Histopathology

Histopathology 2024, **85**, 468–477. DOI: 10.1111/his.15234

Nottingham prognostic x (NPx): a risk stratification tool in ER-positive HER2-negative breast cancer: a validation study

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Date of submission 28 December 2023
Accepted for publication 25 May 2024

Lashen A G, Toss M, Miligy I, Rewcastle E, Kiraz U, Janssen E A M, Green A R, Quinn C, Ellis I & Rakha E A (2024) *Histopathology* **85**, 468–477. https://doi.org/10.1111/his.15234

Nottingham prognostic x (NPx): a risk stratification tool in ER-positive HER2-negative breast cancer: a validation study

Aims: In this study, we validate the use of Nottingham Prognostic x (NPx), consisting of tumour size, tumour grade, progesterone receptor (PR) and Ki67 in luminal BC.

Materials and methods: Two large cohorts of luminal early-stage BC ($n = 2864$) were included. PR and Ki67 expression were assessed using full-face resection samples using immunohistochemistry. NPx was calculated and correlated with clinical variables and outcome, together with Oncotype DX recurrence score (RS), that is frequently used as a risk stratifier in luminal BC.

Results: In the whole cohort, 38% of patients were classified as high risk using NPx which showed significant association with parameters characteristics of aggressive tumour behaviour and shorter survival ($P < 0.0001$). NPx classified the moderate Nottingham Prognostic Index (NPI) risk group ($n = 1812$) into two distinct prognostic subgroups. Of the 82% low-risk group, only 3.8% developed events. Contrasting this, 14% of the high-risk patients developed events during follow-up. A strong association was observed between NPx and Oncotype Dx RS ($P < 0.0001$), where 66% of patients with intermediate risk RS who had subsequent distant metastases also had a high-risk NPx.

Conclusion: NPx is a reliable prognostic index in patients with luminal early-stage BC, and in selected patients may be used to guide adjuvant chemotherapy recommendations.

Keywords: breast cancer, endocrine therapy, Ki67, Nottingham PX, PR, risk

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Abbreviations: BC, breast cancer; BCSS, BC-specific survival; DMFS, distant metastasis-free survival; ER, oestrogen receptor; LI, labelling index; LN, lymph node; NPI, Nottingham Prognostic Index; NPx, Nottingham Prognostic x; PR, progesterone receptor; RS, recurrence score.

Introduction

Breast cancer (BC) is a heterogeneous disease with diverse morphological and biological features and variable therapy response.[1] BC classification aims to provide a precise diagnosis and prediction of tumour behaviour to aid in management decision-making.[2] Oestrogen receptor-positive (ER+), HER2-negative (HER2−) luminal BC constitutes 70% of the newly diagnosed BC cases. Early-stage luminal BC patients are offered adjuvant endocrine therapy; however, some remain at higher risk for distant recurrence and may benefit from the addition of chemotherapy.

The Nottingham Prognostic Index (NPI) is a well-known and validated prognostic tool which includes tumour size, grade and lymph node status that classifies early-stage neoadjuvant naïve BC patients into low-, intermediate- and high-risk groups for management purposes. Adjuvant chemotherapy is usually recommended to high-risk patients, while low-risk patients can avoid such toxic systemic therapy.[3,4] However, the management of patients classified as NPI intermediate risk is an ongoing challenge, and refined prognostic stratification tools are required to ensure that each patient receives the optimal therapy.[5–7]

Recently, the use of multigene assays, such as Oncotype DX recurrence score (RS), MammaPrint, PAM50 and others, to predict recurrence and classify luminal BC patients into low- and high-risk groups has significantly increased in the clinical setting.[8,9] These signatures are used in association with the clinical profile to aid the selection of candidates for whom chemotherapy is probably indicated and have potential prognostic benefit.[9,10]

Although the use of these multigene assays provides additional beneficial information to further risk-stratify luminal BC patients[11] they are expensive, with low concordance between various tests.[12,13] National Institute for Health and Care Excellence (NICE) guidelines indicate performing Oncotype DX on tumours from a subset of patients who have intermediate NPI, which represents only a third of luminal BC patients, reflecting the clinical importance of NPI as a first line for risk stratification.[6,14]

Nottingham Prognostic x (NPx) includes both conventional morphological and biological variables that provides important prognostic information when assessed adequately.[15]

The NPx integrates tumour size, grade, progesterone receptor (PR) status and Ki67 labelling index (LI) into a risk score to stratify patients with clinically indeterminate BC into those with excellent and poor prognostic groups.[15] In our previous study, NPx stratified lymph node-negative (LN−) luminal BC into two clinically relevant classes more effectively than NPI. NPx was developed in a limited-sized BC cohort; therefore, in this study, we aimed to validate the prognostic utility of NPx using two independent large cohorts of patients with early-stage luminal BC.

