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PRECLINICAL STUDY

Impact of the EndoPredict genomic assay on treatment decisions for oestrogen receptor-positive early breast cancer patients: benefits of physician selective testing

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Abstract

Purpose Genomic tests improve accuracy of risk prediction for early breast cancers but these are expensive. This study evaluated the clinical utility of EndoPredict®, in terms of impact on adjuvant therapy recommendations and identification of parameters to guide selective application.

Methods Patients with ER-positive, HER2-negative, and early-stage invasive breast cancer were tested with EndoPredict®. Two cohorts were recruited: one consecutively and another at clinical team discretion. Systemic treatment recommendations were recorded before and after EndoPredict® results were revealed to the multidisciplinary team.

Results 233 patients were recruited across five sites: 123 consecutive and 110 at clinical team discretion. In the consecutive cohort 50.6% (62/123) cases were classified high risk of recurrence by EndoPredict®, compared with 62.7% (69/110) in the selective cohort. A change in treatment recommendation was significantly more likely ($p < 0.0001$) in the selective cohort (43/110, 39.1%) compared to the consecutive group (11/123, 8.9%). The strongest driver of selective recruitment was intermediate grade histology, whilst logistic regression modelling demonstrated that nodal status ($p < 0.001$), proliferative rate ($p = 0.001$), and progesterone receptor positivity ($p < 0.001$) were the strongest discriminators of risk.

Conclusion Whilst molecular risk can be predicted by traditional variables in a high proportion of cases, EndoPredict® had a greater impact on treatment decisions in those cases selected for testing at team discretion. This is indicative of the robust ability of the clinical team to identify cases most likely to benefit from testing, underscoring the value of genomic tests in the oncologists' tool kit.

Keywords Prognostic signatures · Early breast cancer · Prognosis · EndoPredict · Endocrine therapy · Treatment decision

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Introduction

Breast cancer is the most commonly diagnosed cancer and remains the leading cause of female cancer-related deaths worldwide [1]. The majority of breast cancers are oestrogen receptor (ER)-positive and HER2-negative. A significant issue that has emerged in ER-positive breast cancer is the identification of those patients at sufficient risk of distant metastasis to warrant chemotherapy. In the Australian public health setting, pathological variables including tumour size, grade, and nodal status have been used to estimate prognosis and guide adjuvant treatment selection. This has been further enhanced by estimation of proliferative activity using immunohistochemical measurement of Ki67 protein. Commercial gene expression panels are not in routine use in Australia, as is true in many countries globally.

Prognostic gene panels provide information independent of clinicopathological features. Tumours assessed as clinically high risk but genomic low risk, treated with endocrine therapy alone, have an outcome similar to that of clinically low-risk patients [2, 3]. This finding has impelled the use of these commercial assays to become widespread. One such test, EndoPredict®, provides a molecular score (EP index) that is algorithmically combined with tumour size and nodal status to provide a comprehensive risk score (EPclin). EndoPredict® has been robustly clinically validated through retrospective and prospective–retrospective clinical trial cohorts of postmenopausal women treated with endocrine therapy only and in women who received chemo-endocrine treatment. The EPclin score is proven to be significantly associated with both early and late recurrences [4–8]. Moreover, EPclin is not only prognostic of breast cancer disease recurrence but can also predict which patients are likely to benefit from chemotherapy [9]. This is supported by both the National Comprehensive Cancer Network and European Society for Medical Oncology guidelines, which recommend the application of molecular signatures, including EndoPredict®, to gain additional prognostic information to complement pathology assessment and to predict benefit from adjuvant chemotherapy for early-stage ER-positive breast cancer [10, 11]. Both guidelines consider EndoPredict® to be supported by moderate level evidence, which would be further strengthened by additional prospective randomised trial testing.

EndoPredict® measures eight disease-relevant gene transcripts (three associated with tumour cell proliferation and five with ovarian hormone receptor function) normalised to three control gene transcripts and one DNA reference gene [12]. The resulting EP index is a score on a continuous scale from 0 to 15, which is positively correlated with risk of distant recurrence in patients treated with endocrine therapy alone [7]. A further refinement of this score compared with other commercially available panels is the incorporation of tumour size and nodal status, providing the EPclin score, which estimates risk of distant recurrence at 10 years [6]. The values are dichotomised into low and high risk at an EPclin score threshold of 3.3287 (equivalent to a 10% risk of recurrence at 10 years) [5, 7, 13].

This study examined the impact of EndoPredict® on multidisciplinary team (MDT) treatment recommendations for early breast cancer patients at five Australian sites. The primary aim of this study was to identify the impact of EndoPredict® on MDT treatment recommendations measured by a change in recommendations following review of test results. In Australia and indeed many health systems worldwide, the cost of such molecular assays is not reimbursed or is only partially subsidised. Therefore, identification of patients that would most benefit from a prognostic gene panel would be advantageous, enabling more selective use of a costly test. Therefore, two cohorts of patients were recruited: a consecutive group and a second group recruited at clinical team discretion based on a perceived ambiguous clinical risk profile to identify those patients who would most benefit from the test. A second objective was correlation of EndoPredict® and traditional clinicopathological features in order to determine which features most closely predicted risk. Moreover, the impact of the test on actual treatment undergone by the patient was reviewed to determine overall benefit from the molecular test.

