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ORIGINAL REPORT

PAM50 Risk of Recurrence Score Predicts 10-Year Distant Recurrence in a Comprehensive Danish Cohort of Postmenopausal Women Allocated to 5 Years of Endocrine Therapy for Hormone Receptor–Positive Early Breast Cancer

Anne-Vibeke Lænkholm, Maj-Britt Jensen, Jens Ole Eriksen, Birgitte Bruun Rasmussen, Ann S. Knoop, Wesley Buckingham, Sean Ferree, Carl Schaper, Torsten O. Nielsen, Taryn Haffner, Torben Kibøl, Maj-Lis Møller Talman, Anne Marie Bak Jylling, Tomasz Piotr Tabor, and Bent Ejlertsen

Author affiliations and support information (if applicable) appear at the end of this article.

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Corresponding author: Anne-Vibeke Lænkholm, MD, Department of Surgical Pathology, Zealand University Hospital, Ingemannsvej 48, 4200 Slagelse, Denmark; e-mail: anlae@regionsjaelland.dk.

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ABSTRACT

Purpose

The PAM50-based Prosigna risk of recurrence (ROR) score has been validated in randomized clinical trials to predict 10-year distant recurrence (DR). The value of Prosigna for predicting DR was examined in a comprehensive nationwide Danish cohort consisting of postmenopausal women with hormone receptor–positive early breast cancer treated with 5 years of endocrine therapy alone.

Patients and Methods

Using the population-based Danish Breast Cancer Cooperative Group database, follow-up data were collected on all patients diagnosed from 2000 through 2003 who, by nationwide guidelines, were treated with endocrine therapy for 5 years. Primary tumor blocks from 2,740 patients were tested with Prosigna and, after determination of human epidermal growth factor receptor 2 (HER2) status, data from 2,558 hormone receptor–positive/HER2-negative samples were analyzed, including 1,395 node-positive patients. Fine and Gray models were applied to determine the prognostic value of ROR for DR.

Results

Median follow-up for recurrence was 9.2 years. Twenty-six percent of the node-positive patients were classified as low ROR (n = 359) with a DR risk of 3.5% (95% confidence interval [CI], 1.9% to 6.1%) versus a DR risk of 22.1% (95% CI, 18.6% to 25.8%) at 10 years for patients classified as high ROR (n = 648). Node-negative patients classified as low and high ROR had a risk of DR of 5.0% (95% CI, 2.9% to 8.0%) and 17.8% (95% CI, 14.0% to 22.0%), respectively. Luminal B tumors (n = 947; DR risk, 18.4% [95% CI: 15.7% to 21.3%]) had a significantly worse outcome than luminal A tumors (n = 1,474,;DR risk, 7.6% [95% CI: 6.1% to 9.2%]; $P < .001$).

Conclusion

Prosigna ROR score improved the prediction of outcome in this nationwide Danish population. In a real-world setting, Prosigna can reliably identify node-negative patients and a significant proportion of patients with one to three positive nodes who can be spared treatment with adjuvant chemotherapy.

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INTRODUCTION

Estrogen receptor–positive human epidermal growth factor receptor 2 (HER2)–normal disease is the most frequently occurring type of early-stage breast cancer in postmenopausal women. Patient and tumor characteristics have been used to guide decisions about adjuvant chemotherapy generally indicated for younger patients with node-positive or highly proliferative tumors.[1] The recent overview from the Early Breast Cancer Trialists' Collaborative Group suggested that, on average, chemotherapy reduces relative 10- year breast cancer mortality by about one-third.[2] Importantly, the proportional benefits were similar in older and younger women and independent of age, nodal status, estrogen receptor status, and type of chemotherapy regimen.

Several multigene assays have now been evaluated for the prognostication of early-stage, node-negative disease and have confirmed the clinical utility of these tests to robustly identify patients at sufficiently low risk of 10-year distant recurrence (DR) that they have no capacity for a significant additional benefit from cytotoxic adjuvant chemotherapy.[3-11] A similar prognostic capability

ASSOCIATED CONTENT

See accompanying Editorial on page 725

Appendix

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has been demonstrated for Prosigna (NanoString Technologies, Seattle, WA) and Endopredict (Myriad Genetics, Salt Lake City, UT) in patients with one to three positive nodes.[9,12,13] Although these are included in the European guidelines for biomarker analyses, ASCO has not considered the evidence sufficient to recommend the use of any multigene test for risk stratification in node-positive disease.[14,15]

In this large, nationwide, population-based study, we evaluated the ability of Prosigna to predict distant recurrence at 10 years in postmenopausal women with breast cancer with zero to three positive nodes who were allocated to 5 years of endocrine therapy alone without chemotherapy. Here, we report the 10-year follow-up data on distant recurrence by the Prosigna ROR score and number of positive nodes.