Materials and methods

This study was conducted on two cohorts, as follows.

1- NOTTINGHAM COHORT

A large cohort of 2557 ER+/HER2− BC patients who were diagnosed and treated at Nottingham City Hospital, Nottingham, UK from 1996 to 2018 with long-term follow-up.[16] Clinical parameters and tumour characteristics, including tumour size, histological grade, tumour type, lymphovascular invasion (LVI), LN status and NPI, were collected. A total of 75% (2108 of 2557) of patients were LN− and 25% (715 of 2557) were LN-positive (LN+). Oncotype DX score was available for 302 patients as part of their clinical care.[10] Information regarding ER status was collected from patient records. ER was assessed as published previously and scored according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, with expression in 1% or more of invasive tumour cell nuclei considered positive.[17]

Patients were uniformly treated according to local protocols.[4,18,19] Adjuvant endocrine therapy was offered to 88% of patients. Seven per cent of patients were given both endocrine therapy and chemotherapy. Twelve per cent of patients did not receive endocrine therapy, as they were diagnosed prior to 2000 when ER+ early-stage BC in the good NPI prognostic group were not offered endocrine therapy according to local protocols.[4] Outcome data were calculated and maintained prospectively, including BC-specific survival (BCSS), defined as the time in months from primary surgery to death due to BC, and distant metastasis-free survival (DMFS), defined as the time in months between primary surgery and the development of DM. The mean follow-up time was 84 months, the median was 85 months and ranged between 2 and 180 months.

Ki67 proliferation index

Immunohistochemistry (IHC) staining for Ki67 was performed on formalin-fixed paraffin-embedded (FFPE)

tissue using the clinically validated, Dako Cytomation EnVision+ detection system (Dako, Glostrup, Denmark), according to standard protocols. Full face tissue sections were utilised to accommodate intratumoural heterogeneity. Briefly, sections were deparaffinised, rehydrated and microwaved in citrate buffer (pH 6.0) for antigen retrieval. Primary antibody (anti-human Ki67 monoclonal antibody MIB1; Dako) was applied and incubated for 30 min. Positive tissue control of normal tonsil was included in each staining run, while a negative control was included by omitting the primary antibody. Ki67 was assessed using eyeballing examination of the stained sections with a light microscope (ECLIPSE Ni-U; Nikon Instruments Inc., Tokyo, Japan) with 10× ocular subjective lens (eyepieces). Invasive tumour cells only were assessed, while foci of necrosis, areas with poor section quality, non-viable tissue and positivity within ductal carcinoma *in situ* (DCIS) or inflammatory cells were excluded.[20] Sections were evaluated and the percentage of Ki67 positivity was assessed by counting 1000 invasive tumour cells in hot-spot areas (areas with high Ki67 expression compared to the surrounding tissue).[21–23]

PR assessment

A similar IHC protocol was utilised for PR staining, using full face FFPE tissue sections. Primary antibody (anti-PR mouse monoclonal antibody; Dako) was applied and incubated for 30 min. The percentage of PR expression was visually assessed by estimation of the proportion of invasive tumour cells that showed nuclear staining through scanning of the whole tumour at low-power magnification using 4× or 10× objectives. Staining in normal terminal duct lobular units or associated *in-situ* carcinoma was not considered. A 10% cut-off, which was found to be the optimal cut-off for PR in our previous study, was used to categorise negative and positive groups.[24,25]

NPx index

NPx indices for BCSS at 15 years and DMFS at 10 years was calculated using the originally developed algorithm[15] using histological tumour grade, tumour size and the continuous percentage expression of Ki67 and PR. This equation used beta (β) values from the multivariate Cox regression-based analysis for each of the variables.[15] NPx for two separate indices (BCSS and DMFS) were calculated using the following formulae: NPx for BCSS at 15 years = (grade 1–3 × 0.389) + (size cm × 0.957) − (PR × 0.542) + (Ki67 × 0.752). NPx for DMFS at 10 years = (grades 1–3 × 0.332) + (size cm × 0.916) + (Ki67 × 0.729) − (PR × 0.541). PR and Ki67 expression were used as continuous scores.

