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Histopathologic biomarkers in breast cancer diagnosis

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Abstract
Tumor biomarkers are products which are secreted either by the tumor or are produced by the body during the formation and progression of the tumors, they have been proven efficient and hopeful in cancer screening, early detection, risk assessment, recurrence and response to treatment. The function of molecular markers has become crucial in diagnosing metastatic breast cancer for appropriate treatment modalities. Commonly used histopathology biomarkers in breast cancer diagnosis include eestrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. These biomarkers provide the right plan of action for treating patients with breast cancer. The rise in molecular understanding of breast cancer and reference point therapy has led to the present review on histopathology biomarkers to improve the evolution of personalized treatments.

Keywords: Breast cancer, immunohistochemistry, biomarkers

Introduction

Breast cancer is the leading cancer among woman in the world and in 2018, it killed approximately 620 000 women. In 2022, 2.3 million women were diagnosed with breast cancer, and 670,000 died worldwide. Breast cancer affects women of all ages after puberty, but the incidence rises later in life [1]. Tumor biomarkers are products which are secreted either by the tumor or are produced by the body during the formation and progression of the tumors, they have been proven efficient and hopeful in cancer screening, early detection, risk assessment, recurrence and response to treatment. During their formation and progression, they have shown significant and promising potential in cancer screening and early diagnosis, predicting prognosis, detecting recurrence, and monitoring the effectiveness of treatment. They help in the diagnosis, in the identification of relapses, in the orientation of treatment applications and in the outlook for prognostic classification, especially for high-grade tumors. The molecular markers have been established to play a critical role in the diagnosis of metastatic breast cancer in addition to helping determine the appropriate chemotherapeutic and management approaches [2].

Histological biomarkers are particularly used in the diagnosis and treatment of cancer through the revelation of certain characteristics of the tumor and its likely behavior or response to therapy guide targeted treatment applications, and provide insights for prognostic stratification, particularly for aggressive tumor types. The role of molecular markers has become pivotal in diagnosing metastatic breast cancer, facilitating the selection of appropriate chemotherapy and treatment modalities [3].

There are details about mammography, its weaknesses, and this is why the investigation of new biomarkers for breast cancer detection and treatment is important. These biomarkers derived from cancer cells or from other tissues are in fact indispensable tools to prognosticate the behavior of tumors and their reactions to therapies. This situation underlines the necessity for identification of stable noninvasive diagnostic molecular markers for more efficient and individual approach to the treatment of breast cancer [4]. The Aim of this work is to present an overview of biomarkers applicable in breast cancer management.

Methodology

A total of 350 publications were identified by searching Google Scholar, Science Direct, PubMed, Scopus, and Research gate. Keywords for the search theme were “Breast cancer”, “Biomarkers AND Breast cancer”, “Molecular markers AND Breast cancer”. Duplicate scientific papers were excluded only 27 papers were used and referenced in the present study. Discrepancies in the data extraction were resolved through discussion and consultation with the team of reviewers. The search results were limited to only full articles published in English language (Fig. 1).

Histopathology biomarkers of breast cancer

Hormone receptors and the proliferation marker ki-67 are two histological biomarkers relied on in breast cancer diagnosis and assessment of treatment response. Breast cancer ERs, PRs, and HER2 are nuclear receptors and Transcription Factors that are involved in the progression of breast carcinoma. These receptors exhibit post-translational modifications (PTMs) that control their localization, function and protein-protein interactions (PPIs).

1.1 Estrogen Receptors

Estrogen receptor is among the most vital biomarkers in breast cancer, with approximately 70 – 80 % of breast tumor cases being ER+. ER+ cancers for the most part is somewhat less threatening and have a favorable prognosis than ER-negative (ER-) cancers. ER testing is now required to determine how treatment will affect cancer and how endocrine treatments will be received since 55-60% of ER+ and 8% of ER- cancers respond to them. ER is best detected by IHC and using this method we consider tumors that show one to one hundred percent positive nuclei to be ER+. This testing is crucial for defining adjuvant endocrine therapy, which is a mainstay of management following diagnosis of the malignant disease [5].