PATIENTS AND METHODS

The organization of the Danish Breast Cancer Cooperative Group (DBCG) and current study cohort has been described in detail.[16,17] In brief, a treatment algorithm is automatically generated by the clinical DBCG database using diagnostic and surgical information entered in real-time from all patients with early breast cancer and these data are used as a reference for treatment recommendations at the multidisciplinary conference. Subsequent registration of detailed clinical information concerning definitive surgery, radiotherapy, systemic treatment, and follow-up is mandatory for 10 years.[17] The study was approved by the National Danish Ethics Committee (approval no. H-D-2007-0034) and all participants provided written, informed consent before original biobanking.

Study Patients

In the current study cohort, we prospectively included all postmenopausal patients in Denmark who, in January 2000 through December 2003, were allocated to 5 years of endocrine treatment as the only systemic treatment after a first diagnosis of estrogen receptor (ER)–positive invasive breast cancer. All pathology departments completed external quality assurance programs for ER assessment.[18,19] Eligible patients were $\geq$ 50 years and met at least one of the following risk criteria: a tumor size $>$ 20 mm (any histologic subtype), ductal histology with malignancy grade 2 or 3, or one to three positive nodes (any histologic subtype). Surgery, radiotherapy, and systemic treatment were predetermined and have been described previously, as has the external quality insurance program for ER.[16,20]

Central Assessment of HER2 and Prosigna

Formalin-fixed, paraffin-embedded tumor samples from primary excisional surgery specimens were collected at the Department of Surgical Pathology, Zealand University Hospital, and tissue microarrays (TMAs) were constructed with 4 $\times$ 1.5-mm tumor tissue cores split into two separate recipient blocks. HER2 status was established centrally using these TMAs by standard recommendations.[21]

RNA extraction and Prosigna testing were performed blinded and according to standard operating procedures[5,9,22,23] at the Department of Surgical Pathology, Zealand University Hospital. Results from 2,558 HER2-negative samples were included in the statistical analyses (Appendix Fig A1, online only). Once testing of all samples was completed, Prosigna results were transferred to the data manager, who also was blinded to patient data, for preparation of the analysis data set. The analysis data set was then transferred to the DBCG for merging with clinical data and survival analysis. ROR cutoffs varied by the number of positive lymph nodes to identify populations of approximately $<$ 10% (low), 10% to 20% (intermediate), and $>$ 20% (high) risk of DR. The cutoffs used to define the risk groups were prespecified in the study protocol. These cutoffs were developed on the basis of a combined analysis of the transATAC (translational substudy of the Arimidex, Tamoxifen, Alone or in Combination Trial) and ABCSG8 studies before the start of the current study[4-6,9] and are presented in Appendix Table A1 (online only).

End Points

The primary end point was time to DR, defined as the interval from breast cancer surgery until DR or death as a result of breast cancer. Secondary end points included overall survival (OS) and time to any recurrence (TR). For DR and TR secondary carcinomas, contralateral breast cancer and death as a result of causes other than breast cancer were considered competing risk events (Appendix Table A1). For OS, complete follow-up was achieved until October 1, 2014, by linkage to the Danish Central Population Registry.

Statistical Analyses

The statistical analysis was executed by the DBCG statistical office as predefined in a written statistical analysis plan that was fully determined before tumor samples were analyzed.

Follow-up time was quantified in terms of Kaplan-Meier estimates. Univariate and multivariate regression analyses were performed for DR, TR, and OS. For competing risk analysis, the Fine-Gray proportional, subdistribution hazard model was used, whereas the Cox proportional hazard model was used for overall survival. Models were fitted for all patients, for node-positive patients only, and individually for each of the node-count–specific groups.