Methods

Patients and recruitment

The study cohort comprised 233 patients across five Australian sites, including 123 patients from Westmead Breast Cancer Institute, Sydney (Site 1), 28 patients from Monash Health, Melbourne (Site 2), 19 patients from Peter MacCallum Cancer Centre, Melbourne (Site 3), 35 patients from Royal Melbourne Hospital (Site 4), and 28 patients from St. Vincent's Hospital, Sydney (Site 5). Recruitment occurred between April 2017 and March 2020. All patients gave informed consent for the use of their clinical data and biospecimens for the project, and the study was reviewed and approved by the Western Sydney Local Health District Human Research Ethics Committee (HREC/17/WMEAD/211, SSA/17/WMEAD/220). Westmead Breast Cancer Institute was the coordinating centre and sponsor of the study.

Patients were recruited either at the pre-surgical (based on invasive carcinoma on core biopsy) or post-surgical visit

depending on the routine practice of each site. Patients were eligible if they presented with an ER-positive (> 1% ER-positive nuclei by immunohistochemistry), HER2-negative (on in situ hybridisation or immunohistochemistry) invasive breast cancer with clinical TNM stage T1c-T2/N0-N1/M0 (tumour size > 10 to 50 mm, 0 to 3 involved axillary lymph nodes), aged 18–80 years, and ECOG status 0–1. Fifteen cases that were downsized to 10 mm or less after pathology review were retained in the study. All patients consented to EndoPredict® testing of their surgical specimen. A protocol of consecutive recruitment was applied at Site 1, where all eligible patients were invited to participate in the study (Cohort 1). At Sites 2 to 5, a selective recruitment strategy was applied, where the multidisciplinary team selected patients for recruitment to the study, based on eligibility and likely benefit from EndoPredict® testing (Cohort 2).

Histopathological evaluation and EndoPredict® testing

A standardised dataset was collected from all sites, including histopathology data from pathology reports (tumour size, modified Nottingham grade, nodal status, surgical margin status, ER, PR, HER2 status, presence of lymphovascular space invasion, mitotic rate, and Ki67 overall and hotspot estimation where available). Ki67, which was stained at local laboratories of participating sites, was assessed in most cases, using the method outlined by Hida et al. [14].

All EndoPredict® tests were performed on formalin-fixed paraffin-embedded tumour tissue from the surgical excision specimens. A pathologist from each site selected the appropriate tumour material from the formalin-fixed paraffin-embedded surgical specimen for testing with EndoPredict®, by marking invasive tumour regions on an H&E-stained slide. Tumour material was collected from 5 µm sections (in general 1–2 were sufficient) for extraction of total RNA for EndoPredict® evaluation. The materials and clinical data were submitted for centralised molecular testing at Australian Clinical Labs in Melbourne, Victoria.

Documentation of MDT treatment recommendations

All enrolled patients were discussed at multidisciplinary team (MDT) meetings within 14 days of surgery. Treatment recommendations regarding adjuvant chemotherapy and hormonal therapy were documented, based firstly on standard histopathology results, and recorded again following review of EndoPredict® test results. Information regarding specific chemotherapy and endocrine therapy regimens and intended treatment duration were recorded where available or supplied by the study centre.

The final MDT recommendations were discussed with patients. Final treatment decisions made by patients in consultation with oncologists and/or surgeons were also recorded. The type of adjuvant therapy and whether it was actually administered were also recorded. If the treatment plan varied from the final recommendation, a reason for the deviation was recorded where possible.

Statistical analysis

Analysis was performed by a statistician using IBM SPSS Statistics version 23. The pre- and post-EndoPredict® recommendations were collated and compared at the conclusion of the study using a Chi-square test to compare the difference in recommendations without and with EndoPredict® test results.

Fisher's exact test and Mann–Whitney U Test were performed to evaluate differences in histopathological variables between groups. Additional comparisons were made using Prism Version 9 (GraphPad Software).

Results

Clinicopathological features of the cohort

A total of 233 patients were recruited to receive EndoPredict® testing: 123 consecutively recruited at Site 1 (Cohort 1) and 110 selectively recruited by the multidisciplinary team at Sites 2 to 5 (Cohort 2). The clinicopathological characteristics of all 233 patients are summarised in Table 1. Patient age ranged between 29 and 80 years (mean = 57) and age distribution was not different between Cohort 1 and 2. Tumour size distribution was slightly higher in Cohort 2 than Cohort 1 ($p = 0.028$, Supplementary Fig. 1). Most cases were lymph node negative (69.5%) and node status was independent of recruitment pattern. Whilst there was a predominance of grade 2 tumours in both cohorts, the distribution of grade was significantly different between Cohort 1 and 2, with fewer grade 1 and more grade 2 cases in the selectively recruited cohort ($p = 0.010$, Table 1, Supplementary Fig. 2), consistent with a greater demand for the guidance provided by molecular tests for intermediate grade cases ($p = 0.010$, Table 1). Whilst most patients in both cohorts were post-menopausal (65.2%), there were fewer pre-menopausal and more peri-menopausal cases in Cohort 1 than 2 ($p = 0.005$).

