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Prognostic factors in breast cancer: one fits all?

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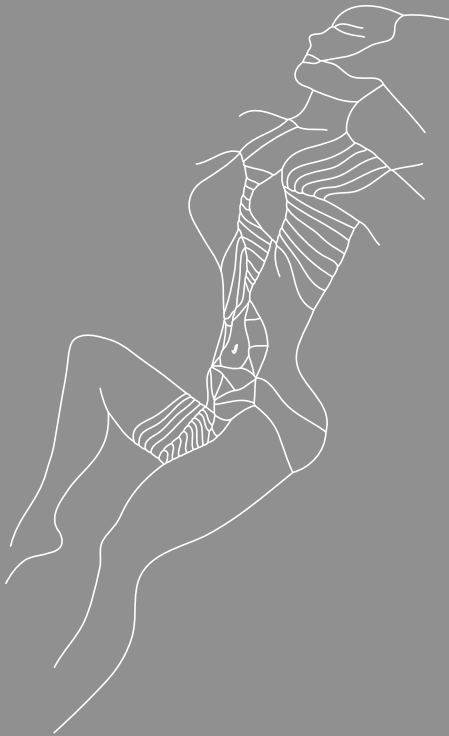
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Chapter 1

Introduction and outline



Introduction

Breast cancer

Breast cancer is the most frequently diagnosed malignancy in women worldwide. In the Netherlands in 2008, 13,005 women were diagnosed with invasive breast cancer and 3,327 patients died of the disease.¹ Although there is an increase in breast cancer incidence, breast cancer mortality is decreasing in the last decennia.²⁻⁴ This decrease in mortality is mainly caused by both the introduction of breast cancer screening and the improvement and more extensive use of adjuvant systemic therapy.^{2,3,5-9} Currently, approximately 2/3 of the patients who are diagnosed with breast cancer do not have nodal involvement at diagnosis and about 2/3 of the patients are 55 years of age or older at diagnosis.¹⁰

Treatment of breast cancer

The treatment of early stage breast cancer consists of two aspects. The first is loco-regional control, which is primarily achieved by surgery with or without radiotherapy. The second part of breast cancer treatment focuses on preventing the development of distant metastases. Distant metastases account for the majority of breast cancer deaths and are thought to develop from undetectable micrometastases or circulating tumor cells that are already present at time of diagnosis. Adjuvant systemic therapy (*i.e.* chemotherapy, hormonal therapy and/or targeted therapy) can help eradicate micrometastases and circulating tumor cells, thereby preventing distant metastases to occur and thus improving survival. The incurable nature of metastatic breast cancer emphasizes the importance of selecting patients for adjuvant systemic therapy who are at risk of developing distant metastases. In patients with lymph node-negative disease, adjuvant chemotherapy improves survival on average by 25%.¹¹ On the other hand, especially chemotherapy can cause a wide range of acute and long-term side effects.¹²

Adjuvant systemic therapy

Since the introduction in the early 1980s, there is a steady increase in the use of adjuvant systemic therapy (AST) in the Netherlands.⁹ This increase is supported by data from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) overviews showing a significant benefit of adjuvant systemic therapy for disease-free and overall survival.^{11,13-15} In the 1990s, adjuvant systemic therapy was recommended mainly for patients with lymph node-positive breast cancer. In 2000, the National Breast Cancer Consultation Netherlands (NABON) developed the first national guideline for adjuvant systemic therapy.¹⁶ Tamoxifen was recommended for lymph node-positive, estrogen receptor (ER)-positive tumors in postmenopausal patients. For lymph node-positive premenopausal patients and

for lymph node-positive postmenopausal patients with an estrogen receptor negative tumor chemotherapy was recommended. In addition, it was recommended to consider adjuvant systemic therapy for a subgroup of patients with lymph node-negative tumors depending on tumor size and tumor grade. The use of AST for Dutch patients with early stage breast cancer increased significantly over time, from 37% in the period 1990–1997, to 53% in 2002–2006.^{17,18} Currently, Dutch breast cancer patients are treated according to the NABON and Dutch Institute for Healthcare Improvement (CBO) guidelines and adjuvant systemic therapy is recommended for > 80% of all patients.¹⁹ As in the Netherlands, the administration of AST increased substantially in the US, where the use of chemotherapy or hormonal therapy tripled from 1987 to 2000 in women with node-negative disease.²⁰ Only 1 in 5 women with node negative disease did not receive any form of adjuvant systemic therapy in the US in the year 2000.²⁰

