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BIOMARKERS IN BREAST CANCER.

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Abstract:

Biomarkers are the molecules or tissue based processes that indicate future behavior of the cancer. Broadly classified as tissue-based and blood-based biomarkers, these need special assays over and above the routine clinical, radiological and histopathological examination. Potential of tumor biomarker ranges from risk determination through screening, differential diagnosis, prognosis and prediction to monitoring the disease. Breast cancer is the leading cause of cancer related deaths among women. With progressively better understanding of tumor biology, clinical implications of the molecular behavior are being explored for better therapeutic gains in such patients. Though, to date we have limited range of clinically useful biomarkers in breast cancer; their potential may guide us for cancer therapy in future.

INTRODUCTION

Breast cancer is a diverse spectrum disease in women and one of the leading causes of death, worldwide. Breast cancer starts from the breast cells, grows into surrounding tissues and tends to spread to distant parts. Breast cancer is commonly found in women, but may also develop in men, although this is rare. Breast cancer occurs mainly in middle aged and elderly women. It is the common cancer affecting more than twice the cancers at any other site.[1]

As per GLOBOCAN 2018 estimates, breast cancer incidence is more in Europe and America than in Asia. So, we may say that breast cancer is a disease of developed world. About 50% of breast cancer related deaths occur in less developed countries. Lowest incidence is found in most of the African countries. In developing countries, incidence of breast cancer is below 40 per lac.[2]

In India, breast cancer is most common cancer among females. In year 2018, breast cancer led with 1,62,468 new cases (accounting for 27.7% of all cancers) and 87,090 breast-cancer related deaths.[2] Breast cancer in the urban areas is three times higher than in rural parts of India. There has been a significant rise in the incidence of breast cancer. By year 2025, the number of breast cancer patients in India is predicted to get doubled.

Biomarkers play an important role in disease detection to treatment and outcomes. Tumor biomarkers are a range of molecules from nucleic acids to metabolites. However, protein biomarkers convert most readily into targeted therapies (as most pharmaceuticals tend to target proteins) and clinical diagnostic assays using standard existing platforms.[3] The panels of breast cancer biomarkers can be designed in anticipation to phenotypic heterogeneity and some have emerged with sufficient sensitivity and specificity for use in prediction of treatment response.[4]

Biomarkers may be measured at different levels- protein, RNA, DNA, cell and/ or tissue. These may be identified and evaluated in the tissue of origin, regional lymph nodes, at distant sites or even in blood circulation. Even the implications of biomarkers are complex and may vary as per the end user.

Potential uses of biomarkers include-

- Risk determination, Susceptibility and Carcinogenesis
- Screening
- Differential diagnosis and Staging
- Prognosis (Future risk without treatment)
- Prediction (Likelihood of response to treatment)
- Monitoring (Response evaluation)

Table 1 enlists some of the known biomarkers those are relevant to breast cancer.

Table 1

Potential Use	Marker	Use/Recommendation	Remarks
Risk determination/ Susceptibility and Carcinogenesis/ Screening	Gene Assays (Oncotype DX, Mammaprint etc), BRCA1, BRCA2, PTEN, TP53, CDH1, STK11, PALB2, CHEK2, ATM	Risk of recurrence in early, node negative, ER+ cases.	No single biomarker to date being used for risk stratification, preventive strategy, early detection of primary disease and developing screening protocols in breast cancer
Differential Diagnosis and Staging	ER, PR HER-2/neu GCDP	Indicates tissue of origin/ specificity	Some Lung and uterine malignancy also express
Prognosis	Tumor tissue uPA, PAI-1	Future risk in absence of treatment	Explains natural course of disease
Predictive	ER	Primary invasive breast cancer	Response to Endocrine therapy
	HER-2/neu	Primary/ Recurrent/ Metastatic Breast Cancer	Response to agents like Trastuzumab/ Lapatinib etc.
Monitoring	CA15-3, CA27.29, CEA, Circulating Tumor Cells	Monitoring in selected patients with metastatic disease	Data insufficient to recommend routine use in breast cancer
Others	Ki-67, cyclin D, cyclin E, p21, p27, p53,Cathepsin D, Proteomic analysis, Epigenomics and Genomics	Ki-67 distinguishes Luminal A versus B in ER/PR+ lesions	Lack of standardization limits the clinical use Data insufficient to recommend routine use in breast cancer

