Prognostic factors in breast cancer: one fits all?
Mook, S.

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Chapter 4

The 70-gene prognosis signature predicts early metastasis in breast cancer patients between 55 and 70 years of age

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Abstract

Background

The majority of breast cancer patients are postmenopausal women who are increasingly being offered adjuvant chemotherapy. Since the beneficial effect of chemotherapy in postmenopausal patients predominantly occurs in the first 5 years after diagnosis, a prognostic marker for early events can be of use for adjuvant treatment decision-making. The aim of this study was to evaluate the prognostic value of the 70-gene prognosis-signature for early events in postmenopausal patients.

Methods

Frozen tumor samples from 148 patients aged 55-70 years were selected (T1-2, N0) and classified by the 70-gene prognosis signature (MammaPrint™) into good or poor prognosis. Eighteen percent received hormonal therapy.

Results

Breast cancer-specific survival (BCSS) at 5 years was 99% for the good-prognosis signature versus 80% for the poor-prognosis signature group ($p = 0.036$). The 70-gene prognosis signature was a significant and independent predictor of BCCS during the first 5 years of follow-up with an adjusted hazard ratio of 14.4 (95% confidence interval 1.7-122.2; $p = 0.01$) at 5 years.

Conclusion

The 70-gene prognosis signature can accurately select postmenopausal patients at low risk of breast cancer-related death within 5 years of diagnosis and can be of clinical use in selecting postmenopausal women for adjuvant chemotherapy.
Introduction

Approximately two-thirds of the newly diagnosed breast cancer patients are ≥ 55 years of age. These postmenopausal women are increasingly being offered adjuvant chemotherapy despite the more favorable biological characteristics of their tumors and their known favorable breast cancer-specific outcome in general. The Early Breast Cancer Trialists’ Collaborative Group meta-analysis has shown that the benefit from chemotherapy is influenced by age, with less benefit in older patients. Moreover, those data have shown that the time course of chemotherapy efficacy differs between pre- and postmenopausal patients; the benefit of chemotherapy in postmenopausal breast cancer patients occurs predominantly in the first 5 years, while in premenopausal patients, the benefit sustains throughout the first 10 years. Therefore, a prognostic marker that can accurately identify postmenopausal patients who are at low risk of developing an early breast cancer-related event can be of clinical use for selecting postmenopausal patients for adjuvant chemotherapy. One of the prognostic markers in the field of breast cancer is the 70-gene prognosis signature (MammaPrint™), which can accurately identify patients who have a good prognosis and therefore might be safely spared chemotherapy. This signature has been developed in a predefined subset of patients, i.e. women under the age of 55 years at diagnosis with stage I or II, node-negative breast cancer. Therefore, the aim of our study was to evaluate the prognostic value of the 70-gene prognosis signature in postmenopausal women with node-negative breast cancer. Specifically, we investigated whether the signature could select postmenopausal patients who are at low risk of developing an early breast cancer-related event and thus can be safely spared chemotherapy, without jeopardizing disease outcome.

Methods

Patient selection

A consecutive series of patients treated at the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (NKI-AVL) from 1984 to 1996 were selected according to the following criteria: female, unilateral T1 or T2 primary invasive breast carcinoma, negative nodal status, aged between 55 and 71 years at diagnosis, no adjuvant chemotherapy and fresh frozen tumor material available in the comprehensive NKI-AVL tissue bank. Patients without complete axillary staging, patients with prior malignancies (except for non-melanoma skin cancer and dysplasia of the cervix), bilateral synchronous breast tumors or patients treated with neoadjuvant therapy were not included.

All patients (n = 148) had been treated by modified radical mastectomy or breast-conserving surgery, including axillary lymph node dissection, followed by radiotherapy...
if indicated. Twenty-seven patients (18%) received endocrine therapy, which consisted of
tamoxifen for a median duration of 2.0 years (range 0.06-7.0 years). Patients were treated
according to consensus guidelines, taking into account patients’ will and consent. The
study was approved by the ethical review board of the NKI-AVL.

