transduction to direct nuclear translocalization", *Breast Cancer Res. Treat.*, Vol. 28 (2005), pp. 1–8.

[40] H. Rochefort, M. Garcia, M. Glondu, V. Laurent, E. Liaudet, J. Rey and P. Roger: "Cathepsin D in breast cancer: mechanisms and clinical applications, a 1999 overview", *Clin. Chim. Acta*, Vol. 291, (2000), pp. 157–170.

[41] A.M. Billgren, L.E. Rutqvist, H. Johansson, T. Hagerstrom and L. Skoog: "The role of cathepsin D and PAI-1 in primary invasive breast cancer as prognosticators and predictors of treatment benefit with adjuvant tamoxifen", *Eur. J. Cancer* , Vol. 36, (2000), pp. 1374–1380.

[42] R. González Cámpora, M. Galera Ruiz, F. Vázquez Ramírez, J.J. Rios Martín, J.M. Fernández Santos, M.M. Martos and A. Gómez Pascual: "Apoptosis in breast carcinoma", *Pathol. Res. Pract.*, Vol. 196, (2000), pp. 167–174.

[43] B. Agrawal, M. Krantz, M. Reddish and B. Longenecker: "Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2". *Nat. Med.,* Vol. 4, (1998), pp. 43–49.

[44] E.A. Rakha, R.W. Boyce, D. Abd El-Rehim, T. Kurien, A.R. Green, E.C. Paish, J.F. Robertson and I.O. Ellis: "Expression of mucins (MUC1, MUC5AC and MUC6) and their prognostic significance in human breast cancer", *Mod. Pathol.*, Vol. 8, (2005), pp. 1295–1304.

[45] N. Berois N, M. Varangot and C. Sonora: "Detection of bone marrow-disseminated breast cancer cells using an RT-PCR assay of MUC5b mRNA", *Int. J.Cancer,* Vol. 103, (2003), pp. 550–555.

[46] K. Yoshikawa, T. Ogawa, R. Baer, H.Hemmi, K. Honda, A. Yamauchi, T. Inamoto, K. Ko, S. Yazumi, H. Motoda, H. Kodama, S. Noguchi, A.F. Gazdar, Y. Yamaoka and R. Takahashi: "Abnormal expression of BCRA1 and BCRA2- interactive DNA-repair proteins in breast carcinomas", *Int. J. Cancer,* Vol. 88, (2000), pp. 28–36.

[47] J. Rogozinska-Szczepka, B. Utracka-Hutka, E. Grzybowska, B. Maka, E. Nowicka, A. Smok-Ragankiewicz, H. Zientek, J. Steffen and A. Wojciechowska-Lacka: "BRCA1 and BRCA2 mutations as prognostic factors in bilateral breast cancer patients", *Ann. Oncol.,* Vol. 15, (2004), pp. 1373–1376.

[48] P.K. Auvinen, P.K. Lipponen, V.V. Kataja, R.T Johansson and K. J. Syrjanen: "Prognostic significance of TGFα expression in breast cancer", *Acta Oncologica*, Vol. 35, (1996), pp. 995–998.

[49] M.J. Duffy: "Urokinase plasminogen activator and its inhibitor, PAI-1, as prognostic markers in breast cancer: from pilot to level 1 evidence studies", *Clin. Chem.*, Vol. 48, (2002), pp. 1194–1197.

[50] P. Chrzan, J. Skolowski, A. Karmolinski and T. Pawelczyk: "Amplification of c-myc gene and overexpression of c-Myc protein in breast cancer and adjacent non-neoplastic tissue", *Clin. Biochem.*, Vol. 34, (2001), pp. 557–562.

[51] V. Masciullo, K. Khalili and A.Giordano: "The Rb family of cell cycle regulatory factors: clinical implications" (Review), *Int. J. Oncol.,* Vol. 17, (2000), pp. 897–902.

[52] S. Zhang, S.C. Liu, L.F. Al-Saleem, D. Holloran, J. Babb, X. Guo and A.J. Klein-Szanto: "E2F-1: a proliferative marker of breast neoplasia", *Cancer Epidemiol. Biomarkers Prev.,* Vol. 9, (2000), pp. 395–401.

[53] S. Coronato, V. Di Girolamo, M. Salas, O. Spinelli and G. Laguens: "Biología de las proteínas del shock térmico", *Medicina, Buenos Aires,* Vol 59, (1999), pp. 477–486.