The significance of biomarker in breast cancer can be appreciated by the fact that recently, in Eighth edition AJCC, use of biomarker status is recommended for prognostic staging along with anatomic stage grouping and influences the prognosis and/ or management decisions; resources permitting. Table 2 compares few changes in 8th AJCC over 7th AJCC staging system.[5,6]

Table 2

TNM	Grade	HER	ER	PR	Oncotype DX	AJCC-7	AJCC-8
T1N0M0	1-3	-	-	-		IA	IIA
	3	-	+	-		-do-	-do-
	3	-	-	+/-		-do-	-do-
T2N0M0	1-3	-	+	+/-	<11	IIA	IA
	2	-	-	-		-do-	IIIA
	3	-	+	-		-do-	IIIA
	3	-	-	+/-		-do-	IIIA
T01N1M0	2	-	-	-		-do-	IIIA
T2N1M0	1	-	+	+		IIB	IB
	2	+	+	+		-do-	IB
	1-2	-	-	-		-do-	IIIB
	3	-	+	-		-do-	IIIB
	3	-	-	+/-		-do-	IIIC
T2N2M0	1-2	+	+	+		IIIA	IB
	1	-	-	-		-do-	IIA
	2-3	-	+/-	+/-		-do-	IIIC
T3	1-3	+	+	+		-do-	IB
	1-3	-	+/-	+/-		-do-	IIIC
N3	1	+	+	-		IIIC	IIIA

It may be inferred here that the markers found positive, predict good response to hormone/ targeted therapies and favor prognosis. Hence markers positivity downstages in latest stage grouping whereas markers found negative lead to upstaging of corresponding stage in previous stage grouping.

Based on the source, biomarkers can broadly be categorized as- tissue based biomarkers and blood based biomarkers. Following section pertains to the commonly used biomarkers in breast cancer for various purposes.

TISSUE BASED PROGNOSTIC BIOMARKERS

ER, PR and HER-2/neu

A standard clinical practice guideline prefers measurement of ER, PR, and HER2/neu expression in all primary invasive breast tumors to determine treatment course.[7]

As per ASCO/CAP guidelines 2010, ER/PR is considered positive if ≥1% tumor cells overexpress/ are immune-reactive.

As per ASCO/CAP guidelines 2013, HER-2/neu is considered; positive, if- IHC 3+ or FISH amplified (HER-2neu/CEP17 ratio of ≥2.0 or average HER-2neu gene copy number ≥6 signals/nucleus); equivocal, if- IHC 2+ or FISH amplified (HER-2neu/CEP17 ratio of <2.0 and average HER-2/neu gene copy number ≥4 but <6 signals/nucleus); and negative, if- IHC 0-1+ or FISH amplified (HER-2neu/CEP17 ratio of <2.0 or average HER-2/neu gene copy number <4 signals/nucleus)

Based on the expression of markers, several genomic subtypes of breast cancer have been identified that differ in prognosis and treatment protocols **(Table 3)**.

Table 3

Subtype	Proportion	ER	PR	HER-2	Other	Prognosis (5-yr DFS)	Treatment
Luminal A	50-60 %	+	+	-	Low Ki-67 Low grade CK 8,18	79-85%	ET
Luminal B	15-20 %	+	+	-/+	Mod Ki-67 High grade CK 8,18	60-75%	ET±CT±TT
HER Enriched	15-20 %	-	-	+	High Ki-67	41-65%	CT+TT
Basal like	10-15 %	-	-	-	High Ki-67 EGFR CK 5,14,17	48-72%	CT, May benefit from Gefitinib like agents
Normal Like	5-10 %	-/+	-/+	-	High Ki-67	72-81%	CT±ET

ET-Endocrine therapy, CT- Chemotherapy, TT- Targeted therapy

Hormone receptor expression is predictive of response to endocrine therapies, such as selective estrogen receptor modulators (SERM's), aromatase inhibitors (AI).

The tumor that does not express ER, PR or HER-2/neu is termed as triple negative. Triple-negative phenotype is characterized by its unique molecular profile, distinct patterns of metastasis, aggressive behavior and lack of benefit to targeted therapies.[8]

Some studies have shown that the triple negative tumors which expressed basal markers were different from those cells which do not express it. Basal like breast cancer has better prognostic value than triple negative phenotype.[9] These phenotypes generally show no response to the available endocrine and anti-HER2/neu targeted therapies like trastuzumab and lapatinib etc.