**Tumor samples, RNA extraction and gene expression analysis**

Frozen tumor samples were evaluated for MammaPrint™ (FDA 510(K) cleared) at Agendia's
laboratories (ISO17025 certified and CLIA accredited; Amsterdam, the Netherlands)
blinded to clinical data, as previously described. Briefly, frozen sections were stained with
hematoxylin and eosin; only samples that contained at least 30% tumor cells were used
for RNA isolation. Labeled complementary RNAs were hybridized together with a standard
breast cancer reference pool to the custom-designed MammaPrint™ microarray. Tumors
were classified according to their cosine correlation coefficient with the MammaPrint™
template. Tumors with a correlation coefficient above the threshold were classified as
good prognosis signature, whereas all other tumors were classified as poor prognosis
signature.

**Clinicopathological and follow-up data**

Clinical data were retrieved from medical patient records, blinded to the 70-gene prognosis
signature. Follow-up was completed until October 2007. End points considered were time
from surgery to distant metastasis as first event [distant metastasis-free survival (DMFS)]
and breast cancer-specific survival (BCSS), defined as the time from surgery to breast cancer-
related death. For the analysis of DMFS, we considered distant metastasis as first event as
failure; patients were censored on the date of local or regional recurrence, development
of a second primary, including contralateral breast cancer, death from any cause or date
of last follow-up visit. Tumor grade was defined according to Bloom-Richardson. Estrogen
receptor (ER) expression was estimated using ER messenger RNA levels as determined by
the microarray. Clinical risk was evaluated using Adjuvant! software version 8.0 (available at www.
adjuvantonline.com). Adjuvant! calculates 10-year survival probability based on patient’s
age, co-morbidities (set to ‘average for age’), tumor size, tumor grade, ER-status and
number of positive axillary lymph nodes. Patients were classified as having low clinical
risk when the predicted 10-year BCSS was > 88% for ER-positive tumors and > 92% for ER-
negative tumors, respectively.
**Statistical analyses**

Analyses were carried out using SPSS version 15.0 (SPSS Inc., Chicago, IL). Cox proportional hazards regression analyses were used to calculate hazard ratios (HRs) and their 95% confidence intervals (CIs). HRs for the risk groups as defined by the 70-gene signature were estimated with stratification for clinical risk as defined by Adjuvant! (adjusted HRs). Additionally, HRs for the risk groups as defined by Adjuvant! were estimated with stratification for genomic risk as defined by the 70-gene prognosis signature. The impact of duration of follow-up on HRs was analyzed by censoring observations at increasing time points.

**Results**

The 70-gene prognosis signature (MammaPrint™) risk classification was assessed in tumor tissues of a consecutive series of 148 postmenopausal patients with early-stage, lymph node-negative, invasive breast cancer. Tumor samples of 173 patients fulfilled the selection criteria, of which 25 contained insufficient tumor cells (n = 22) or had insufficient RNA quality (n = 3). All 148 samples eligible for genomic profiling were successfully hybridized. There was no difference in tumor or patient characteristics between the 25 samples that could not be hybridized and the 148 analyzed samples with regard to age, tumor size, histology, overall survival and BCSS (data not shown). The median duration of follow-up was 12.5 years (range 0.4-20.2) for the 114 patients who did not die of breast cancer and 7.2 years (range 0.8-17.7) for the 34 patients who died of breast cancer. During follow-up, 83 patients had at least one event, among which were 42 distant metastases including 36 distant metastases as first event and 57 deaths of which 34 were breast cancer-related. Twelve of the 34 breast cancer-related deaths occurred within 5 years after diagnosis.

**Classification by 70-gene prognosis signature and disease outcome**

The 70-gene prognosis signature classified 91 (61%) patients as good prognosis, whereas 57 (39%) patients were classified as poor prognosis. A good prognosis signature was associated with smaller, well-differentiated and ER -positive tumors (Table 1).