Impact of EndoPredict® testing on adjuvant treatment recommendations

In total, 103 patients (44.2%) were classified as low risk according to EndoPredict® EPclin score and 130 patients (55.8%) were classified as high risk (Table 2). The

Table 1 Cohort characteristics

Variable	Characteristic	Total cohort $N=233$	Cohort 1 $N=123$	Cohort 2 $N=110$	p value[e]
Consecutive recruitment			Yes	No	Cohort 1 vs Cohort 2
Age at diagnosis	Mean (SD)	57.0 (10.4)	58.1 (10.1)	55.7 (10.5)	0.107
	Min/Max	29, 80	30, 80	29, 74	
Menopausal status, *N* (%)	Pre-	62 (26.6)	26 (21.1)	36 (32.7)	0.005
	Post-	152 (65.2)	81 (65.9)	71 (64.5)	
	Peri-	19 (8.2)	16 (13.0)	3 (2.7)	
Tumour size, *N* (%)	≤ 1 cm (pT1a/b)	15 (6.4)	8 (6.5)	7 (6.4)	0.017
	> 1–≤ 2 cm (pT1c)	114 (48.9)	70 (56.9)	44 (40.0)	
	> 2–≤ 5 cm (pT2)	104 (44.6)	45 (36.6)	59 (53.6)	
	> 5 cm (pT3)	0	0	0	
Positive lymph nodes, *N* (%)	0	162 (69.5)	90 (73.2)	72 (65.5)	0.423
	1	61 (26.2)	28 (22.8)	33 (30.0)	
	2	9 (3.9)	5 (4.1)	4 (3.6)	
	3	1 (0.4)	0	1 (0.9)	
Tumour grade, *N* (%)	1	36 (15.5)	27 (22.0)	9 (8.2)	0.010
	2	125 (53.6)	58 (47.2)	67 (60.9)	
	3	72 (30.9)	38 (30.9)	34 (30.9)	
PR status, *N* (%)[a]	Positive	216 (92.7)	112 (91.1)	104 (94.5)	0.309
	Negative	17 (7.3)	11 (8.9)	6 (5.5)	
Ki67	Median (SD)	20 (23.1)	25 (26.1)	15 (16.5)	< 0.0001
	< 14%, *n* (%)	79 (34.3)	33 (26.8)	46 (41.8)	
	≥ 14%	151 (65.7)	90 (73.2)	60 (54.5)	
	14–30%	89 (38.7)	42 (34.1)	46 (41.8)	
	> 30%	62 (27.0)	48 (39.0)	14 (12.7)	
	Not reported	4 (1.7)	0	4 (3.6)	
LVI present[b]	No	176 (76.5)	93 (75.6)	82 (74.5)	0.853
	Yes	54 (23.5)	30 (24.4)	28 (25.5)	
Mitotic score[c]	1	69 (29.5)	51 (41.5)	18 (16.4)	0.143
	2	49 (20.9)	35 (28.5)	14 (12.7)	
	3	42 (17.9)	36 (29.3)	5 (4.5)	
	Not reported	74 (31.6)	1 (0.8)	73 (66.4)	
EPclin risk class, *N* (%)[d]	Low	103 (44.2)	61 (49.6)	42 (38.2)	0.081
	High	130 (55.8)	62 (50.4)	68 (61.8)	

[a]PR: progesterone receptor

[b]LVI: lymphovascular invasion

[c]Mitotic score: categories according to modified Nottingham grading system 3 component score

[d]EPclin: EndoPredict risk of recurrence score incorporating clinical variables

[e]p values represent results of Mann–Whitney test comparing continuous variables in the two cohorts, Fisher's exact test comparing two categories, or Chi-square test for categorical variables with greater than two categories

distribution of EPclin scores was not significantly different regardless of site or recruitment pattern (Fig. 1a, $p=0.72$). In the subset of consecutively recruited cases (Cohort 1) there was a change in the adjuvant treatment recommendation for 11 of 123 (8.9%) cases (Fig. 1b), reflecting a recommended treatment escalation from endocrine therapy (ET) alone to ET plus chemotherapy (CT) in 8/123 (6.5%) cases and de-escalation of recommendation from ET plus CT to ET alone in 3/123 (2.4%). In the selectively recruited cohort (Cohort 2) there was a substantially greater proportion of change in treatment recommendations ($p<0.0001$), with escalation to ET plus CT recommended for 15/110 (13.6%) and de-escalation to ET alone recommended for 28/110 (25.5%) cases, reflecting a total recommendation change of 39.1% in this group (Fig. 1b).