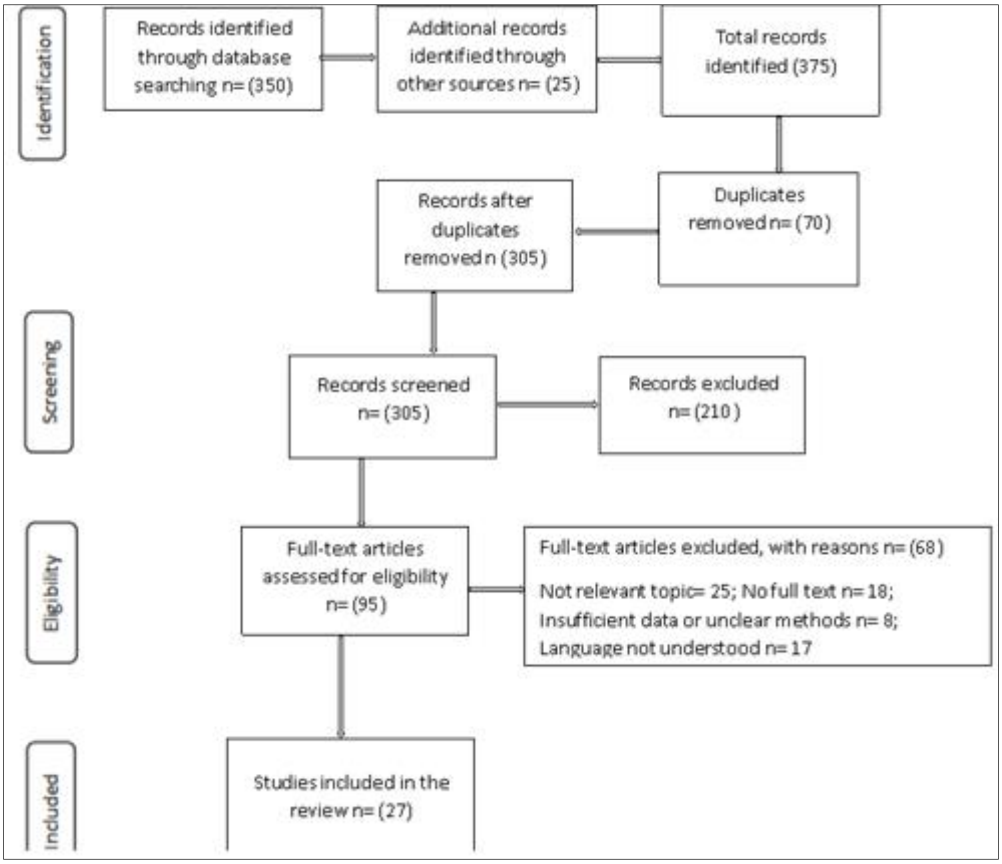

Fig 1: A PRISMA flowchart showing the search, selection, retrieval of relevant studies

1.2 Progesterone Receptors

The progesterone receptor (PR) is one of the most important markers in breast cancer with a significant bearing in treatment as well as in outcome. Despite the important of PR expression for anticipating the response to endocrine therapies, there is controversy surrounding its measurement, with some laboratories quantifiable naming it as optional since the value of PR expression is uncertain in predicting response to therapy. ER-/PR-: discrepant expression, around 5-10% of cancers are ER-/PR- While ER-/PR+ accounts for approximately 10-15% of breast cancer Approximately 65-75% of breast cancers are hormone receptor positive of which 45-55% are PR-positive and 50-60% of these are PR-ER-co-positive. There are two isoforms which are PR-A and PR-B; PR-A has poorer prognosis since it has high level. In general, therefore, the assessment of PR status continues to be relevant in evaluating disease-free and overall survival in breast cancer patients [6].

1.3 Human epidermal growth receptor 2

Her2 is another important protein that has a key role in cell signaling and growth, and amplified in 25-30% of metastatic breast cancer cases. This over expression is also associated with poor prognosis and malignant phenotype of the tumor, as well as recurrence. Email notification for HER-2 status involves IHC and FISH in choosing the right treatment for the patient. Developed drugs such as Trastuzumab (Herceptin) have been developed to target the HER-2 positive breast cancer and have benefited the patients tremendously. HER-2 works as an adverse prognostic indicator and is, in particular, used to predict the positive outcomes after treatments, which target HER-2 positive tumors. According to [7].

