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Prognostic factors in breast cancer: one fits all?

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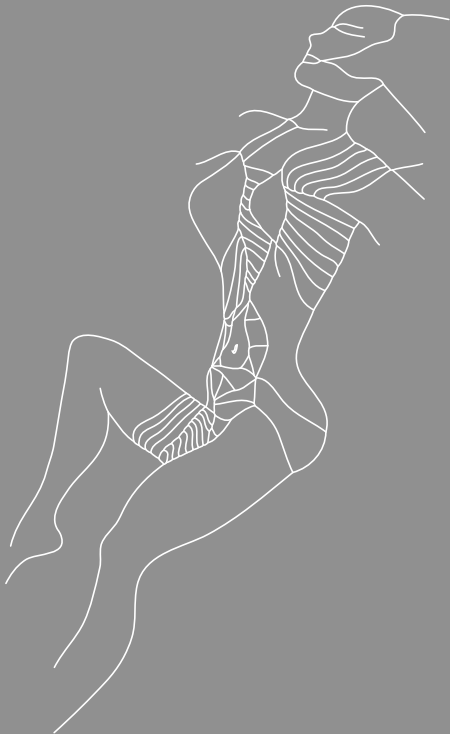
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Chapter 4

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 BCSS 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.¹ 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.²⁻⁵ 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.⁶ 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.⁷⁻¹¹ 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.^{7,12} 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.¹² 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.^{7,12}

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.⁸

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.^{13,14} 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.⁹

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

Characteristics	70-gene prognosis signature				P value
	Good prognosis signature (n=91)		Poor prognosis signature (n=57)		
	No.	%	No.	%	
Age (years)					0.25
< 60	26	28.6	24	42.1	
60 - 64	34	37.4	15	26.3	
65 - 71	31	34.1	18	31.6	
Surgery					0.32
Breast conserving surgery	46	50.5	24	42.1	
Mastectomy	45	49.5	33	57.9	
Tumor size					0.007
pT1 (\leq 20 mm)	59	64.8	24	42.1	
pT2 ($>$ 20-50 mm)	32	35.2	33	57.9	
Histological tumor type					0.02
Invasive ductal carcinoma	67	73.6	52	91.2	
Invasive lobular carcinoma	17	18.7	0	0	
Mixed IDC ILC	4	4.4	3	5.3	
Other	3	3.3	2	3.5	
Histological grade					< 0.001
Grade I	52	57.1	3	5.3	
Grade II	28	30.8	15	26.3	
Grade III	11	12.1	39	68.4	
Estrogen-receptor status					< 0.001
Negative	3	3.3	29	50.9	
Positive	88	96.7	28	49.1	
Adjuvant endocrine therapy					0.86
No	74	81.3	47	82.5	
Yes	17	18.7	10	17.5	

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*).