Who to treat; prognostic factors

Patients who are at high risk of developing distant metastases are candidates for AST. Prognostic factors help identify patients who are at high risk of distant metastases in the absence of AST.²¹ An ideal prognostic factor tells us exactly 'who to treat', by reliably distinguishing patients who are at high risk of developing distant metastases from those who are at low risk. Nowadays, the selection of patients who are at high risk of recurrence is based on clinical and pathological prognostic factors, such as age, menopausal status, co-morbidity, tumor size, tumor grade, lymph node status and hormonal receptor status.²² These clinicopathological criteria are often combined into guidelines or models such as the St. Gallen recommendations, the Nottingham Prognostic Index, the Dutch CBO guideline or the Adjuvant! tool.^{19,23-25} However, tumors with the same clinicopathological characteristics can have strikingly different outcomes. Consequently, AST recommendation according to these guidelines is far from accurate. Although 60-70% of patients with lymph node-negative breast cancer are likely to be cured by surgery and radiotherapy alone, the majority of patients is currently treated with chemotherapy, hormonal therapy and/or targeted therapy (*Figure 1*).¹¹ As a result, a substantial proportion of patients will unnecessarily receive AST and will be needlessly exposed to its toxicity. This overtreatment is due to the lack of accurate identification of patients with a low risk of developing distant metastases, who are unlikely to benefit from adjuvant systemic therapy. Apparently, better prognostic factors are urgently needed. Although, a number of single parameter prognostic biomarkers have been studied, few have achieved the level of supporting evidence required for routine clinical use.²⁶ One of the more recently developed techniques that provides us with promising new prognostic tools is the microarray gene expression technique.

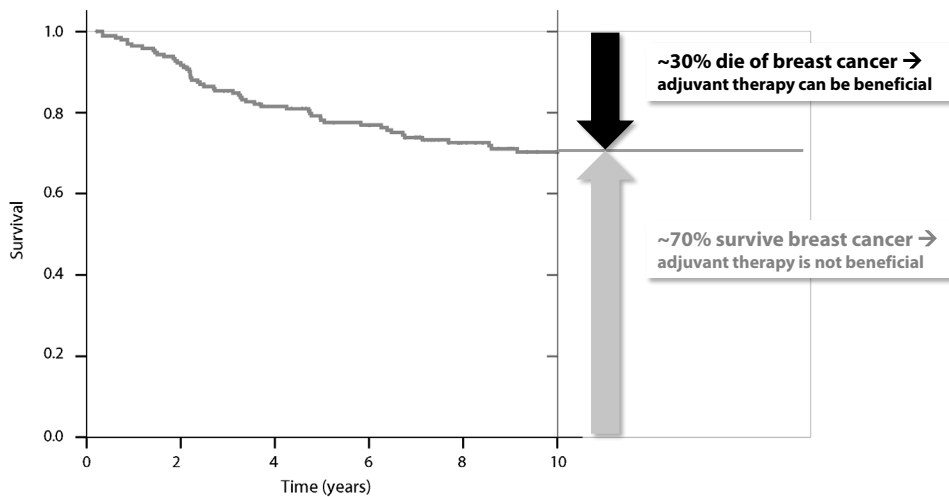


Figure 1. Survival of early stage breast cancer patients after loco-regional treatment.

Gene expression profiling

The introduction of the new high-throughput microarray technology at the beginning of this century, has introduced a new era of multi-parameter prognostic tests and caused a revolution in medicine, particularly in the oncology field.²⁷ In contrast to the single-parameter biomarker, microarray analyses can measure the expression of thousands of genes in the tumor simultaneously.²⁸⁻³⁰ The expression level of all genes together gives insight in tumor biology and in this way provides the possibility to subdivide breast cancer based on its biology. Since tumor behavior and clinical outcome depend largely on tumor biology, gene expression profiles are anticipated to refine the prognostication of breast cancer.

