Gene signature for risk stratification and treatment of breast cancer: Incorporating tumor biology in clinical decision-making

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Chapter 1
Introduction and outline
Breast cancer
Breast cancer is the most frequently diagnosed cancer among women worldwide. Over the past two decades, the incidence rate in the Netherlands increased from 56 per 100,000 women diagnosed with invasive breast cancer in 1991 to 83 per 100,000 in 2011. This increasing incidence may (partly) be explained by the introduction of population-based screening programs in 1990, which resulted in an increase in the detection of early stage breast cancer after full coverage was achieved in 1997. Another important observation is a decrease in breast cancer mortality-rates, from 45 per 100,000 women in 1991 to 38 per 100,000 women in 2011, which may be explained by early detection due to the implementation of screening programs as well as the improvement and more extensive use of adjuvant systemic treatment.

Adjuvant systemic therapy
After primary treatment consisting of surgery with or without radiotherapy to achieve loco-regional control most breast cancer patients are nowadays systemically treated in the adjuvant or neoadjuvant setting. Adjuvant systemic therapy (AST), including endocrine therapy, chemotherapy and/or trastuzumab, is used to control micrometastatic disease and improve long-term outcome. Data from the Early Breast Cancer Trialists’ Collaborative Group (EBCTCG) confirmed the survival benefit of AST by showing a significant better disease-free and overall survival for patients treated with chemotherapy and/or endocrine therapy in different subgroups.

Guidelines in breast cancer treatment
The selection of those patients at a high risk of recurrence who are most likely to benefit from AST has traditionally been based on clinicopathological factors such as age, tumor size, grade, estrogen-receptor status (ER), progesterone-receptor status (PR), Human Epidermal growth factor Receptor-2 (HER2) and the status of the axillary lymph nodes. There are multiple breast cancer guidelines and clinical tools that use these clinicopathological factors to estimate the risk of recurrence and provide a related recommendation for AST. The components used in these guidelines are all very similar, but show slight differences in their definitions of high and low risk. Most guidelines only identify a small group of patients who are at a low risk of recurrence and for whom AST is of limited value. Consequently, a majority of patients are classified as high risk and therefore become eligible for AST. This will result in a substantial number of patients being treated with AST while they are unlikely to derive significant benefit from it. The differences in the definitions of low risk used by established guidelines create a non-overlapping group of patients at a low or high risk, indicating a suboptimal predictive accuracy for the individual patient. For example, the online decision-making tool Adjuvant! Online uses age, tumor size, grade, ER and nodal status to estimate the 10-year survival probability of a given patient and the possible survival-benefit that can be derived from adjuvant endocrine therapy and chemotherapy, while the Nottingham Prognostic Index (NPI) only provides a high or low risk estimation based on a
score which is calculated using only tumor size, grade and nodal status. Detailed information on prognostic factors used by established clinicopathological guidelines is summarized in Table 1.

Even when using extensively validated clinicopathological factors, predicting the risk of recurrence for the individual patient remains challenging. Already for a long time, pathologists, clinicians and researchers are aware that breast cancer is a heterogeneous disease. Morphology, receptor expression and molecular subtypes all contribute to the clinical course of breast cancer in the individual patient. Variations in clinical behaviour and outcome have been described for several decades.\(^{12}\) Guidelines and clinical tools have improved over the past years and are now including clinicopathological factors such as HER2 status and Ki67.\(^{13,14}\) Nevertheless, HER2 and Ki67 only account for a small part of this heterogeneity and still most guidelines do not adjust for the heterogeneity entirely. Therefore, clinicopathological guidelines have only limited ability to predict individual patient outcomes.\(^{15}\)

**Insight in the biology of breast cancer: introducing gene signatures**

Over the past decades, researchers identified many single genes involved in the proliferation and metastatic capacity of breast cancer. Breast cancer progression is a result however of multiple genetic aberrations, and thus one gene will never be responsible for the entire cancer process.\(^{16,17}\) Therefore, researchers were looking for methods to evaluate the relationships among and within different cellular pathways. The introduction of micro-array analyses provided a way to evaluate multiple genes in multiple pathways at once in a more robust manner.