Factors included in the multivariable analyses were age (continuous), tumor size [transformed to log (cm)], number of positive lymph nodes (none, one, two, or three), grade (1, 2, 3, and not graded), and ER expression (continuous, percentage of positive tumor cells). The original continuous variables were examined for evidence of nonlinearity. As a result, tumor size was log transformed; the other two continuous variables required no transformation. The multivariable analyses, including age and ER expression as continuous variables, were based on the statistical model developed as previously described.[16] The assumption of proportional hazards was assessed by Schoenfeld residuals. The hazard rates of age, ER, and grade were not proportional over the entire 10-year period, and were each modeled for early and late period (ie, $<$ 5 years, $\geq$ 5 years). Cumulative incidences were calculated for DR and TR, using the Gray test for comparison, and the Kaplan-Meier method and log-rank test were used for OS. All P values are two-sided. Statistical analyses were done using SAS, version 9.4 (SAS Institute, Cary, NC) and R, version 3.2.2 (R Project for Statistical Computing, https://www.r-project.org/).

RESULTS

We identified 2,971 patients who had early breast cancer and who, after breast-conserving surgery or mastectomy between January 2000 and December 31, 2003, initiated adjuvant treatment with tamoxifen or an aromatase inhibitor (AI) without chemotherapy and were eligible for this cohort study. An archived formalin-fixed, paraffin-embedded tumor block was available from each of 2,740 patients; of these blocks, 2,726 (99.5%) passed the PAM50 quality assessment. HER2 status was established on TMAs for 2,730 patients. The median follow-up was 9.2 years for DR and 12.5 years for OS. Of the 2,726 patients, 2,558 HER2-normal patients were included in the statistical analyses (Appendix Fig A1).

At 10 years, 228 patients (8.9%) had a distant breast cancer recurrence, and 46 (1.8%) died of breast cancer during the study period. The patients' baseline characteristics are provided in Table 1. The median age of patients was 63 years (range, 50 to 89 years); 45.5% were node negative, and 54.5% had one to three positive nodes. Tumor characteristics including nodal status, tumor size, malignancy grade, and lymphovascular invasion were all associated with DR, whereas treatment adherence was not.[16]

In node-negative disease and in patients with one, two, or three positive nodes, Prosigna showed a significant association ($P <$ .001 in

each case) of the continuous ROR with risk of DR at 10 years (Fig 1). In the overall population, compared with the high ROR risk group, the risk of a DR event was significantly ($P < .001$) lower in the intermediate ROR and low-risk groups (Table 2). Figure 2 shows, for the group of node-negative patients, an absolute 10-year DR of 5.0% (95% CI, 2.9% to 8.0%) for the low-risk group versus 7.3% (95% CI, 4.8% to 10.6%) in the intermediate-risk group and 17.8% (95% CI, 14.0% to 22.0%) in the high-risk group ($P < .001$, low *v* high). In the group of patients with positive lymph nodes (one to three positive nodes), the absolute 10-year risk of DR was 3.5% (95% CI, 1.9% to 6.1%) for the low-risk group versus 11.5% (95% CI, 8.0% to 15.6%) in the intermediate-risk group versus 22.1% (95% CI, 18.6% to 25.8%) in the high-risk group ($P < .001$) when risk groups were tailored to the number of positive nodes (Fig 2). The results of the Prosigna analysis using the commercial product cutoff for node-positive disease combining one to three positive nodes (ROR ≤ 40) also identified a very low-risk population with an absolute risk of DR at 10 years of 4.8% (95% CI, 3.1% to 6.9%) compared with a high-risk group with a 21.9% risk of DR (95% CI, 18.9% to 25.1%). At 10 years, patients with any nodal status (ie, zero to three positive nodes) and a low ROR score who, without chemotherapy, were allocated to 5 years of adjuvant endocrine treatment had a DR rate of 4.3%.

Table 1. Patient Characteristics

Characteristic	No.	(%)
Age at diagnosis, years		
50-59	939	37
60-69	1,082	42
≥ 70	537	21
No. of positive lymph nodes		
0	1,163	46
1	779	30
2	393	15
3	223	9
Tumor size, mm		
≤ 10	241	9
11-20	1,087	43
21-30	862	34
> 30	368	14
Histologic subtype		
Ductal	2,126	83
Lobular	340	13
Other	92	4
Malignancy grade		
I	628	25
II	1,362	53
III	306	12
Unknown	262	10
Lymphovascular invasion		
Present	280	11
Absent	2,278	89
ER expression level		
10-59	259	10
60-89	529	21
90-99	674	26
100	1,064	42
Positive*	32	1
Molecular subtype		
Luminal A	1,474	58
Luminal B	947	37
HER2 enriched	110	4
Basal-like	27	1
ROR group		
Low	720	28
Intermediate	763	30
High	1,075	42

Abbreviations: ER, estrogen receptor; ROR, risk of recurrence.
*At least 10% of the exact ER expression level percentage unknown.

