REVIEW ARTICLE

Prognostic Markers in Breast Cancer: A Review

Ritwik Kishore, Vivek Srivastava*, Datta S. Subramanyam, Kumar Kaushik, Vipul Srivastava, Harikesh Yadav, Mumtaz Ansari

Department of General Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

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ABSTRACT

Prognostic markers are indicators of aggressiveness, invasiveness, extent of spread of tumours, and correlate with survival independent of systemic therapy and can be used to select patients at risk. They could be traditional or clinical, molecular or investigational prognostic markers. There are newer prognostic markers but newer emerging biomarkers. However, will have to undergo analytical and clinical validation before entering clinical use. By combining established prognostic factors with validated prognostic biomarkers, we can begin the journey toward personalized treatment for every newly diagnosed patient with breast cancer.

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INTRODUCTION

Breast cancer is the most common malignancy in the world, and its incidence rate is about 1.3 million, and the death rate is 0.5 million worldwide.[1] The prognosis depends on its stage, typically graded as I to IV with sub-stages. A biomarker, a portmanteau of 'biological marker', is a characteristic that is objectively measured as an indicator of normal biological processes, pathological processes, pharmacological responses to therapeutic intervention, or to predict the incidence or outcome of a disease. Prognostic markers are indicators of aggressiveness, invasiveness, extent of spread of tumors, and correlate with survival independent of systemic therapy and can be used to select patients at risk. Prognostic biomarkers focus on identifying the likelihood of a clinical event in the disease setting. Unfortunately, sometimes prognostic biomarkers are a blunt measure of stratifying outcomes, and their reliability is limited through interindividual variability (i.e., differing values for a spectrum of patients), intraindividual variability (i.e., differing scoring by histopathologists providing Ki-67 measurement), and sensitivity and specificity implications. Consequentially, biomarkers are not always absolute in predicting outcomes.[2]

Traditional Biomarkers

The most important prognostic biomarker is the presence and number of axillary node metastases. However, the extent of axillary lymph node involvement doesn't determine the disease outcome. Tumor size and tumor grade are also one among the two traditional biomarkers widely used.

Lymph Node Metastasis

The presence and number of axillary node metastases has been and remain the single most important prognostic factor in breast cancer. Indeed, a direct relationship exists between the number of metastatic axillary lymph nodes and risk of metastasis. This relationship is independent of tumor size.[3] Accurate lymph node staging is utilized mainly to estimate prognosis and contributes to determining treatment strategies.[4]

Tumor Size

As with the number of axillary node metastases, measurement of tumor size is also mandatory in assessing prognosis for breast cancer, as the likelihood of the formation of metastases increases with increasing tumor size, regardless of the number of lymph node metastases.[5] Breast cancer staging is a classical and even outdated proposition. The traditional tumor stage is becoming less important with the development of molecular subtyping and precision treatment. The latest version of the AJCC prognostic staging system is a fusion of molecular subtypes and traditional pathological indicators. However, in recent years, some studies on T and N stages have been performed to explore the correlation between these two factors and their influence on prognosis. Yu et al. found that in LN-negative diseases, the relationship between tumor size and breast cancer-specific mortality (BCSM) was piecewise. More interestingly, Jennifer Y. Wo's work indicated that small tumors with four positive LNs might predict higher BCSM compared

*Author for Correspondence: vivekims97@gmail.com

with larger tumors. In extensive node-positive disease, very small tumor size may be a surrogate for biologically aggressive disease.[6]

Tumour Grade

Like lymph node metastases and tumor size, tumor grade is widely used to determine prognosis in breast cancer patients.[7,8] The grading of breast cancer is based on the microscopic similarity of breast cancer cells to normal breast tissue. The Nottingham system is one of the most widely used and best validated grading systems. This system utilizes 3 microscopic features to assign grade to a tumor, nuclear pleomorphism, gland or tubule formation and the number of dividing cells. Each of these factors is assigned a score from 1 to 3 (with 1 being the closest to normal breast and 3 the least close). These scores are then added together. If the combined tumor score is between 3 and 5, it is assigned to be grade 1. If the combined score is 6 or 7, the tumor is designated as grade 2; if the combined score is 8 or 9, it is designated as grade 3. In 2017, the Nottingham tumor grading system was incorporated into the American Joint Committee on Cancer for breast cancer staging.[9]