Micro-array technology is used to develop gene signatures that are related to the metastatic potential of an individual breast cancer. These signatures can refine risk estimations based on standard clinicopathological guidelines.\(^{18}\) One of these signatures is the 70-gene signature (MammaPrint\(^\circledast\)), which was developed by van ‘t Veer and colleagues at the Netherlands Cancer Institute (NKI) in Amsterdam, the Netherlands. The 70-gene signature measures the level of expression of a set of genes by semi-quantitatively determining the level of messenger RNA (mRNA) transcripts.\(^{19}\) The intensity of the nucleic acids that hybridize to the individual gene probes are commonly shown in a two-color array.\(^{19}\) Green reflects low expression and red reflects high expression of that gene in the tumor (Figure 1). After hybridization the slides are scanned with a dual laser scanner (Agilent Technologies) and the data is processed using a specific algorithm providing an index-score which originally ranges from 0 to 1.\(^{20}\) The 70-gene signature was developed using frozen tumor samples from 78 patients who were diagnosed at the NKI with lymph node-negative breast cancer and who were up to 55 years of age at the time of diagnosis. 44 of these 78 patients remained free of distant metastases for at least 5 years. These patients were defined as good prognosis or low risk.
Table 1. Clinicopathological factors used by breast cancer guidelines to estimate the risk of recurrence

<table>
<thead>
<tr>
<th>Guideline</th>
<th>Age</th>
<th>Size</th>
<th>Grade</th>
<th>Hist. type</th>
<th>ER/PR</th>
<th>HER2</th>
<th>Ki67</th>
<th>Nodal status</th>
<th>Other factors</th>
<th>Low risk is defined as</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOL</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Ductal, in case of other hist. type, information is available online</td>
<td>ER</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Co-morbidities, CT regimen</td>
<td>Not specified.</td>
</tr>
<tr>
<td>St. Gallen expert panel 2011</td>
<td>Pre- or post menopausal</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>ER/PR</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes, more than 3+ nodes is high risk</td>
<td>Biological subtype</td>
<td>Luminal A; ER/PR +, HER2 -, low Ki67</td>
</tr>
<tr>
<td>NPI</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[0.2 x Size] + Number of nodes + Grade; low risk = score &lt; 3.4</td>
</tr>
<tr>
<td>NABON 2012</td>
<td>&lt;35 or ≥ 35</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10-years survival probability ≥85%. N0, &lt;35, grade I tumor ≤ 1 cm OR ≥35 yrs, grade I tumor ≤ 2 cm.</td>
</tr>
<tr>
<td>PREDICT plus</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>ER</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Method of detection, CT regimen</td>
<td>Not specified. Suggested; &lt;3% survival benefit in 10-years no chemotherapy; 3-5% chemotherapy discussed as possible option</td>
</tr>
</tbody>
</table>

AOL = Adjuvant! Online; NPI=Nottingham Prognostic Index; NABON=Nationaal Borstkanker Overleg Nederland; ER=estrogen receptor; PR=progesterone receptor; HER2=Human Epidermal Growth factor Receptor 2; CT=chemotherapy.
The remaining 34 patients developed distant metastases within 5 years after diagnosis and were defined as poor prognosis or high risk. Tumors with an index-score >0.4 are classified as 70-gene signature low risk and tumors with an index-score <0.4 as 70-gene signature high risk. The signature was validated by van de Vijver et al. in a consecutive series of 151 lymph node-negative and 144 lymph node-positive breast cancer patients, diagnosed at the NKI, aged up to 53 years at the time of diagnosis. Buyse et al. performed an independent validation in 302 lymph node-negative patients from 5 European hospitals, aged up to 60 years at the time of diagnosis. The prognostic value of the 70-gene signature has also been retrospectively confirmed in several patient subgroups, such as postmenopausal patients, patients with positive axillary lymph nodes and in case of HER2-positive disease. Aside from the 70-gene signature, a few other gene signatures have found their way to the clinic. The characteristics of these tests, including PAM 50, Oncotype Dx, EndoPredict, Breast Cancer Index and MapQuant Dx, are described in Table 2. The analyses presented in this thesis focus on the 70-gene signature.

