E20. Do we really need sophisticated diagnostic tests?

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The treatment of primary operable breast cancer is comprised of a combination of surgery, radiation therapy and systemic therapy.

The diagnostic tests that guide optimal surgery and radiation therapy are histopathological analyses of biopsies and resection specimens. Prognostic and predictive factors are used to guide adjuvant systemic therapy.

For all treatment modalities, further refinement of diagnostic tests will continue to result in improved treatment for individual patients. The areas where most improvements in diagnostic tests are needed are the tests that guide adjuvant systemic treatment.

At present, the factors that guide adjuvant systemic treatment include: age, lymph node status, tumour size, histological grade, oestrogen receptor (ER) status and HER2 status.

It has been shown that treatment with adjuvant chemotherapy, adjuvant hormonal therapy for ER positive tumours and trastuzumab for HER2 positive tumours results in improved survival.

Clinicopathological prognostic factors are presently used to select patients for adjuvant systemic treatment. All lymph node positive breast cancer patients and a very large proportion (up to 90%) of lymph node negative breast cancer patients are eligible for adjuvant systemic treatment, and while this adjuvant systemic treatment improves survival, 70–80% of breast cancer patients receive adjuvant systemic treatment without any benefit from it (because they would also have survived without adjuvant systemic therapy). Therefore, there is a great need for more sophisticated prognostic tests to select patients for adjuvant systemic therapy. In addition, it would be of great benefit to develop tests that can guide the systemic drugs for adjuvant treatment.

Scientific advances in the field of genetics and gene-expression profiling have greatly increased the possibilities to develop such prognostic and predictive tests in breast cancer and other malignancies.. Analysis of differential gene-expression patterns across thousands of biological samples in a single experiment (as opposed to hundreds to thousands of experiments measuring the expression of one gene at a time) can help define the best therapeutic regimes for particular patient subgroups.

For breast cancer, three relevant gene-expression profiles associated with prognosis have been identified: a 70-gene classifier,[1,2] a 21-gene signature,[3] and a 76-gene expression profile.[4]

In 2002, a 70-gene prognostic signature was identified by the Netherlands Cancer Institute in Amsterdam. The investigators initially studied the frozen samples of 78 tumours on an Agilent platform (containing 25,000 probes). Using supervised classification, the investigators found that in patients under the age of 53 years the expression of a set of 70 genes best correlated to distant metastases as a first event in node-negative cancer. This 70-gene expression signature was subsequently validated in a second partially independent validation series of 295 breast cancer patients from the same institute. This 70-gene signature outperformed the traditional clinicopathological features with a hazard ratio of 4.6 (95% C.I. 2.3–9.3, $P<0.001$) in a multivariable analysis. A 'good' prognosis signature was present in 39% of the patients, and was associated with a 94.7% probability of freedom from distant metastases in the first 10 years, and an overall survival of 97.4% independent of nodal status. A 'poor' signature was present in 61% of patients and, at 10 years, 60.5% of these patients remained free of distant metastasis, and an overall survival of 74.1%. A gene-expression profiling-based commercial test is offered by Agendia and is called the Mammaprint®.

In 2004, the American National Surgical Adjuvant Breast and Bowel Project (NSABP) in cooperation with the company Genomic Health identified a recurrence score including 21 genes that quantified the likelihood of distant recurrence in tamoxifen-treated patients with node-negative, oestrogen-positive breast cancer. Fixed, paraffin-embedded tumour tissue was assessed and gene-expression measured and has resulted in the development of the Oncotype DX assay (Genomic Health). The list of 21 genes (16 cancer related genes and five controls) in this assay and the recurrence-score algorithm were designed by analysing data from three independent preliminary studies involving 447 patients and 250 candidate genes identified in earlier (including microarray based) studies. To test the prognostic value of the recurrence score, RT-PCR was successfully carried out in 668 paraffin-embedded tumour blocks out of a larger study population of tamoxifen-treated patients in the B-14 study of the NSABP. Tumour and patient

characteristics in this subpopulation were similar to those of the total study population. The patients were of all ages, and had tumours that were pT1–T2 ER-positive, and patients were treated with tamoxifen. Using this 21 gene recurrence-score, 338 patients (51%) had a low risk, 22% an intermediate risk and 27% a high risk profile. Multivariable analysis showed that the hazard ratio of this recurrence score was 2.81 (95% C.I. 1.70–4.64, $P < 0.001$).

In 2005, the Erasmus Medical Centre in Rotterdam in cooperation with the American company Veridex, identified a signature of 76-genes, which could distinguish node-negative breast cancer patients at high risk of distant recurrence eligible for adjuvant systemic therapy. In a retrospective study design, the Affymetrix-chip U133a that contains 22,000 genes was used on frozen samples of 286 untreated node-negative T1–T3/4 breast cancer patients of all ages. This prognostic signature was identified using a training series of 171 tumours and consists of two separate profiles, one for ER-positive (60 genes) and one for ER-negative breast carcinomas (16 genes). The gene expression levels were analysed using the log rank test and validated on an independent validation set of 115 tumours without any overlap with the training set. The distant metastasis-free survival of a 'poor' profile present in 65% of the patients after 60 months was 53%, and this number dropped to 49% after 80 months. A 'good' profile was observed in 35% of patients whose disease-free survival was 93% after 60 months, and 88% after 80 months. The overall survival is 70% after 60 months for patients with a 'poor' profile, and this decreased to 63% after 80 months, and, conversely for patients with a 'good' profile, the overall survival rates for 60 months and 80 months were 97% and 95%, respectively.

For tests requiring frozen material, prospective studies are needed for validation. At present, the 70-gene signature is being used in the MINDACT-trial (TRANSBIG consortium in collaboration with the EORTC); the 21 gene recurrence score is being studied in the TAILORX study.

A key concern is when these gene-expression-based prognostic classifiers will be ready for clinical use. In the past 15 years, there have been hundreds, even thousands of studies attempting to identify novel prognostic factors in breast cancer, often employing molecular techniques. The reason that none of the factors have been implemented in clinical practice is that validation of the prognostic value often failed, and few prospective studies were properly planned. It appears that the gene expression based classifiers are more appealing to clinicians and patients, leading to a pressure to implement them in the clinic as soon as possible. However, prospective validation of each of these classifiers in sufficiently large representative patient cohorts is required before these tests can be used in the clinic. Premature use of these tests may lead to inappropriate advice on adjuvant systemic treatment, resulting in both under- and overtreatment of patients with breast cancer patients.

Conflict of interest statement

The author is named as co-inventor on a patent to use microarray technology to ascertain breast-cancer prognosis.

References

[1] van 't Veer, LJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;415:530–36.
[2] van de Vijver MJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002;347:1999–2009.
[3] Paik S, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351:2817–26.
[4] Wang Y, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005;365:671–9.