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Breast Cancer: A Heterogeneous Pathology. Prognostic and Predictive Factors – A Narrative Review

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Rezumat

Cancerul mamar: o patologie heterogenă. Factori de prognostic și predicție – review al literaturii

Neoplasmul mamar reprezintă una dintre principalele malignități în rândul femeilor la nivel mondial. Heterogenitatea sa remarcabilă reprezintă o caracteristică definitorie, determinând atât tipare diverse de progresie a bolii, cât şi răspunsuri terapeutice variate. Acest review al literaturii explorează evoluția clasificărilor cancerului mamar, concentrându-se pe factorii prognostici şi predictivi. Sunt examinate metodele tradiționale de evaluare a bolii, cum ar fi sistemul de stadializare TNM şi diferențierea histologică, şi în plus sunt prezentate şi elemente moderne, precum subtipurile moleculare, modificările genomice şi testele diagnostice avansate. Combinarea cunoştințelor clinicopatologice clasice cu tehnologiile de ultimă oră în domeniul molecular genetic şi genomic permite un diagnostic de precizie, avansând astfel atât înțelegerea, cât şi managementul acestei boli complexe. Articolul subliniază, de asemenea, importanța unei perspective exhaustive, deoarece atingerea principalelor obiective ale tratamentului - supraviețuirea prelungită şi îmbunătățirea calității vieții - implică abordarea bolii într-un context mai larg şi mai cuprinzător. Complexitatea cancerului mamar, determinată de variabilitatea semnificativă atât între, cât şi în cadrul tumorilor, generează provocări majore pentru abordările diagnostice şi terapeutice convenționale. Cu toate acestea, progresele din cercetarea genomică, cum ar fi profilarea moleculară şi testarea genetică, au aprofundat înțelegerea noastră asupra naturii complexe a acestui cancer. Aceste progrese au condus la identificarea unor modificări genetice - incluzând mutațiile în BRCA1/2, TP53, PALB2, PTEN şi PIK3CA - care influențează profund comportamentul tumorii, eficacitatea tratamentului şi prognosticul pacientului. Teste genomice precum Oncotype DX, MammaPrint, PROSIGNA şi EndoPredict oferă informații valoroase despre riscurile de recidivă şi opțiunile de tratament, subliniind importanța tot mai mare a medicinii de precizie. Mai mult, activitatea comisiei de diagnostic şi indicație terapeutică, a cărei decizii se bazează pe toate aceste elemente moderne de prognostic şi predicție, contribuie la îmbunătățirea strategiilor de tratament personalizate, ducând la optimizarea rezultatelor, fapt ce se reflectă atât

prin creşterea ratei de supravieţuire, cât şi prin creşterea calităţii vieţii. Acest articol subliniază importanţa abordărilor terapeutice personalizate şi evidenţiază natura dinamică a cercetării în domeniul cancerului mamar cu impact în practica clinică.

Cuvinte cheie: neoplasm mamar, factori prognostici, factori predictivi, subtipuri moleculare, teste genomice

Abstract

Breast cancer (BC) is recognized as one of the leading malignancies affecting women worldwide. Its remarkable heterogeneity is a defining characteristic, contributing to both diverse patterns of disease progression and varied therapeutic responses. This review explores the evolution of breast cancer classifications, focusing on key prognostic and predictive factors. It examines traditional systems, such as the TNM staging and histological differentiation, while also incorporating modern elements like molecular subtypes, genomic alterations, and advanced diagnostic assays. By combining classical clinicopathological insights with cutting-edge molecular genetic technologies, the goal is to refine the precision of treatment strategies, ultimately advancing both our understanding and management of this complex disease. The review also emphasizes the importance of a global perspective, as achieving the primary treatment goals – prolonged survival and enhanced quality of life – requires addressing the disease in a broader, more comprehensive context. The complexity of breast cancer, driven by significant variability both across and within tumors, presents major challenges to conventional diagnostic and therapeutic approaches. However, breakthroughs in genomic research, such as molecular profiling and genetic testing, have deepened our understanding of the intricate nature of this condition. These advances have led to the identification of critical genetic alterations – Including mutations in BRCA1/2, TP53, PALB2, PTEN, and PIK3CA – that profoundly impact tumor behavior, treatment efficacy, and patient prognosis. Genomic assays like Oncotype DX, MammaPrint, PROSIGNA, and EndoPredict offer valuable insights into recurrence risks and treatment choices, underscoring the growing importance of precision medicine. Moreover, the implementation of Molecular Tumor Boards further enhances personalized treatment strategies, contributing to improved patient outcomes and survival rates. This review highlights the significance of tailored therapeutic approaches and the dynamic evolution of breast cancer in clinical practice.

Key words: breast cancer, prognostic factors, predictive factors, molecular subtypes, genomic assays

Introduction

Breast cancer is one of the most common malignancies affecting women worldwide and is characterized by a high degree of biological and clinical diversity. This heterogeneity significantly impacts its evolution, therapeutic response and overall prognosis. This variety of characteristics is observed both among different patients (inter-tumor heterogeneity) and within individual tumors (intra-tumor heterogeneity). Inter-tumor heterogeneity includes differences in histopathological subtypes, molecular profiles, and responses to treatment among patients. In contrast, intra-tumor heterogeneity refers to the spatial and temporal variations within a single tumor. These variations can manifest as differences in genomic, epigenetic, and phenotypic features, as well as alterations arising during tumor progression or in response to therapeutic interventions (1).

The intricate and multifactorial nature of breast cancer necessitates a comprehensive approach to treatment that integrates clinical, histological, and molecular data. By leveraging advanced diagnostic modalities and molecular characterizations, personalized therapeutic strategies can be developed to address the unique biological properties of each tumor and the individual needs of patients (1).

Nowadays, the established and long-lasting staging and classification systems are complemented by innovative technologies. New concepts, such as PIK3CA, newly discovered pathogenic mutations in susceptibility genes and emerging genomic assays, highlight the dynamic and intricate nature of this disease (2).

A key feature of this illness is that it cannot be defined by a single characteristic; rather, it consists of a diverse array of pathologies that show significant variations in genetic mutations, molecular subtypes, and patient-specific factors. These differences affect not only how the disease

progresses but also the effectiveness of various treatments (3).

Recent advancements in genomic profiling and molecular diagnostics have enhanced our understanding of breast cancer and have ushered in an era of personalized treatment options.The continuous development of genomic assays, such as Oncotype DX, MammaPrint, PROSIGNA, and EndoPredict, combined with the expertise of multidisciplinary teams (Molecular Tumor Board) reflects a shift towards more tailored and effective therapeutic approaches (4).

This article delves into the key prognostic and predictive factors that influence the clinical progression of breast cancer, emphasizing their crucial role in refining personalized treatment strategies for this complex disease. By integrating genetic, molecular, and clinical perspectives, this review highlights the dynamic interplay between clinicopathological features and genomic markers. The aim is to clarify the mechanisms driving tumor behavior and to illustrate how these insights enhance our understanding of prognostic and predictive frameworks. Ultimately, the review provides a comprehensive overview of the evolving approaches to breast cancer management.