Patients classified as good prognosis by the signature had a 5-year DMFS probability of 93% (standard error (SE) 3%), compared with 72% (SE 6%) in the poor-prognosis signature group (Figure 1A). DMFS at 5 years was significantly worse in the poor-prognosis signature group, with a univariate HR of 4.6 (95%CI 1.8-12.0; p = 0.001). Over the entire follow-up period, the HR for DMFS was 1.8 (95% CI 0.9-3.5; p = 0.07).
Table 1. Baseline characteristics and association between clinicopathological characteristics and the 70-gene prognosis profile

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Good prognosis signature (n=91)</th>
<th>Poor prognosis signature (n=57)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>26 (28.6)</td>
<td>24 (42.1)</td>
<td>0.25</td>
</tr>
<tr>
<td>60 - 64</td>
<td>34 (37.4)</td>
<td>15 (26.3)</td>
<td></td>
</tr>
<tr>
<td>65 - 71</td>
<td>31 (34.1)</td>
<td>18 (31.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Surgery</strong></td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td>Breast conserving surgery</td>
<td>46 (50.5)</td>
<td>24 (42.1)</td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>45 (49.5)</td>
<td>33 (57.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>pT1 (≤ 20 mm)</td>
<td>59 (64.8)</td>
<td>24 (42.1)</td>
<td></td>
</tr>
<tr>
<td>pT2 (&gt; 20-50 mm)</td>
<td>32 (35.2)</td>
<td>33 (57.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Histological tumor type</strong></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>67 (73.6)</td>
<td>52 (91.2)</td>
<td></td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>17 (18.7)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mixed IDC ILC</td>
<td>4 (4.4)</td>
<td>3 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (3.3)</td>
<td>2 (3.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Histological grade</strong></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Grade I</td>
<td>52 (57.1)</td>
<td>3 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Grade II</td>
<td>28 (30.8)</td>
<td>15 (26.3)</td>
<td></td>
</tr>
<tr>
<td>Grade III</td>
<td>11 (12.1)</td>
<td>39 (68.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Estrogen-receptor status</strong></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (3.3)</td>
<td>29 (50.9)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>88 (96.7)</td>
<td>28 (49.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Adjuvant endocrine therapy</strong></td>
<td></td>
<td></td>
<td>0.86</td>
</tr>
<tr>
<td>No</td>
<td>74 (81.3)</td>
<td>47 (82.5)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17 (18.7)</td>
<td>10 (17.5)</td>
<td></td>
</tr>
</tbody>
</table>

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

The 5-year BCSS probability was 99% (SE 1%) for the good-prognosis signature group and 80% (SE 5%) for the poor-prognosis signature group (Figure 1B). In addition, the difference in BCSS between the poor-prognosis signature group and the good-prognosis signature group over the entire follow-up period was significant with a univariate HR of 2.0 (95% CI 1.0-4.0; p = 0.04). This difference was most pronounced at 5 years, with a univariate HR of 19.1 (95% CI 2.5-148; p = 0.005) (Figure 2A).
Figure 1.
Kaplan-Meier curves by 70-gene prognosis signature.
A. Time to distant metastases as first event.
B. Breast cancer-specific survival.
Clinical risk assessment and discordance with 70-gene prognosis signature

Using the predefined cut-off, Adjuvant! classified 74 patients (50%) as clinical low risk and 74 patients (50%) as clinical high risk. Concordance was observed between Adjuvant! and the 70-gene prognosis signature for 62 (42%) low-risk/good prognosis patients, whereas 45 patients (30%) were classified as high-risk/poor prognosis according to both risk assessments. The clinical risk assessment was discordant with the genomic prognosis for 41 patients (28%); 12 (8%) were classified as clinical low risk and poor prognosis signature and 29 (20%) were classified as clinical high risk though good prognosis signature.