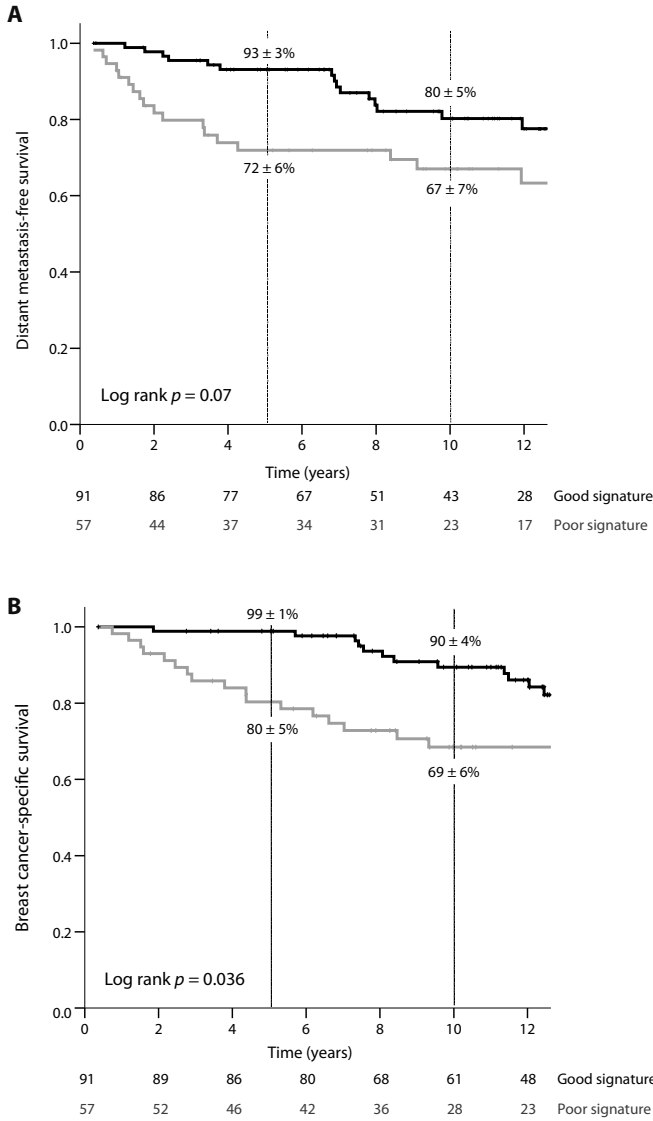


Figure 1.

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).

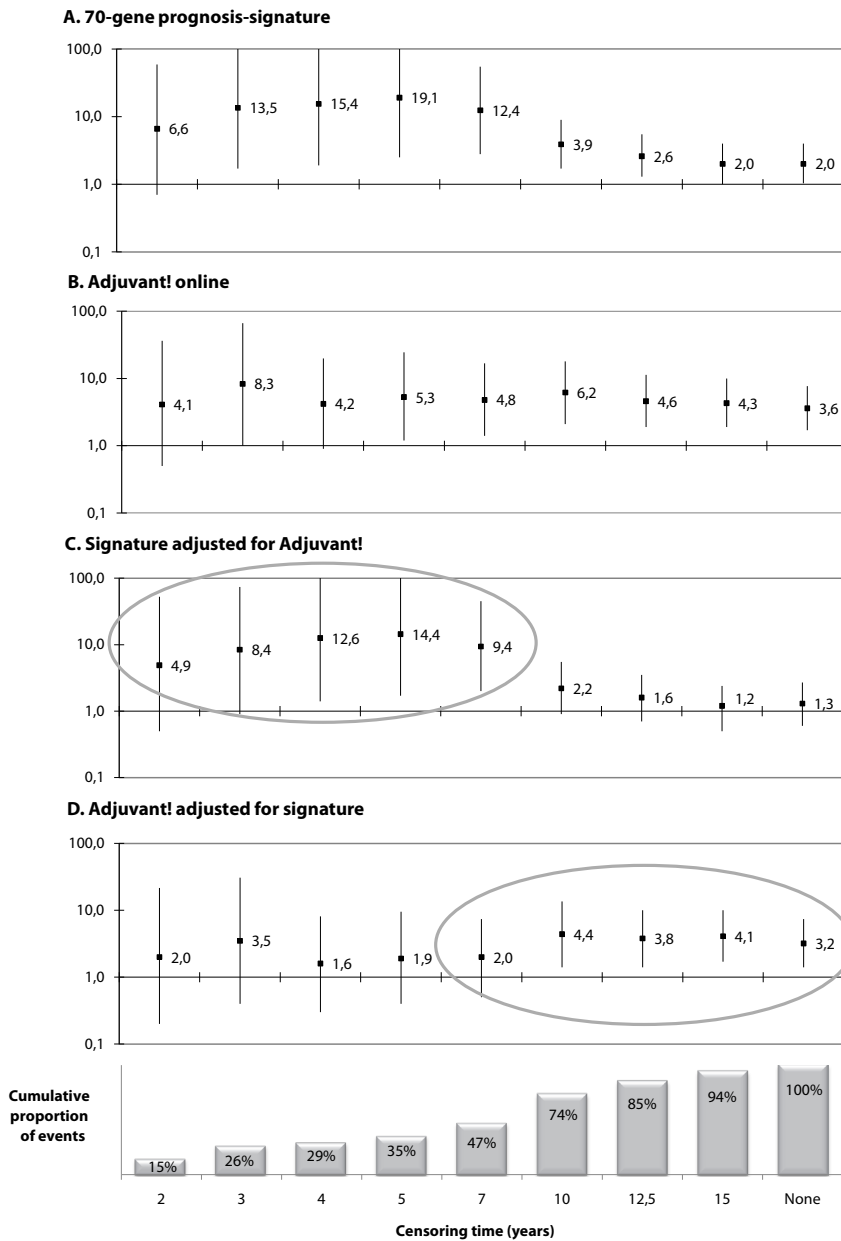


Figure 2.