1.4 Ki-67

Ki67 is a protein that in different concentrations is used to measure proliferation activity in a tumor to establish its malignant potential in breast cancer. Its assessment by IHC is a valuable prognostic marker and therapeutic predictor, especially in luminary breast cancer. Ki67 is better in chemotherapy, particularly in triple-negative breast cancer. However, Ki67 has practical drawbacks, which are based on the differences in Ki67 assessment techniques and the lack of stability of studies with predictive Ki67 data. The most recent trials have revealed that an LRI of Ki 67 percent of 20 or above helps define the high risk of recurrence and affecting treatment choices and final FDA endorsement of some or all treatments [8]

1. Emerging Biomarkers

In more current investigations, a number of new biomarkers for breast cancer have been found that have the possibility of improving the detection of early disease as well as the selection of individualized therapies [8]

1. **Fatty Acid Synthase (FASN):** Present study suggests that FASN may enhance the survival of triple negative breast cancer when MET gene (HGF receptor) positive [8].
2. **Androgen Receptor (AR):** AR is diagnosed in high breast cancer and is characterized by favorable associations with survival in luminal A but may be an indicator of growth in ER-negative and HER-2 positive breast cancer [9].
3. **BRCA1 and BRCA2**: These tumor suppressor genes are mediating DNA repair processes. Patients with aggressive disease show decreased staining of BRCA1, and IHC can reveal protein deficiencies in BRCA [5]

4. **Epithelial Cell Adhesion Molecule (EpCAM)**: Significantly overexpressed in various cancers including TNBC, EpCAM has been associated with poor survival and could be a potential marker [5]

5. **Cyclin D1: This** cell cycle regulator is overexpressed in about 50% of breast cancers and is considered to be related to tamoxifen resistance; however, its prognostic value is an issue [5]

6. **Mammaglobin-A:** A secretory protein utilized in IHC for metastatic breast cancer which prognostic worth is still questionable [5]

7. **Human Epididymis Protein 4 (HE4):** Overexpressed in breast cancer, HE4 is associated with a poor outcome and may be used for diagnosis and the detection of relapse [5]. These biomarkers suggest potential avenues of raising the efficiency of the illness treatment and diagnosis methods of breast cancer.

3. Techniques for Identifying Histological Biomarkers

3.1 Immunohistochemistry

IHC is very important in the field of oncology and cancer research since it offers several valuable advantages for diagnosing, prognosis, as well as for treatment of cancer patients. This technique takes advantage of the humanness of antibodies and cellular antigens, where by the antibodies are used to identify and locate specific proteins in tissue samples, IHC remains critical in determining the receptors status of breast cancer especially estrogen and progesterone which are very vital in management decisions [6].

