Comprehensive comparison of the performance of six prognostic signatures for estrogen receptor positive breast cancer

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Key points

Question: What is the comparative performance of prognostic multigene signatures for prediction and risk stratification of overall and late distant recurrence in ER positive/HER2 negative breast cancer?

Findings: In this retrospective biomarker analysis, we found that a combination of multigene expression test with clinical information improved the prognostic value for the prediction of distant recurrences and risk stratification, specifically in women with node-positive disease. Clear differences for the prediction of late distant recurrence were observed where these tests may be valuable for decision-making with regards to extended endocrine treatment.

Meaning: The combination of clinical and molecular information enhanced the prognostic value for the prediction of distant recurrence and risk stratification for ER-positive, HER2-negative breast cancer, particularly for women with node positive disease.

Abstract

Importance: Multiple molecular signatures are available for managing estrogen receptor positive (ER+) breast cancer patients but little direct comparative information to guide their choice.

Objective: To conduct a within-patient comparison of the prognostic value of Oncotype *Dx* (RS), ProSigna (ROR), EndoPredict (EPclin), Breast Cancer Index (BCI), and IHC4 in women with early ER+ breast cancer treated with 5 years' endocrine therapy.

Design: Retrospective biomarker analysis.

Setting: Randomized clinical trial of 5 years' of anastrozole versus tamoxifen (ATAC) with 10-year follow-up.

Participants: 774 postmenopausal women with ER+ /HER2-negative breast cancer with results for all tests.

Main Outcomes: The primary objective was to compare the added prognostic value of these signatures on top of the Clinical Treatment Score (nodal status, tumor size, grade, age, endocrine treatment) for distant recurrence (i) for years 0-10, and (ii) for years 5-10 after diagnosis. Likelihood ratio statistics (LR-χ^2) were used to assess the prognostic value of each signature.

Results: In women with node-negative disease (N=591), the signatures providing the most prognostic information were ROR, followed by BCI, and EPclin. Each provided significantly more information than CTS, RS, and IHC4. Substantially less information was provided by all of the molecular tests for patients with 1-3 node-positive disease (N=183), but BCI and EPclin provided more additional prognostic information than the other signatures.

Conclusions and Relevance: For women with node-negative disease, ROR, BCI, and EPclin were significantly more prognostic for overall and late distant recurrence. For women with 1-3 positive nodes limited independent information was available from any test. These data will help oncologists and patients to choose the most appropriate test to aid considerations of chemotherapy use and/or extended endocrine therapy.

Introduction

Almost all women with estrogen receptor (ER) positive primary breast cancer are offered adjuvant endocrine therapy and a highly relevant clinical question is who remains at high risk for distant recurrence despite completion of primary adjuvant therapy. Multigene expression profiles have significantly increased our ability to predict distant recurrence in ER-positive breast cancer following surgery and endocrine treatment [1]. These signatures are used in combination with different clinical characteristics to aid the selection of patients for whom chemotherapy may be appropriate based on prognosis. Several of these signatures such as the Oncotype Dx Recurrence Score (RS) (Genomic Health), PAM50-based Prosigna Risk of Recurrence Score (ROR) (NanoString), Breast Cancer Index (BCI) (Biotheranostics), EndoPredict (EPclin) (Myriad Genetics), and the NKI 70-gene signature (Mammaprint) (Agendia) are commercially available, endorsed by several guidelines [2-5] and routinely used by clinicians.

The TransATAC cohort was previously used to develop two prognostic algorithms, the Clinical Treatment Score (CTS), which includes clinicopathological information, and the immunohistochemical score (IHC4), which combines prognostic information of four widely used IHC markers [6]. We have furthermore evaluated four gene expression based signatures in the TransATAC cohort: RS [6], ROR [7], BCI [8], and EPclin [9]. RS and BCI include only molecular information in their signatures, while ROR (tumour size) and EPclin (tumour size and number of positive nodes) integrate clinical information. All of these signatures significantly predicted the risk of distant recurrence, particularly in women with node-negative disease, but with varying amount of prognostic information for late distant recurrence (5-10 years). An

important area of research remains to accurately predict the risk of late distant recurrence in women with ER positive disease, as over 50% of recurrences occur after five years of endocrine treatment. Gene expression based signatures should show an improvement in prediction when compared to standard clinical parameters [8, 10, 11].