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!.

C). HRs for 70-gene prognosis signature adjusted for clinical risk. D). HRs clinical risk assessment

by Adjuvant! adjusted for prognosis signature.

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,^{6,15} 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.*⁹ 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).⁶ 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.^{16,17} 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.¹⁸

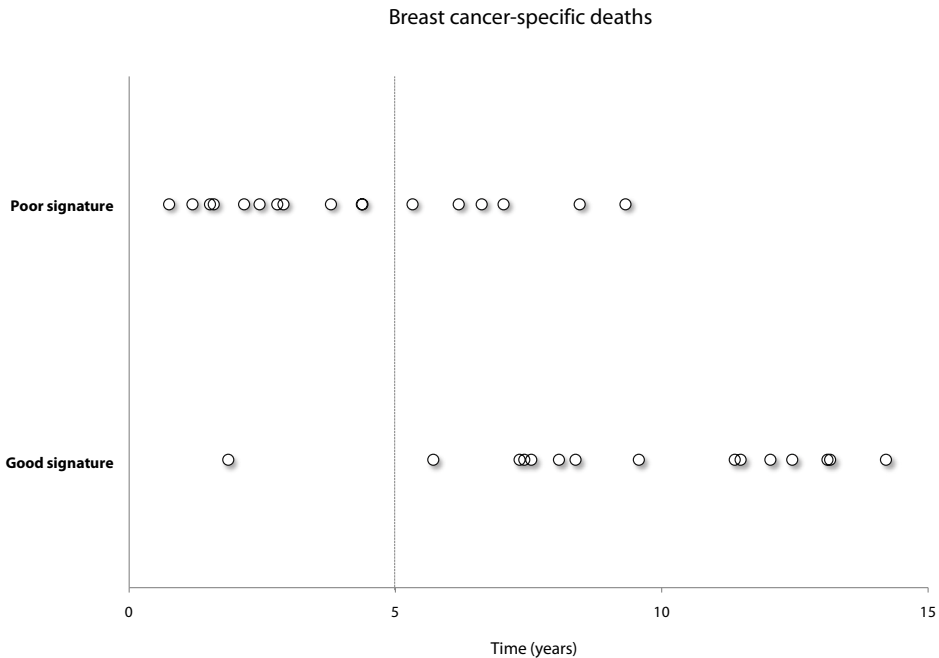


Figure 3.

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.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.²⁻⁴

Recently, an independent validation study of the 70-gene prognosis signature in predominantly postmenopausal women was published.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, *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 *et al.*,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.^{20,21} Both profiles have been developed and so far validated in

a mixed population of pre- and postmenopausal women.^{22,23} 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.*⁵ 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.²⁴⁻²⁶ 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.^{27,28}

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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