Table 2 EPclin risk compared to histopathological variables

Histopathology variables		High risk $N=130$	Low risk $N=103$	Total $N=233$	p value
Cases		N (%)	N (%)	N (%)	
Tumour grade	1	7 (5.4)	29 (28.2)	36 (15.5)	< 0.0001
	2	65(50)	60(58.3)	125 (53.6)	
	3	58(44.6)	14(13.6)	72(30.9)	
LVI present	No	86 (66.2)	89(86.4)	175 (75.1)	< 0.001
	Yes	44(33.8)	14(13.6)	58 (24.9)	
Ki67	Mean (SD)	36.7 (24.7)	15.1 (13.9)	27.1 (23.2)	< 0.0001
Mitotic score	Missing	41 (17.6)	32(13.7)	73(31.3)	< 0.0001
	1	21 (16.2)	48 (46.6)	69 (29.6)	
	2	29 (22.3)	20 (19.4)	49 (21)	
	3	39 (30.0)	3 (2.9)	42 (18)	
Lymph node involvement	Neg	78 (60.0)	84(81.6)	162 (69.5)	0.0005
	Pos	52 (40.0)	19(18.4)	71 (30.5)	
Tumour size, N (%)	≤ 2 cm (pT1)	57 (43.8)	72 (69.9)	129 (55.4)	< 0.0001
	> 2–≤ 5 cm (pT2)	73 (56.2)	31 (30.1)	104 (44.6)	

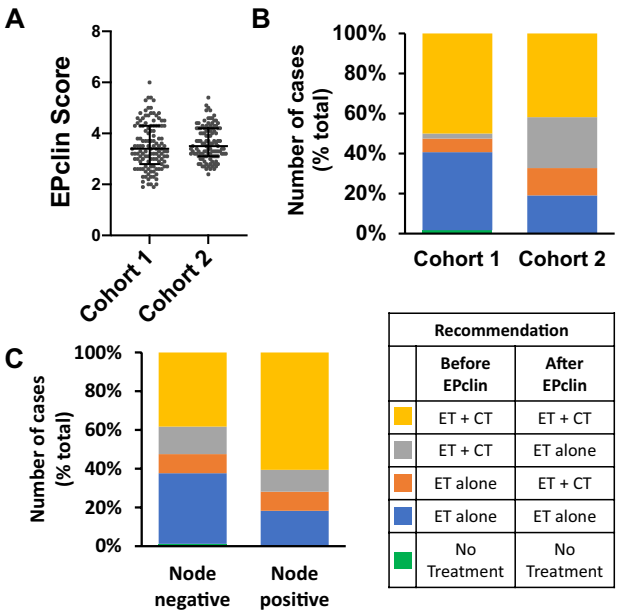

Fig. 1 Pattern of adjuvant treatment recommendation before and after EndoPredict® testing in Cohort 1 (consecutive recruitment) and Cohort 2 (selective recruitment). **a** Distribution of EPclin scores. Bars = median and inter-quartile range. **b** Treatment recommendation patterns in the two cohorts. **c** Treatment recommendation pattern in the combined cohort according to node status. *ET* endocrine therapy, *CT* chemotherapy

Given that the number of positive nodes is a component of the EPclin score, there was a positive relationship between nodal involvement and EPclin risk ($p=0.0005$), with 52 of 71 node-positive cases contributing 40% of high-risk cases, compared with 19 node-positive cases contributing just 18.4% of low-risk cases (Table 2). Whilst there was an association between node status and initial recommendation, with ET plus CT recommendation more common in node-positive cases, there was no association between node status and change in treatment recommendation (Fig. 1c, $p>0.05$). Treatment escalation from ET alone to ET plus CT was recommended after EndoPredict® for 9.8% node negative and 9.9% node-positive cases and de-escalation from ET plus CT to ET alone was recommended for 14.1% node negative and 11.3% node-positive cases (Fig. 1c). Of the 23 node-negative cases where the recommendation was changed from ET plus CT to ET alone, 17 were designated low risk by EndoPredict® and 6 were high risk. All 16 node-negative patients who were initially recommended for ET alone, but escalated to ET plus CT after EndoPredict® were reported to be high risk by the molecular test.

Prior to review of EndoPredict® results, 137 patients were recommended to have ET plus CT, this reduced to 127 in final recommendations after EndoPredict® results were reviewed. Of the 103 patients found to be low risk by EndoPredict®, 35 had initially been recommended for ET plus CT, with this number reducing to 10 in the final treatment recommendations following review of the EndoPredict® assay (Fig. 2). Of the 130 EndoPredict® high-risk patients, 102 were initially recommended to have ET plus CT and this increased to 117 in the final treatment recommendations. When the recommendations were discussed in consultation with patients, this reduced to 97 in the actual treatment administered, for the high-risk group but remained unchanged for the EndoPredict® low-risk group.

