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EDITORIAL

Emerging gene-based prognostic tools in early breast cancer: First steps to personalised medicine

Umar Wazir, Kefah Mokbel

Umar Wazir, Kefah Mokbel, The London Breast Institute, Princess Grace Hospital, London W1U 5NY, United Kingdom
Author contributions: Wazir U and Mokbel K contributed equally to this work.
Supported by Grants from the Breast Cancer Hope Foundation (London, United Kingdom)
Correspondence to: Kefah Mokbel, Professor, The London Breast Institute, Princess Grace Hospital, 45 Nottingham Place, London W1U 5NY, United Kingdom. kefahmokbel@hotmail.com
Telephone: +44-207-9082040 Fax: +44-207-9082275
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Abstract

Breast cancer remains a major cause of neoplastic disease in much of the developed world. The majority of cases are diagnosed with oestrogen receptor (ER)-positive and human epidermal growth factor receptor-2 negative invasive ductal carcinoma and are treated predominantly by surgery which includes sentinel node biopsy and adjuvant endocrine therapy ± adjuvant radiotherapy. It is believed that an indeterminate subset of the patient population is needlessly incurring chemotherapy related morbidity without attaining any increase in survival due to therapy. Furthermore in the era of extended adjuvant endocrine therapy it is important to identify those patients who can be safely treated with 5 years rather than 10 years of endocrine therapy thus optimising the benefit-risk balance. This perception has propelled the development of more personalised prognostic tools for newly diagnosed cases of ER-positive breast cancer. In this article, we shall review the evidence regarding the currently available gene assays for human breast cancer.

Key words: Personalised medicine; Breast cancer; Prognosis; Polymerase chain reaction

Core tip: Recurrence score, Prosigna and EndoPredict (EP) currently have the most convincing evidence available, of which Prosigna and EP have a significant degree of external validation. In terms of cost and turnover, EP has an advantage over its competitors, being designed to be performed at a local laboratory rather than at a central facility. The results of the MINDACT and TailoRx trials are awaited.

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INTRODUCTION

Breast cancer is remains a major cause of neoplastic disease in much of the developed world, comprising of 30.7% of cancers diagnosed in 2011. The 41523 cases were registered during that year[1]. The majority of cases are diagnosed with oestrogen receptor (ER) positive and human epidermal growth factor receptor-2 (HER2) negative invasive ductal carcinoma, which predominantly undergo surgery and staging, including sentinel node biopsy (SNB)[2]. Subsequent decisions by the multi-disciplinary team regarding the use of chemotherapy, radiotherapy and endocrine therapy are determined by the perceived risk of recurrence. Conventionally, the risk of recurrence is estimated based on histology, receptor status and the result of the SNB, or by composite prognostic tools, such as the Nottingham Prognostic Index[3], and Adjuvant! Online (Adjuvant, Inc., San Antonio, TX)[4].

However, it is believed that an indeterminate subset of the patient population is needlessly incurring chemotherapy related morbidity without attaining any increase

in survival due to therapy. This perception has propelled the development of more personalised prognostic tools for newly diagnosed cases of breast cancer[3]. Furthermore, the results of the ATLAS randomised trial suggests that the survival benefit of continuing adjuvant tamoxifen for 10 years may be superior to stopping at 5 years after diagnosis of ER positive breast cancer. This finding has necessitated the development of new tools that could identify the subset of women who would not benefit from extended adjuvant endocrine therapy beyond 5 years[5].

Central to these developments was the identification of sub-types of breast cancer based on so-called "molecular patterns" or "ignatures". These classifications are now referred to as intrinsic sub-types, and have a broad if imperfect concordance with breast cancer classifications based on histology and receptor status. Initially, breast cancers were typed as luminal, HER2 enriched and basal. Luminal are further sub-typed into luminal-A and -B[6]. The intrinsic typing of breast cancers continues to be an area of continuous research. As of the time of writing, 7 intrinsic types have been identified thus far. Luminal-A is characterised as strongly ER positive, while luminal-B is less so, with a greater preponderance of proliferative genes. Broadly speaking, luminal-A corresponds with ER positive and HER2 negative tumours, which are characterised as relatively low risk for recurrence[7].

With a suitable prognostic test, it may be possible to treat a portion of luminal-A patient with post-resection endocrine therapy rather than chemotherapy with endocrine therapy. This subset of patient has been the target of the majority of the extant prognostic and predictive assays.

The major gene-based prognostic assays for breast cancer have been discussed below (Table 1).

MAMMAPRINT

This the oldest test available, developed by Agendia BV (Netherland). This is a 70 gene DNA microarray test performed on frozen or formalin-fixed tissue by a central reference laboratory, which returns a score which stratify patients into a high and low risk categories[8].