Patients were categorised into low- and high-risk groups using a cut-off score of 1.0, as previously described.[15] The best cut-off to dichotomise NPx scores into two risk groups was generated using X-tile software, which defines the optimum cut-off based on the association with BCSS. In this study, the intermediate-risk group was defined as patients with moderate NPI scores (3.41–5.4).

2- INDEPENDENT EXTERNAL COHORT

This cohort consisted of 307 ER+/HER2− LN− BC patients, who were diagnosed and treated at the Stavanger University Hospital, Norway. All patients were treated according to the National grade, ER, PR and Ki67 expression levels were assessed.[29] Ki67 Guidelines of the Norwegian Breast Cancer Group.[28–30] These guidelines were based on the results of several-randomised clinical trials. Information regarding histological was assessed in a hot-spot area of 1.59 mm.[29,31] Outcome data in the form of BCSS were also available. NPx was calculated in the validation cohort using the same formula as used in the Nottingham cohort.

Oncotype DX recurrence score

Oncotype DX RS classified patients into three risk groups according to the modified cut-off levels used in the Tailor X study[26,27]; low-risk < 11, moderate-risk (11–25) and high-risk > 25.[27] Head-to-head comparison was performed between NPx and Oncotype DX RS.

Statistical analysis

The Statistical Package for the Social Sciences software version 27.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. In this study, NPx for BCSS predicted DMFS with an estimated hazard ratio (HR) of 1.99 ($P < 0.0001$) and NPx for DMFS predicted BCSS with an HR of 2.1 ($P < 0.0001$). Therefore, we applied NPx for DMFS for predicting both DMFS and BCSS to be more clinically relevant. The association of NPx with the response to adjuvant endocrine therapy was also evaluated. Cox regression models were used for the multivariate analysis. Tumour grade, size and Ki67 were not included in the Cox regression models, as they are components of the NPx. Estimated HR and 95% confidence intervals (95% CI) were calculated. For all tests, $P < 0.05$ (two-tailed) were statistically significant.

This study was approved by the Yorkshire and the Humber—Leeds East Research Ethics Committee (REC Reference: 19/YH/0293) under the IRAS Project ID: 266925. Data collected were fully anonymised.

Results

1- NOTTINGHAM COHORT

In the overall cohort, 71% (1816 of 2557) of patients had smaller tumour size (< 2 cm), 56% (1422 of 2557) had grade 2 tumours and 43% had a moderate NPI, with a median follow-up time of 85 months. The clinicopathological characteristics of the study cohort are summarised in Supporting information, Table S1.

Of the whole cohort, 72% (n = 1833 of 2557) had low-risk NPx while 28% were classified as high-risk. There was a significant association between high-risk NPx and poor NPI ($P < 0.0001$), where 78% (1045 of 1337) of patients with low NPI had low NPx. No association was observed in terms of LVI or LN status.

Outcome analysis

In the whole luminal BC cohort, there was a significant association between high-risk NPx and poor outcome in terms of shorter BCSS and DMFS (both $P < 0.0001$) (Figures 1 and 2); 94% (1649 of 1758) of patients who had low-risk NPx did not have an event during follow-up, while 6% of patients developed distant metastasis. When the cohort was classified based on LN status, the association between NPx and outcome was observed in both LN− and LN+ subgroups ($P < 0.001$; Supporting information, Figure S1). When the cohort was classified based on the endocrine therapy status, there was no association between NPx and patient outcome in patients who did not receive endocrine therapy. However, in endocrine therapy-treated patients, there was a significant association between high NPx and shorter BCSS ($P < 0.0001$; Figure 2).

Evaluation of the cumulative survival probabilities during the follow-up period for patients with low-risk NPx versus high-risk NPx revealed a 5% difference at 5 years was observed, with the high-risk group having a worse outcome. This difference increased gradually on yearly progression rate to a 10% difference at 15 years (Supporting information, Table S2 and Figure 3).