A comprehensive literature search was conducted across major scientific databases, including PubMed, Scopus, and Web of Science, focusing on studies published within the past two decades. Key search terms included "breast cancer classifications," "prognostic factors," "predictive biomarkers," "genomic alterations," "molecular subtypes," "precision medicine," "genetic testing," and "molecular tumor boards." The review prioritized peer-reviewed articles, meta-analyses, clinical trials, and comprehensive reviews to ensure the inclusion of high-quality evidence.

Brief History: The Evolution of Understanding Breast Cancer

The understanding and treatment of cancer have evolved significantly over time, dating back to prehistoric times. The earliest written records about cancer originate from Ancient Egypt, with the Smith Papyrus documenting cases of breast cancer and identiying negative prognostic factors. It noted that if tumors felt cold to the touch, were prominent, and spread throughout the entire gland, no treatment would be successful. During the Greco-Roman period, physicians such as Leonides of Alexandria, Hecateus of Abdera, and Paulus Aegineta expanded on these observations. They detailed methods for diagnosing and surgically treating breast cancer, including the empirical technique of breast amputation. In the 17th century, Bernardino Ramazzini (1633-1714) identified an epidemiological link between breast cancer and the lifestyles of nuns, suggesting that their lack of sexual activity - which would have led to pregnancy and breastfeeding - contributed to the disease. Jean-Louis Petit (1674-1750), the first president of the French Academy of Surgery, advocated for the removal of tumors along with a segment of the breast while aiming to preserve the nipple. He also contributed to the understanding of lymphatic dissemination and supported total mastectomy with axillary lymph node (LN) dissection as a way to prevent recurrences (1).

A significant advancement in the understanding of cancer was introduced by William Halsted, who proposed the concept of radical surgery. For breast cancer, this involves removing the entire breast along with the regional lymph nodes. The Halsted Radical Mastectomy served as the primary surgical treatment for breast cancer for over 50 years. After the mid-20th century, more conservative and selective surgical techniques for breast cancer began to emerge and gain acceptance. These methods, which include conservative breast treatment, sentinel lymph node biopsy, oncoplastic surgery, skin-sparing mastectomy, nipple-sparing mastectomy, and breast reconstruction, have reduced the morbidity associated with surgery and improved postoperative esthetic outcomes. When combined with systemic treatments and radiotherapy, these surgical techniques have enhanced both survival rates and quality of life for patients (5).

The trajectory of understanding and managing breast cancer and cancer in general has been shaped by several paradigm shifts. The Halstedian Theory states that breast cancer arises regionally within the breast tissue, spreading locally and gradually in a predictable manner, resembling the evolution of an oil stain. The invasion of regional lymph nodes occurs systematically, serving as the primary pathway for dissemination, while distant metastases develop later in the disease progression through vascular dissemination following the invasion of regional lymph nodes. This theory laid the groundwork for radical cancer surgery (5). A change of paradigm was the Systemic Theory proposed by B. Fisher which asserts that breast cancer is a systemic disease from its very onset, with neoplastic cells capable of circulating through both blood and lymphatic systems, even in the early stages. Fisher believed that metastases are

not merely a consequence of primary tumor progression but may arise in parallel with the growth of the initial tumor (6). The Spectrum Theory of Breast Cancer was introduced in 1994 by Samuel Hellman. He proposed a new concept suggesting that the progression of breast cancer does not strictly adhere to a purely local or systemic model. Instead, changes in malignant tumor biology occur along a continuous spectrum. For instance, small tumors (≤ 2 cm) with more favorable biological characteristics suggest a localized form of the disease that typically requires less aggressive treatment. Conversely, as tumor size increases, the likelihood of metastasis rises. Larger tumors contain more cells capable of spreading, and their biological behavior tends to be more aggressive. This integrative approach emphasizes the importance of tailoring therapies to the specific stage and aggressivity of the disease (7).

These evolving theories reflect the complex nature of breast cancer and the ongoing efforts to the understanding of the disease and improve treatment strategies.

Content

TNM Staging

The TNM staging method allows the stratification of patients into groups that share similar prognoses and treatment options. This system was developed to enhance the understanding of the clinical behavior of cancers, providing a universal framework for communication among clinicians. It also serves as a basis for selecting initial therapeutic approaches and assessing the need for potential neoadjuvant treatment. Without this standardized language, conducting meaningful interventional or observational therapeutic studies would be challenging (8).

The TNM classification describes the anatomical extent of cancer based on the idea that both treatment selection and patient survival rates are directly related to the original extent of the tumor (T), the presence or absence of metastases in locoregional lymph nodes (N), and the existence of distant metastases (M). Tumors must be classified before treatment (clinical staging, cTNM) and after surgery (pathological staging, pTNM). The TNM system offers a quite precise description of the apparent extent of the disease (9).

History of TNM Staging

The process of cancer staging has significantly evolved over the last century, beginning with early systems such as the Dukes classification and advancing to Pierre Denoix's TNM classification. In 1958, the International Union Against Cancer (UICC) published the first guidelines for breast and laryngeal cancer, which expanded to cover 23 body sites by 1968. Subsequently, between 1974 and 1987, further editions standardized the cancer staging process globally in collaboration with the American Joint Committee on Cancer (AJCC). By 2018, eight editions had been published, with the ninth edition planned for release in 2025. Currently, the eighth edition of this staging system represents the most significant departure from previous classifications (8).

Recent updates to cancer staging have created a need for a clearer and more accurate approach in various situations. The standard procedures for assessing the dimensions of primary tumors have been refined, discouraging the rounding of sizes for very small tumors and clarifying T size measurements in multifocal tumors. Specifications for the N category now include detailed guidelines for measuring nodal metastases. Additionally, the term pM0 has been deemed invalid, while cM1 and pM1 have been reaffirmed. Post-neoadjuvant therapy classifications have been expanded to exclude surrounding fibrosis, redefine the dimensions of residual nodal metastasis and reassess the definition of pathological complete remission, particularly in cases involving M1 disease. These updates aim to enhance accuracy and consistency in staging practices (8).

TNM Stage as a Prognostic Factor

The TNM system has demonstrated its reliability over time and remains a crucial prognostic tool for predicting overall survival in cancer patients. A prognostic factor is a measurable variable whose presence influences the natural progression of the disease. Tumors typically grow locally and expand in a locoregional pattern, often involving regional lymph nodes. However, over time, tumors can metastasize to visceral organs, with or without impacting nearby lymphovascular structures (9). Staging, based on either clinical or pathological data, provides only a snapshot of the disease at a specific point in time. Within each stage, there can be significant variability in biological behavior, rate of progression, and prognosis among cases. While the size of the primary tumor and the burden of regional lymph node involvement are independent variables, they are closely interrelated. As tumor size increases, the likelihood of metastasis to regional lymph nodes and distant sites also

rises. Additionally, tumor size is associated with a higher average number of involved axillary lymph nodes and an increased probability of recurrence and metastasis. Notably, when treated as continuous variables, the T (tumor size) and N (regional lymph node involvement) classifications provide a more precise evaluation of prognosis compared to traditional anatomic staging (10).

