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Genomic assays in breast cancer: Issues yet to settle?

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ABSTRACT

Breast cancer today has emerged as the most commonly diagnosed malignancy in women world wide, accounting for 1 in 4 of every cancer diagnosed in women today. It is the leading cause of cancer death in women in the developing world and second leading cause of cancer (following lung cancer) in the developed world. Introduction of novel high through-output gene expression profiling technologies such as Next Generation Sequencing (NGS) and Genome wide association studies (GWAS) has led to the genetic profiling of breast cancer and to the development of genomic assays that ushered in an paradigm shift in the management of breast cancer from single individual variable to multivariate prediction models encompassing the tumors gross, microscopic and genetic variables. Oncotype DX, MammaPrint assay, MammoStrat assay, & Prosigna kit are some of the commercially available assays in various stages of validation. But various studies have reported discordance in risk stratification when the different tests is applied to the same patient cohort leading to a therapeutic quagmire. Tumor genetic signatures are not concordant but highly variable with each carrying its own unique set of genes dictating its growth, response to chemotherapy and risk of recurrence. Similarly triple negative breast cancers (TNBC), risk of late recurrence (> 5 years), validity of these over different population groups and quality control are some of the other issues which are yet to settle.

Key words: Gene expression profiling, Prognostic assays, Multigene assays.

Cancer care in the last decade has been revolutionized by the introduction of genomic assays, and much knowledge has been accrued in understanding the molecular pathology of breast cancer. Gene expression profiling has aided in the identification of specific genomic markers and has been used to construct multigene assays that predict the risk of early or late recurrences and need for adjuvant chemotherapy. Some of the commonly used assays in the market are Oncotype DX (Genomic Health Inc., Redwood City, CA, USA), MammaPrint (Agendia, Irvine, CA, USA), MammoStrat assay (Clarient Diagnostic Services Inc., CA, USA), Prosigna kit (Nanostring Technologies, Seattle, WA, USA), and EndoPredict assay (Sividon Diagnostics, GmbH, Koln, Germany).[1-7]

The assays are designed to evaluate the expression of various candidate genes via technologies such as DNA microarray, polymerase chain reaction, or indirectly using immunohistochemistry [Table 1].[1-7]

Amidst the multitude of available tests, a very pertinent question that arises is regarding the concordance in the risk assignment. When multiple tests are applied to the same patient cohort, risk assignment discordance will have therapeutic and prognostic implications.

Maroun *et al*.[8] reassessed 86 node negative estrogen receptor-positive (ER+) breast cancer tissues tested originally on Oncotype DX with MammaPrint, BluePrint, and TargetPrint assays. Of the 50 patients with a low Oncotype DX recurrence score; 33 were low risk on MammaPrint (66% concordance), and 7 out of 9 cases classified as high risk by Oncotype DX were also high risk on MammaPrint (78% concordance). Of the 27 cases classified as intermediate risk by Oncotype DX, 14 (52%) were MammaPrint low risk and 13 (48%) were high risk. In a similar comparative study, Dabbs *et al*.[9] compared the Oncotype DX recurrence score of 437 patients with MammaPrint, bluePrint, and TargetPrint assays. Their results showed that of the 301 MammaPrint low-risk cases, 191 (63% concordance) were assigned low risk by Oncotype DX; of the 136 MammaPrint high-risk cases, 63 (46%) were high risk by Oncotype DX; of the 161 intermediate risk cases, 57 were reclassified as high risk and 104 as low risk on MammaPrint assay.

Shivers *et al*.[10] reassessed 135 patients on Oncotype DX and 129 patients on MammoStrat assay out of the primary cohort of 148 patients stratified on MammaPrint assay. Of the 121 patients who were evaluated on all 3 assays, only 22% were concordant for low risk and 9% for high risk, while overall, 30% of cases had a major discordance in their risk stratification.

One can only imagine the plight of the patient who has been identified by one genomic assay as low risk and not requiring chemotherapy, while another genomic assay suggests the exact opposite.