When the cohort was classified based on patient age, high-risk NPx was significantly associated with shorter DMFS and BCSS in those younger (< 50) or older than 50 (≥ 50) years ($P < 0.0001$; Supporting information, Figure S2). In terms of menopausal status, there was a significant association between high-risk NPx and poor outcome in postmenopausal patients ($P < 0.0001$). In premenopausal patients,

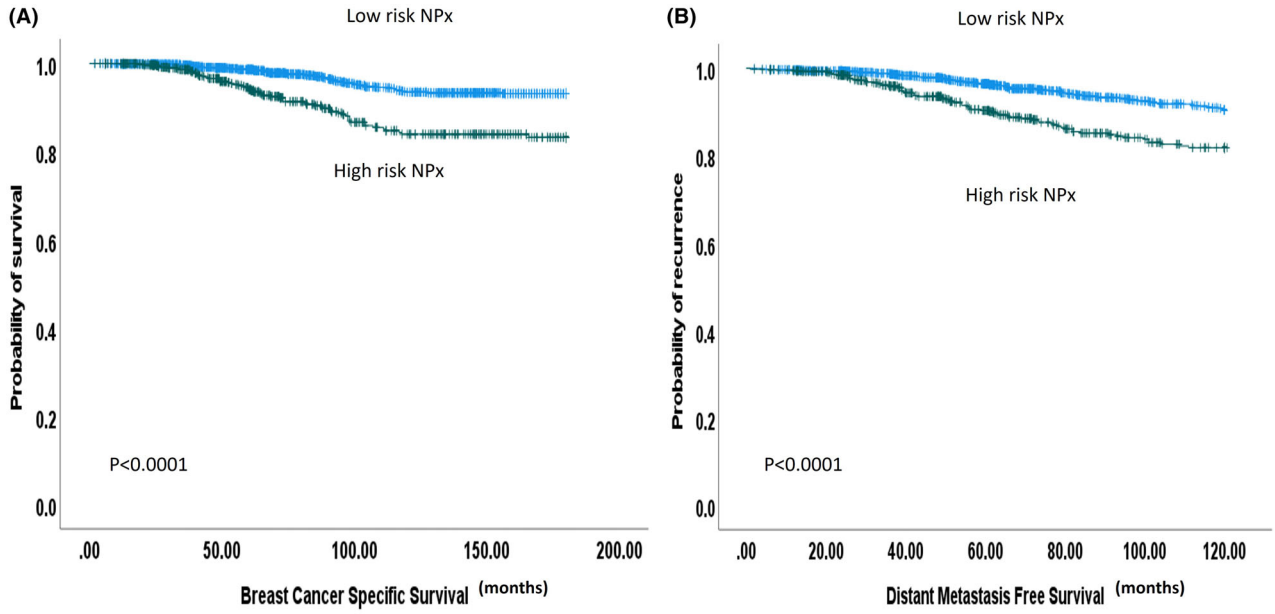

Figure 1. Kaplan–Meier curves show the association of Nottingham prognostic x (NPx) with breast cancer-specific survival (**A**) and distant metastasis-free survival (**B**).