The more favorable prognosis of non-palpable invasive carcinomas compared to palpable ones, as well as cancers detected through screening versus those identified otherwise, can be attributed to their smaller size. For example, one study found that axillary metastasis occurred in only 7.7% of tumors measuring between 0.1 and 5 mm and in 12.5% of tumors measuring 6 to 10 mm in diameter. Tumors of the same size tend to have similar prognostic outcomes, regardless of whether they are palpable or detected through screening (11).

The impact of primary tumor size on prognosis is evident in both node-negative and node-positive cases, highlighting the relationship between tumor growth and the likelihood of dissemination. This is especially significant in node-negative cases, where tumor size serves as a practical indicator for distinguishing between patients at low and high risk of recurrence (10). However, the clinical examination of lymph nodes is not as reliable as expected in determining the presence or absence of metastases, which makes it a poor criterion for accurate staging. Literature reports indicate false-negative results up to 38.6% and false-positive results up to 27.3% (12).

The detection of metastasis in axillary lymph nodes through histological examination confirms that the tumor has already spread and may have affected distant sites as well. Multivariate analyses consistently show that the presence or absence of axillary lymph node metastasis is one of the most important predictors of both recurrence and disease-free survival. A study conducted by B. Fisher indicated that without systemic adjuvant therapy, the likelihood of recurrence within 10 years is 24% for patients without nodal metastasis, compared to 76% for those with nodal metastasis (13). Another study involving 25,315 patients assessed the relationship between the number of affected lymph nodes and the 5-year overall survival rate. Patients were classified into three categories based on the number of positive lymph nodes: 1-3 positive lymph nodes, 4-9 positive lymph nodes, and 10 or more positive lymph nodes. The overall survival rate decreased proportionally as the number of affected lymph nodes increased, with survival rates of 84% for the first category, 74% for the second, and 55% for the third category (14).

Breast cancer related mortality is predominantly attributed to metastatic progression. The mortality rate for patients with breast cancer is higher than that of the general population, even 20 years after diagnosis. Monitoring the characteristics - such as the number and molecular features - of circulating tumor cells (CTCs) in breast cancer patients, particularly those with early-stage disease, may help detect the potential for relapse. This early detection test could enable timely therapeutic interventions to delay or possibly prevent the evolution of the disease. Such an approach could lead to more personalized and effective treatments, although this concept has yet to be perfected (15).

A common criticism of the TNM staging system is its biological simplicity, as it does not fully capture the complexity of tumor behavior. While TNM staging provides valuable insights, it necessitates the integration of additional biological factors. Ongoing research aims to provide a clearer understanding of cancer behavior (10).

Histological Grade

History of Histological Grading

In 1893, Von Hansemann was the first to identify a connection between the cellular characteristics of invasive breast cancer and the progression of the disease, comparing to the normal breast cells. He hypothesized that anaplastic tumors, which are characterized by a loss of differentiation, have a significantly higher likelihood of metastasizing – a theory he confirmed in 1902. Twenty years later, researchers established a link between the degree of differentiation and the post-mastectomy survival rate. Following this, Greenough developed the first grading system for tumors, classifying them into three grades of malignancy based on factors such as tubular differentiation, cell and nucleus size, hyperchromatism, and mitotic activity (16).

Until the late 1950s, tumors were classified only by clinical stage, which did not consider the wide range of biological behaviors seen in breast carcinomas. Bloom and Richardson noted that while clinical staging was useful, it could not predict the presence of hidden metastases, particularly in early-stage cases, nor could it anticipate the speed at which metastases might develop. This led them to create a histological grading system. In their system, tumors were assigned a score from 1 to 3 based on three components: tubule formation

differentiation, pleomorphism, and "hyperchromatosis" (or the presence of mitotic nuclei). A total score of 3-5 indicated a low-grade (Grade I) tumor, 6-7 signified an intermediate (Grade II) tumor, and 8-9 denoted a high-grade (Grade III) tumor. Bloom and Richardson emphasized that these grades represented divisions on a continuous scale of malignancy rather than distinct pathological entities, and their scoring system was intended as a guide for prognosis, rather than a mathematically precise classification. Despite these insights, histological grading was not widely adopted as a routine practice for many years due to concerns about reproducibility (17).

Basics of Histologic Grading

Breast cancer is a complex and diverse disease characterized by variations in its morphology, molecular features, behavior, and treatment responses. Effective management of breast cancer depends on both clinical and pathological prognostic and predictive factors. These elements are crucial for guiding therapeutic decisions, especially as more treatment options become available. Systemic therapy was for a long time based primarily on three main prognostic determinants: tumor size, lymph node status, and histological grade (17).

The Nottingham (Elston-Ellis) system, commonly referred to as the Nottingham Grading System (NGS), is the universally recommended grading system by organizations such as the World Health Organization (WHO), the American Joint Committee on Cancer (AJCC), the European Union (EU), and the Royal College of Pathologists in the UK. NGS has independent prognostic value and is a key component of various prognostic indices. For instance, the Nottingham Prognostic Index (NPI) gives equal weight to both the NGS and lymph nodestages. As a straightforward, cost-effective, and widely applicable method, the NGS provides essential information regarding the intrinsic biological characteristics and clinical behavior of tumors. It complements other important prognostic factors, including tumor size and LN status (18).

Histological Grade as a Prognostic Factor

The histological grade is a well-established prognostic factor that evaluates the biological characteristics of tumors based on their morphological features. It offers valuable insights into the clinical behavior of the tumor. However, there are limitations due to variability in result interpretation, which can be influenced by factors such as laboratory resources, the physician's expertise, and the integrity of the specimen (16).

Artificial intelligence (AI) has transformed the field of oncologic pathology by offering standardized and objective data analysis algorithms. This represents a more effective approach to handling various pathological variables. AI serves as a powerful tool for classifying histological differentiation grades in breast cancer. The advantage of these advanced detection technologies, which are based on convolutional neural networks and deep learning algorithms, is that they eliminate human subjectivity by applying consistent evaluation criteria across all analyzed cases. Furthermore, AI allows for rapid and efficient analysis of large volumes of data, facilitating the early identification of signs of malignancy. It also significantly reduces errors associated with human observation. As AI gradually integrates into the diagnostic aspects of medicine, it has the potential to enable more accurate and standardized diagnoses, ultimately leading to improved patient outcomes (19).

Despite its limitations, NGS is a straightforward and cost-effective method that should not be overlooked. Its importance is particularly evident in countries with limited health resources, where access to expensive innovative technologies may be unavailable. In such settings, NGS provides a basic yet accessible means for assessing tumor biology (16).