There has not been a direct and comprehensive comparison of multigene signatures in the same patient population with long-term follow-up data. Here, we compare the prognostic performance of six signatures for distant recurrence in (i) the 10 years period following diagnosis to assess the potential value of the addition of chemotherapy versus endocrine therapy alone, and (ii) for late distant recurrence in years 5-10 to investigate the potential value of extended adjuvant endocrine therapy. Furthermore, the comparison was performed separately for women with node-negative and 1-3 node-positive disease, since the most significant prognostic clinical indicator for early stage breast cancer is the presence or absence of lymph node involvement.

Methods

Study design and patients

In this comparative analysis, tumour blocks from the TransATAC study were used from patients with hormone receptor positive early stage breast cancer treated with five years of tamoxifen or anastrozole in the ATAC randomized clinical trial [12]. Micro-dissection of the tumours and RNA extraction was done by Genomic Health Inc. and residual RNA was provided to collaborators for RNA expression profiling. Women were excluded from the analysis if they received chemotherapy, did not have ER-positive disease, received the combination treatment (i.e. anastrozole plus tamoxifen), or had 4 or more positive lymph nodes. All women consented for their tissue to be used in translational research. This study was approved by the South-East London Research Ethics Committee.

Procedures

The CTS and IHC4 were developed in TransATAC and have been described in detail previously [6]. In brief, the CTS contains information on nodal status, grade, tumour size, age, and treatment (tamoxifen versus anastrozole). The IHC4 combines four commonly used IHC markers: estrogen receptor (ER), progesterone receptor (PgR), Ki67, and HER2. The commercial signatures are all based on RNA expression profiling and were performed according to specifications by the individual commercial collaborators, who were all blinded to clinical outcome data. The Oncotype Dx RS [13] is a 21-gene signature that was developed in ER-positive, node-negative breast cancer patients. RS risk groups were determined in node-

negative patients as previously described [13], using predefined cut-offs of 18 and 31 to determine low, intermediate, and high risk groups, respectively. The RS-Pathology-Clinical (RSPC) score was calculated using the website tool for node-negative patients [14, 15]. BCI [16, 17] combines the HOXB13/IL17BR ratio with the molecular grade index (MGI; five proliferation genes) in a linear model and was developed in postmenopausal breast cancer patients with ER-positive, lymph-node negative disease [8]. Cut-off points for BCI were determined in a node-negative population (low risk < 5.0825, high risk >6.5025) [18]. The Prosigna ROR score [7] incorporates 46 genes and was developed in pre- and postmenopausal women treated without any adjuvant systemic therapy, and includes information on tumour size. The TransATAC cohort was used to determine the cut-off points for Prosigna ROR for risk stratification in node-negative and node-positive patients separately. They correspond approximately to a point estimate of up to 10% distant recurrence rate for low risk, and more than 20% rate for high risk after ten years of follow-up [19]. EPclin was developed in pre- and postmenopausal tamoxifen-treated patients with ER-positive, HER2-negative breast cancer. It incorporates the expression of 12 genes plus information on tumour size and nodal status [20]. A pre-defined cut-off point (EPclin=3.3, based on [20]) was used for risk stratification, which corresponds to a 10% distant recurrence risk at 10 years.

Statistical analysis

The primary endpoint was time to distant recurrence. Distant recurrence was defined as metastatic disease, excluding contralateral disease, and locoregional and ipsilateral recurrences. Death before distant recurrence was treated as a censoring

event. We defined two primary analysis populations: firstly patients with ER-positive, HER2-negative, node-negative breast cancer, and secondly patients with ER-positive, HER2-negative breast cancer with 1 to 3 positive lymph nodes. The primary objective was the comparison of prognostic signatures in node-negative and node-positive patients separately, for two specific follow-up periods: overall (0-10 years) and late (5-10 years).