Gene expression profiles/ Genome Assay

Many diagnostic genome assays are now done, mainly on paraffin embedded tissue, like Oncotype DX, Mammaprint etc. Commonly used is Oncotype DX, a 21 gene panel based on reverse transcription polymerase chain reaction (RT-PCR) and is used to quantify the likelihood of recurrence in early stage, ER positive breast cancer. Recurrence score determined by this assay has been found to be a better predictor of outcome and guides well of the treatment options. Low risk (score ≤18) patients of early stage ER positive may be offered endocrine therapy only, intermediate risk group (score 18-30) endocrine with/ without chemotherapy and high risk (score ≥31) are best managed with combination of both endocrine and chemotherapy. TAILORx study has addressed the utility.[10]

BLOOD BASED PROGNOSTIC BIOMARKERS

These biomarkers indicate physiologic state and provide information about occurrence and progression of disease. These include DNA, RNA, protein and different metabolites. Advancement of sequencing technologies leads to development of blood analyses of tumors, mainly based on circulating tumor DNA. This kind of cancer cells are found in the peripheral blood and play an important role in tumor progression.

Many researchers established tumor DNA as a prognostic biomarker for breast cancer. It has been observed that biomarker in blood may be superior for monitoring tumor response to therapy

in breast cancer. Some studies have confirmed the ability of blood markers measurement in predicting breast cancer survival.[11]

Circulating tumor DNA are small fragments of cell-free DNA circulating in blood, containing some tumor specific sequence modifications. Recent studies observed that circulating tumor DNA acts as highly sensitive biomarker for breast cancer.[11] An important advantage of circulating tumor DNA is its ability to test for specific mutations or modifications and acquired resistance mechanisms by using only a few milliliters of venous blood. This may be helpful in identifying the non-responders to a particular therapy and selection of second-line therapies in future. Standardization of assay for circulating tumor DNA is currently on its way.

Recent advances in high-throughput technologies; in genomics, proteomics and metabolomics; have facilitated biomarker discovery. As more potential biomarkers are discovered, further studies are needed to validate these markers. The ultimate use of these biomarkers is in clinical applications for effective cancer management and outcomes.

OTHER BIOMARKERS

There are certain other prognostic and predictive factors that have not been widely adopted in routine clinical use. Such markers include- proliferation markers (S-phase fraction, percentage of cells labeled with thymidine or bromo-deoxy-uridine, over expression of Ki-67 or MIB and mitotic index), plasminogen activator inhibitor-1 (PAI-1), detection of micro-metastases in the bone marrow, and measures of the plasminogen activator system (Mammostrat and Urokinase Plasminogen Activator k.a. MUPA).[12]

Some molecular biomarkers are also used to study other important aspects like genetic polymorphism associated with alteration in receptor expression, function and evolving as important determinant of breast cancer susceptibility. The association of genetic polymorphism in estrogen receptor-α, cyclin-D1 and other related genes has been a subject of increasing interest. Several studies have addressed association of polymorphism and breast cancer, representing either increased or decreased risk of breast cancer.[13]

BIOMARKERS IN CLINICAL TRIALS

Clinical trials determining maximum tolerated dose (MTD) of systemic therapies are in early phases. However, in case of targeted therapies, optimum dose may not depend on initial dose. This is determined by various biomarkers that identify successful target binding. ^{18}F-FES biomarker shows an important role in the designing clinical trials. It is used as a pharmacodynamic biomarker in monitoring estrogen and progesterone engagement in targeted therapies and also determines optimal dose for novel therapies. ^{18}F-FES PET/CT and degrader GDC-0810 are available biomarkers which are applied to the ER antagonist (study registered as NCT01377324 in clinicaltrials.gov).[14]

CHALLENGES IN USE OF BIOMARKERS

The use of many molecular biomarkers is in its early stages and prospective data are generally lacking. Before receiving acceptance as a clinically valuable biomarker, molecular techniques face several challenges, like documentation of test reproducibility in many clinical trials, exhibiting a strong correlation between the test and clinical outcome. Perhaps the most challenging thing is the evidence of better survival.[15]

CONCLUSION

Although significant progress has been made, in both understanding and treatment of breast cancer over the last three decades, it is still a leading cause of death in women worldwide. To fight against this disease, not only better therapies are required but also improved methods with marker study are needed to assess individual's risk of developing cancer, to detect cancers at early stages, to treat more effectively and to monitor recurrence and response to therapy.

Only a few breast cancer biomarkers have proven to be of clinical utility. These biomarkers are ER, PR, HER-2/neu and gene panels like Oncotype DX which are used in estimating risk of disease recurrence and predicting the benefit of chemotherapy and targeted therapy. But clinical utility of some biomarkers still requires large prospective multicenter clinical trials.

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