The assay was developed in a non-randomised cohort of 78 patients treated at the Netherland Cancer Institute, in which the median age was 55[9]. Subsequent internal studies characterised this assay to be an independent prognostic assay in the primary target group, outperforming clinical parameters[10].

However, it is yet to be validated externally. The studies pertaining to this assay were performed in only one market. Furthermore, owing to the lack of randomisation, the cited studies do not qualify as level 1 evidence. In addition, the population in which it was used is considerably younger than that seen in many countries where this assay may potentially be utilised[11]. Furthermore, the test seems to be a reliable predictor of recurrence occurring in the early follow up period. In meta-analysis of published studies, a high MammaPrint score was found to predict a 12% distant disease-free survival benefit from the addition of chemotherapy[12]. Prospective validation is awaited with the results of the ongoing MINDACT (Microarray In Node negative and 1-3 positive lymph node Disease may Avoid ChemoTherapy) trial[13].

ONCOTYPE-DX

Oncotype-DX (Genomic Health Inc., CA, United States) is currently the most widely available prognostic assay for breast cancer. It is a 21-gene assay in which quantitative real-time polymerase chain reaction (qRT-PCR) is performed on formalin-fixed breast cancer tissue samples taken during initial surgical resection and processed in a central laboratory, which returns a recurrence score (RS) out of a maximum score of 100. It is quoted to have a turnaround time of 7-10 d. It is primarily advised in early ER positive and HER2 negative disease with negative SLN[14]. In addition, it is also recommended in ER positive and HER2 negative disease in elderly patients with positive SNB[15].

The RS score was formulated and validated in patients enrolled in the National Surgical Adjuvant Breast and Bowel Project (NSABB) trials. Specifically, the predictive value of the score was initially validated by the NSABB trial B14, in which patients were randomly allocated into placebo and tamoxifen groups[16]. This was followed up by the NSABB trial B20, in which patients on tamoxifen alone were compared with patients receiving tamoxifen with chemotherapy[17]. A subsequent retrospective study is cited for validation of Oncotype-DX for predicting prognosis in relatively elderly patients treated with tamoxifen with SNB positive disease[18].

These initial studies stratified RS scores into low, intermediate and high risk groups, with RS score below 18 being labelled as low risk of recurrence (< 5% risk), and 31 and above as high risk (39.5%)[16]. Since the beginning, the clinical implications of an intermediate score has been ambiguous. Furthermore, the thresholds have been revised downwards to 11 and 25. Validation for the new thresholds is less clear[8]. The results of the Trial Assigning Individualized Options for Treatment (TAILORx) are awaited to help clarify the recommendations for the intermediate group[19].

There have been several studies suggesting that Oncotype-DX is cost effective as a prognostic test[20]. Furthermore, this assay has been recommended by a number of regulatory bodies[11,21]. However, the cost and turnover time of the test are not insignificant primarily due to test centralization. Although the Oncotype-DX was validated by randomised controlled trials, it must be emphasised that these studies were supported by funding from industry, and are regarded as internal trials by regulatory bodies[11]. It should be also highlighted that only 26% and 29% of patients (some of whom had HER2 positive tumours) in the B-14 and B-20 trials respectively were available for analysis thus reducing the effect of randomi-

Table 1 Comparison of gene-based prognostic assays for early oestrogen receptor + breast cancer

Prognostic assay	Manufacturer	Underlying technology	No. of genes	Test induction	Output/score	Comments
MammaPrint	Agendia BV, The Netherland	DNA microarrays	70	Reference lab	Risk category for recurrence (low risk *vs* high risk)	Prospective validation is awaited with the results of the ongoing MINDACT trial
Oncotype-DX	Genomic Health Inc., CA, United States	qRT-PCR	21	Reference lab	RS scores (1-100) stratified into low, intermediate and high-risk groups for recurrence	Oncotype-DX has been included in several guidelines, and has been validated by internal industrial studies (NSABB trial B14). The characterisation of intermediate risk group awaits the results of the TAILORx trial
PAM50/ Prosigna	NanoString Technologies, Inc., WA, United States	DNA microarrays and qRT-PCR using nCounter technology	50	Reference lab	ROR scores (1-100) stratified into low, intermediate and high risk groups	The assay was been validated in studies based on the ATAC and ABCSG-8 trials
EndoPredict	Sividon Diagnostics GmbH, Köln, Germany	qRT-PCR	8	Local lab	Low or high risk groups on the basis of EP or EPClin scores	EndoPredict has been validated in ABCSG-6 and ABCSG-8 trials, and has been included in German guidelines. Potentially shorter turnover at lower cost, as there is no need for dispatching samples to a reference laboratory

qRT-PCR: Quantitative real-time polymerase chain reaction; ROR: Risk of recurrence.

sation. This significantly weakens the evidence regarding the predictive role of Oncotype-DX in adjuvant chemotherapy, so much so that the evidence does not reach level 1 as per the Marker Utility Grading System[22]. Moreover, the Oncotype-DX is not specific to HER2 negative disease and does not incorporate any clinicopathological features which could improve its prognostic ability of longer term clinical outcome. Although the test has not been validated externally for reproducibility and reliability due to industrial centralization, the internal industry reports suggest that the test is reliable.