We assessed overall distant recurrence in the first 10 years after diagnosis (N=774) and late distant recurrence within the subset of patients who remained distant recurrence free for the first five years after diagnosis (N=689). Partial likelihood ratio tests based on Cox regression models were used to test the prognostic information of all signatures. The amount of prognostic information provided by each signature alone was assessed by C-indices. Furthermore, partial likelihood ratio χ^2 value (LR-χ^2), with a two-side 5% significance level (LR-χ^2=3.84) are also presented. The improvement in distant recurrence prediction of each signature over clinical and pathological variables (CTS) was quantified by the increase of the likelihood ratio χ^2 value (ΔLR-χ^2; two-sided 5% significance level). Pre-defined cut-off points were used to determine risk stratification for the four commercially available signatures. Kaplan–Meier curves were used to estimate the average risk of distant recurrence after 10 years of follow up in pre-defined risk groups. To compare the prognostic performance of all signatures, continuous scores were normalised to have unit variance and the hazard ratios and associated 95% confidence interval (CI) were estimated from Cox models. All statistical analyses were two-sided, and a P-value of less than .05 was regarded as significant. All analyses were performed with STATA version 13.1 (College Station, Texas, USA).

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Results

A total of 774 postmenopausal women with ER-positive, HER2-negative disease for whom we had all signatures available were included in this analysis (eFigure 1). 591 women had node-negative disease, with a mean age of 63.4 years (standard deviation (SD) 7.9) and a mean tumour size of 17.6mm (SD 8.5). A total of 58 distant recurrences (9.8%) were recorded for this population, with approximately half of distant recurrences (N=34) occurring in the late follow-up period (eTable 1). In contrast, women with 1-3 node-positive disease (N=183) were significantly older (mean age 66.4 (SD 8.3)) and had significantly larger tumours (mean size 24.2mm (SD 12.2)) than node-negative women (eTable 1). 40 distant recurrences were recorded over 10 years of follow-up, with 21 of them occurring 5 years post diagnosis. Results of the prognostic performance of all six signatures for the overall population (node-negative and node-positive combined) and C-indices are shown in eTable 2.

<u>Years 0-10</u>

Node-negative population

All six signatures provided statistically significant prognostic value for distant recurrence in years 0-10 and all HRs and C-indices are shown in Table 1.ROR (HR=2.56 (95% CI 1.96-3.35)), BCI (HR=2.46 (95% CI 1.88-3.23)), and EPclin (HR=2.14 (95% CI 1.71-2.68)) provided statistically more prognostic than the other signatures in this patient population. The CTS (HR=1.99 (95% CI 1.58-2.50)) and IHC4 (HR=1.95 (95% CI 1.55-2.45)) provided similar amounts of prognostic information in this time period (Table 1, eFigure 2). All signatures provided

independent prognostic information beyond the CTS for women with node-negative disease, in particular BCI and ROR provided the most prognostic value (eFigure 2).

We determined 10-year DR risks for the four commercially available multigene signatures using pre-defined cut-off points (Figure 1). All four signatures identified a large proportion of women who were at low risk of developing a distant recurrence (<10%) after 10 years of follow-up. EPclin only has two risk groups, and categorised 429 (73%) of women into the low risk group of which 27 (10-year DR=6.6%) developed a distant recurrence (Figure 1). Only 10% of patients were categorised into the high risk group by RS, and they had a 10-year DR risk of 27%. EPclin, BCI, and ROR identified larger proportions of women as high risk, who had a 10-year DR risk of 22%, 27%, and 32%, respectively (Figure 1). For 507 women we also had information for the RSPC and the incorporation of clinical parameters into the RS substantially improved the prognostic performance for the prediction of distant recurrence compared to the molecular RS alone (data not shown).