Invasive carcinomas can be classified morphologically based on their growth patterns and the degree of differentiation. This classification reflects how closely tumor cells resemble normal breast epithelial cells. The subdivision process involves assessing both the histological type and the histological grade of the tumor. It is well established that the type of tumor provides important prognostic information. Notably, a significant majority of breast cancers (60% to 75%) fall under the category known as Invasive Ductal Carcinoma of No Special Type (NST), which is much more common than the special types that exhibit specific characteristics and thus have more defined prognostic value. The histological tumor grade is determined by analyzing three morphological features, as noted by Greenough in 1925: the extent of tubule or gland formation, nuclear pleomorphism, and mitotic count. These features result in three distinct histological categories: G1, well-differentiated tumors that closely resemble healthy breast tissue, displaying mild nuclear

pleomorphism and approximately 75% tubule formation; G2, moderately differentiated tumors; G3, poorly differentiated tumors characterized by significant cellular pleomorphism, lack of tubule formation, and frequent mitoses (20).

Numerous independent studies have established that the NGS is a more reliable predictor of prognosis than tumor size and is comparable to LN status. A large-scale study analyzing survival data from nearly 23,000 breast cancer cases found that patients with histological grade 1, stage II disease had survival rates similar to those with grade 3, stage I disease. Furthermore, it was observed that patients with grade 1 tumors smaller than 2 cm had an excellent prognosis, with a 99% five-year survival rate, even when positive LN involvement was present. These findings strongly indicate that histological grade is a more dependable predictor of tumor behavior, especially in smaller, early-stage tumors, compared to other time-dependent prognostic factors like tumor size. Importantly, the relationship between tumor size and histological grade as prognostic factors should be considered when assessing outcomes for patients diagnosed with this condition (21).

A retrospective study involving a cohort of 355 patients demonstrated the prognostic value of histological grade. The analysis revealed that patients with G3 tumors (poorly differentiated) had significantly worse outcomes in both overall survival and 5-year recurrence-free survival compared to those with G2 or G1 tumors (22).

Grade 2 Tumors

Over time, misclassification between the extremes of differentiation categories - grades 1 and 3 - has been rarely reported. However, grade 2 tumors consistently show the lowest concordance rates. This issue arises from the inherent challenges in classifying regions with histological overlap, which was anticipated when evaluating biological variables. Consequently, grade 2 tumors are often associated with intermediate-risk factors, such as tumor sizes ranging from 2 to 5 cm, a limited number of affected lymph nodes, or inconclusive scores from multigene assays. As a result, these "intermediate" or "neutral" characteristics often lead to the decision to utilize chemotherapeutic treatments (23).

Many studies have sought to further classify grade 2 tumors into two distinct categories. One subgroup resembles grade 1 tumors and is characterized by a favorable prognosis, making it possible to forgo neoadjuvant therapy. The other subgroup resembles grade 3 tumors and requires a more aggressive approach to systemic treatment (23).

Despite ongoing efforts, there remains variability among the factors that influence the classification of tumors into three differentiation grades. To address this issue, the classification system has been progressively refined by incorporating additional molecular tumor characteristics. This refinement supports pathologists in selecting and justifying appropriate, disease-specific treatment regimens. Furthermore, these molecular profiles emphasize the idea that each unique combination of tumor para-meters defines a distinct pathological identity (22).

Predictive and Prognostic Biomarkers

There are significant limitations in determining the overall prognosis of a breast cancer patient based solely on the anatomical extent of the disease or the histological differentiation of the tumor. One major concern for clinicians is the heterogeneity of breast cancer and the numerous pathogenic variants that can arise. As technology and our understanding of this condition continue to advance, factors such as tumor size, lymph node involvement, and histological differentiation grade have become inadequate for effectively managing patients (24).

ER/PR Status

Currently, one of the most widely used prognostic factors in breast cancer is the presence of hormone receptors in tumors, specifically the expression of nuclear estrogen receptors (ER) and progesterone receptors (PR). Initially, ligand-binding biochemical assays were employed to detect both ER and PR; however, this method was limited by the requirement for fresh tissue samples. As a result, there has been a transition to immunohistochemical (IHC) assays, which have now become the standard practice. The mechanism of action of ER involves its interaction with regulatory elements, such as Cyclins, which promote tumor cell growth. The presence of PR serves as a marker of functional ER, as its expression is induced by ER activity (24).

Different methods are employed to evaluate the level of expression in tissue samples. One widely used system for this assessment is the Allred score. It is important to note that the currently accepted threshold for ER and PR positivity is only 1%. As a result, patients who show any level of nuclear expression above this threshold are considered likely to respond to

hormonal therapy and are therefore candidates for this treatment option (25).

Human Epidermal Growth Factor/ HER 2

The HER-2/neu oncogene encodes a protein that serves as a receptor for a specific growth factor, stimulating cell proliferation. Normal epithelial cells, found throughout the body, contain two copies of the HER-2/neu gene and produce low levels of the HER-2/neu protein on their cell membranes. However, in a significant percentage of invasive breast cancer cases, as well as in other cancers like ovarian and bladder cancer, the HER-2/neu gene undergoes amplification. This leads to an overexpression of the HER-2/neu protein. Tumors that exhibit this overexpression tend to grow more aggressively and are resistant to both endocrine therapy and certain chemotherapeutic agents (26).

HER2 testing has advanced significantly in the past decade to improve the identification of HER2-positive patients who are eligible for anti-HER2 therapy. The NCCN and ASCO/ESMO guidelines recommend that all patients with invasive breast cancer be tested for HER2 expression and/or gene amplification to inform the selection of first-line anti-HER2 therapy (26).

HER2 marker expression can be classified into four distinct subgroups: IHC 0: No membrane staining indicative of HER2 protein presence is observed, or only weak staining is seen (less than 10% of tumor cells); IHC 1+: Weak staining of the cellular membrane, yet insufficient to be considered positive, representing an ambiguous result (more than 10% of tumor cells); IHC 2+ (borderline): Weak to moderate staining of the cellular membrane, yet still insufficient to be considered positive; IHC 3+: Intense expression of the HER2 protein with complete and intense staining of the cellular membrane; this is the only result considered directly positive (26).

If the IHC result shows a score of 2+, the positive expression status of this receptor must be confirmed through an additional test: either FISH (fluorescence in situ hybridization) or SISH (silver in situ hybridization). FISH utilizes special "tags" that bind to components of the HER2 genes. When bound, these tags emit fluorescence in the dark, enabling the pathologist to count them accurately. The results of this test will determine whether the sample is classified as HER2-negative or HER2-positive breast cancer, which will then inform the subsequent treatment plan (27).

If we classify breast cancer based solely on the HER2 marker, the classification would be straightforward: HER2-negative breast cancer includes IHC 0, IHC 1+, and IHC 2+ (FISH/ISH negative), while HER2-positive breast cancer includes IHC 3+ and IHC 2+ (FISH/ISH positive)(27).