Table 1: The genomic assay platforms and the number of genes assessed

Assay	Number of genes assessed	Platform
Oncotype DX	21	PCR
MammaPrint	70	DNA microarray
MammoStrat assay	5	IHC
Prosigna kit	50	m-RNA expression using novel nanostring technology
EndoPredict assay	11	RT-PCR

RT-PCR: Reverse transcription polymerase chain reaction

Gene expression profiling has ushered in a new era of cancer care, signaling a paradigm shift from the conventional single prognostic variable to the development of multivariate prediction models.

Gene transcription and translation is a complex process involving a coordinated expression of thousands of genes and subject to multiple regulatory mechanisms such as microRNA and epigenetic regulation. Although a majority of the predictive models developed assume that patient and tumor genetic signatures are concordant over different population groups, data from Next Generation Sequencing (NGS) and gene expression profiling studies suggest that each cancer contains a variable and unique assortment of genetic signatures that dictate its growth response to chemotherapy and behavior.[7] Thus, molecular prognostic models especially when combined with tumor characteristics such as size, grade, and nodal status along with the treatment protocol offered far better prediction models than pure molecular assays.

Oncotype DX was validated recently for the first time in tamoxifen-treated, ER+, node-negative early-stage breast cancer patients from the clinical study National Surgical Adjuvant Breast and Bowel Project (NSABP B-14 trial), thus validating the prognostic value of recurrence score, but its clinical validity gets questioned in Her2-neu + patients treated with trastuzumab or patients with advanced disease.[11]

The results of the lowest risk arm of the the trial assigning individualized options for treatment (TAILORx) trial have recently been announced and are pretty impressive. The trial, which began in 2006, had enrolled a total of 10,253 women between the ages of 18 and 75 with ER+, Her2negative, node negative, 1.1–5 cm breast cancers who were tested on the Oncotype DX platform, the results of which were presented at the European Cancer Congress 2015.[11] The lowest risk arm of the cohort with a recurrence score of 0–10, that constituted about 15.9% (1626) of the study group, was assigned to receive hormonal therapy alone with aromatase inhibitors (60%), tamoxifen (33%), and tamoxifen followed by aromatase inhibitors (1%). The thresholds were deliberately kept low over the traditional values (low risk <18, intermediate 18–25, and high risk >25) to mitigate the risk of under-treatment. At 5 years, 93.8% of the participants were free of invasive disease, 99.3% were free of distant relapse, and the overall 5-year survival was an impressive 98%. The results were concordant irrespective of patient age, tumor size or grade, with the final results of TAILORx are expected by late 2017.[11]

Recurrences in more than 50% of all ER+ breast cancer cases develop after more than 5 years. As per current treatment standards, almost all hormone receptor positive patients are offered hormonal therapy and most high-risk cases are offered chemotherapy. Thus, the question of residual risk prediction arises. Newer prognostic assays such as MammaPrint, EndoPredict, Prosigna kit claim to predict the risk of late recurrence, but this has yet to be validated with blinded cohort studies MINDACT trial (Microarray In Node negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy). Microarray in node negative and 1–3 positive lymph node disease may avoid ChemoTherapy, a large prospective trial involving 6600 study subjects will be testing the clinical utility of MammaPrint, especially in predicting late recurrences. It has been proposed that MammaPrint offers superior presage in terms of late recurrences than Oncotype DX.[7,12]

On a similar note, triple negative breast cancers are another diagnostic niche, and there is need for the development of novel genetic signatures for risk stratification and prediction of response to taxol and platinum-based regimes.

Breast cancer has emerged as the most common cancer in Indian women, and there are a number of private players who are offering these tests, but interpolating a set of markers validated on a western population on the indigenous cohort without proper clinical validation renders these tests somewhat apocryphal.[13,14] Finally, analytical validity is another issue. With the emergence of new centers across the world, issues of quality control as well as intra- and inter-laboratory reproducibility of tests are paramount. Therefore, the development of standardized operating guidelines, central pathological review of specimens, stringent quality control, and reference databases can help improve the overall performance of the tests.

A large amount of data is being generated by the plethora of ongoing trials, along with the development of genomic assays for other tumors such as Non-Hodgkins lymphoma, colon cancer, and medulloblastoma and of novel technologies such as circulating tumor DNA and next-generation sequencing (NGS). Periodic appraisal of the accrued data can help formulate better multivariate prediction models.

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