PAM50

Parker *et al*[23] developed a risk of recurrence (ROR) score (also called Prosigna) which is applicable to all tumour types including those that are ER positive. The score is derived by analysing of the expression levels of a set of 50 genes using qRT-PCR and DNA microarrays. The ROR score was developed as a prognostic tool in a cohort of 761 patients[23]. The DNA microarray cluster partitioning and analysis was done using the partitioning around medoid or microarray (PAM) methodology[24]. In addition, a related test was developed primarily as an intrinsic sub-type classifier for breast cancer. This test was termed PAM50 (NanoString Technologies, Inc., WA, United States)[25].

Currently, the ROR score and PAM50 test are performed on formalin fixed samples by a central laboratory utilising proprietary nCounter technology[26,27]. Like Oncotype-DX, ROR scores (1-100) are stratified into low, intermediate and high risk groups. The ROR score has predictive value in the neoadjuvant setting, as well as in the case of newly diagnosed patients with node negative disease[23]. The assay was validated in studies based on the ATAC[28] and ABCSG-8 trials[29]. In addition, a recent study validated the Prosigna assay for use at local laboratories[26]. Dowsett *et al*[28] found Prosigna to be superior to immunohistochemistry and RS in ER positive node negative patients receiving endocrine therapy.

ENDOPREDICT

EndoPredict (EP) is a relatively new assay developed by Sividon Diagnostics GmbH (Köln, Germany), which until recently was largely limited to German-speaking markets. It is an 8-gene qRT-PCR assay performed on formalin fixed breast tissue, design in the first instance to be performed at a local laboratory. Remarkably, whilst these genes are related to proliferation and hormone receptor activity, the assay does not include ER, PR, or HER2 status[30]. It was validated on 1702 samples taken from two randomised control trials, ABCSG-6 and ABCSG-8[31].

There is a level Ib evidence showing that EP is an independent prognostic parameter in patients with ER-positive, HER2 negative breast cancer. Patients with a low EP score can be safely treated with endocrine therapy as the only adjuvant systemic treatment, therefore, they can be spared chemotherapy[32]. The level of evidence regarding its independent prognostic role is similar to that of Oncotype-DX[33]. Furthermore, a hybrid score incorporating clinical parameters (EpClin) has been shown to be superior to purely clinical assessment tools[32]. In addition, Muller *et al*[34] found that use of EP resulted in change in clinical decision in 37.7% of patients when applied to a cohort of 167 patients. The effects of the change in therapy are to be assessed.

A further consideration is the inherent costs and logistics such a test may incur. In this regard, EP has an advantage over other similar test, being designed to be performed at a local laboratory rather than at a central facility. Proponents of this assay cite the fact that EndoPredict can be performed on-site resulting in a faster result at a lower cost. In addition, it also has the advantage of

dividing tumours into two categories: low and high thus avoiding the immediate group or grey zone of characterisation, which can create anxiety and dilemma to both the oncologist and the patient. EP has achieved CE certification, and has been included in German guidelines[35]. In addition to reliably identifying patients who can be safely treated with adjuvant endocrine therapy only, EP has other potential applications including further stratification of tumours with intermediate RS (18-31) in order to make final recommendations regarding the need for chemothery and selection of patients for 5 years *vs* 10 of adjuvant endocrine therapy. Finally, the hybrid score EpClin is applicable to patients with node positive ER-positive breast cancer.

However, owing to its relative novelty, other regulatory bodies are yet to consider EP in their recommendations.

CONCLUSION

The recent developments in our understanding of intrinsic sub-types within breast cancer, and the explosion in the use of PCR and DNA microarrays have resulted in a growing number of promising prognostic tools for human breast cancer. OncoType-DX, Prosigna and EP currently have the most convincing evidence available, of which Prosigna and EP have a significant degree of external validation. EpClin is the only tool available that combines molecular signature with important clinicopathological parameters with the potential advantage of superior prognostication regarding the longer term clinical outcome. The RS is the only assay that has been investigated in a randomised trial population as a predictive tool of chemotherapy benefit. However the evidence in this context is considered to be of low quality[22].

Whilst some products are more mature than others, the results of several ongoing trials, such as MINDACT and TailoRx, can be expected to have profound implications for the selection of the optimal test.

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