1-3 node-positive population

CTS (HR=1.63 (95 CI 1.20-2.21)), BCI (HR=1.67 (95% CI 1.21-2.29)), ROR (HR=1.58 (95% CI 1.16-2.15)), and EPclin (HR=1.69 (95% CI 1.29-2.22)) provided significant prognostic information in this patient population (Table 1). The prognostic performance of all signatures, while significant, was much weaker than for node-negative disease as evidenced by the smaller HRs and C-indices in this patient group. IHC4 did not provide any prognostic value for the prediction of distant recurrence. Apart from the IHC4, all signatures provided independent prognostic

information, with BCI and EPclin showing largest improvements beyond the CTS (eFigure 2).

Risk group stratification is shown in Figure 1. ROR identified a small group of women (N=15) as low risk of whom none developed a distant recurrence at 10 years (Figure 1). EPclin categorised 43 women (23.5%) into the low risk group of whom 5.6% had a distant recurrence at ten years. Both signatures identified most women as high risk with an average ten year distant recurrence risk of more than 30%. In contrast, BCI and RS categorised a high proportion of women into the low risk group with a high risk of distant recurrence at 10 years (Figure 1).

Years 5-10

Node-negative population

To assess the prognostic power of each signature for late distant recurrence, 535 women who were alive and without distant recurrence after five years of follow-up were included. HRs and C-indices are shown in Table 2. ROR (HR=2.77 (95% CI 1.93-3.96), BCI (HR=2.30 (95% CI 1.61-3.30)), and EPclin (HR=2.19 (95% CI 1.62-2.97)) provided significant prognostic value for late distant recurrence (Table 2, eFigure 3), and substantially more than the CTS alone (HR=1.95 (95% CI 1.43-2.65)). IHC4 and RS were not significant predictors for late distant recurrence when added to CTS (eFigure 3). BCI, EPclin, and in particular ROR, provided significant independent prognostic information for late distant recurrence beyond the CTS

(eFigure 3). RSPC provided twice as much prognostic information for late distant recurrence compared to the RS alone in the univariate analysis, but no additional prognostic value for late distant recurrence above CTS (data not shown).

All four signatures categorised the majority of women into the low risk group, who on average had a very low distant recurrence risk in years 5-10 of less than 5% (Figure 2). EPclin categorised 26.5% of patients into the high risk group, which had the lowest 10-year distant recurrence risk of 14.6%. In contrast the ROR identified over 14% of women as high risk, and they had the highest 10-year DR risk of any test (23%) (Figure 2).

1-3 node-positive population

154 women who were alive and did not recur within the first five years of follow-up were included (Table 2, eFigure 3). EPclin provided the most prognostic value for the prediction of late distant recurrence on its own, followed by ROR, and BCI (Table 2). IHC4 and RS did not provide any prognostic information for late distant recurrence univariately or in addition to the CTS (eFigure 3). EPclin and BCI added significant but limited independent prognostic information to CTS (eFigure 3).

Good risk stratification in this patient group was observed for BCI, ROR, and EPclin (Figure 2). ROR categorised 9.7% of women into the low risk group of whom none developed a late distant recurrence. EPclin identified a larger proportion of women

295 as low risk (26%), of which only one patient developed a distant recurrence by year
296 10. No clear risk stratification was observed for the RS (Figure 2).

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Discussion

Multigene signatures have become increasingly important for the prognostic evaluation of ER-positive, HER2-negative breast cancer [6, 21, 22]. Here we compared six prognostic signatures for the prediction of distant recurrence in the TransATAC cohort. In years 0-10, all signatures provided significant prognostic information in women with node-negative disease in addition to clinical variables. For women with 1-3 node-positive disease, the independent prognostic strength of the investigated signatures was much weaker. It should be noted that even though there were fewer patients with node-positive than node-negative disease, the number of distant recurrences was similar and hence provided similar power. For the prediction of late distant recurrence, BCI, ROR, and EPclin provided independent prognostic information among women with node-negative and 1-3 node-positive disease.