The vast majority of this pathological entity is currently classified as HER2-negative, accounting for 80%-90% of cases, and therefore is not eligible for anti-HER2 targeted therapy. Recent studies have identified a potential new category of HER2 classification known as HER2-Low, which includes cases with IHC 1+ and IHC 2+/non-amplified results. However, there is still no conclusive evidence to define this category as a distinct subtype with specific prognostic and predictive implications. Additionally, there have been reports of IHC 0 cases where low levels of HER2 expression were detected using more sensitive testing methods. A phase II study suggests that both IHC 0 and HER2-Low may respond similarly to Trastuzumab deruxtecan (28).

Currently, there is not enough concrete evidence to change all existing HER2 reporting systems or to completely embrace the new concept of HER2-Low. Additionally, ongoing studies are focused on collecting more data regarding specific IHC 0 specimens that might have sufficient levels of HER2 to produce a clinical response (28).

Ki 67- Proliferation Index

Ki67 is a biological marker used to evaluate and quantify the rate of cellular proliferation in tumors. It is a nuclear protein that is expressed only in cells actively engaged in the cell cycle phases (G1, S, G2, and mitosis) and is absent in cells that are in a quiescent phase (G0). The Ki67 proliferation index has been studied for its prognostic and predictive implications in this pathology. It is routinely assessed through immunohistochemical techniques, with results expressed as percentages that represent the proportion of positively staining tumor cells out of 100 analyzed cells. The values can range from 0% (indicating no proliferation) to 100% (indicating intense proliferation) (29).

From a prognostic perspective, a high Ki67 index is typically associated with more aggressive tumor behavior, a significantly increased risk of recurrence, and shorter overall survival. In contrast, tumors with low Ki67 levels tend to show less aggressive behavior and are often more responsive to less intensive therapies. This index is crucial for the molecular stratification of breast cancer, helping to identify the biological subtypes of the disease, each with distinct prognostic and

therapeutic implications. It is especially useful for differentiating between Luminal A and Luminal B subtypes, which are often difficult to distinguish based solely on hormonal receptor status. Currently, a threshold of 15-20% is recommended to differentiate these two subtypes, aiding in clinical decision-making (30,31).

Molecular Subtypes of Breast Cancer

History

Traditional clinicopathological characteristics, such as the anatomical extent of the disease, lymph node status, and histological grade, were the initial efforts toward classifying breast cancer. In the 1990s, research began to focus on the role of hormonal receptors, specifically estrogen and progesterone receptors, as well as the overexpression of the HER2 receptor (32).

The influential studies conducted by Perou et al., known as the "founding study," and later by Sorlie et al., established the concept of molecular classification and identified four main subtypes of breast cancer: LUMINAL A, LUMINAL B, HER2-ENRICHED, and TRIPLE NEGATIVE/BASAL-LIKE. Each subtype exhibits distinct biological, prognostic, and predictive characteristics (33).

The findings from the METABRIC and TCGA projects (2012-2016) reinforce this categorization, demonstrating that the molecular differences among the four subtypes are linked to variations in therapeutic response and the clinical outcomes for patients (33).

Characteristics and Clinical Features

LUMINAL A - The LUMINAL A subtype accounts for approximately 40-50% of all breast cancer cases. It is characterized by high expression of hormonal receptors, specifically ER and PR, low cellular proliferation (KI-67 < 15%), and the absence of HER2 amplification. Luminal A tumors have the best long-term survival rates, with a recurrence rate of less than 10% at 10 years. The risk of metastasis primarily occurs in the bones. This subtype responds well to endocrine therapy, including tamoxifen and aromatase inhibitors. Studies such as ATAC and BIG 1-98 have shown that adjuvant chemotherapy provides limited benefits for this subtype, as the risk of recurrence can be effectively managed with endocrine monotherapy (34).

LUMINAL B - The LUMINAL B subtype is more aggressive than the previous subtype and accounts for approximately 20-30% of cases. This category is characterized by variable expression of hormonal receptors. A key feature of LUMINAL B is the elevated level of cell proliferation, indicated by a KI-67 percentage greater than 15-20%. This subtype may also include HER2-positive tumors. Compared to the first subtype, patients with LUMINAL B have a higher risk of recurrence and shorter overall survival. Metastases are commonly observed in the bones, but can also occur in other organs.Patients typically require a combination of hormonal therapy and adjuvant chemotherapy. For cases of Luminal B with HER2-positive tumors, studies such as CLEOPATRA and APHINITY have shown that adding anti-HER2 therapy (such as trastuzumab and pertuzumab) significantly improves disease-free survival (35).

HER2-enriched subtype - The HER2-enriched subtype of breast cancer accounts for approximately 15-20% of cases and is distinguished by the overexpression of the HER2 receptor, along with the absence of hormonal receptors (ER/PR negative). The gene expression profile of this subtype is largely driven by genes related to cell proliferation and HER2 signaling. Before the advent of anti-HER2 therapies, this subtype was associated with a poor prognosis, characterized by a high recurrence rate and reduced overall survival. However, targeted therapies have significantly changed this outlook, improving outcomes for patients in this category. Key studies on HER2, such as HERA and CLEOPATRA, have demonstrated that the combination of trastuzumab with chemotherapy notably enhances patient prognosis. Additionally, dual therapy with trastuzumab and pertuzumab, as well as the use of T-DM1 in residual disease, offers new hope for managing this condition (36).

Basal-like subtype (triple-negative) - This category of breast cancer represents approximately 15% of cases and is often associated with BRCA1/2 mutations. It features a complex molecular profile characterized by the expression of basal epithelial genes and a high rate of cellular proliferation. This subtype is the most aggressive, with a high risk of early metastasis to the lungs and brain. The median overall survival following the onset of metastasis is approximately 12 to 18 months. Due to the absence of receptors that can be targeted by specific therapies, chemotherapy remains the gold standard for treatment. Recent studies, such as KEYNOTE-355, have shown that immunotherapy with PD-1 inhibitors, like pembrolizumab, significantly improves the prognosis in advanced cases. Additionally, PARP inhibitors (such as olaparib and talazoparib) have proven effective in

tumors with BRCA1/2 mutations (37).

In a meta-analysis that included 2,546 cases of breast cancer, researchers evaluated the correlation between the expression of PD-L1 and various clinicopathological characteristics. The results indicated that the presence of this surface protein is more frequently associated with triple-negative breast cancer (TNBC), lymph node invasion, and a high histological grade. On the other hand, PD-L1 has a dual role in breast cancer. While it serves as a negative prognostic factor that contributes to more aggressive tumor properties, its presence may also enhance therapeutic outcomes through targeted immunotherapy treatments using monoclonal antibodies, such as Atezolizumab and Avelumab (38).

The molecular subclassification of breast cancer has transformed the approach to this disease, allowing for precise patient stratification and personalized treatment strategies. The development of advanced technologies holds promise for uncovering new subtypes and understanding the underlying pathogenic mechanisms. Recent studies indicate that an expanded classification, which includes immunophenotyping and microenvironment characterization, could provide deeper insights into the complexity of the disease (3).