Molecular Biomarkers Classical Biomarkers Ki67

Antigen Ki-67, also known as Ki-67 or Marker of Proliferation Ki-67 (MKI67), is a protein in humans encoded by the MKI67 gene. Ki-67 encoded two protein isoforms with molecular weights of 345 and 395 kilodaltons and was initially identified in Hodgkin lymphoma cell nuclei 1983 by Gerdes and Scholzer. The name of this biomarker is derived from its city of origin, Kiel, and its location within the 96-well plate. The quantity of Ki-67 present at any time during the cell cycle is regulated by a precise balance between synthesis and degradation, as indicated by its short half-life of 60–90 minutes.[10] A nuclear non-histone protein and is expressed only in cells in the proliferative phases of the cell cycle (G1, S, G2, and M phases). It is tightly controlled and regulated and is vital for cell proliferation. The expression is usually estimated as the percentage of tumor cells positively stained by the antibody, with nuclear staining being the most common criterion of the proliferative index. A strong correlation has been noted between the percentage of cells positive for Ki67 and the nuclear grade, age, and mitotic rate. Multiple studies have indicated that breast cancer overexpresses Ki67 in more than 20–50% of the cells are at high risk of developing recurrent disease, showing a statistically significant correlation with clinical outcome, such as disease-free survival or overall survival. To compare the prognostic significance of Ki67 expression in breast cancer before and after neoadjuvant chemotherapy expression of Ki67 was assessed using immunohistochemistry (IHC) in pre-therapy core-needle biopsy and post-therapy surgical excision specimens. On multivariate analysis for overall survival, pre- and excision Ki67 expression were significant independent predictors, but the latter showed a stronger prognostic impact. In a cohort of 284 patients with only excision samples, post-therapy Ki67 was a significant independent prognostic factor. They concluded that post-chemotherapy Ki67 is a strong predictor of outcome for patients not achieving a complete pathological response. In addition, Ki67 correlates with other well-characterized proliferation markers, such as the proliferating cell nuclear antigen, which is a target of E2Fs. Ki67 staining will continue to be used as a useful laboratory test to predict the prognosis of breast cancer patients since it is technically easier and more closely associated with clinical outcomes than DNA ploidy analysis or S phase measurement by flow cytometry.[11]

Oestrogen and Progesterone Receptors (ER and PR)

The ER and PR are dimeric, gene-regulatory proteins. Estrogen and progesterone are well-established endocrine steroid regulators that modulate multiple aspects of mammary gland pathology. These two hormones work together to direct mammary epithelial growth, differentiation, and survival. Although, both steroids are commonly thought to be of primary importance for tumors arising in the reproductively competent years, between puberty and menopause, local aromatization of adrenal androgens provides additional estrogens in the postmenopausal years. Estrogen and progesterone act through their nuclear receptors to modulate the transcription of target genes. Genes encoding the receptors for each class of steroids are members of a single large superfamily of transcription-modulating factors. ERs may exist either in homodimeric or heterodimeric species, composed of α and β receptors acting as hormone-dependent transcriptional regulators. The ER pathway plays a critical role in the pathophysiology of human breast cancer. Although it is known that ERα is of key importance in the mammary ductal elongation of puberty, PR and ERβ appear to be more involved with lactational differentiation of the lobules. From knockout studies in mice, it is apparent that PR plays an important role in normal mammary gland's ductal and lobuloalveolar development.

Overexpression of ERα is a well-established prognostic factor in breast cancer patients. Generally, ERα-positive breast cancers are associated with slow tumor growth, lower histology grade, DNA diploidy, and thus a better overall prognosis. More than 90% of lobular breast carcinomas are ER-positive, while medullary and inflammatory carcinomas are predominantly ER-negative. ER/PR-negative tumors are often associated with aggressive disease, and these tumors frequently show amplification of HER2, c-Myc, and Int2 oncogenes and mutations of the p53 tumor suppressor gene.

In addition, because adjuvant or palliative hormone therapy is a common treatment for patients with ER-positive tumors receives, it is difficult to evaluate the prognostic value of their ER status alone. In some studies, the longer duration of disease-free survival (DFS) and overall survival rates of patients with ERα-positive tumors are seen only in the presence of hormone therapy. In addition, the favorable effect of ERα-positive status as a discriminant often is lost after several years, suggesting that treatment benefit is temporary. When node-positive patients not receiving adjuvant hormone therapy were studied, the 5-year DFS rate was 20% higher for ER-positive patients

compared with that for ER-negative patients. Among node-negative patients, small but statistically significant differences in DFS and overall survival rates have been found between ER-positive and ER-negative cases after various follow-up periods. The results of a multivariate analysis of prognostic factors in over 3,000 patients showed ER status to be more important for prognosis than tumor size in node-negative cases, but not in node-positive cases. Allred and colleagues showed that tamoxifen decreased the risk of local-regional recurrence in patients with ER-positive ductal carcinoma in situ.