We have previously published the results of the individual evaluations of the four commercial signatures and have shown that all provide significant and similar prognostic information in the first 5 years after diagnosis [6-9]. In this study we have shown that the difference in prognostic performance between signatures over ten years of follow-up is largely due to their differential ability to predict distant recurrence between 5 and 10 years. Thus BCI [8, 23], ROR [24] and, EPclin [9, 10] clearly have molecular components in their signatures that specifically predict late recurrence better than IHC4 or RS. An important finding is that combined genomic and clinical models showed enhanced prognostic performance, particularly for patients with 1-3 positive lymph nodes, and are thus the preferred approach for the decision making process for this patient group. This was furthermore underlined by

the finding that the RSPC provided significantly more prognostic value for distant recurrence in node-negative patients than the molecular RS alone.

In the adjuvant setting, the need for chemotherapy or extended endocrine therapy (for late recurrence) are important clinical questions. We used pre-defined cut-off points to determine the 10-year distant recurrence risk for the commercial scores in years 0-10 (chemotherapy) and years 5-10 (extended endocrine therapy). For node-negative disease, the majority of women were categorised into the low risk group by all four signatures and women had a low average risk<7% where chemotherapy might not be indicated. The two signatures that contain clinical variables in their scores (ROR and EPclin) identified a sizeable group of women with 1-3 node-positive disease who had a very low risk of distant recurrence at ten years (average risk<6%), suggesting that chemotherapy would be of very limited benefit in these women.

None of the signatures were specifically developed to predict late distant recurrences. However, BCI, ROR, and EPclin demonstrated accurate prediction of these late events in our analysis. Wolmark and colleagues reported that the RS was significantly prognostic for the prediction of late distant recurrence, but only in patients with high *ESR1* levels [25]. However, we did not observe any relationship between high *ESR1* levels and prediction of late distant recurrence with RS in our dataset. A few studies have investigated a series of extended endocrine therapy (EET) with aromatase inhibitors to address the question what the ideal length of extended treatment is. The MA17.R trial [26] showed that ten years of letrozole

resulted in significantly higher rates of disease-free survival compared to placebo. In the NSABP-B42 [27], DATA [28], and IDEAL [29] trials no significant improvement in disease-free or overall survival with EET was observed. These data raise the question whether patients need to be specifically selected for EET (i.e. based on high risk for late distant recurrence or high likelihood of benefit from extended therapy).

Strengths of our study include the mature clinical data with clinical outcome and long-term follow-up, well characterised tissue samples, and data on six prognostic signatures for breast cancer. For all RNA analyses the same extraction of RNA used. For all commercial signatures standardised quantitative methods and analyses were used, and all collaborators were blinded to clinical outcome. Limitations include that our results are only applicable for chemotherapy-free and postmenopausal women. An unintended selection bias might have occurred as sample analyses might have only been possible where sufficient amounts of RNA were available, but all assays yielded reportable results. IHC4, CTS and partially RSPC were trained in the TransATAC cohort thus slightly overestimating their performance in this analysis. The risk group cut-off points of the ROR score were defined in the TransATAC cohort for node-negative and node-positive women separately, therefore optimising the cut-offs to identify a low-risk group with less than 10% risk and high risk group with greater than 20% risk. Finally, our current analysis wasn't able to assess the ability of these signatures to predict the benefit from chemotherapy or extended endocrine therapy.

In summary, the prognostic signatures evaluated provided significant information to help determine appropriate candidates for whom chemotherapy and extended endocrine therapy might not be indicated in patients with ER-positive, HER2-negative breast cancer. In patients with node negative disease, all multigene signatures provided significant and clinically meaningful prognostic information beyond clinical factors. The combination of clinical and molecular information enhanced prognostic performance, particularly for women with node positive disease. All signatures performed similarly in the first 5 years of follow-up, but clear differences in years 5-10 were seen, where these tests may be valuable for decision-making with regards to extended endocrine treatment.

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IS had full access to all the data in the study and takes the responsibility for the integrity of the data and the accuracy of the data analysis.