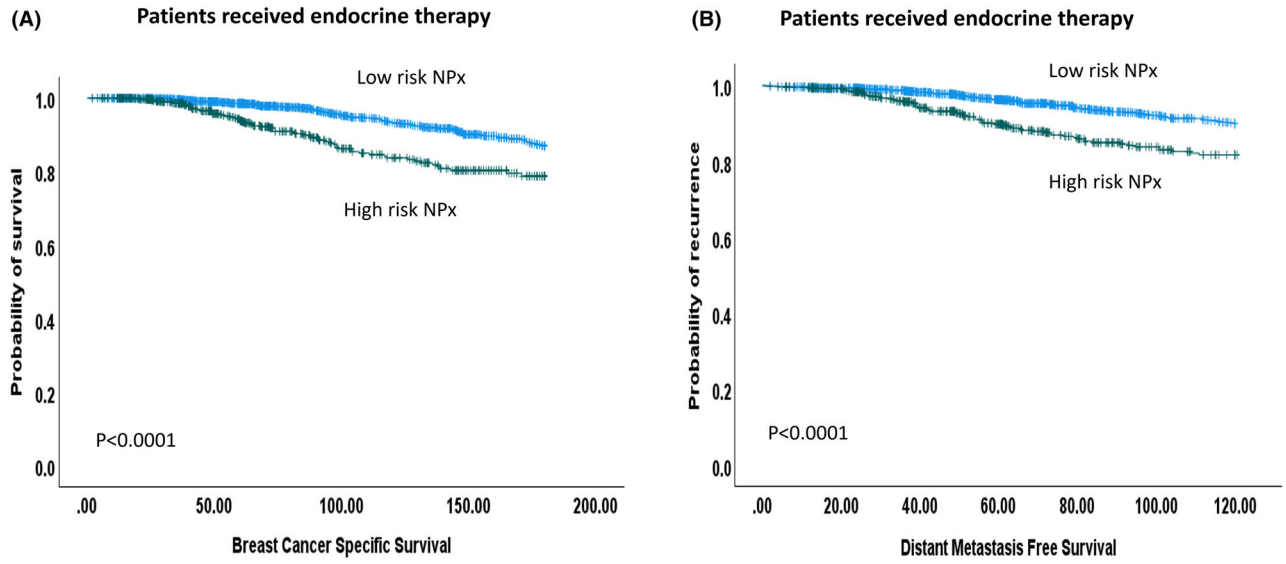

Figure 2. Kaplan–Meier curves show the association of Nottingham prognostic x (NPx) with breast cancer-specific survival (**A**) and distant metastasis-free survival (**B**) in patients who received endocrine therapy.

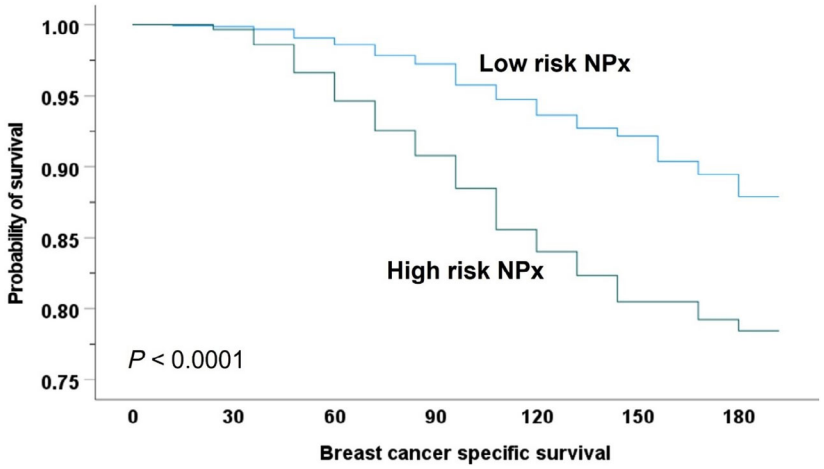

Figure 3. Cumulative proportion surviving for Nottingham prognostic x low-risk versus high-risk during the follow-up period.

there was significant association with shorter DMFS ($P = 0.01$) but not BCSS ($P = 0.05$; Supporting information, Figure S3).

As the moderate-NPI risk group is considered clinically indeterminate, we tested the ability of NPx to stratify these patients into risk groups. NPx further classified BC patients with the moderate NPI group who received endocrine therapy into two prognostically relevant risk groups in terms of BCSS and DMFS (both $P < 0.0001$; Figure 4). Of these intermediate-risk patients, 82% (1477 of 1812) were classified as low-risk NPx and 18% (335 of 1812) as high-risk NPx. In low-risk patients, 96% did not develop events during 15 years of follow-up. In contrast,14% (46 of 335) of the moderate NPI patients who showed high-risk NPx developed events during the follow-up period.

Multivariate analysis including LN stage and LVI showed that NPx had a strong independent association with both BCSS and DMFS (Table 1). In this model, grade, size and Ki67 were not included, as they are components of the index. When restricting the multivariate analysis to the intermediate-risk group NPx showed independent association with