One notable example is the emerging "HER2-LOW" category in breast cancer. This group is characterized by low expression of the HER2 receptor without amplification, as detected by additional tests such as FISH or ISH. This subtype cannot be clearly categorized as either HER2-POSITIVE or HER2-NEGATIVE. Patients with HER2-LOW tumors typically have an intermediate prognosis - better than that of triple-negative tumors but worse than that of patients with appropriately treated HER2-POSITIVE tumors. The DESTINY-Breast04 trial has demonstrated the efficacy of Trastuzumab Deruxtecan, an antibody-drug conjugate, in the treatment of metastatic HER2-LOW patients, showing a significantly higher response rate compared to standard therapies. This new molecular subtype marks a significant advancement in the personalization of breast cancer treatment (39).

Molecular classification captures only one aspect of the complexity of breast cancer. Within a single tumor, various cellular populations with different molecular profiles often coexist. This diversity contributes to tumor plasticity and the development of treatment resistance. Furthermore, the tumor microenvironment - including stromal components and immune cells - plays a crucial role in the progression of the disease (40).

Genetic Alterations

With the advancement of technology and a deeper understanding of the complexities of breast cancer, new strategies for analyzing tumor characteristics have emerged. Genomic classification goes beyond the limitations of traditional clinical and pathological methods, offering a detailed molecular perspective. The identification of specific gene expression patterns has become feasible with the development of genetic profiling techniques such as microarray and RNA sequencing (41).

Multigene sequencing can identify a variety of genetic anomalies that may increase the risk of developing breast cancer. These anomalies are categorized into two types: germline mutations, which are present in all of an individual's cells, and somatic mutations, which only occur in tumor cells. Various testing panels typically include high-penetrance genes along with genes associated with moderate or low risks for the disease. The number of genes included and the laboratory techniques used can vary from one panel to another (41).

BRCA1, BRCA2

Mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 significantly increase the risk of developing breast and/or ovarian cancer in women. Specifically, women with BRCA1 mutations have an estimated lifetime risk of developing breast cancer ranging from 57% to 65%, and a risk of ovarian cancer between 39% and 40%. In contrast, BRCA2 mutations are associated with a 45% to 49% risk of breast cancer and an 11% to 18% risk of ovarian cancer. Tumors linked to BRCA mutations are often of higher clinical grade and stage, exhibiting a greater potential for metastasis. Notably, around 70% of breast tumors with germline BRCA1 mutations are classified as triple-negative breast cancer (TNBC), which is a particularly aggressive and highly metastatic subtype (41).

The BRCA1 and BRCA2 genes are crucial for the DNA damage response and repair pathways, playing a vital role in maintaining genomic integrity. Mutations that disrupt the normal functions of these genes not only contribute to the initiation and progression of cancer but also increase the risk of developing contralateral breast cancer. Additionally, these mutations can affect therapeutic outcomes, particularly in targeted treatments. Due to their

deficient DNA repair mechanisms, cells with BRCA mutations are particularly vulnerable to DNA cross-linking agents, such as platinum-based drugs, making these agents effective treatments for various cancers. In vitro studies have shown strong evidence of an enhanced response to platinum drugs among individuals with BRCA mutations. This is further supported by clinical studies, which indicate that patients with BRCA mutations experience improved survival rates and longer disease-free intervals when treated with platinum-based therapies, such as cisplatin. These findings suggest favorable treatment outcomes for this patient population (42)

Recent studies suggest that overall survival in women with BRCA1/2 mutations may be improved by the use of PARP inhibitors. Additionally, not implementing a treatment plan for this type of early-stage breast cancer could reduce overall survival by three years (43).

TP53 (Tumor Protein 53)

The TP53 gene encodes the p53 protein, which plays a crucial role in regulating the cell cycle, repairing DNA, and inducing apoptosis in cells with damaged or altered DNA. Due to its function in preventing the proliferation of cells with defective DNA, it is often referred to as the "guardian of the genome". Germline mutations in the TP53 gene are commonly associated with Li-Fraumeni syndrome, a rare condition that significantly increases an individual's risk of developing multiple types of cancer, including breast cancer, which often manifests at a young age (between 30 and 40 years old) (44).

Research indicates that somatic mutations of the TP53 gene are found in approximately 40% of sporadic breast cancers. These mutations are linked to more rapid disease progression and a poorer response to chemotherapy (45).

Additionally, another study has shown that around 90% (ranging from 80% to 88%) of triple-negative breast cancers exhibit TP53 mutations. These mutations are associated with a worse prognosis and an inadequate response to standard therapies (46).

PALB2

The Partner and Localizer of BRCA2 (PALB2) gene plays a crucial role in DNA repair, and mutations in this gene can increase the risk of developing breast cancer. The risk is notably higher when the inherited mutation is heterozygous. PALB2 is classified as a high-penetrance gene, with risk levels that can sometimes be similar to those associated with BRCA1/2 mutations, although it generally has a more favorable prognosis. Approximately 1 in every 1,000 cases involves PALB2 mutations, which confer a 35% risk of developing breast cancer (often of the Triple Negative type) by the age of 70 (47).

Research has shown an association between PALB2 mutations and an increased risk of developing breast cancer at younger ages, specifically before the age of 50 (48). Cancers linked to PALB2 mutations have also been found to be sensitive to PARP inhibitors, such as Olaparib. These drugs specifically target DNA repair mechanisms in cancer cells, offering a promising therapeutic option for patients with these mutations (49).

PTEN

Phosphatase and Tensin Homolog (PTEN) is a tumor suppressor gene that codes for a protein essential in regulating critical cellular functions, such as cell cycle progression, DNA repair, and apoptosis. The mechanism by which PTEN operates is vital for managing cell growth, survival, and metabolism. Mutations in PTEN or the complete loss of its expression can lead to uncontrolled cellular proliferation and increased resistance to apoptosis, which ultimately contributes to tumor development (50).

A meta-analysis involving 10,231 patients found that the loss of PTEN expression is associated with more aggressive tumors, larger sizes, and a higher frequency of lymph node involvement. Additionally, this mutation correlates with advanced TNM stages, Grade 3 (G3) tumors, and Triple Negative Breast Cancer (50).

PIK3CA – Somatic alteration

The phosphoinositide 3-kinase (PI3K) signaling pathway plays a crucial role in regulating various cellular processes, including cell growth, proliferation, and metabolism. Numerous studies have shown that this pathway is upregulated in more than 70% of human tumors. Furthermore, it is the most commonly altered pathway in patients with hormone receptor-positive (HR+) breast cancer. PIK3CA gene mutations occur in 20-40% of early breast cancer cases (51).

A 2020 study demonstrated that the PIK3CA mutation imparts specific characteristics to affected patients, including reduced sensitivity to chemotherapy. Notably, only 51% of individuals with this mutation showed an objective response

to chemotherapy induction. The study also found that chemoresistance associated with the PIK3CA mutation was independent of other clinical parameters. Additionally, the presence of the PIK3CA mutation was linked to poorer survival outcomes. The study revealed that persistent levels of cell-free circulating PIK3CA mutations in plasma during chemotherapy correlate with an unfavorable prognosis (51).