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Figure legends

Figure 1: Kaplan-Meier curves and 10-year distant recurrence risks according to signature and nodal status for years 0-10.

Figure 2: Kaplan-Meier curves and 5-10 year distant recurrence risks according to signature and nodal status for years 5-10.

503 **Table 1:** Univariate Hazard Ratios (95% CI) and C-indices for all prognostic
504 signatures according to nodal status in years 0-10. All HRs are for a change in one
505 Standard Deviation.

	Node-negative (N=591)		Node-positive (N=227)	
	HR (95% CI)	**C-index (95% CI)**	**HR (95% CI)**	**C-index (95% CI)**
CTS[a]	1.99 (1.58-2.50)	0.721 (0.668-0.774)	1.63 (1.20-2.21)	0.640 (0.554-0.726)
IHC4[b]	1.95 (1.55-2.45)	0.725 (0.665-0.785)	1.33 (0.99-1.78)	0.601 (0.511-0.690)
RS[c]	1.69 (1.40-2.03)	0.667 (0.585-0.750)	1.39 (1.05-1.85)	0.603 (0.513-0.693)
BCI[d]	2.46 (1.88-3.23)	0.762 (0.704-0.820)	1.67 (1.21-2.29)	0.652 (0.566-0.739)
ROR[e]	2.56 (1.96-3.35)	0.764 (0.707-0.821)	1.58 (1.16-2.15)	0.636 (0.552-0.719)
EPclin[f]	2.14 (1.71-2.68)	0.765 (0.716-0.814)	1.69 (1.29-2.22)	0.671 (0.590-0.752)

506 HR=Hazard Ratio, CI=Confidence Interval, a) CTS=Clinical Treatment Score, b) IHC4=Immunohistochemical Score, c)
507 RS=Recurrence Score, d) BCI=Breast Cancer Index, e) ROR=Risk of Recurrence Score, f) EPclin=EndoPredict clinical
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510 **Table 2:** Univariate Hazard Ratios (95% CI) and C-indices for all prognostic
511 signatures according to nodal status in years 5-10. All HRs are for a change in one
512 Standard Deviation.

	Node-negative (N=535)		Node-positive (N=154)	
	HR (95% CI)	**C-index (95% CI)**	**HR (95% CI)**	**C-index (95% CI)**
CTS[a]	1.95 (1.43-2.65)	0.721 (0.654-0.788)	1.61 (1.05-2.47)	0.644 (0.534-0.753)
IHC4[b]	1.59 (1.16-2.16)	0.660 (0.576-0.745)	1.20 (0.79-1.81)	0.579 (0.460-0.697)
RS[c]	1.46 (1.09-1.96)	0.585 (0.467-0.702)	1.24 (0.81-1.90)	0.555 (0.418-0.693)
BCI[d]	2.30 (1.61-3.30)	0.749 (0.668-0.830)	1.60 (1.04-2.47)	0.633 (0.514-0.751)
ROR[e]	2.77 (1.93-3.96)	0.789 (0.724-0.854)	1.65 (1.08-2.51)	0.643 (0.528-0.758)
EPclin[f]	2.19 (1.62-2.97)	0.768 (0.701-0.835)	1.87 (1.27-2.76)	0.697 (0.594-0.799)