The prognostic significance of ERβ is not well defined. Honma *et al.* studied archival materials from 442 invasive breast cancers treated with adjuvant tamoxifen monotherapy and with a long follow-up period (median: 11.1 years) using three antibodies to detect ERβN, ERβ1, and ERβcx (ERβ2). Positive staining for ERβN or ERβ1 was associated with significantly better survival. By contrast, ERβcx status showed no association with length of survival. ERβ1status was significantly associated with longer survival in postmenopausal, but not premenopausal, women. ERβ1 positivity was associated with significantly better survival in patients with ERα(–) (–) or triple-negative tumors, which are widely believed to have a poor prognosis. Differential expression of ERβ had no prognostic value in patients with ERα/PR negative breast cancer.

Because female sex steroids often regulate the growth of breast cancer, determinations of the cellular concentrations of ER and PR in the tumor continue to be used as predictors of good prognosis and of potential benefit from anti-hormonal therapy. To improve the value of ER determinations for tumor prognosis tests for the presence of the estrogen-regulated PR protein are routinely performed. In many breast tumor cell lines—and in normal tissues containing ER, such as the endometrium and brain—PR expression is induced by estrogen. It is still unknown whether ER regulates PR in the normal human mammary epithelium in the same subpopulation of ductal and lobular luminal cells, although this assumption is likely. The ER and PR appear to be strongly up-regulated in ductal carcinoma in-situ and in hormone-dependent breast cancer, relative to the normal mammary epithelium.

PR is a heterodimeric protein with A and B subunits. Overexpression of the PR indicates that the ER pathway is intact, even if the tumor is reported as ER-negative. These results correlate closely with biochemical ligand binding assays and clinical response rates to endocrine therapy. Importantly, higher PR levels are negatively correlated with tumor size and grade. In summary, ER will be used as a marker to predict the response to hormonal therapy and PR will be used as a predictor of the response to hormonal therapy as well as a prognostic factor in ER+ breast cancers.[10]

HER2

HER2-positive breast cancer (BC) represents 10–20 % of all breast tumors and are characterized by the amplification of the ERBB2/HER2 gene by constitutive activating the MAPK and PI3K/AKT signaling pathways, which in turn enhances cell proliferation, invasion, and metastasis. A further mechanism by which HER2 overexpression may promote oncogenesis or tumor progression is by deforming cell membranes. According to Chung *et al.,* this deformation can result in cells becoming less attached to their surrounding extracellular matrix/neighboring cells and thus more likely to acquire an invasive phenotype. It is strongly associated with increased disease recurrence and poor prognosis. HER2 is the target of the monoclonal antibody Trastuzumab (Herceptin). Another monoclonal antibody against HER2 is Pertuzumab, which inhibits the dimerization of HER2 and HER3 receptors. Two other drugs which are permitted for antiHER2 therapy are Lapatinib and Transtuzumab Emtansine (TDM-1). The extracellular domain is shed from the tumor cells and enters the circulation. The measurement of HER2 can be performed by using ELISA. Earlier, HER2 was supposed to be only the prognostic biomarker of carcinoma breast but now changes in serum concentrations may be useful in predicting response to Herceptin therapy.[11]

Investigational Prognostic Biomarkers

Prognostic mi-RNAs

MicroRNAs are a class of naturally occurring, small non-coding RNA molecules, about 21-25 nucleotides in length. They were first described in 1993 by Lee and colleagues. MicroRNAs alterations are associated with metastasis and tumor genesis and are used as biomarkers for breast cancer diagnosis and prognosis.[12] miRNAs have been found to be dysregulated in case of carcinoma breast. They can either get up-regulated or downregulated. Several miRNAs have been identified which correlate with overall survival (OS) like miR210, miR21, miR221 and miR652. miRNAs associated with time to metastasis are miR-127-3p, miR-210, miR−185, miR−143 and miRlet-7b. Some miRNAs like miR-210, miR−21, miR-106b, miR−197 and miRlet-7i are common to both prognostic signatures. Patients over-expressing miR-210 showed shorter overall survival and earlier time to metastasis and is also associated with tumor aggressiveness and is a poor predictor of disease-free survival and relapse-free survival. miR-148 and miR-210 were found to be associated with shorter relapse-free survival. A better prognosis has been observed associated with miR-497 overexpression.[13-16] Among the downregulated miRNAs, miR-30a, miR-31, miR34, miR-93, miR-125, miR-126, mR-146a, miR-195, miR-200, miR-205, miR-206, miR-503, and let-7 have been shown to have a role in breast cancer pathogenesis through the loss of their tumor suppressor properties. The main mechanisms affected by downregulated miRNAs are cell cycle, proliferation, and metastasis diffusion. It was found by a study done by Roth *et al.* that miR-155 can be detected in serum of patients with breast carcinoma and not in healthy controls.[10] These miRNA signatures have also been used to predict carcinoma breast response to Herceptin. The let-7 family and miR-125a-5p/b-5p are important predictors of therapeutic response.[17]