Using the 70-gene signature in the daily clinical practice
To prospectively evaluate the feasibility of implementation of the 70-gene signature in the community-based setting, the MicroarRAY PrognoSTics in Breast CancER (RASTER) study was conducted. Between 2004 and 2006 427 eligible patients were included in 16 hospitals in the Netherlands. Implementation of the 70-gene signature appeared feasible, even though the test could only be performed on fresh frozen tumor samples at the time. Recently, also formalin fixed paraffin embedded (FFPE) tumor samples can be used to perform the 70-gene signature.
<table>
<thead>
<tr>
<th></th>
<th>MammaPrint</th>
<th>Pam50</th>
<th>Oncotype Dx</th>
<th>EndoPredict</th>
<th>Breast Cancer Index</th>
<th>MapQuant Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay</td>
<td>70-gene signature</td>
<td>55-gene signature</td>
<td>21-gene recurrence score</td>
<td>11-gene signature</td>
<td>2-gene ratio HOXB13 and IL17R and molecular grade index (MGI)</td>
<td>97-gene (micro-array) or 8-gene (qRT-PCR) signature</td>
</tr>
<tr>
<td>Technique</td>
<td>Micro-array</td>
<td>qRT-PCR</td>
<td>qRT-PCR</td>
<td>qRT-PCR</td>
<td>qRT-PCR</td>
<td>Micro-array/ qRT-PCR</td>
</tr>
<tr>
<td>Provided by</td>
<td>Agendia (Amsterdam, NL)</td>
<td>ARUP Laboratories (Salt Lake City, USA)</td>
<td>Genomic Health (Redwood City, USA)</td>
<td>Sividon Diagnostics (Keulen, DU)</td>
<td>Biotheranostics (San Diego, USA)</td>
<td>Ipsogen (Marseille, FR)</td>
</tr>
<tr>
<td>Tissue sample</td>
<td>Frozen or FFPE</td>
<td>Frozen or FFPE</td>
<td>Frozen or FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
<td>Frozen or FFPE</td>
</tr>
<tr>
<td>Training set</td>
<td>78 pt, T1-2N0, &lt;55 yrs ER +/-</td>
<td>189 pt, ER +/-, HER2 +/-, T1-2N0-1</td>
<td>447 pt, ER+ from NSABP B-20 study (tamoxifen-treated arm)</td>
<td>964 pt, ER+, HER2-</td>
<td>588 pt, ER+, N0, treated with tamoxifen for 2-gene ratio. 410 pt ER+, N0 for MGI</td>
<td>64 pt, ER+</td>
</tr>
<tr>
<td>Validation set</td>
<td>295 pt, T1-2N0-1, &lt;53 yrs, ER +/-</td>
<td>761 pt for prognosis 133 pt for prediction ER+/-, HER2 +/-, T1-2N0-1</td>
<td>668 pt, ER+ from NSABP B-14 study (treated with tamoxifen)</td>
<td>1702 pt, ER+, HER2-, treated with tamoxifen (2 series)</td>
<td>265 pt, ER+, N0, treated with tamoxifen</td>
<td>597 pt, ER+</td>
</tr>
<tr>
<td>Output</td>
<td>High and Low risk</td>
<td>Continuous variable</td>
<td>Continuous variable divided in 3 groups; High, Intermediate, Low risk</td>
<td>Continuous variable divided in 2 groups; High and Low risk (EP score)</td>
<td>Continuous variable divided in 3 groups; High, Intermediate, Low risk</td>
<td>Low grade GGI or high grade GGI</td>
</tr>
<tr>
<td>Initially developed for</td>
<td>Prognosis prediction of T1-2N0 pt, ER+/-, &lt;61 yrs</td>
<td>Prognosis prediction of N0 pt, ER+, treated with endocrine therapy</td>
<td>Prognosis prediction of ER+, N0 pt treated with tamoxifen</td>
<td>Prognosis prediction of ER+, HER2- pt treated with tamoxifen</td>
<td>Prognosis prediction of ER+, N0 pt treated with tamoxifen</td>
<td>Molecular grading of ER+, grade 2 tumors</td>
</tr>
<tr>
<td>Additional information</td>
<td>mRNA levels ER, PR and HER2 (TargetPrint), intrinsic subtypes (BluePrint)</td>
<td>xx</td>
<td>mRNA levels ER, PR and HER2</td>
<td>EP clin score by combining EP score with clinicopathological factors</td>
<td>xx</td>
<td>xx</td>
</tr>
<tr>
<td>Prognostic value in other subgroups</td>
<td>Patients with up to 3 pos. axillary lymph nodes, patients who are 55-70 yrs, and for HER2+ disease</td>
<td>xx</td>
<td>ER+ and patients with up to 3 pos. axillary lymph nodes Postmenopausal ER+ pt, treated with aromatase-inhibitors</td>
<td>ER+ tumor in postmenopausal women</td>
<td>Possibly also prognostic for late recurrences</td>
<td>ER+ pt, treated with aromatase-inhibitors</td>
</tr>
</tbody>
</table>