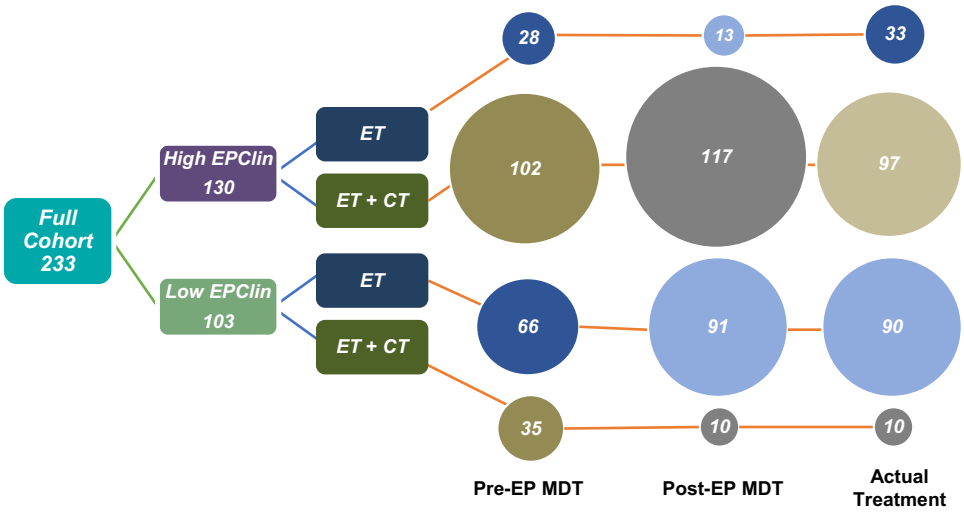

Fig. 2 Distribution of cases according to EndoPredict® risk prediction and recommended and actual adjuvant treatment. Numbers of cases recommended ET or ET plus CT in the low and high EndoPredict risk groups, before and after consideration of the EPclin result, and after discussion with the patient (Actual Treatment)

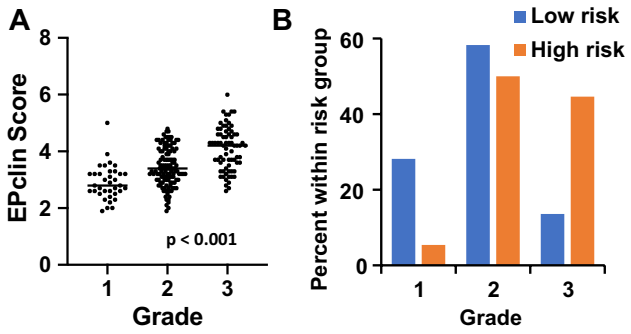

Fig. 3 Distribution of EPclin scores according to tumour grade. **a** Distribution of individual EPclin scores stratified by tumour grade. **b** Proportion of low and high-risk cases in each tumour grade category

In 32 cases the actual treatment administered was different from final MDT recommendation. In 24 cases the final MDT recommendation was for ET plus CT, including 8 cases where the treatment recommendation had changed from ET to ET plus CT after MDT discussion of the EndoPredict® results. In all these cases the addition of CT was declined by the patient. The mean EPclin score (3.85, SD 0.39) was not significantly different ($p = 0.69$) from the mean score in cases where the changed recommendation to ET plus CT was accepted (3.80, SD 0.46) and both were only modestly above the risk cut-off (Supplementary Fig. 3). In contrast the mean EPclin score in cases where ET plus CT was recommended both before and after EndoPredict, was significantly higher in cases where recommendations were followed (4.21, SD 0.62), than in cases where CT was declined (3.78, SD 0.61, $p = 0.030$), suggesting that the EPclin score influenced patient decisions.

Correlation between histopathological factors and EndoPredict® risk scores

The EPclin score showed a significant positive correlation with tumour grade ($p < 0.0001$, Table 2; Fig. 3a). However, whilst grade 1 accurately predicted low risk in the majority of cases, grade 3 overestimated risk compared to the EndoPredict® assay with 13.4% low-risk cases being grade 3. Moreover, grade 2 demonstrated approximately equal numbers of cases assessed as low and high risk (Fig. 3b), suggesting a benefit from EndoPredict® testing in both grade 2 and grade 3 cases.

Markers of proliferation (mitotic rate and Ki67 immunohistochemistry) were significantly higher in the EndoPredict® high-risk group than in the low-risk group. Significant positive relationships were observed between EPclin score and Ki67 positivity, as both overall ($p < 0.0001$, $R^2 = 0.3538$) (Fig. 4a) and hotspot estimates ($p < 0.0001$, $R^2 = 0.5180$, Supplementary Fig. 4) and with mitosis score ($p < 0.0001$, Table 2, Supplementary Fig. 5). The median overall Ki67 score was significantly higher in the EPclin high-risk group ($p < 0.0001$) compared to the low-risk cases (Fig. 4b; Table 2). However, whilst Ki67 below 14% or above 30% positive cells accurately predicted low and high EPclin, respectively, an intermediate Ki67 (14–30%) did not predict risk, with 33.3% low-risk cases and 43% high-risk cases falling in this category (Fig. 4c). Similarly, cases with an intermediate mitosis score were similarly distributed between high and low risk (Table 2, Supplementary Fig. 5). Cases that were classified as high risk by EndoPredict were significantly more likely to be characterised by the presence of lymphovascular invasion, lymph node involvement, and higher tumour size category (Table 2), suggesting that these clinical features could be combined with histological markers to more accurately classify a proportion of cases into risk groups.