Prediction of early breast cancer-specific death: time dependency

Since the benefit of chemotherapy in postmenopausal patients is predominantly seen in the first 5 years and given the long follow-up time in this study compared with the original validation study (11.6 versus 6.7 years, respectively), we calculated unadjusted HRs for the signature and clinical risk assessment with censoring of all observations at increasing time points (Figure 2, panels A and B). Remarkably, the 70-gene prognosis signature was a strong predictor for early breast cancer-specific death (BCSD) with the strongest prognostic value at 5 years as shown by the highest HR (HR 19.1; 95% CI 2.5-148; \(p = 0.005\)), whereas the clinical risk classification predicted disease outcome more evenly with a tendency to predict better after 5 years, with the strongest prognostic capacity at 10 years (HR 6.2; 95% CI 2.1-18.0; \(p = 0.001\)). To further evaluate the clinical utility of the 70-gene prognosis signature, we adjusted its performance for the clinical risk assessment, which showed that the signature is a powerful predictor of early BCSD independent of the clinical risk, with an adjusted HR at 5 years of 14.4 (95% CI 1.7-122; \(p = 0.01\)) (Figure 2, panel C). The reverse analysis, i.e. HRs for the clinical risk classification adjusted for the gene signature, showed that the clinical risk classification is a prognostic factor of BCSD after 10 years independent of the signature, with an adjusted HR of 4.4 (95% CI 1.4-13.6; \(p = 0.01\)) for BCSS at 10 years (Figure 2, panel D).
The 70-gene signature in postmenopausal patients

Figure 2.
Hazard ratios (HRs) for breast cancer-specific death at increasing censoring times.
A). Univariate HRs for poor-prognosis signature versus good-prognosis signature groups.
B). Univariate HRs for clinical high-risk versus clinical low-risk groups as calculated by Adjuvant!
Discussion

The present validation study shows that the 70-gene prognosis signature, which was
developed in premenopausal patients with early-stage breast cancer, is also a prognostic
factor in postmenopausal women, with especially strong prognostic capacity in the first
5 years after diagnosis. Since the beneficial effect of chemotherapy in postmenopausal
women mainly occurs in the first 5 years after diagnosis, accurate identification of
early events by the 70-gene prognosis signature can be of great value in selecting
postmenopausal patients for adjuvant chemotherapy.

In a previous validation study, Buyse et al. showed a strong time dependency of the signature.
Since we were especially interested in predicting early events which might be prevented by
chemotherapy, we also investigated the effect of duration of follow-up on the prognostic
value of the signature. The prognostic value of the signature was most pronounced within
the first 5 years of diagnosis, even after adjustment for clinicopathological risk classification
by Adjuvant! (Figure 2, panel C). These results demonstrate the additional value of the 70-gene
prognosis signature over and above the clinical risk assessment in predicting early BCSD.
First, the signature enlarged the group of low-risk/ good prognosis patients as compared
with the clinical risk classification (from 50% to 61%). Secondly, despite this increase in
the low-risk group, the signature accurately classified 11 of 12 (92%) patients who died of
breast cancer within 5 years of diagnosis as poor prognosis (Figure 3), compared with 10 of 12
(83%) correctly classified by Adjuvant!