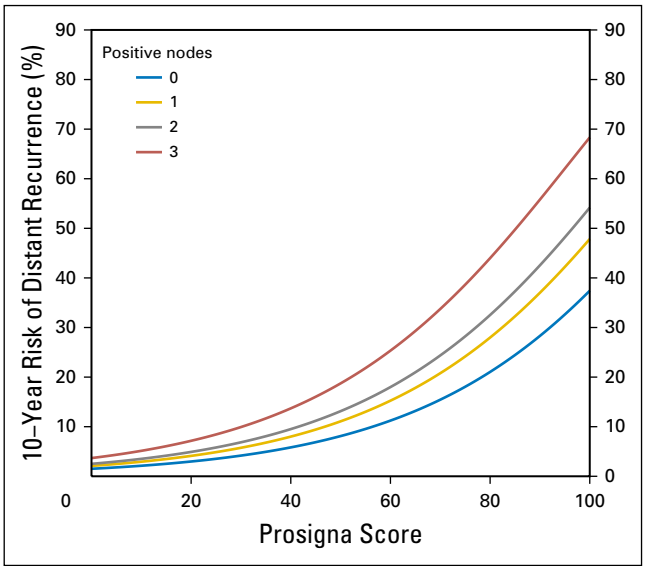

Fig 1. Continuous relationship between the 10-year risk of distant recurrence and the risk of recurrence score by number of positive nodes (zero to three) based on univariate Fine-Gray model.

Most patients (n = 1,474 [57.6%]) were assigned a luminal A subtype, whereas 947 (37.0%) were assigned a luminal B subtype by Prosigna. Only a minor proportion of the patients (n = 110 [4.3%]) had an HER2-enriched subtype or a basal-like subtype (n = 27 [1.1%]; Table 1). Regardless of lymph node status, DR was significantly lower ($P < .001$) in patients with a luminal A subtype as compared with the luminal B or HER2-enriched subtypes, with an absolute 10-year DR of 7.6% (95% CI, 6.1% to 9.2%) for the luminal A subtype versus 18.4% (95% CI, 15.7% to 21.3%) for the luminal B subtype versus 26.0% (95% CI, 17.8% to 35.0%) for the HER2-enriched group ($P < .001$). The difference in 10-year DR for the luminal subtypes is illustrated in Fig 3.

Compared with patients in the ROR intermediate-risk group, TR was significantly lower in the low-risk ROR group (multivariate hazard ratio [HR], 0.56; 95% CI, 0.37 to 0.85) and significantly higher in the ROR high-risk group (multivariate HR, 1.67; 95% CI, 1.26 to 2.23; $P < .001$). Compared with patients with a luminal A subtype, TR was significantly higher in patients with a luminal B subtype (multivariate HR, 1.92; 95% CI, 1.47 to 2.49; $P < .001$) or a HER2-enriched subtype (multivariate HR, 2.40; 95% CI, 1.48 to 3.89; $P < .001$). Of the 2,558 patients in the study, a total of 795 (31.1%) died during the study period. The 10-year OS was 86.1% (95% CI, 83.6 to 88.7) in ROR low-risk group; the mortality rate was higher in the ROR intermediate-risk group (multivariate HR, 1.18; 95% CI, 0.96 to 1.45) and significantly higher in the ROR high-risk group (multivariate HR, 1.65; 95% CI, 1.36 to 2.00).