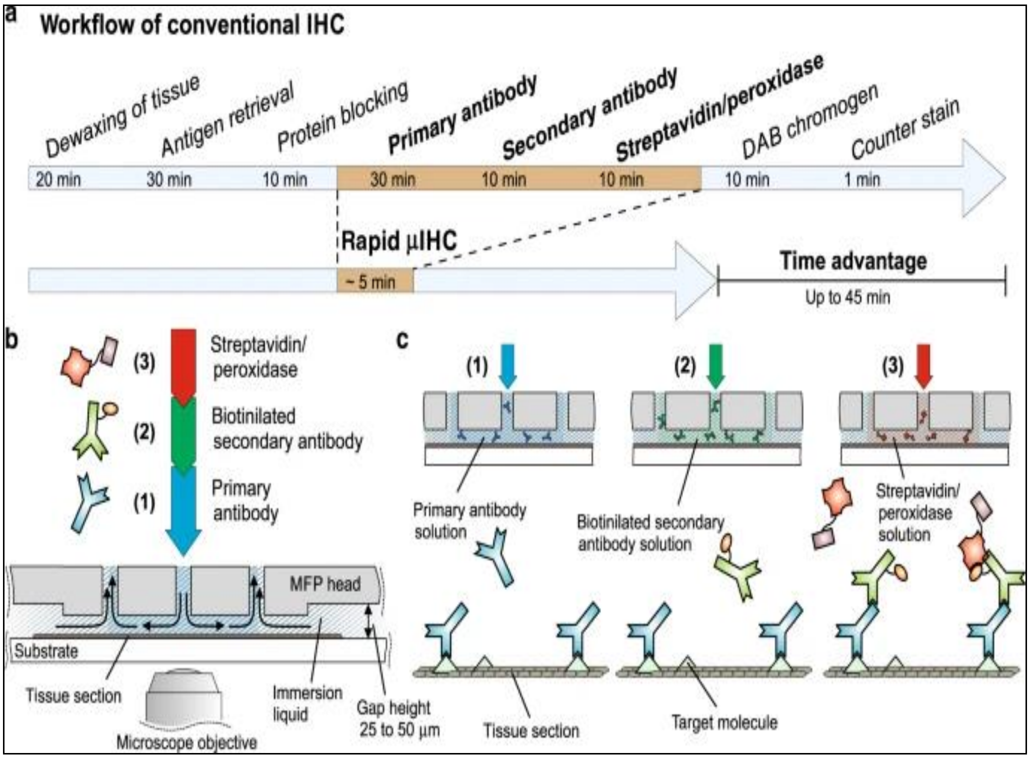

Fig 2: Immunohistochemistrytry Flow chart [7]

The IHC process involves a series of standardized steps to ensure accurate detection of biomarkers:

1. **Tissue Fixation and Preparation:** Breast tissue samples include the primary tumor tissue or metastatic tissue, which is often collected by biopsy or surgical resection, fixed in formalin and then embedded in paraffin wax to maintain tissue structure as well as proteins.

2. **Sectioning:** Sections of the sample used here are obtained with the paraffin, and then the sections are placed on the glass slides to be stained.

3. **Antigen Retrieval:** Due to formalin fixation, target epitopes can be hidden, which is why heat or enzymatic treatment is performed to satisfy the population of antibodies and allow them bind to their matching antigens.

4. **Blocking**: To avoid this type of binding, which is otherwise known as non-specific binding, the tissue is first washed in blocker solutions such as serum.
5. **Primary Antibody Application:** Primary antibodies are specific to the target biomarkers in the tissue section, for instance estrogen/progesterone receptors or HER2.

6. **Secondary Antibody and Visualization:** A second antibody, linked to an enzyme (e.g. horseradish peroxidase), or a fluorescein label is introduced. In enzyme based detection DAB is used to develop a visible color at the antigen sites, so that they can be observed. Fluorescent dyes emit light under some fixed wavelength for visualization purpose.

7. **Counterstaining and Mounting:** Histological slides can be stained with a counterstain which is hematoxylin used to stain the cell nuclei and then mounted for microscopy.

4. Hormone Receptor Status

Steroids defined as regulatory substances, consisting of hormones estrogen and progesterone are secreted primarily by the ovaries in women and by the other tissues in both males and females. These hormones may stimulate the development of the hormone receptor-positive breast cancer that contains receptors for estrogen and/or progesterone at their cells surface: the estrogen receptors (ER) and the progesterone receptors (PR). Breast tumors characterized by the presence of these receptors are called estrogen and/or progesterone receptor-positive (ER+ / PR+,), if they are absent, tumors are called estrogen and/or progesterone receptor-negative (ER- / PR-). A large number of ER-positive tumors are also PR-positive. Evaluating hormone receptor status by examining cancer tissue is necessary to determine a course of treatment because HR+ cancers can be treated with drugs that block the hormones that stimulate cancerous cell growth [5].

5. Application of IHC in breast cancer diagnosis

Histological biomarkers play a crucial role in cancer diagnosis and treatment by providing insights into tumor characteristics that guides prognosis and therapy decisions. They assist in treatment outcomes anticipation and disease status, such as its progress or relapse, therefore promoting tailored medicine [8]. Tissue pathology biomarkers is use in cancer diagnosis and treatment especially by helping in determining tumors behavior in the body to enable prognosis and therapy plans. They assist to determine treatment outcomes and simultaneously track diseases progression or potential relapse, allowing so called ‘individualized approach’ to therapy [8].