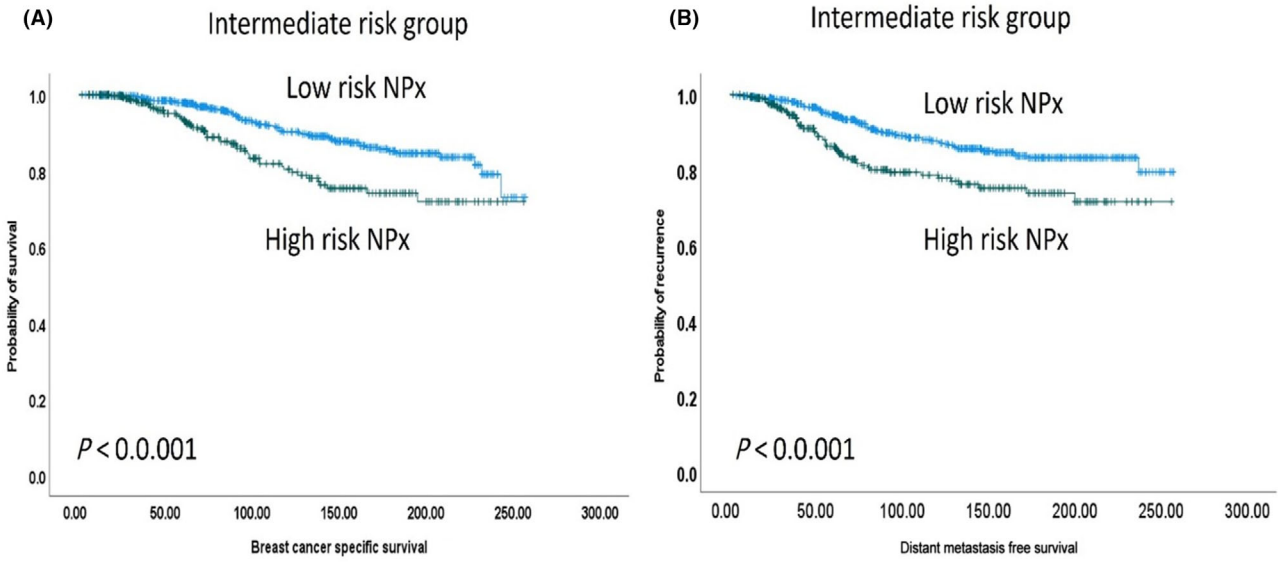

Figure 4. Kaplan–Meier curves show the association of Nottingham prognostic x (NPx) with poor breast cancer-specific survival (**A**) and distant metastasis-free survival (**B**) in the intermediate-risk group of patients (moderate Nottingham prognostic index).

Table 1. Multivariate analysis of Nottingham PX (NPX) in early-stage luminal breast cancer

	BCSS			DMFS		
Parameters	Hazard ratio	95% (CI)	*P*-value	Hazard ratio	95% (CI)	*P*-value
NPX	2.4	1.7–3.2	< **0.0001**	2.3	1.7–3.1	< **0.0001**
Lymph node status	2.0	1.5–2.8	< **0.0001**	2.3	1.7–3.2	< **0.0001**
Lymphovascular invasion	2.4	1.7–3.3	< **0.0001**	2.4	1.8–3.2	< **0.0001**

Significant *P*-values are shown in bold type.
95% CI, 95% confidence interval; BCSS, breast cancer-specific cancer; DMFS, distant metastasis-free survival.

BCSS, while LN lost the association with BCSS (Table 2).

Adding NPI to the multivariate analysis both NPx and NPI showed similar prognostic value, with an HR of 2.1 and 2.3, respectively, in the whole cohort. However, in a head-to-head comparison between NPI and NPx in LN+ patients who received endocrine therapy, NPx showed a stronger association with BCSS with an HR of 2.4 for NPx compared with 1.6 for NPI (Table 3).

Table 2. Multivariate analysis of Nottingham PX (NPX) in intermediate risk early-stage luminal breast cancer

	BCSS			DMFS		
Parameters	Hazard ratio	95% (CI)	*P*-value	Hazard ratio	95% (CI)	*P*-value
NPX	2.3	1.6–3.4	< **0.0001**	2.3	1.5–3.4	< **0.0001**
Lymph node status	1.4	0.9–2.1	0.091	2.3	1.7–3.2	**0.008**
Lymphovascular invasion	1.6	1.7–3.3	**0.012**	2.4	1.0–2.3	**0.027**

Significant *P* values are shown in bold type.
95% CI, 95% confidence interval; BCSS, breast cancer-specific cancer; DMFS, distant metastasis-free survival.