DISCUSSION

We present here a comprehensive, nationwide, population-based study of a multigene expression assay for determination of prognosis

Table 2. Multivariate Analyses for Distant Recurrence

Variable	One to Three Positive Nodes		Zero to Three Positive Nodes	
	HR* (95% CI)	*P*	HR* (95% CI)	*P*
ROR				
Continuous	1.68 (1.42 to 1.99)	< .001	1.67 (1.46 to 1.92)	< .001
Low *v* Intermediate	0.39 (0.20 to 0.77)	< .001	0.53 (0.33 to 0.85)	< .001
High *v* Intermediate	1.54 (1.04 to 2.26)		1.81 (1.33 to 2.44)	
Subtype				
Luminal B *v* luminal A	1.97 (1.38;2.82)	< .001	1.93 (1.45 to 2.56)	< .001

Abbreviations: HR, hazard ratio; ROR, risk of recurrence.
*HR estimates are from Fine-Gray multivariate modeling of distant recurrence for one to three and zero to three positive nodes.

for patients with early-stage breast cancer. We demonstrated that Prosigna adds significant prognostic information in an inclusive population of postmenopausal patients with ER-positive and HER2-negative early breast cancer comprising 1,163 node-negative and 1,395 node-positive patients. At 10 years, patients with any nodal status (ie, zero to three positive nodes) and a low ROR score who, without chemotherapy, were allocated to 5 years of adjuvant endocrine treatment had a DR rate of 4.3%. Furthermore, a significant separation of prognosis was observed by molecular subtype.

This heterogeneity in prognosis in a population-based cohort confirms the observations made in the retrospective analysis of the randomized TransATAC and ABCSG-8 trials, and in their combined analysis.[5,6,9] Both trials included postmenopausal patients with ER-positive breast cancer who were allocated to different adjuvant endocrine treatments. In both, the Prosigna ROR score provided information beyond classic clinicopathological factors, identifying a group with a very low 10-year risk of DR, demonstrable in both lymph node–negative and lymph node–positive patients.

