Gene signature for risk stratification and treatment of breast cancer: Incorporating tumor biology in clinical decision-making
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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, which resulted in an increase in the detection of early stage breast cancer. Another important observation is a decrease in mortality rates, which may be explained by early detection, but also by the improvement and more extensive use of adjuvant systemic treatment. Adjuvant systemic therapy (AST) improves survival, but not all patients who receive AST derive significant benefit from it. This indicates that the selection of patients eligible to receive AST based on clinicopathological factors as used nowadays is not sufficient. Knowledge about the biological background of breast cancer has grown tremendously over the past years. This knowledge was used to develop gene signatures, such as the prognostic 70-gene signature.

**Chapter 1** provides an introduction to breast cancer, clinicopathological factors used to predict the risk of recurrence and the introduction of gene signatures. Furthermore, the rationale and outline of this thesis are described.

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. In **Chapter 2** we provide an overview of the prognostic value of the 70-gene signature in different subgroups of patients as described in recently published, retrospective studies. This overview shows that the 70-gene signature was initially developed for patients < 55 years old with stage 1 or 2, ER-positive or -negative, node-negative breast cancer. Shortly after, the test was retrospectively validated for breast cancer patients who are 55-70 years of age, patients with HER2-positive disease, and patients with 1-3 positive nodes in the axilla. At this point in time, only retrospective data on patient outcome was available for the 70-gene signature. Fortunately, as described in chapters **2** and **3**, the 5-year follow-up results of the RASTER study became available, providing the first prospective data on patients for whom the 70-gene signature was used in the decision whether or not to treat a patient with adjuvant chemotherapy. A total of 427 women with early stage, node-negative breast cancer were included in this observational study conducted between 2004 and 2006. After 5 years of follow-up an excellent distant-recurrence-free-interval of 100% is seen in patients who did not receive adjuvant chemotherapy based on their low risk 70-gene signature result, despite unfavorable clinicopathological factors and therefore a high risk assessment by Adjuvant! Online (AOL). All clinical risk estimations in the first analyses were based on AOL. To put the results of the RASTER study in a wider context, the analyses were not only performed compared to AOL, but also compared to Nottingham Prognostic Index (NPI), the St. Gallen expert panel recommendations of 2003, the Dutch National guidelines of 2004 and the updated version of 2012, and the relatively new online risk estimation tool PREDICT plus (chapter 4). The results of our analyses show that adding the 70-gene signature to clinical guidelines significantly improves...
risk predictions. The most accurate risk predictions were seen when using the PREDICT plus tool in combination with the 70-gene signature (c-index of 0.662). In chapter 5, we evaluated the agreement among oncologists and the impact of the 70-gene signature on risk estimations and treatment recommendations in early stage, node-negative breast cancer. Only a moderate agreement on risk estimation was seen ($\kappa=0.55$; range: 0.20-0.88). This could slightly be improved by providing the 70-gene signature result ($\kappa=0.61$; range: 0.14-1.00; $p=0.035$), indicating that the 70-gene signature may be a useful tool to provide patients with more standardized, but individualized treatment. Overall, when adding the 70-gene signature the proportion of patients classified as high risk was reduced with 7.4% (range: 6.9-22.9%; $p<0.001$) and the proportion of chemotherapy that was recommended was reduced with 12.2% (range: 5.4-29.5%; $p<0.001$).

To evaluate the effect of the 70-gene signature on long-term outcome, we updated follow-up for the 295 patients included in the consecutive validation cohort of van de Vijver et al. in chapter 6. The median follow-up for this cohort was prolonged to 18.5 years. A significant difference was seen in distant-metastasis-free-survival (DMFS) for patients with a low and a high risk 70-gene signature ($p<0.0001$), for node-negative ($n=151$; $p<0.0001$) as well as node-positive patients ($n=144$; $p=0.0004$). The 25-year Hazard Ratio (HR) for DMFS and OS were 3.1 (95%CI: 2.02-4.86) and 2.9 (95%CI: 1.90-4.28), respectively. The HR for DMFS and OS was largest in the first five years after diagnosis (9.6 (95%CI: 4.2-22.1) and 11.3 (95%CI: 3.5-36.4) respectively).

The second part of this thesis focuses on new areas in which the 70-gene signature may be helpful. A current problem is the increasing incidence of breast cancer after implementation of population-based mammographic screening programs. This has been suggested to be partly due to the detection of slow growing tumors that would never have caused symptoms or death, i.e. breast cancer overdiagnosis. Only rough estimates have been made of the proportion of patients that are overdiagnosed and identification of those patients is difficult. Therefore, the aim of the study described in chapter 7 was to evaluate whether tumor biology can help identify patients with screen-detected tumors at such a low risk of recurrence that concerns regarding overdiagnosis might be raised. We hypothesized that screen-detected cancers have a more favorable tumor biology, aside from more favorable clinicopathological factors. Our results show that screen-detected cancers had significantly more often a low (68%), of which 54% even an ultralow risk tumor biology compared to interval cancers (53% low, of which 45% ultralow risk ($p=0.001$) with an OR of 2.33 ($p<0.0001$; 95% CI: 1.73-3.15). Especially for patients with screen-detected cancers the use of tools, such as the 70-gene signature, to differentiate breast cancers by risk of recurrence may minimize overtreatment.
Second, we evaluated the impact of the recent transition from film-screen mammography (FSM) to full-field digital mammography (FFDM) on the biological background of the tumors detected in the nation-wide screening program. FFDM detected significantly more high risk tumors (35%) compared to FSM (27%; p=0.011), suggesting that screening with this imaging modality is more efficient.

The 70-gene signature was developed to predict the risk of distant metastases. Given the strong association between loco-regional and distant recurrence, we hypothesized that the 70-gene signature will also be able to predict the risk of loco-regional recurrence (LRR). In chapter 8 it is shown that a high risk 70-gene signature is associated with a 2.89 times higher risk of LRR compared to a low risk 70-gene signature (95%CI: 1.80-4.63). Adding the 70-gene signature to known clinicopathological factors significantly improved the risk prediction model (multivariable HR 2.27 (95%CI: 1.24-4.15); p=0.007). This effect was seen in patients treated with breast conserving surgery as well as patients treated with mastectomy.

In chapter 9 the results presented in this thesis are discussed and put in perspective of current clinical practice. In general, the results described in this thesis expand our knowledge on the use of the 70-gene signature in clinical practice and the impact this gene-signature has on clinical decision-making. Not only did the results show that the 70-gene signature is an important prognostic factor in long-term retrospective and observational prospective data, they also revealed new areas in which the 70-gene signature can be used.