FFPE=formalin fixed paraffin embedded; pt=patient; ER=estrogen receptor; HER2=Human Epidermal Growth Factor receptor 2
Shortly after confirmation of its feasibility in the RASTER study, the 70-gene signature was subjected to an international, multicenter, randomized-controlled trial called Microarray in Node-negative and 1-3 lymph node positive Disease may Avoid Chemotherapy (MINDACT). Patients enrolled in the MINDACT trial had their risk of recurrence assessed by the known online decision making tool Adjuvant! Online and the 70-gene signature. In case of a concordant low risk estimation patients would only receive endocrine therapy, while in case of a concordant high risk estimation patients would receive adjuvant chemotherapy with or without endocrine therapy. If the clinicopathological risk estimation was discordant with the 70-gene signature risk estimation patients were randomized between treatment according to the risk estimation by the 70-gene signature or treatment according to the clinicopathological risk estimation. On July 1st 2011, the required 6673 patients were successfully enrolled in the trial. The MINDACT trial will evaluate whether adjuvant chemotherapy can safely be omitted in patients with a tumor that is low risk according to the 70-gene signature, while clinical guidelines (in this case Adjuvant! Online) assessed this tumor as high risk. Meanwhile, better prognostication is desired in routine clinical practice and for this reason the 70-gene signature is increasingly applied when there is uncertainty regarding the indication of AST. Several studies from the Netherlands Cancer Institute have shown the impact of the introduction of the 70-gene signature on the quality of life patients and the cost-effectiveness of genomic testing was confirmed multiple times. On the other hand, the effect on clinical decision-making had not systematically been studied.

Rationale and outline of this thesis

The aim of this thesis is to evaluate outcome prediction and clinical relevance of the 70-gene signature for locoregional and distant recurrence, its influence on risk assessment and AST recommendations, and its additional value to established clinical guidelines used in breast cancer treatment. In addition, we used the 70-gene signature to gain better insight in the biological background of tumors detected in a population-based screening program.

The first part of this thesis focuses on the current applicability of the 70-gene signature in daily clinical practice and the impact of the 70-gene signature on clinical decision-making. Chapter 2 of this thesis provides a current overview of the prognostic value of the 70-gene signature in different subgroups of patients as described in recently published, retrospective studies. Chapter 3 provides the first prospective evidence of the prognostic value of the 70-gene signature. The 5-year follow-up data of the RASTER study shows the outcome of patients for whom the 70-gene signature was used to decide whether or not an individual patient should receive adjuvant systemic treatment. In this chapter the 70-gene signature is compared to Adjuvant! Online. Because Adjuvant! Online is the most commonly used, but not the only guideline in breast cancer, we also compared the additional value of the 70-gene signature to other established guidelines in chapter 4. As described earlier, clinicopathological guidelines vary in their risk estimations. At
this point in time, there is no data on the agreement among oncologists using clinicopathological factors for risk estimations and the impact of the 70-gene signature on clinical decision-making. Therefore, agreement among oncologists before and after providing the 70-gene signature result was evaluated in chapter 5. Also, we aimed to evaluate long-term outcome of patients for whom a 70-gene signature result was available. Therefore we updated the original consecutive series as published by van de Vijver et al. in 2002 (chapter 6).

The second part of this thesis focuses on new areas where the 70-gene signature may improve the biological understanding of breast cancer. Method of detection has proven to be an independent prognostic factor in breast cancer. Patients with a screen-detected cancer have more favorable outcome, independent of known clinicopathological factors such as age, size and ER-status. To investigate whether this observation is supported by a more favorable tumor biology in screen-detected cancers, we described the proportions of high, low and ultralow risk according to the 70-gene signature among screen-detected and interval cancers in the Dutch MINDACT cohort in chapter 7. Since a transition from film-screen mammography (FSM) to full field digital mammography (FFDM) took place at the same time as the MINDACT trial was conducted in the Netherlands we were also able to evaluate the impact of this transition on the biological background of the tumors detected in the nation-wide screening program.

The 70-gene signature was developed to predict the risk of distant recurrence in breast cancer. Because of the correlation between distant and locoregional recurrence, we hypothesized that the 70-gene signature would also be able to predict the risk of locoregional recurrence after both breast conserving surgery and mastectomy. The results of analyzing this hypothesis in a pooled dataset of all patients included in one of the 70-gene signature validation studies, who were diagnosed and treated at the Netherlands Cancer Institute, is described in chapter 8.*

This thesis ends with a general discussion and future prospects in chapter 9 and a summary of all results is presented in chapter 10.

*All studies described in this thesis are performed in accordance with the FEDERA codes of conduct.34
References


2. Nederlandse Kankerregistratie beheerd door IKNL.