A meta-analysis conducted in 2022 found that in patients with HR+/HER2- metastatic breast cancer (mBC) who are receiving non-PIK3CA-targeted therapies, the presence of a PIK3CA mutation acts as a negative prognostic factor. This mutation is associated with a significant reduction in progression-free survival (PFS) by about 2 months and overall survival (OS) by roughly 8 months. These findings highlight the considerable clinical impact of the PIK3CA mutation and the need for effective treatment options for this patient population (52).

Predictive and Prognostic Genomic Assays

These genomic tests evaluate breast cancer risk, predict tumor behavior, and guide therapy based on tumor characteristics. They are essential for precision medicine and enable individualized treatment for each patient (53).

ONCOTYPE DX

Oncotype DX is a molecular diagnostic test that measures the activity of 21 genes associated with the risk of breast cancer recurrence and the response to adjuvant therapies, including chemotherapy and hormonal therapy. This test is endorsed by prominent clinical practice guidelines, such as those from NCCN, ASCO, ESMO, NICE, and St. Gallen. Oncotype DX evaluates two main components: the recurrence score and the expression of genes involved in cellular proliferation, survival, angiogenesis, and inflammatory responses (53). The recurrence score is represented as a numerical value ranging from 0 to 100, indicating the relative risk of breast cancer recurrence over the next 10 years. Based on this score, patients are categorized into three risk groups: high, intermediate, and low (*Fig. 1* and *Fig. 2*) (53).

The TAILORx study (Trial Assigning Individualized Options for Treatment) is a phase III clinical trial that showed how useful the ONCOTYPE DX test is in determining the recurrence risk for patients with ER-positive and HER2-negative breast cancer. The study found that patients with a recurrence score below 11 did not gain any additional benefits from adjuvant chemotherapy when compared to hormonal therapy alone (54).

ONCOTYPE DX is effective, but it is not universally applicable. It is mainly utilized for hormone receptor-positive, HER2-negative tumors in early-stage breast cancer (53).

MAMMAPRINT

MAMMAPRINT measures the expression of 70 genes that are associated with the risk of breast cancer recurrence. These selected genes play crucial roles in important biological processes, such as cellular proliferation, cellular stress response, and cell cycle regulation. The test uses microarray technology to analyze gene expression profiles in tumor cells. Based on this analysis, MAMMAPRINT can estimate the risk of both early and late recurrence in patients with early-stage breast cancer (55).

The risk score generated by MAMMAPRINT is

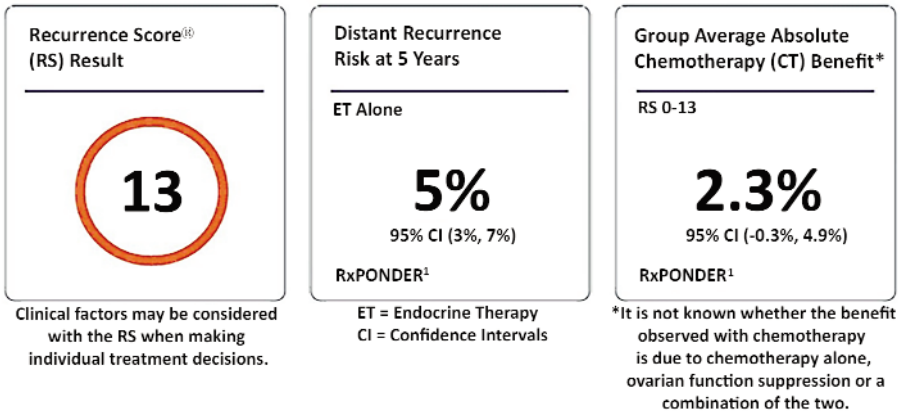

Figure 1. ONCOTYPE DX – Low Recurrence Score

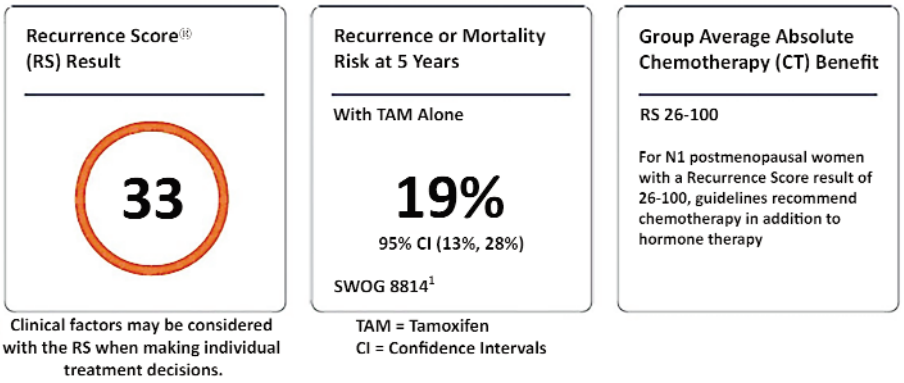

Figure 2. Figure 2. ONCOTYPE DX– High Recurrence Score

categorized as either "low risk" or "high risk". This classification helps clinicians determine whether a patient is likely to benefit from adjuvant chemotherapy or if hormonal treatments alone might be sufficient (55). Unlike other genetic tests that focus on a limited number of genes, MAMMAPRINT provides broader coverage by evaluating a diverse range of genes. This comprehensive approach captures the complexity of tumor behavior more effectively (55) (*Fig. 3*).

A key study supporting this test is MINDACT (Microarray in Node-Negative and 1-3 Positive Lymph Node Disease: Assessment of a Multigene Expression Test). This study evaluated the effectiveness of genomic testing in managing breast cancer. It found that patients with low MAMMAPRINT scores did not gain additional benefits from adjuvant chemotherapy. This suggests that hormonal therapies alone may be sufficient for these patients, helping them avoid the risks and side effects associated with chemotherapy. In contrast, patients with high-risk scores showed a higher likelihood of recurrence and could significantly benefit from adjuvant chemotherapy, which aligns with findings from ONCOTYPE DX (56, 57).

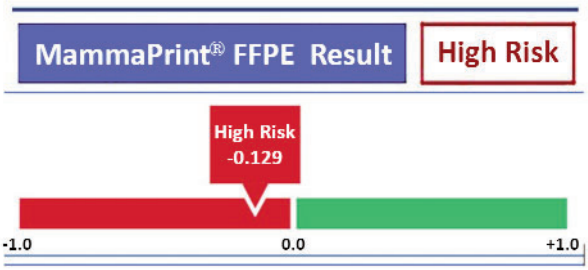

Figure 3. MammaPrint

PROSIGNA

PROSIGNA, also known as the PAM50 gene signature, is a genomic test designed to assess the risk of breast cancer recurrence. It plays a crucial role in personalizing treatment strategies. Unlike Mammaprint, which evaluates the expression of 70 genes to stratify risk, PROSIGNA (PAM50) analyzes 50 genes linked to the biological and molecular characteristics of breast tumors. This analysis categorizes breast cancer into specific molecular subtypes and estimates the likelihood of disease recurrence (58) (*Fig. 4*).