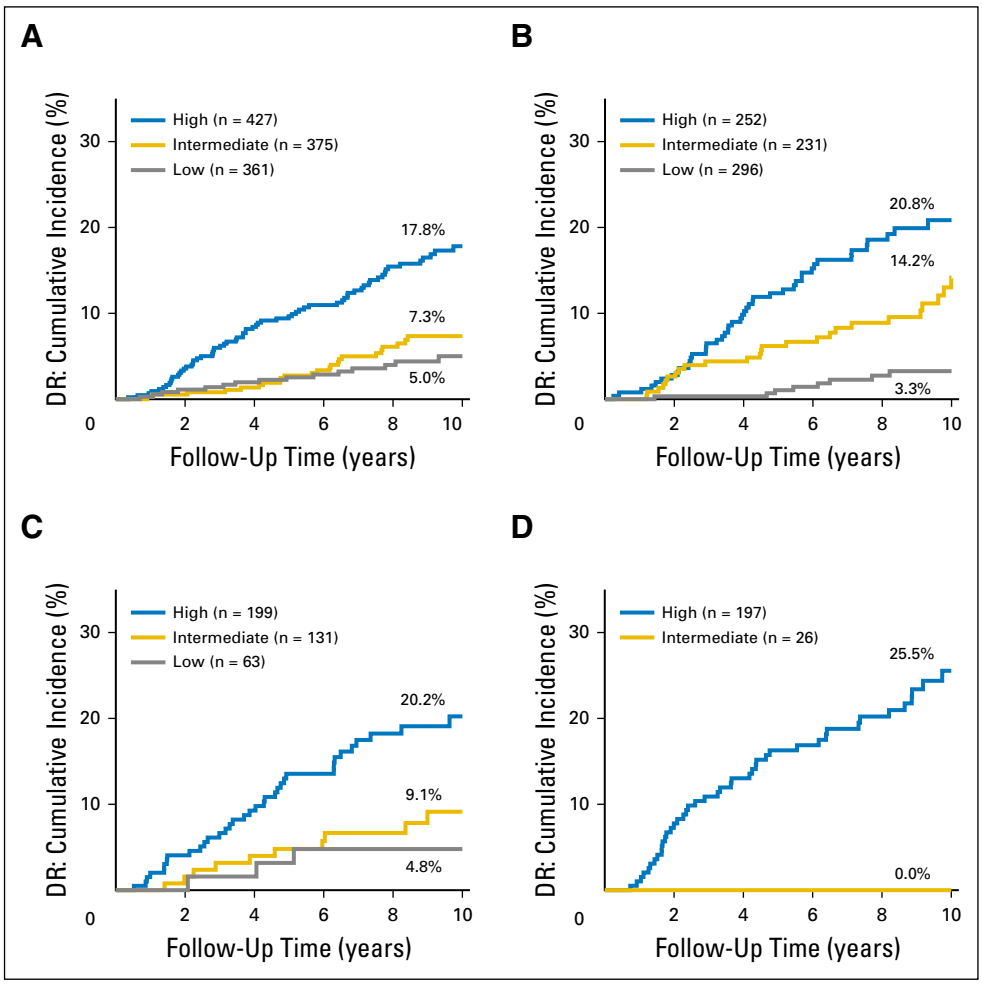

Fig 2. Cumulative incidence by risk of recurrence groups for zero, one, two, and three positive nodes. (A) Node negative, (B) one positive node, (C) two positive nodes, and (D) three positive nodes. DR, distant recurrence.

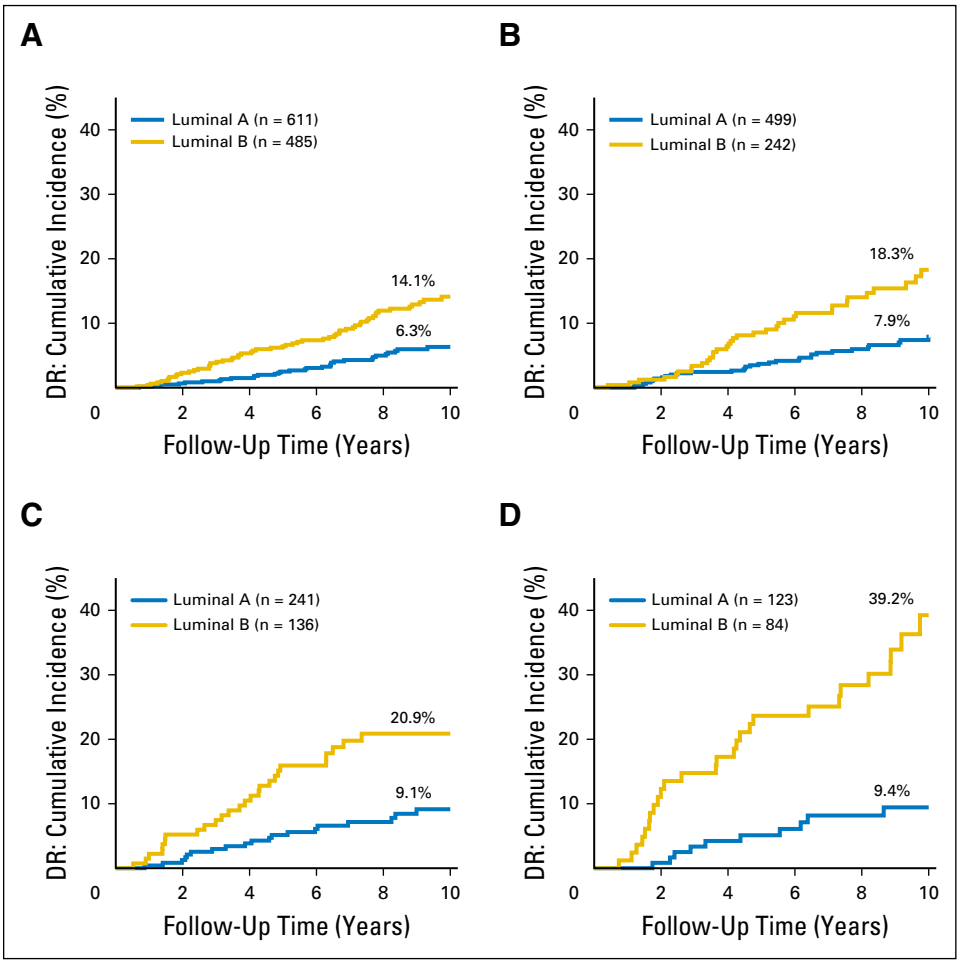

Fig 3. Cumulative incidence by subtype for zero, one, two, and three positive nodes. (A) Node-negative, (B) one positive node, (C) two positive nodes, and (D) three positive nodes. DR, distant recurrence.