A. Hormone Receptors:

- **Estrogen Receptor (ER):** ER is essential in breast cancer, primarily because ER positive malignancies require estrogen to grow. IHC tests for ER presence, and it can also determine which patient is likely to respond positively to hormone treatment such as tamoxifen and aromatic inhibitors.

- **Progesterone Receptor (PR):** PR is usually combined with ER testing since the absence of both hormones determines hormone-dependent cancer more precisely. This status of PR has added value for patient prognosis and treatment consideration.

Clinical Implication: ER and PR have also been shown to be positive for breast cancer patient overall survival and sensitivity to hormone therapies.

b. HER2

1. **HER2 over expression**: HER2 is an antigen that tends to boost the growth of cancer cells. In this context, IHC is applied to determine the intensity of expression of HER2 protein on the membrane of Breast Cancer cells. HER2 is over expressed in approximately 20% of breast cancer cases and is considered to be aggressive.

2. **Scoring HER2 Expression**: The IHC score ranges from 0 to 3+ depending on the level of intensity of staining. A score of 3 plus means that the tumor is HER2 positive and patients are now candidates for HER2-targeted therapies, such as trastuzumab (Herceptin).

3. **Clinical Implications:** HER2-positive breast cancers are more malignant but target therapies are available that enhance the prognosis dramatically.

c. Ki-67 Proliferation Index

Ki-67 concerns cell proliferation, that is, how fast cancer cells are reproducing. High dosage of Ki-67 proves the high chances of tumor while low Ki-67 landmark low rate of cancer progression. Ki-67 index is useful when sorting out the prognosis and whether certain patient should undergo chemotherapy at all, and in breast cancer at stages I and II [9].

Clinical Application of immunohistochemistry

IHC is central to the molecular subtyping of breast cancer, which helps guide treatment decisions:

Luminal A: ER/PR positive, HER2 negative and low ki-67 index. Estrogen and progesterone receptors positive, HER2 negative, and low Ki-67 index. This subtype is less aggressive and can be treated by means of hormone therapy [2, 9].

Luminal B: Luminal A include ER/PR positive HER 2 negatives with low Ki-67 or luminal B include ER/PR negative or HER 2 positive or HER 2 negatives with high Ki-67. Even more advanced than Luminal A; they may need chemotherapy and HER2-targeted therapy in case if they are HER2 positive [2].

HER2-Enriched: It is HER2-positive and is negative for estrogen receptors and progesterone receptors. These cancers are malignant but guide HER2 targeted therapies [9].

Triple-Negative: Estrogen receptor negative/ER negative, progesterone receptor negative /PR negative, human epidermal growth factor receptor 2 negative/ HER2 negative. There is a somewhat higher likelihood of this occurring and the tumors are not hormone or HER2 positive, however, chemotherapeutic agents are usually helpful [2].

Fluorescence in Situ Hybridization (FISH)

FISH stands for Fluorescence in Situ Hybridization and is a molecular cytogenetic technique that is used to visualize a particular DNA target on chromosomes in samples such as tissue sections or cells. That is why it is very effective in revealing of gene amplification, translocations or deletions, and is widely used for diagnostics of cancer, especially breast cancer [10].

HER2 Gene Amplification in Breast Cancer

HER2 (Human Epidermal Growth Factor Receptor 2) is a gene that increases the growth of cells. Overexpression of HER2 protein is due to gene HER2 amplification and occurs in 15-20% of breast cancer patients and correlates with worse prognosis. Determining HER2 status is important because women with HER2 positive disease will respond well to drugs like trastuzumab (Herceptin) or pertuzumab that produce good results when used [11].