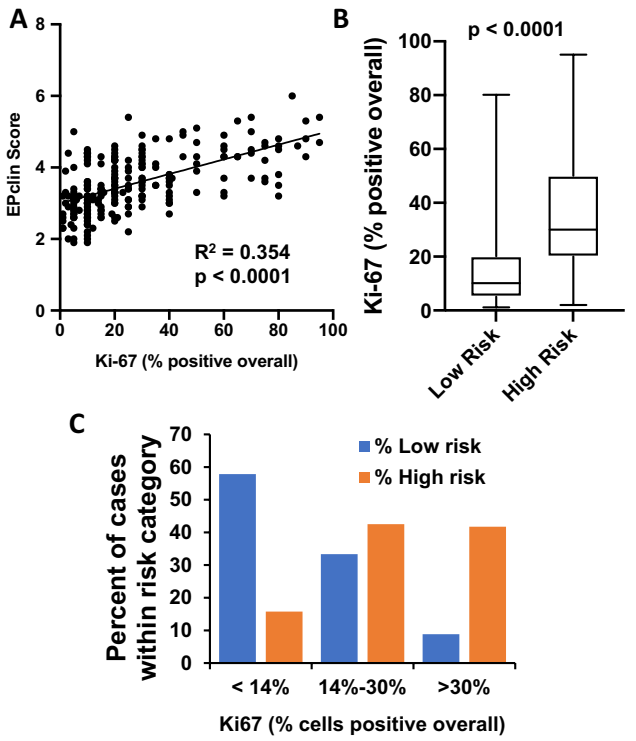

Fig. 4 Relationship between proliferation levels and EndoPredict® result. **a** Association between individual EPclin scores and Ki67 overall positivity in the full cohort. **b** Distribution of Ki67 levels in low and high EPclin risk groups. **c** Proportion of low and high-risk cases according to EndoPredict® EPclin, in low, intermediate and high Ki67 cases

Given that several histopathological factors were independently correlated with EPclin risk, multivariate logistic regression was used to test the ability of combined variables to predict risk status in an individual patient (Supplementary Table 1). Combining lesion size, number of involved lymph nodes, grade, Ki67, lymphovascular invasion presence, mitotic rate, and hormone receptor levels gave rise to a weighted formula (Fig. 5a) which accurately predicted the odds of a high-risk classification in 81% of cases (Supplementary Table 2 and Fig. 5b). The strongest contributions to the model were from lymph node positivity ($p=0.0002$), tumour size ($p=0.008$), Ki67 level ($p=0.001$), and PR positivity ($p=0.0002$). ER positivity made a weak non-significant contribution to the model. This was consistent with the observation that there was not a significant relationship between ER percent positivity and ER score with EPclin score (Supplementary Fig. 6a–b). When the distribution of ER and PR expression scores were compared across the cohort, PR scores had a more heterogeneous distribution, whereas most cases were reported to have strong ER expression, resulting in ER scores above 250 out of a possible 300. A clear biphasic distribution of ER score was observed around a cut point of 250 (Supplementary Fig. 6c). Comparing EPclin scores for cases stratified into low and high ER using the 250 cut-off revealed no significant difference in EPclin scores ($p=0.46$, Supplementary Fig. 6d). Similar non-normal distributions of ER score were seen in the low and high EPclin risk groups and there was no significant difference in ER expression between the risk groups ($p=0.65$, Supplementary Fig. 6e). Amongst the EPclin low-risk cases, 76 of 95 (80%) cases were correctly classified by the multivariable model and 94 of 116 (82%) cases that were EPclin high risk were correctly classified (Supplementary Table 2, Fig. 5b). Where the observed risk was different from what was predicted by the model, the features fell in an intermediate range characterised by Ki67 between 10 and 35%, tumour size between 16.5 and 22 mm, and PR score in a range of 120 to 255 (Fig. 5c–e, Supplementary Fig. 7) and it is suggested that this is where the EndoPredict® test would be most likely to add value.