Circulating Tumour Cells (CTCs)

Circulating tumor cells (CTCs) are tumor cells that have sloughed off the primary tumor and extravasate into and circulate in the blood. CTCs were first described in 1869 by Ashworth who observed "some cells" in the blood of a metastatic cancer patient with an appearance like tumor cells in the primary tumors. Although they originate from the primary tumor, yet they are distinct from the primary tumor cells. It is technically very difficult to isolate these CTCs from the massive circulating blood cell pool. Epithelial-to-mesenchymal (EMT) is the process by which epithelial tumor cells lose their intercellular adhesion and acquire mesenchymal and invasive properties. During dissemination, tumor cells detach themselves from the basement membrane through EMT activation and directly enter the circulation, serving as CTCs traveling to distant sites. After extravasation, they undergo a reverse process termed Mesenchymal-to-epithelial transition (MET) and proliferate to form macro-metastasis. Clinically, combining the total CTC count and the proportion of mesenchymal CTCs can be used to monitor therapeutic resistance and predict prognosis in cancer patients. Different numbers of total CTCs and EMT CTCs were found to play an important role in determining the prognosis of breast cancer patients. CTC EMT-positive patients with neutrophil-to-lymphocyte ratios ≥3 had 8.6 times increased risk of disease recurrence compared with CTC EMT-negative patients with lower neutrophil levels; inflammation-based scores increased the prognostic value. It is a really challenging process for the detection of CTCs in the blood and many methods have been proposed to do the same. The core strategies for CTC detection technologies are (1) capture and enrichment, (2) detection and identification, and (3) release. Some of these are CellSearch System, Onquick system (density-dependent technique), Apostream (Dielectric electrophoresis technique), MagsWeeper System, CTC-chip, CTC-iChip system etc. CellSearch system is the only FDA-approved system for detecting CTC in clinical practice. The main target of investigations is to enumerate the CTC counts and the cut-off value is ≥5 for positivity, indicating poor prognosis. Increased CTC expressions are correlated with more metastasis and cancer aggressiveness.[18] The presence of CTCs after neoadjuvant chemotherapy was also found to be relevant to early metastatic relapse and worse disease-free survival. CTCs have also been used for therapeutic evaluation, in which patients with persistent CTCs after completion of (neo) adjuvant chemotherapy have an increased risk of relapse. For patients receiving palliative chemotherapy, CTCs numbers after the first cycle of treatment showed strong relevance to the therapeutic response.[19]

Stromal Tumour-Infiltrating Lymphocytes

Tumour infiltrating lymphocytes (TILs) are mostly composed of T cells but can also include B cells, NK cells, dendritic cells, and macrophages.[20] The presence of TILs in the microenvironment of breast tumors has been proposed to reflect the efficacy of immune therapy and to predict the prognosis of breast cancer. TILs are higher in triple-negative and HER2-positive breast tumors than in other breast cancers. In the neoadjuvant setting, TILs may be used to predict complete pathological response in all molecular subtypes of breast cancers and may be associated with a survival benefit in HER2+ breast cancer, while an increased number of TILs has been associated with shorter overall survival in luminal-HER2-negative tumor.[21] Most studies conclude that high levels of TILs are associated with increased benefit from anti-HER2 therapy. It was found in most studies that TILs and pathological response rates were not linearly related, for every 1% increase in TILs, there was an associated with a 3% decrease in the rate of an event across all treatment groups. Similarly, in the metastatic setting, levels of TILs also increase in trastuzumab, pertuzumab and chemotherapy.[22-24]

CONCLUSION

The prognosis of breast cancer depends on a wide spectrum of factors. Breast cancer has led the way in the introduction of prognostic and predictive biomarkers for cancer patients. Over 40 years ago, ER was first introduced to predict endocrine therapy response. Twenty years later, HER2 became available to identify patients likely to benefit from trastuzumab and other anti-HER2 therapy. In the last decade, several multigene signatures have been proposed for identifying patients with early breast cancer whose prognosis is so good, that they are unlikely to benefit from adjuvant chemotherapy. Currently, a considerable amount of research is focusing on miRNAs and CTCs, with the aim of identifying new prognostic and predictive biomarkers. However, these emerging biomarkers will have to undergo analytical and clinical validation before entering clinical use. By combining established prognostic factors with validated prognostic biomarkers, we can begin the journey towards personalized treatment for every newly diagnosed patient with breast cancer.

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