1. Ries LAG, Melbert D, Krapcho M *et al.* SEER Cancer Statistics Review, 1975-2005. Bethesda, MD: National Cancer Institute 2008; http://seer.cancer.gov/csr/1975_2005/, based on November 2007 SEER data submission, posted to the SEER web site. Last accessed 28 November, 2008.
2. Anderson WF, Chatterjee N, Ershler WB, Brawley OW. Estrogen receptor breast cancer phenotypes in the Surveillance, Epidemiology, and End Results database. *Breast Cancer Res Treat* 2002; 76: 27-36.
3. Diab SG, Elledge RM, Clark GM. Tumor characteristics and clinical outcome of elderly women with breast cancer. *J Natl Cancer Inst* 2000; 92: 550-556.
4. Fisher B, Wickerham DL, Brown A, Redmond CK. Breast cancer estrogen and progesterone receptor values: their distribution, degree of concordance, and relation to number of positive axillary nodes. *J Clin Oncol* 1983; 1: 349-358.
5. Anders C, Hsu D, Broadwater G, *et al.* Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. *J Clin Oncol* 2008; 26: 3324-3330.
6. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005; 365: 1687-1717.
7. Van't Veer LJ, Dai H, Van de Vijver MJ, *et al.* Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; 415: 530-536.
8. Van de Vijver MJ, He YD, Van't Veer LJ, *et al.* A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002; 347: 1999-2009.
9. Buysse M, Loi S, Van't Veer L, *et al.* Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. *J Natl Cancer Inst* 2006; 98: 1183-1192.
10. Mook S, Schmidt MK, Viale G, *et al.* The 70-gene prognosis-signature predicts disease outcome in breast cancer patients with 1-3 positive lymph nodes in an independent validation study. *Breast Cancer Res Treat* 2009; 116: 295-302.
11. Bueno de Mesquita JM, Linn SC, Keijzer R *et al.* Validation of 70-gene prognosis signature in node-negative breast cancer. *Breast Cancer Res Treat* 2009; 117: 483-495.
12. Glas AM, Floore A, Delahaye LJ, *et al.* Converting a breast cancer microarray signature into a high-throughput diagnostic test. *BMC Genomics* 2006; 7: 278-287.
13. Ravdin PM, Siminoff LA, Davis GJ, *et al.* Computer program to assist in making decisions about adjuvant therapy for women with early breast cancer. *J Clin Oncol* 2001; 19: 980-991.
14. Olivotto IA, Bajdik CD, Ravdin PM, *et al.* Population-based validation of the prognostic model ADJUVANT! for early breast cancer. *J Clin Oncol* 2005; 23: 2716-2725.
15. Demicheli R, Miceli R, Moliterni A, *et al.* Breast cancer recurrence dynamics following adjuvant CMF is consistent with tumor dormancy and mastectomy-driven acceleration of the metastatic process. *Ann Oncol* 2005; 16: 1449-1457.
16. Klein CA, Blankenstein TJ, Schmidt-Kittler O, *et al.* Genetic heterogeneity of single disseminated tumour cells in minimal residual cancer. *Lancet* 2002; 360: 683-689.

17. Schmidt-Kittler O, Ragg T, Daskalakis A, *et al.* From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. *Proc Natl Acad Sci USA* 2003; 100: 7737-7742.
18. Saphner T, Tormey DC, Gray R. Annual hazard rates of recurrence for breast cancer after primary therapy. *J Clin Oncol* 1996; 14: 2738-2746.
19. Wittner BS, Sgroi DC, Ryan PD, *et al.* Analysis of the MammaPrint breast cancer assay in a predominantly postmenopausal cohort. *Clin Cancer Res* 2008; 14: 2988-2993.
20. Paik S, Shak S, Tang G, *et al.* A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; 351: 2817-2826.
21. Wang Y, Klijn JG, Zhang Y, *et al.* Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005; 365: 671-679.
22. Esteva FJ, Sahin AA, Cristofanilli M, *et al.* Prognostic role of a multigene reverse transcriptase-PCR assay in patients with node-negative breast cancer not receiving adjuvant systemic therapy. *Clin Cancer Res* 2005; 11: 3315-3319.
23. Foekens JA, Atkins D, Zhang Y, *et al.* Multicenter validation of a gene expression-based prognostic signature in lymph node-negative primary breast cancer. *J Clin Oncol* 2006; 24: 1665-1671.
24. Coates AS, Keshaviah A, Thurlimann B, *et al.* Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. *J Clin Oncol* 2007; 25: 486-492.
25. Mauriac L, Keshaviah A, Debled M, *et al.* Predictors of early relapse in postmenopausal women with hormone receptor-positive breast cancer in the BIG 1-98 trial. *Ann Oncol* 2007; 18: 859-867.
26. Forbes JF, Cuzick J, Buzdar A, *et al.* Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol* 2008; 9: 45-53.
27. Bogaerts J, Cardoso F, Buyse M, *et al.* Gene signature evaluation as a prognostic tool: challenges in the design of the MINDACT trial. *Nat Clin Pract Oncol* 2006; 3: 540-551.
28. Mook S, Van't Veer LJ, Rutgers EJ, Piccart-Gebhart MJ, Cardoso F. Individualization of therapy using MammaPrint: from development to the MINDACT Trial. *Cancer Genomics Proteomics* 2007; 4: 147-155.