Discussion

This study found that application of EndoPredict® resulted in altered treatment recommendations in 8.9% of cases if all eligible patients were tested, but rose to 39.1% of cases when testing was at the clinical team discretion in cases with more equivocal risk features. This gave a combined average change in recommendation of 23% of patients, which is similar to previously published decision impact studies. [15–18]. Importantly, the MDT played a key role in identifying those patients likely to benefit from the additional information provided by the test, since treatment decisions were altered considerably more frequently in the selectively recruited cohort, than when consecutive patients were tested irrespective of clinically assessed risk. The higher rate of decision change in the non-consecutive cohort was comparable to similar studies assessing decision impact of the 21-gene Oncotype DX Breast Recurrence Score assay in cohorts selected according to physician uncertainty about adjuvant treatment [19, 20]. A strength of the current study is the direct comparison to recommendation patterns in a parallel consecutively recruited cohort as a part of the same study. The major impact of the molecular test in the selectively recruited cohort was in de-escalating ET plus CT treatment recommendations in the patient group who had initially been considered likely to benefit from chemotherapy. There was an overall de-escalation to ET alone in 28/110 (25.5%) cases where ET plus CT had originally been recommended and 15/110 (13.6%) escalated from ET to ET plus CT. This contrasted with the consecutively recruited cohort, where there had been a net increase in patients recommended ET plus CT, which suggests that in the absence of the EndoPredict® test, physicians were more likely to take a more conservative approach where there was ambiguity regarding clinical risk.

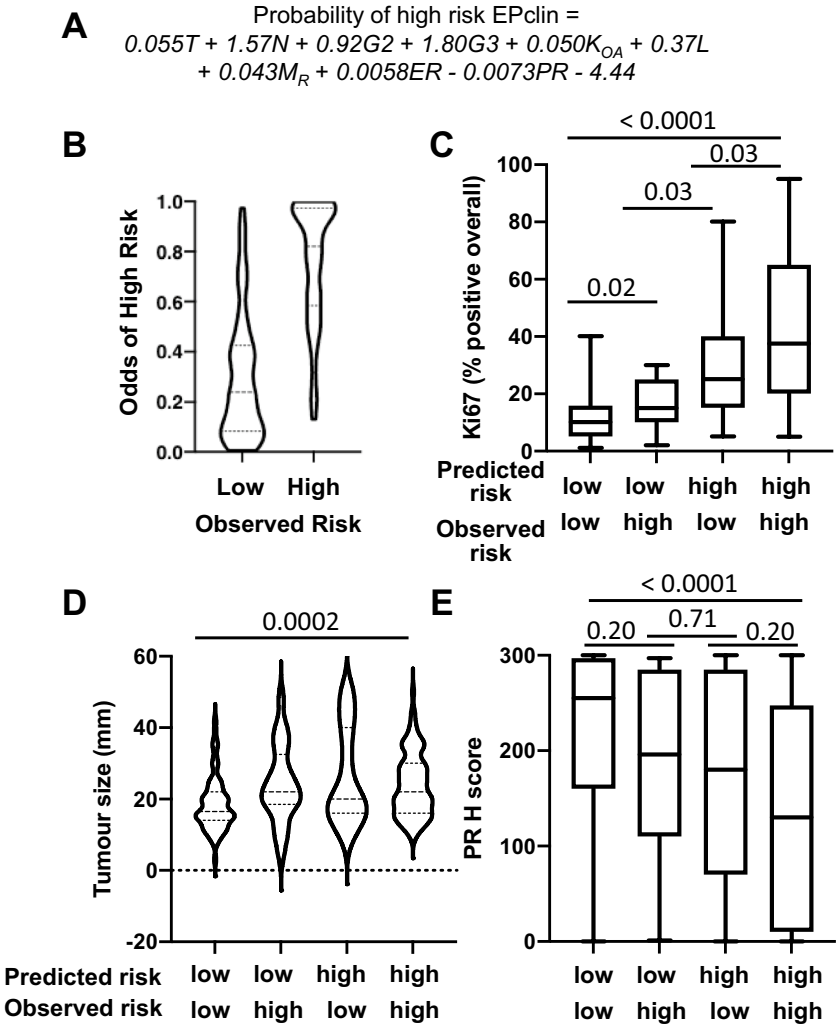

Fig. 5 Multivariate logistic regression modelling of EndoPredict® risk according to histopathalogical and clinical variables. **a** Model equation for probability that a case will return a high-risk EndoPredict® result. N: number of positive lymph nodes; T: tumour size; G2: classified grade 2 (true = 1, false = 0); G3: classified grade 3 (true = 1, false = 0); K_{OA}: Ki67 overall positivity; L: Lymphovascular invasion present (true = 1, false = 0); M_R: mitotic rate (n per 10HPF); ER: ER score (0–300); PR: PR score (0–300), based on % of positive cells multiplied by predominant intensity of staining. **b** Performance of the model demonstrated by distribution of predicted and observed risk classification. **c** Distribution of Ki67 overall levels in correctly and incorrectly classified low and high-risk cases. **d** Distribution of tumour size according to observed and predicted EndoPredict® risk. **e** Distribution of PR positivity (by H score) according to predicted and observed EndoPredict® risk classification

Similar to previous studies, this study demonstrated that chemotherapy could be either added or withdrawn, suggesting that the assay aided in more individualised treatment decisions. In the majority of patients in the consecutive group (91.1%) there was no change in the treatment recommendation, suggesting that clinicopathological variables are highly informative in systemic treatment decisions for most patients. It also confirmed other studies [20] that suggested the selection of patients who would most benefit from a prognostic gene profile were patients where there is equipoise regarding their risk estimation based on traditional clinical and pathological variables. This is further supported by the significant association between grade extremes (grades 1 and 3) and EPclin score and the lack of predictive value in grade 2 cases (Fig. 3).