Although our study confirmed that the signature can correctly predict early BCSD, late
BCSD was less accurately predicted by the signature (Figure 3), resulting in misclassification
of 15 BCSD after 5 years of diagnosis (compared with seven misclassified by Adjuvant!).
Remarkably, all 15 patients who were classified as good signature but died of breast cancer
after 5 years had ER-positive tumors, and only one patient received endocrine therapy.
Consequently, endocrine treatment could potentially have prevented at least part of these
late BCSDs (31% reduction of annual breast cancer death rate by adjuvant tamoxifen). In our series, the 27 patients who received hormonal therapy were equally distributed
between the good- and the poor-prognosis signature groups. Moreover, the median
duration of hormonal therapy was only 2 years (according to Dutch treatment guidelines
in the years of diagnosis concerned), instead of the current standard treatment of at least
5 years. Separate analyses of the 121 hormonal therapy-naive patients showed that the
70-gene prognosis signature was also a predictor for early BCSD in untreated patients
between 55 and 71 years of age (adjusted HR at 5 years 10.8; 95% CI 1.2-94.7; \( p = 0.03 \)).
The strong time dependency of the signature can be explained by the fact that the
signature was built to identify patients with distant metastases within 5 years. Moreover,
it supports the hypothesis that different biological mechanisms are involved in early and
late disease recurrences. ER-negative, high-grade tumors are more likely to metastasize
during the early years after diagnosis, whereas ER-positive, low-grade tumors more often
cause late recurrences.
The 70-gene signature in postmenopausal patients

Breast cancer-specific deaths by 70-gene prognosis signature. Each circle represents a patient who died of breast cancer.

In our study, 61% of the patients were classified as good prognosis by the signature. In previous validation studies, the 70-gene signature consistently classified ~40% to 50% of the (predominantly premenopausal) patients as good prognosis.\textsuperscript{8-11} The increase in patients classified as good prognosis in our series could be the reflection of the intrinsic low-risk nature of breast cancer and mammographic screening in postmenopausal women.\textsuperscript{2-4} Recently, an independent validation study of the 70-gene prognosis signature in predominantly postmenopausal women was published.\textsuperscript{19} In contrast to our study, the majority of their study population was classified as high risk (73\% versus 39\% in our series). This discrepancy in risk classification can be attributed to differences in baseline characteristics, \textit{i.e.} more poorly differentiated tumors in their series. On the other hand, disease outcome in our series is worse compared with the outcome in the series of Wittner \textit{et al.},\textsuperscript{19} which can be caused by the difference in proportion of patients who received adjuvant systemic therapy (18\% hormonal therapy in our series versus 45\% chemo- and/or hormonal therapy in Wittner’s series).

Several other prognostic profiles have been studied, among which are the 76-gene profile and the 21-recurrence score.\textsuperscript{20,21} Both profiles have been developed and so far validated in
a mixed population of pre- and postmenopausal women. To our knowledge, this is the first study that evaluates the prognostic value of a prognostic signature in an exclusively postmenopausal patient series. Recently, Anders et al. showed a significant difference in gene expression patterns between tumors from pre- and postmenopausal breast cancer patients. However, our study indicates that disease outcome in pre- and postmenopausal patients can be determined by common denominators, which are captured by the 70-gene signature.

In conclusion, our study indicates that application of the 70-gene prognosis signature in breast cancer patients between 55 and 71 years of age could result in a more accurate allocation of adjuvant systemic therapy. A poor prognosis signature would imply chemotherapy treatment to prevent early breast cancer deaths, and patients with ER-positive tumors should receive endocrine therapy to prevent late events. Furthermore, given the results from the ATAC (Arimidex, Tamoxifen, Alone or in Combination) and (Breast International Group) BIG 1-98 trial indicating that aromatase inhibitors (AIs) are more effective in preventing early recurrences compared with tamoxifen, patients with ER-positive tumors classified as poor prognosis by the signature might be candidates for up-front AI treatment. This last question will also be addressed in the endocrine therapy randomization of the MINDACT (Microarray for Node Negative and 1 to 3 Positive Node Disease may Avoid Chemotherapy) trial; patients with hormone receptor-positive tumors (good and poor prognosis signature) will be randomized between 2 years of tamoxifen followed by 5 years of letrozole versus 7 years of letrozole up front, therefore endocrine responsiveness can be related to the 70-gene prognosis signature.

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**Disclosures**

LJvV and MJvdV are named inventor on a MammaPrint™ patent. LJvV reports holding equity in Agendia BV. AF and AMG are employees of Agendia BV.
References