[54] D.R. Ciocca and S.K. Calderwood: "Heat shock proteins in cancer: diagnostic, prognostic, predictive and treatment implications", *Cell Stress Chaperons*, Vol. 10, (2005), pp. 86–103.

[55] S.K. Calderwood, M.A. Khaleque, D.B. Sawyer and D.R. Ciocca: "Heat shock proteins in cancer: chaperones of tumorigenesis", *Trends Biochem. Sci.*, Vol. 31, (2006), pp. 164–172.

[56] G.E. Laguens, S. Coronato, O. Spinelli, R.P. Laguens and V. Di Girolamo: "Can breast cancer Hsp27 (Heat Shock Protein 27000) expression influence axillary lymph node status?", *The Breast*, Vol. 10, (2001), pp. 179–181.

[57] A. Stathopoulou, I. Vlachonikolis, D. Mavroudis, M. Perraki, Ch. Kouroussis, S. Apostolaki, N. Malamos, S. Kakolyris, A. Kotsakis, N. Xenidis, D. Reppa and V. Georgoulias: "Molecular detection of cytokeratin-19-positive cells in the peripheral blood of patients with operable breast cancer: evaluation of their prognostic significance", *J. Clin. Oncol.,* Vol. 20, (2002), pp. 3404–3412.

[58] N. Xenidis, I. Vlachonikolis, D. Mavroudis, M. Perraki, A. Stathopoulou, N. Malamos, Ch. Kouroussis, S. Kakolyris, S. Apostolaki, N. Vardakis, E. Lianidou and V. Georgoulias : "Peripheral blood circulating cytokeratin-19 mRNA-positive cells after the completion of adjuvant chemotherapy in patients with operable breast cancer", *Ann. Oncol.*, Vol. 14, (2003), pp. 849–855.

[59] G. Spizzo, G. Gastl, D. Wolf, E. Gunsilius, M. Steurer, D. Fong, A. Amberger, R. Margreiter and P. Obrist: "Correlation of Cox-2 and Ep-CAM overexpression in human invasive breast cancer and its impact on survival", *Br. J. Cancer*, Vol. 88, (2003), pp. 574–578.

[60] G. Spizzo, P. Obrist, C. Ensinger, I. Theurl, M. Dunser, A. Ramoni, E. Gunsilius, G. Eibl, G. Mikuz and G. Gastl: "Prognostic significance of EP-CAM and Her-2/neu overexpression in invasive breast cancer" *Int.J.Cancer*, Vol. 98, (2002), pp. 883–888.

[61] J.A. King, S. Ofori-Acquah and T. Stevens: "Activated leukocyte cell adhesion molecule in breast cancer: prognostic indicator", *Breast Cancer Res.,* Vol. 6, (2004), pp. 478–487.

[62] M.J. Duffy: "Predictive markers in breast and other cancers: a review", *Clin. Chem.,* Vol. 51, (2005), pp. 494–503.

[63] I. Benoy, R. Salgado, H.Elst, P. Van Dam, J. Weyler, E. Van Marck, S. Scharpe, P.B. Vermeulen and L.Y. Dirix: "Relative microvessel area of the primary tumour, and not lymph node status, predicts the presence of bone marrow micrometastases in patients with clinically non-metastatic breast cancer", *Breast Cancer Res.,* Vol. 7, (2005), pp. 210–219.

[64] A. Lindblom and A. Liljegren: "Tumour markers in malignancies" *B.M.J.,* Vol. 320, (2000), pp. 424–427.

[65] R. Molina, V. Barak, A. van Dalen, M.J. Duffy, R. Einarsson, M. Gion, H. Goike, R. Lamertz, M. Nap, G. Soletormos and P. Stieber: "Tumor markers in breast cancer-European Group on tumor markers recommendations", *Tumour Biol.,* Vol. 26, (2005), pp. 281–293.

[66] A. Urruticoechea, I.E. Smith and M. Dowsett: "Proliferation marker Ki-67 in early breast cancer", *J. Clin. Oncol.,* Vol. 23, (2005), pp. 7212–7220.

[67] G. Gasparini, R. Longo, M. Toi and N. Ferrara: "Angiogenic inhibitors: a new therapeutic strategy in oncology", *Nat. Clin. Pract. Oncol.*, Vol. 2, (2005), pp. 562–577.