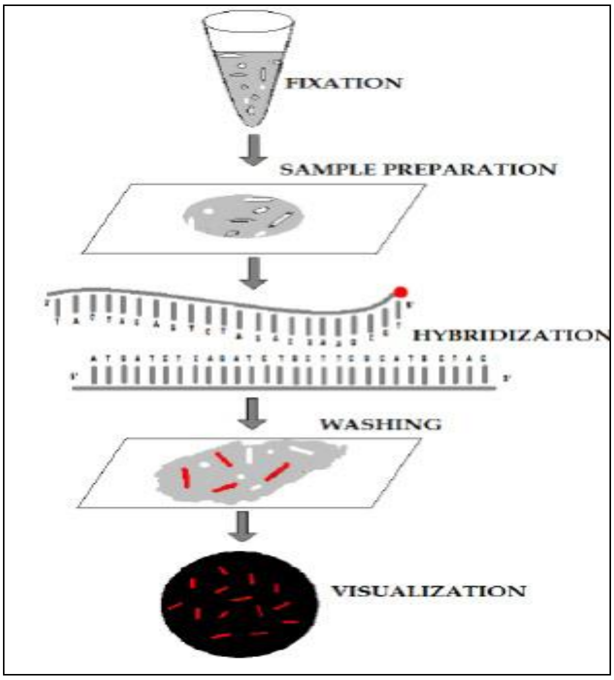

Fig 3: Flow chart of FISH [11]

FISH protocol for HER2 Test as described by Gutierrez *et al*. [12]

1. **Tissue Preparation:** Breast cancer tissue sections in FFPE are used to perform FISH. Thin sections are mounted on glass slide and are used for analysis.

2. **Hybridization:** Fluorescent labelled DNA probes unique to HER2 gene on chromosome 17 are placed on the tissue section. These probes attach to similar sequences located in the HER2 gene. A second fluorescent probe specific to the centromere of human chromosome 17 (CEP17) is used as an internal control to quantify the HER2 gene signal relative to chromosome signals**.**

3. **Visualization:** The slide is then viewed under the fluorescence microscope channel. Here visible fluorescence of only the HER2 gene and CEP17 probes in individual nuclei of cancer cells is counted. In abdominal metastasizing carcinomas with HER2 gene amplification in this study, greater than three fluorescent signals from the HER2 gene probe compared to the standard probe will be detectable.

4. **Interpretation:** For greater comparability of results, the values are usually measured as the HER2/cytokeratin 5/6 to CEP17 ratio. The tumor samples were considered to be HER2 gene amplification if HER2/CEP17 ratio was more than 2.0 or the number of HER2 signals per nucleus was more than 6.

Application of Fish in Breast Cancer

1. **HER2 Testing for Treatment Selection:** FISH stands for Fluorescence in Situ Hybridization to define HER2 gene amplification in the breast cancer patients. Patients with HER2 positive are suitable for HER2 directed therapies including trastuzumab, pertuzumab and T-DM1. It also showed these therapies enhance the patient's survival and halted recurrence of the disease in HER2+ breast cancer [13].

2. **Confirmatory Testing:** FISH is mostly done to complement testing whenever the results of IHC for HER2 are borderline (HER2 score of 2+). Whereas, IHC evaluates the HER2 protein on the cell membranes, FISH gives additional information on the status of the genes on the DNA level [13].

3. **Prognostic and Predictive Value:** Increased HER 2 gene copy number detected by FISH is used to predict more aggressive breast cancer. It identifies the respondent HER2-positivepatients through FISH in order to predict the response to therapies that target the HER2 pathway and their prognosis [14]

4. **Companion Diagnostics:** FISH is an FDA cleared-companion diagnostic for HER2-targeted therapy. It helps to make certain that only the patients who will benefit from these treatments make it to receive such treatments

Emerging techniques

In addition to regular IHC and FISH, there are other methods which have been improved to make the detection of histological biomarkers for breast cancer even better. These techniques provide more accurate methods of distinguishing the nature and location of the tumor as well as the general techniques for treating the disease.

1. Digital pathology and image analysis

The current developments in the field of digital image analysis and especially artificial intelligence are soon expected to fortify breast pathology in terms of speed of diagnosis and quality. Such technologies can serve as tools for pathologists in improving grading and biomarker analysis that are time consuming and can reduce the variability; in addition, these technologies can discover novel patterns associated with patient survival. Mentioned techniques remain limited in their implementation to date, but the area is developing quickly, so the applicability of these digital methods should be verified to confirm the actual positive impact on patient care outcomes [14].

2. RNA Sequencing and Gene Expression Analysis

RNA sequencing (RNA-seq) is a Next-generation sequencing (NGS) tool used for the measurement of gene expression changes in breast cancer tissues. RNA-seq in transcriptome analysis identifies biomarkers at the molecular level which cannot be identified in IHC using protein-based markers such as CD31 [15].