The first published molecular classification of breast cancer using microarray technology displayed the molecular heterogeneity of the disease. Unsupervised analyses of microarray gene expression data of breast cancer patients have resulted in the identification of 4 molecular subtypes, according to gene expression profile: Luminal A, Luminal B, Basal-like and ERBB2 breast cancers.³¹ Those gene expression profiles reflect biological diversity and were shown to be associated with disease outcome as well.^{31,32} Many subsequent studies have discovered several other prognostic gene expression profiles.^{31,33-40} Remarkably, although the prognostic performance of these signatures in terms of individual patient classification was similar, overlap in terms of gene identity was limited.⁴¹ However, it was shown that these signatures reflect overlapping common biological processes and cellular phenotypes that drive breast cancer prognosis.^{42,43}

In addition to unsupervised analyses, supervised analyses can be used to develop a gene expression signature that can predict clinical outcome. In contrast to unsupervised analyses that classify tumors based on the similarity of gene expression, supervised analyses compares gene-expression data from patients with known clinical outcomes (*e.g.* absence or presence of distant metastases) to identify genes that are associated with prognosis. Such classification method was used to identify the 70-gene prognosis signature (MammaPrint™).³⁸ The 70-gene signature has been identified using frozen tumor samples from 78 patients who were diagnosed at the Netherlands Cancer Institute-Antoni van Leeuwenhoek hospital (NKI-AVL) with lymph node-negative breast cancer and who were up to 55 years of age at diagnosis. Among these 78 patients, 44 remained free of distant metastases for at least 5 years (defined as the good prognosis group), whereas 34 patients developed distant metastases within 5 years of diagnosis (poor prognosis group). The signature consists of the top 70 genes that were differentially expressed between the two prognosis groups and most accurately classified tumors in the good- or poor prognosis group. The signature was validated in a consecutive second patient series from the NKI-AVL, consisting of 151 lymph node-negative and 144 lymph node-positive patients up to 53 years at diagnosis, and in a third independent patient series of 302 lymph node-negative breast cancer patients from 5 European hospitals, who were up to 60 years of age at diagnosis.^{44,45} Subsequently, the prognostic value of 70-gene signature has been confirmed by others.⁴⁶⁻⁴⁸

In 2004, another prognostic test has been developed. The OncotypeDX™ is a RT-PCR based assay performed on paraffin-embedded tumor samples that classifies tumors based on the expression of 16 genes into a low Recurrence Score (RS), an intermediate RS or a high RS.³⁶ A community-based validation study demonstrated that the RS could be used to predict the outcome of node-negative patients receiving tamoxifen alone.⁴⁹ Retrospective analysis of the node-negative NSABP B20 and node-positive SWOG 8814 trial showed similar prognostic value for the RS in patients treated with the combination of tamoxifen and chemotherapy.^{50,51}

Validation studies to assess the reliability and reproducibility are of utmost importance to determine a signature's clinical utility. Furthermore, practical issues of the implementation of gene expression microarrays need to be addressed and quality of performance and standardized procedures for a diagnostic test should be monitored by International Organization for Standardization (ISO) or Clinical Laboratory Improvement Amendments (CLIA) certification and should preferably fall under the regulatory oversight such as the US Food and Drug Administration (FDA).⁵² Successful implementation of a gene expression profile requires, in addition to thorough validation studies, the collection of good quality, fresh frozen tumor tissue and close collaboration between different departments in the hospital.

Rationale and outline of this thesis

The overall aim of this thesis is to evaluate the accuracy and clinical utility of a relatively new prognostic microarray test, the 70-gene signature, in several breast cancer subpopulations. In addition, we evaluated the accuracy of the extensively used prognostic tool Adjuvant!, which is based on clinicopathological characteristics. Finally, we evaluated whether the method of detection of a tumor (*i.e.* screen-detected or symptomatic) affect prognosis and should be taken into account to improve patient selection for AST.

The first part of this thesis focuses on the applicability of the 70-gene signature (MammaPrint™) and the potential improvement of patient selection for adjuvant systemic therapy by using this microarray test.