De-escalation of treatment of patients with limited nodal involvement remains an important goal yet a significant challenge in early-stage breast cancer. Of particular note, this study identified a substantial population of patients with one to three positive lymph nodes with sufficiently low risk to safely avoid chemotherapy. By tailoring risk categorization to the number of positive nodes we identified, 37% of patients with a single positive lymph node and 15% of patients with two positive nodes as low risk, by Prosigna, with very favorable outcomes when treated with adjuvant endocrine therapy alone. Although chemotherapy could still provide some relative additional benefit for patients, when the low-risk group carries a residual rate of recurrence of $< 5\%$ at 10 years, the absolute benefit that can be achieved by still giving chemotherapy could be, at best, very small (and more than counterbalanced by toxicities). However, treatment guidelines make recommendations for adjuvant therapy based on the grouping of patients with one to three positive nodes; the clinical Prosigna test, therefore, also provides risk categorization by nodal stage. Prosigna was able to identify a low-risk population of node-positive patients who had $< 5\%$ probability of DR at 10 years and who, therefore, may safely be spared treatment with adjuvant chemotherapy. The ability of gene signatures to identify a low-risk, node-positive population has also been demonstrated for OncotypeDX in the publication by Roberts et al.[24]

The current study has some potential limitations. According to Danish national guidelines at the time of enrollment, the patients identified as having lowest-risk disease by clinical variables (N0, T $\leq$ 20 mm, grade 1 tumors) were ineligible for adjuvant endocrine therapy and, therefore, were excluded from this study. This effect of exclusion of the lowest-risk patients is evident in some of the analyses. For example, the fraction of node-negative patients classified as low risk is smaller than in previous studies[4,5] and the percentage of patients assigned a luminal A subtype was lower in the node-negative group than in the node-positive group. This exclusion also likely explains why the absolute risk of the low ROR category appears higher among node-negative (10-year DR, 5%) than node-positive (10-year DR, 3.5%) women.

Strengths of our study include a formal prospective-retrospective design within a large, comprehensive, population-based cohort with long and detailed clinical follow-up, demonstrating clinical utility of the Prosigna assay in a real-world setting. This original cohort was identified in an unbiased manner by a national cooperative group using algorithm-assisted guidelines on an individual patient basis. The study also demonstrates a high technical success rate for the Prosigna assay ($> 99\%$), confirming the robustness of the analysis and the decentralized applicability of Prosigna under standardized, US Food and Drug Administration-cleared conditions.

In conclusion, in this large comprehensive and population-based cohort, the addition of the Prosigna test to clinical and pathologic factors provided valuable information regarding the use of adjuvant chemotherapy in postmenopausal patients for both node-negative and node- positive ER-positive breast cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Anne-Vibeke Laenkholm, Maj-Britt Jensen, Ann S. Knoop, Sean Ferree, Torsten O. Nielsen, Bent Ejlertsen
Administrative support: Taryn Haffner, Bent Ejlertsen
Provision of study materials or patients: Bent Ejlertsen
Collection and assembly of data: Anne-Vibeke Laenkholm, Jens Ole Eriksen, Birgitte Bruun Rasmussen, Ann S. Knoop, Torben Kibøl, Maj-Lis Møller Talman, Anne Marie Bak Jylling, Tomasz Piotr Tabor, Bent Ejlertsen
Data analysis and interpretation: Anne-Vibeke Laenkholm, Maj-Britt Jensen, Sean Ferree, Carl Schaper, Torsten O. Nielsen, Bent Ejlertsen
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Affiliations

Anne-Vibeke Lænkholm, **Jens Ole Eriksen**, and **Torben Kibøl**, Zealand University Hospital, Slagelse; **Maj-Britt Jensen**, **Ann S. Knoop**, **Maj-Lis Møller Talman**, and **Bent Ejlertsen**, Rigshospitalet, Copenhagen; **Birgitte Bruun Rasmussen**, Herlev Hospital, Herlev; **Anne Marie Bak Jylling**, Odense University Hospital, Odense; **Tomasz Piotr Tabor,** Vejle Hospital, Vejle, Denmark; **Wesley Buckingham**, **Sean Ferree**, **Carl Schaper**, and **Taryn Haffner**, NanoString Technologies, Seattle, WA; and **Torsten O. Nielsen**, University of British Columbia, Vancouver, British Columbia, Canada.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Anne-Vibeke Laenkholm
Honoraria: Roche
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Ann S. Knoop
Consulting or Advisory Role: Novartis/Pfizer, Roche
Travel, Accommodations, Expenses: Novartis, Roche, Pierre Fabre