Mass Spectrometry Based Proteomics

The study of proteomics for breast cancer is evolving in the proteomics methodology showing promising signs in diagnostics as well as in treatment. New in vitro and in vivo combined investigations investigated cultured breast cancer cells at different tumor stages and performed experiments on tissue specimens. This study showed proteomic changes in tumor stage and validated on tissue microarray that revealed structural and metabolic abnormalities in transformed cells. Moreover, the current investigations also state that, plasma proteome in breast cancer represents the proteins from the tumor microenvironment which promotes important physiological functions of healing wound, immune response, and tissue remodeling. [16].

Multiplex Immunohistochemistry (mIHC)
Multiplex immunohistochemistry is a process by which several biomarkers can be identified on a section of tissue at the same time. This is particularly useful in studying the tumor microenvironment since it allows the visualization of infiltration of immune cells and also identification of how the different cells collaborate or not within the tumor [17,18].

Nanomaterial Based Biosensors
Current trends in biomarker sensing include biosensors fabricated from nanotechnology. Employing the properties of the nanomaterial, these devices, can identify biomarkers with a high specificity and sensitivity. It is able to identify circulating tumor DNA (ctDNA), exosomes, and other molecules from the body fluids such as blood samples. Nano biosensors for biomarkers including HER2, ER and PR from liquid biopsy are being developed as a less invasive approach than tissue biopsy in the assessment of disease progression and treatment response. These techniques offer greater precision in identifying and characterizing tumor biology, aiding in prognosis and treatment planning.

Some limitations of biomarkers
The application of biomarkers in breast cancer therapy poses many problems. Economically, advanced detection technologies and targeted therapies are costly and may lag in implementation in facilities with limited resource shops. Furthermore, understanding these biomarkers calls for expert knowledge, which most often, the underdeveloped healthcare systems may not possess. Biomarker data requires strict sample collection and analytical methods for validity, which as a result of the availability of technology unearths disparities in regional treatment. In addition, using genetic data also poses some ethical questions with regard to respect to privacy and informed consent of the patient. Solving these technical and practical concerns is essential for using biomarkers in breast cancer practice [2,19,].

Issues with biomarkers discordance
Although hormone receptors and HER 2/neu were mainly used to study original breast tumors, new research shows that there is a low agreement between the original tumor and its spread. Estrogen receptors differ in 30-40% of cases, and HER 2/neu differs in 10-30% of cases. Such dissociation could be due to different approaches of assessment, evolution of tumor biology during transition from primary cancer to metastatic disease, tumor heterogeneity, or impact of therapies. These disparities sources are not well explained and instead reveal tumor heterogeneity and variability across diagnostic procedures. Perhaps, bringing these variables up to an acceptable standard could improve the dependability of diagnosing outcomes. With the use of therapies against hormone receptors and HER-2/neu being introduced in to practice more often, recognizing changes in a tumor's biomarkers is fundamental in making therapy decisions. They have also found that a down-regulation of hormone receptors or HER-2/neu may signal tumor resistance whereas the gain of these receptors may provide fresh therapeutic avenues which would enhance patient prognosis [20, 21, 22].

Conclusion
Breast cancer is a heterogeneous form of cancer. Commonly used prognostic biomarkers to characterize the disease include estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. These biomarkers create the right strategy for treating patients with breast cancer. Therefore, the rise in molecular understanding of breast cancer and new targeted therapies to improve the development of personalized treatments

Abbreviations
DIA; digital image analysis, AI; artificial intelligence, H&E; hematoxylin and eosin, IHC; immunohistochemistry, ISH; in situ hybridization, FISH; Fluorescence In Situ Hybridization, ER; estrogen receptor α, PR; progesterone receptor, HER2; human epidermal growth factor receptor 2, mRNA; messenger ribonucleic acid, TNBC; Triple negative breast cancer, DNA; deoxyribonucleic acid, NGS; Next-generation sequencing, DBT; digital breast tomosynthesis, AR; Androgen receptor.

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Consent for publication: Not applicable

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