513 HR=Hazard Ratio, CI=Confidence Interval, a) CTS=Clinical Treatment Score, b) IHC4=Immunohistochemical Score, c)
514 RS=Recurrence Score, d) BCI=Breast Cancer Index, e) ROR=Risk of Recurrence Score, f) EPclin=EndoPredict clinical
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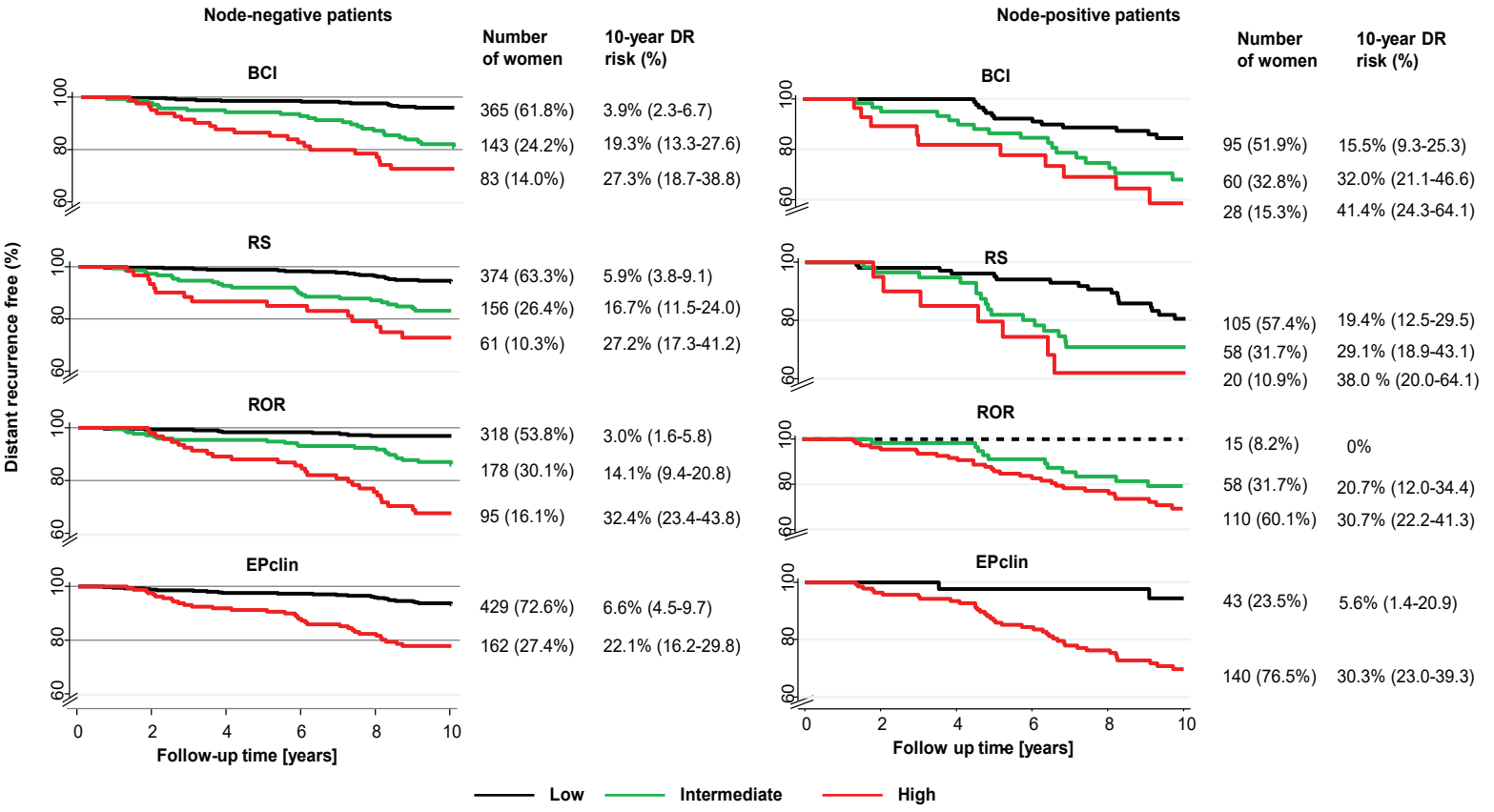
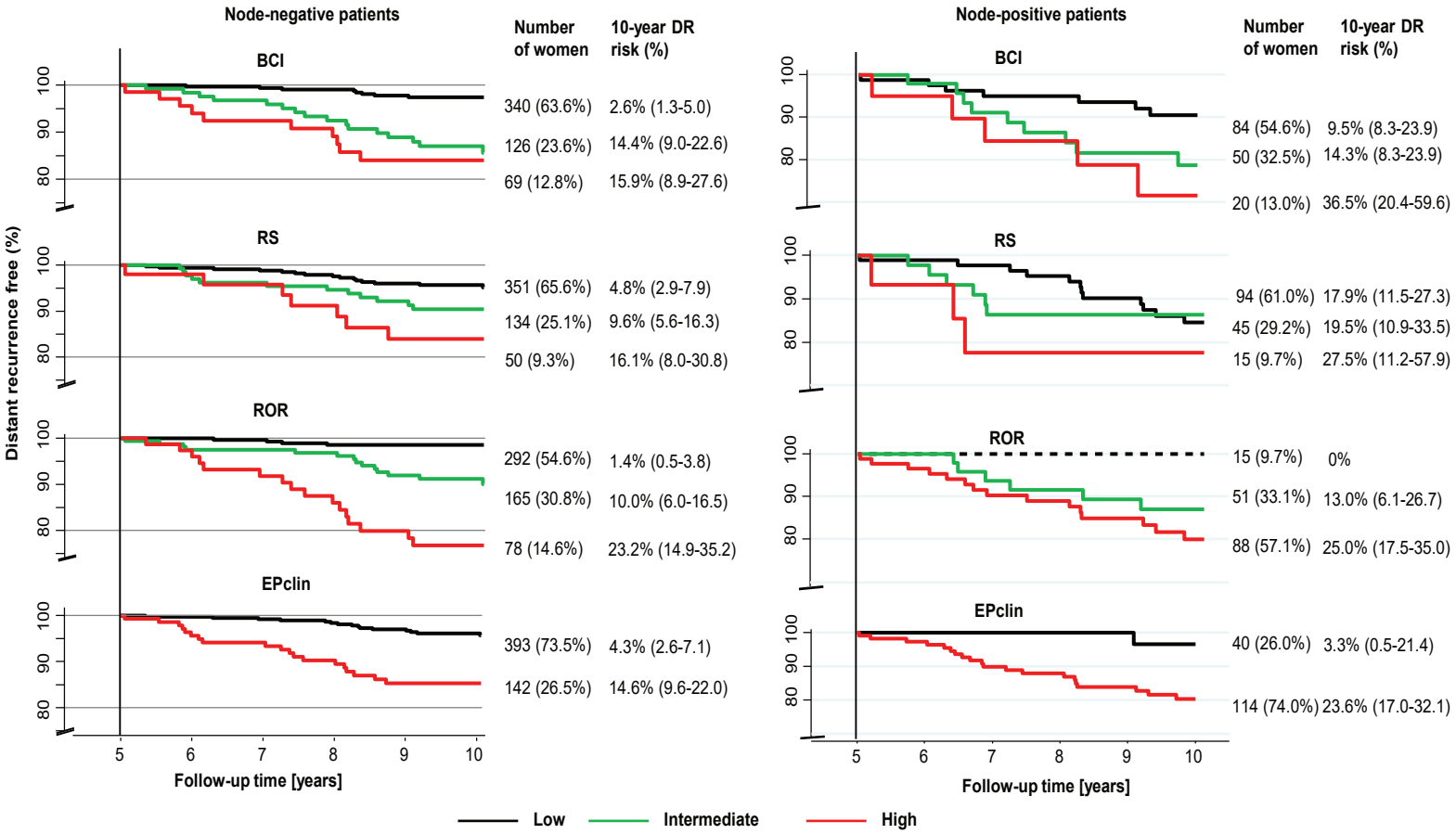


BCI=Breast Cancer Index, RS=Recurrence Score, ROR=Risk of Recurrence Score (cut-off points defined in TransATAC for node-negative and node-positive separately), EPclin=EndoPredict clinical, DR=Distant Recurrence



BCI=Breast Cancer Index, RS=Recurrence Score, ROR=Risk of Recurrence Score (cut-off points defined in TransATAC for node-negative and node-positive separately), EPclin=EndoPredict clinical, DR=Distant Recurrence