Wesley Buckingham
Employment: NanoString Technologies
Stock or Other Ownership: NanoString Technologies

Sean Ferree
Employment: NanoString Technologies
Stock or Other Ownership: NanoString Technologies
Patents, Royalties, Other Intellectual Property: NanoString Technologies

Carl Schaper
Leadership: Vivid Vision (I)
Stock or Other Ownership: Vivid Vision (I)
Consulting or Advisory Role: NanoString Technologies, Medivation, Illumina, Guardant Health, Dako, Kypha, IncellDx, AcceleratedDX, Verax, Biomedical, SysMex

Torsten Nielsen
Stock or Other Ownership: Bioclassifier
Consulting or Advisory Role: NanoString Technologies
Patents, Royalties, Other Intellectual Property: Bioclassifier
Travel, Accommodations, Expenses: NanoString Technologies

Taryn Haffner
Employment: NanoString Technologies
Stock or Other Ownership: NanoString Technologies
Research Funding: NanoString Technologies
Travel, Accommodations, Expenses: NanoString Technologies

Torben Kibøl
No relationship to disclose

Maj-Lis Møller Talman
Honoraria: Roche
Stock or Other Ownership: Roche (I)
Consulting or Advisory Role: Dako/Agilent Technologies
Travel, Accommodations, Expenses: Roche

Anne Marie Bak Jylling
Honoraria: Roche
Travel, Accommodations, Expenses: Visiopharm, Faxitron

Tomasz Piotr Tabor
Honoraria: Roche
Travel, Accommodations, Expenses: Roche

Bent Ejlertsen
Research Funding: NanoString Technologies (Inst), Roche (Inst), Novartis (Inst)
Travel, Accommodations, Expenses: AstraZeneca

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Appendix

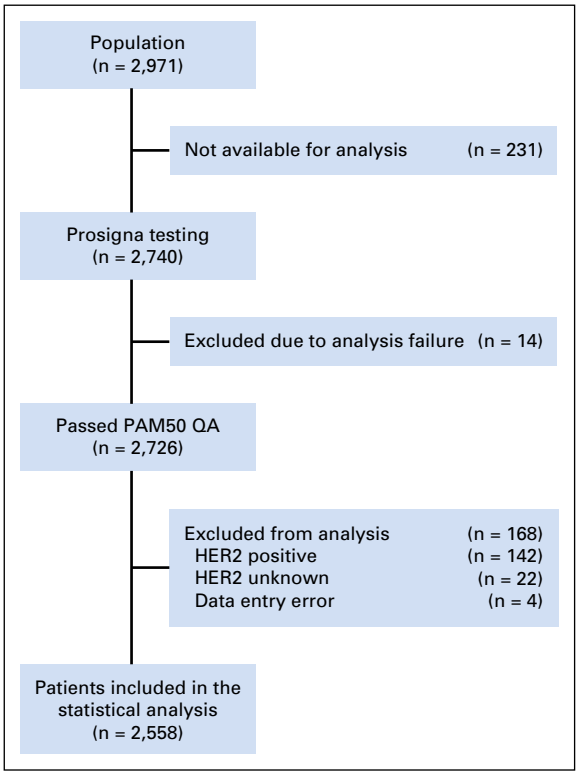

Fig A1. CONSORT diagram (trial profile 2000 through 2003). HER2, human epidermal growth factor receptor 2; QA, quality assessment.

Table A1. Outcome As a Function of Positive Lymph Nodes

Parameter	No. of Positive Lymph Nodes					All
	0	1	2	3	1-3	0-3
Cohort, no.	1,163	779	393	223	1,395	2,258
DR events, no.	105	78	48	43	169	274
10-year DR rate, %	10.5	12.1	14.0	22.7	14.3	12.6
Competing events, no.	177	166	63	26	205	382
10-year competing risk rate, %	17.9	18.4	19.6	14.4	18.1	18.0
10-year DR rate (age < 70 years), %	9.6	10.0	10.8	20.5	11.9	10.9
10-year competing risk rate (age < 70 years)	15.0	15.9	17.7	13.0	16.0	15.5
ROR cutoff						
Low risk	≤ 40	≤ 35	≤ 25	NA		
Intermediate risk	41-60	36-55	26-45	≤ 25		
High risk	> 60	> 55	> 45	> 25		

Abbreviations: DR, distant recurrence; ROR, risk of recurrence.