The PRELUDE study, which analyzed the PAM50 gene signature, showed that PROSIGNA scores are strongly linked to the long-term prognosis (up to 10 years) for patients with early-stage breast cancer. Additionally, this test significantly decreased the unnecessary use of chemotherapy in low-risk patients, highlighting its importance in optimizing treatment decisions (59,60).

ENDOPREDICT

EndoPredict is an advanced genomic assay designed to evaluate the risk of breast cancer recurrence and guide treatment decisions, especially for patients with early-stage, hormone receptor-positive (ER-positive) and HER2-negative breast cancer. This test is based on the expression of 12 key genes that are crucial for predicting recurrence risk and estimating the response to hormonal therapies. Unlike other genetic tests that analyze a larger panel of genes, EndoPredict integrates the biological characteristics of the tumor with clinical factors to generate a risk score. This score is presented in two ways: a general score that reflects the overall risk of tumor recurrence and a 10-year score that estimates long-term risk (61).

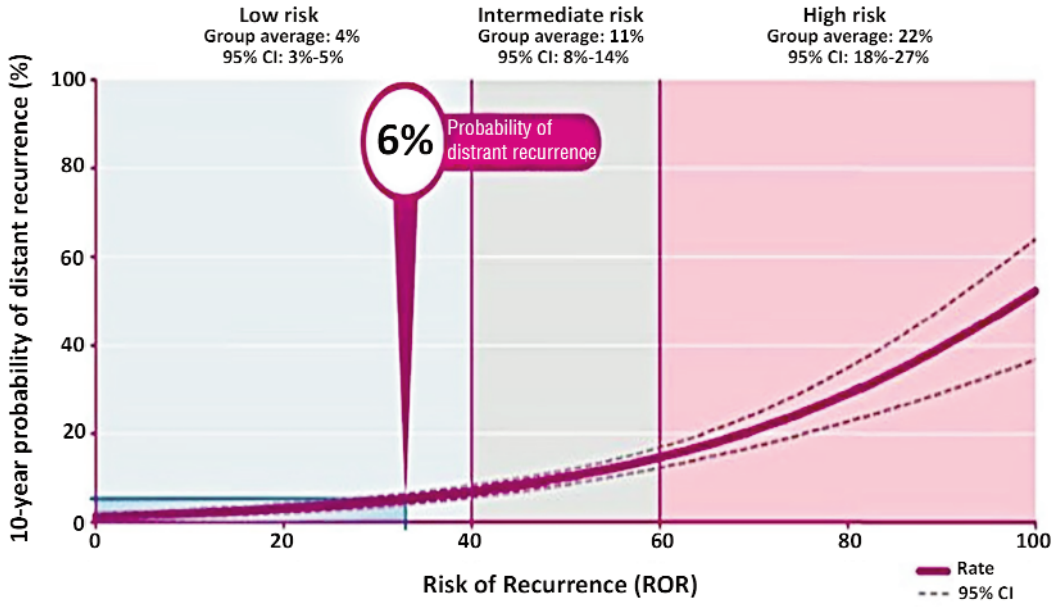

Figure 4. Prosigna – Risk of Recurrence

The ABCSG-8 study, an international clinical trial, assessed the effectiveness of EndoPredict in predicting breast cancer recurrence in ER-positive and HER2-negative patients. The study found that patients with low EndoPredict scores had a significantly reduced long-term recurrence risk. These results suggest that such patients may not benefit significantly from chemotherapy and that hormonal therapies alone may be sufficient for effective management (61).

Molecular Tumor Board

The Molecular Tumor Board (MTB) plays a crucial role in the targeted management of breast cancer by integrating molecular data into clinical practice, thereby providing tangible therapeutic benefits. The MTB is a multidisciplinary team composed of oncologists, geneticists, pathologists, bioinformatics specialists, and molecular biologists, which facilitates effective therapeutic decision-making. One key advantage of the MTB is its ability to offer insights into the potential aggressiveness of tumors, as well as the likelihood of recurrence or metastasis, which helps in determining the appropriate intensity of treatment strategies. Additionally, the MTB enhances the selection of treatment options that may be implemented (62).

Another significant benefit of this approach is risk stratification, which categorizes patients into high-risk and low-risk subgroups. This tailored method allows for the adjustment of treatment intensity, reducing the chances of overtreatment in low-risk cases (62).

In a study conducted in 2024, 30% of participating patients received treatments informed by genomic analysis, which included advanced therapies primarily accessible through clinical trials. The findings of the study indicated that treatments tailored to individual patients based on their unique molecular characteristics significantly increased the chances of a positive therapeutic response compared to standard treatment methods (63).

It is important to highlight that the therapeutic option known as theMTB is both feasible and scalable, as demonstrated by studies conducted in community centers. Research has shown that MTBs can operate effectively even in settings with limited resources while still providing recommendations in a reasonable timeframe (64). Additionally, MTBs offer the opportunity to implement experimental therapeutic options for patients in advanced stages of the disease, which is crucial when standard treatments are no longer effective (65). The strength of MTBs lies in their ability to integrate multidimensional data specific to each patient, enabling the creation of individualized treatment plans. They serve as an essential tool in the personalized management of this condition by continuously adapting recommendations based on

emerging medical evidence and the availability of medications. This dynamic approach enhances patient care and treatment outcomes (66,67).

Conclusions

Breast cancer is a complex disease that requires a multidimensional approach due to its various characteristics. Recent advancements in our understanding and management of this disease have shifted the focus from rigid clinical staging to integrating new molecular and systemic perspectives, which enhances patient stratification.

Each unique combination of prognostic and predictive factors results in a distinct pathological identity that should be treated according to its specific characteristics. The wide range of potential variants in breast cancer remains a significant unknown that needs further exploration.

The topics discussed previously provide a comprehensive analysis of the complex characteristics of breast cancer, emphasizing its clinical and biological diversity and the implications for prognosis, treatment and patient outcomes. The analysis covers both historical perspectives that laid the groundwork for our current understanding of this disease and contemporary discoveries that have transformed therapeutic approaches, particularly through the emergence of personalized medicine.

A key theme of the article is the heterogeneity of breast cancer, highlighted by both inter-tumoral and intra-tumoral variability, as well as the numerous possible combinations of characteristics that this disease can present. The importance of integrating classical clinical and pathological factors - such as tumor size, histological differentiation grade, and lymph node status - with advanced molecular and genomic biomarkers is underscored. This multidimensional approach allows for more precise patient stratification and the personalization of treatment strategies to achieve optimal outcomes. Additionally, breast cancers - plural, because this disease cannot be uniformly defined - remain an imprecisely characterized and constantly evolving subject. This ongoing complexity necessitates continuous research and the regular incorporation of new data into existing treatment protocols to improve patient care.

Conflicts of Interests

The authors declare no conflicts of interests.

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