In **chapter 2** the development of the 70-gene signature, its initial retrospective validation studies and logistical feasibility studies are described. In addition, the currently conducted prospective randomized clinical trial, the so-called MINDACT study (Microarray In Node-negative and 1-3 positive lymph node disease may Avoid ChemoTherapy) which will compare the prognostic value of the 70-gene signature with that of currently available prognostic clinicopathological variables, is discussed. More detailed information about the design of the MINDACT trial is provided in **Appendix 1**.

Chapter 3 presents the results of a European pilot study preceding the MINDACT trial to test the feasibility and to optimize the logistics for the collection of good-quality fresh frozen tumor tissue in order to perform the 70-gene signature.

The 70-gene signature has been developed and so far mostly validated in premenopausal patients with lymph node-negative breast cancer. However, the majority of breast carcinomas is diagnosed in postmenopausal women. Therefore, we evaluated the accuracy of the 70-gene signature in postmenopausal patients, which is described in **chapter 4**. Although lymph node metastases are a strong indicator of a poor prognosis, still approximately 30-40% of patients with 1-3 positive lymph nodes at diagnosis will remain free of distant metastases without adjuvant systemic therapy. Currently, there are no biomarkers available to select these low risk lymph node-positive patients. In **chapter 5** we evaluated the ability of the 70-gene signature to identify patients with 1-3 positive lymph nodes who are at low risk of recurrence in an independent, retrospective validation study. In addition to lymph node status, tumor size is known to be a powerful prognostic factor, with small tumor size being thought to indicate a low risk of recurrence. Nevertheless, small tumors still can metastasize, which leaves us with the question of the necessity of adjuvant systemic therapy in patients with pT1 (≤ 20 mm) tumors. In **Chapter 6** the prognostic value and clinical utility of the 70-gene signature in a pooled retrospective series of patients with pT1 (≤ 20 mm) breast carcinomas are discussed. Adjuvant treatment allocation based on the 70-gene signature seems to be justified when the low risk of recurrence in the good prognosis group is sufficiently low to withhold chemotherapy and the expected benefit

from adjuvant chemotherapy is limited. In addition, administration of chemotherapy in patients classified as high risk is legitimate when the benefit of treatment in these patients is substantial. In **chapter 7** we assessed this predictive value of the 70-gene signature in a pooled analysis.

The 70-gene signature is currently studied in the prospective MINDACT (Microarray In Node-negative and 1-3 positive lymph node disease may Avoid ChemoTherapy) trial, which will evaluate whether patients who are considered high risk according to the currently available prognostic tool Adjuvant! but classified as low risk by the 70-gene signature can be safely spared chemotherapy. Adjuvant! combines clinicopathological characteristics, such as patient age, co-morbidity, tumor size, lymph node involvement, histological grade and estrogen receptor status, to forecast the overall and breast cancer-specific mortality and to predict the benefit of additional chemotherapy and/or endocrine therapy. The Adjuvant! model is based on information from breast cancer patients in the United States who were diagnosed between 1988 and 1992 and recorded in the Surveillance, Epidemiology and End Results (SEER) registry.²⁴ In 2005, the model was retrospectively validated in breast cancer patients from British Columbia.⁵³ Since the European breast cancer populations may differ from those in the US and Canada, the question remains whether outcome predictions of the Adjuvant! model are applicable to the European population. Therefore, we conducted a retrospective validation study to test the accuracy of Adjuvant! in a Dutch breast cancer cohort of 5,830 patients, which is described in **chapter 8**. The aim of this study was to assess both the ability of Adjuvant! to predict outcomes in (sub)groups of Dutch breast cancer patients (calibration) and its ability to distinguish individuals who will experience different outcomes (discriminatory accuracy).

Awaiting the incorporation of gene expression profiles in prognostic tools, models such as Adjuvant! are still suboptimal. Incorporation of other prognostic markers may also improve these tools. It has been shown that mammographic screening detects breast cancer at an earlier stage.⁵⁴⁻⁵⁶ Therefore, we investigated whether method of detection has additional prognostic value that could improve the estimation of disease outcome, assuming that screen-detected carcinomas are of a different tumor biology. This question is addressed in **chapter 9**, where we studied the accuracy of Adjuvant! in patients with a screen-detected carcinoma as well as assessed the independent prognostic value of screen-detection in a retrospective patient cohort.

This thesis ends with concluding remarks and future prospects in **chapter 10** and a summary of the results presented in **chapter 11**.

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