Table 3. Head-to-head comparison of Nottingham PX (NPX) versus Nottingham prognostic index (NPI) in early-stage luminal breast cancer in terms of breast cancer-specific survival

	The whole cohort			Lymph node-positive tumours		
Parameters	Hazard ratio	95% (CI)	*P*-value	Hazard ratio	95% (CI)	*P*-value
NPX	2.1	1.3–2.9	**<0.0001**	2.4	1.5–3.6	**<0.0001**
NPI	2.3	1.7–3.0	**<0.0001**	1.6	1.1–2.4	**0.016**
Lymphovascular invasion	1.9	1.4–2.8	**0.012**	2.2	1.3–3.4	**<0.0001**

95% CI, 95% confidence interval. Significant *P*-values are shown in bold type.

Association of NPx with Oncotype DX RS

There was a strong association between NPx and Oncotype DX risk groups; 68% of patients with a high-risk Oncotype DX RS had a high-risk NPx ($P < 0.0001$). However, 11 patients with a low-risk Oncotype DX RS had a high-risk NPx (Table 4). Six patients with intermediate-risk Oncotype DX RS developed distant metastases, four of which had a high-risk NPx. In the group of patients who had Oncotype DX testing there was a significant association between high-risk NPx and poor outcome ($P = 0.03$). This association was not observed using the Oncotype DX RS score (Supporting information, Figure S4).

In patients with intermediate-risk Oncotype Dx, there was a significant association between high-risk NPx with larger tumour size, high grade and low PR status. However, no significant association was observed between NPx and patient outcome ($P = 0.25$).

2- EXTERNAL VALIDATION COHORT

The patient characteristics of the external cohort are summarised in Supporting information, Table S3; 69% (213 of 307) of patients had smaller tumour size (< 2 cm), 44% (136 of 307) had grade 2 tumours and 57% had a moderate NPI. Median age at diagnosis for the included patients was 55 (range = 28–70) years, with a median follow-up time of 152 months (range = 11–189 months).

Low-risk NPx was observed in 77% (236 of 307) of patients. There was a strong correlation between NPx and NPI in this cohort ($P < 0.0001$). There was a significant association between high-risk NPx and shorter survival ($P = 0.04$; Supporting information, Figure S5). Like the internal cohort, there was no significant difference in survival between patients with low-risk NPx versus high-risk when assessing the cumulative survival probabilities NPx after 1 year of follow-up, but a 2% difference at 5 years was observed with the high-risk group having a worse outcome. This difference increased gradually on a yearly progression rate to a 16% difference at 15 years (Supporting information, Figure S6). However, in the intermediate-risk group, there was no association between NPx and patient survival ($P = 0.17$; Supporting information, Figure S7).

Table 4. The relationship between Nottingham prognostic x (NPx) and Oncotype DX recurrence score

Variables	Low-risk (0–10)	Intermediate-risk (11–25)	High-risk (26–100)	χ^2 (*P* value)
Low-risk NPx	52 (27%)	131 (69%)	8 (4%)	64.5 **< 0.0001**
High-risk NPx	11 (10%)	57 (51%)	44 (39%)	

Significant *P*-value is shown in bold type.

Discussion

Luminal BC is a heterogeneous disease with considerable variation in prognosis and response to therapy. Prognosis is the probability or risk that an outcome/event develops over a specific time. It is estimated using clinical and non-clinical profiles,[32] and used in clinical practice to assist decision-making regarding adjuvant therapy recommendations. Luminal BC patients with a good prognosis are likely to receive adjuvant endocrine therapy alone while those considered to have a poor prognosis may be offered additional adjuvant chemotherapy. The latter is associated with both short- and long-term side effects and substantial financial costs to health service providers.[33]

Various BC prognostic indices have been developed, but few have been well validated in different clinical settings. PREDICT is a BC prognostic and treatment

benefit model implemented online and it incorporates clinicopathological parameters and molecular markers.[34] However, it underestimates breast cancer-specific mortality in women diagnosed under the age of 40 years.[35] NPI, which takes account of tumour size, tumour grade and LN status, stratifies patients into three prognostic groups and is used to guide recommendations regarding adjuvant treatment following surgery.[36] However, the NPI and other available prognostic tools tend to be of most clinical value in the low- and high-risk groups, with less applicability in patients with intermediate risk BC. Additionally, these indices generally do not include BC biomarker values, which may limit their utility.[15]

The NPx was developed by combining selected histopathological features and biomarkers and incorporates histological tumour grade, tumour size, PR and Ki67, with separate indices calculated for BCSS and DMFS. In contrast to other indices, the index stratifies BC patients into two groups rather than three, and provides additional prognostic information on patients classified as moderate/intermediate-risk according to other prognostic indices. This study validates the prognostic performance of NPx in two cohorts of luminal BC, categorised into distinct high- and low-risk groups in terms of patient outcome.