When histopathological factors were combined into a multivariable predictive model, all factors contributed to risk prediction, with lymph node involvement, tumour size, grade, Ki67, and PR level being significantly correlated with EPclin risk classification in the model. Whilst nodal involvement and tumour size would be expected to show a correlation to EPclin given their inclusion in score calculation, the additional features independently strengthened the model to accurately predict risk in 81% cases. Whilst it is acknowledged that this model was developed and tested in the same dataset, application of such a model could support clinicians to identify those patients most likely to benefit from EndoPredict® testing. The recent data emerging from the MINDACT [2] and TAILORx [3] trials advocate routine use of molecular tests demonstrating that adjuvant treatment can be safely de-escalated in molecular low risk and clinical high-risk cases, in all but younger women; however, selective use of these costly tests will benefit the health economy.

EndoPredict® generated prognostic information that was independent of ER level. ER expression had a negligible impact on the predictive model and there was no association between ER level and EPclin score, demonstrating that ER positivity is not a driver of test outcome. This is important given recent debate regarding a biologically meaningful cut-off for ER positivity, with the suggestion that cases

reported between 1 and 9% ER-positive are more similar to cases with ER < 1%, than to cases with ER > 10%, in terms of systemic treatment response and disease-free and overall survival [21, 22]. Although the number of cases in the cohort with ER between 1 and 9% was small, the lack of relationship between ER level and EndoPredict result suggests that the choice of ER cut-off would not influence the prognostic value of the test.

The substantial difference in treatment recommendation change levels between the consecutive and selective cohorts confirms the capability of the clinical team in identifying cases likely to benefit from a molecular test. Multivariable modelling of risk demonstrated that cases that were classified at the extreme ends of the spectrum of risk could be accurately predicted by a combination of histopathological and clinical factors and genomic testing added little, but there was a particular value in EndoPredict® testing in cases where there were intermediate scores across multiple traditional variables. This suggests that whilst the model may not accurately predict risk category in all cases, it could serve to develop an algorithm to select cases that should be recommended to receive the genomic test. In settings where such assays are not widely available or subsidised, this strategy would have health economic benefits by identifying a smaller group of patients that would most benefit from testing.

A limitation of our study is that, as a prospective study, it was not possible to determine the prognostic or predictive value of EndoPredict® in our cohort. Although this was not the primary objective of the study and there is considerable published evidence of the prognostic strength of the EPclin [4–8], it is the goal of the study to also report on this outcome measure. The participants in the study remain in clinical surveillance by their treating centres and have provided consent for their clinical follow-up information to be reported in the longer term. A limitation of the study design, which involved consecutive and selective recruitment across the same time period, was that the two cohorts were recruited by different multidisciplinary teams. This was mitigated by recruiting the selective cohort across four separate sites, which would reduce individual selective bias. Ideally, this would be further reduced by the recruitment of larger cohorts for both the selective and consecutive groups; however, the high cost of molecular testing limited cohort size.

Finally, in some cases, the final treatment decisions were not altered despite contradicting EndoPredict® results. For example, not all patients designated as high risk had final recommendations to have chemotherapy. This demonstrates that individual patient factors as well as the actual numerical percentage of risk (several of these cases were borderline between low and high risk) also influenced decisions. The latter emphasises the importance of considering risk as a continuous variable rather than a binary decision. In addition assessment of the EPclin risk in cases where ET plus CT was recommended by the MDT but not agreed to after patient consultation revealed the genomic test result was generally borderline high risk. Further, high-risk cases that received concordant treatment had higher EPclin risk scores than this group, suggesting patient decisions were also influenced by EndoPredict® results, including the actual risk estimate as a continuous variable.

Conclusion

EndoPredict® impacted systemic treatment decisions and enabled more precise individualisation of systemic therapies with the potential to improve health outcomes in a significant proportion of ER-positive, HER2-negative early breast cancer patients. However, the high cost of these tests limits their accessibility. The higher rate of treatment recommendation change in cases where physicians selectively recruited patients for genomic testing based on perceived need demonstrates that the clinical team is proficient in recognising those cases most likely to benefit from the additional information provided by genomic testing. This study demonstrates that a care model where standard histological and clinical parameters are used to guide selective application of commercial genomic tests to the subset of patients most likely to benefit from them is both feasible and advisable. This will enhance the health outcomes for patients at the same time as optimising the use of increasingly limited health budgets.

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Declarations

Conflict of interest All authors confirm that they have no conflicts of interest to declare.

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