In this study, high NPx was significantly associated with poor outcome in the whole cohort and this association was maintained in patients who received endocrine therapy, but not in endocrine therapy-naïve patients. This suggests that the NPx may be predictive of response to endocrine therapy. We originally started this study by validation of our previous study, which was developed on ER-positive, HER2-negative LN– BC patients, as shown in the Kaplan–Meier curve (Supporting information, Figure S1a,b). However, considering that Oncotype DX was first developed for LN– patients and was then eventually used for stratification of patients with positive (1–3) lymph nodes, we added a group of patients with positive (1–3) lymph nodes to test whether NPx can be also used as a prognostic tool to stratify patients in that group. Notably, NPx further stratified the LN+ tumours–intermediate-risk patients into two distinct prognostic groups. This could help to prioritise patients with LN+ to receive additional chemotherapy. With the acceptance that Oncotype DX yields potentially informative risk assignments in indeterminate-risk LN– tumours,[10,37,38] NPx can add value to predict the outcome of indeterminate risk, LN+ tumours.

In recent years, there has been a major increase in the utility of multigene assays in the clinical setting to guide the use of adjuvant chemotherapy by assessing the likelihood of disease recurrence and potential therapeutic benefit.[39–41] In this study, there was a strong association between NPx and Oncotype Dx risk groups. Discordant results between the various multigene assays and risk assessment tools are well documented.[42–44] In the current study, a small number of patients with discordant results who developed distant recurrences were classified as NPx high-risk. When we reviewed the patients with low-risk Oncotype RS and high-risk NPx, most of those patients had a high proliferation status or showed a low PR status. These parameters can therefore predict patient outcome; NPx can be reliably used to refine the number of patients requiring multigene testing.

In conclusion, the results of this study validates NPx as a powerful prognostic indicator in BC and confirms our previous data regarding the role of NPx in early-stage BC.[15] The NPx stratifies patients with luminal BC into two risk groups and provides useful prognostic information on patients classified as intermediate-risk using other indices. In this era of multigene testing, the NPx may also be used to refine the candidate patients requiring more advanced genomic testing.

LIMITATIONS

The cohort used in this study had few events, because it included early-stage luminal BC patients. In addition, this study was carried out using two retrospective cohorts.

Acknowledgements

We thank the PathLAKE digital pathology consortium. These new centres are supported by a £50m investment from the Data to Early Diagnosis and Precision Medicine strand of the government's Industrial Strategy Challenge Fund, managed and delivered by UK Research and Innovation (UKRI).

Funding information

A L is supported by and funded by the Egyptian Ministry of Higher Education and Scientific Research.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Data availability statement

All data used in this study are archived and could be available on a reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinicopathological characteristics of Nottingham study cohort.

Table S2. Life table analysis of Nottingham prognostic x (NPx) risk groups (low versus high risk).

Table S3. Clinicopathological characteristics of external validation cohort.

Figure S1. Kaplan Meier curves show the association of Nottingham prognostic x (NPx) with breast cancer-specific survival and distant metastasis free survival in lymph node negative (a, b) and in lymph node positive (c, d) luminal breast cancer patients.

Figure S2. Kaplan Meier curves show the association of Nottingham prognostic x (NPx) and breast cancer-specific survival and distant metastasis free survival in patients aged below 50 years (a, c) and in patients aged above 50 years (b, d).

Figure S3. Kaplan Meier curves show the association of Nottingham prognostic x (NPx) and breast cancer-specific survival and distant metastasis free survival in postmenopausal patients (a, c) and in premenopausal patients (b, d).

Figure S4. Kaplan Meier curves show the association of Nottingham prognostic x (NPx) with poor breast cancer-specific survival (BCSS) (a) while Oncotype DX failed to show association BC patients who were tested for Oncotype DX.

Figure S5. Kaplan Meier curve shows the association of Nottingham prognostic x with breast cancer-specific survival in the external validation cohort.

Figure S6. Cumulative proportion surviving for Nottingham prognostic x (NPx) low risk versus high risk during the follow up period in the external cohort.

Figure S7. Kaplan Meier curve shows the association of Nottingham prognostic x with breast cancer-specific survival in the intermediate risk group of external validation cohort.

