Nottingham Clinico-Pathological Response Index (NPRI) after neoadjuvant chemotherapy (Neo-ACT) accurately predicts clinical outcome in locally advanced breast cancer

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STATEMENT OF TRANSLATIONAL RELEVANCE

We developed new pathological response index which can divide our patients into four distinct prognostic groups based on breast cancer specific survival, the index was validated in an internal and external independent cohorts.

Patients with higher NPRI scores showed statistically significant associations with shorter survivals. The specificity and sensitivity of NRPI as a prognostic tool is superior to currently used prognostic indexes (e.g. pathological complete response (pCR)). For example, the good prognosis groups included 52% of all patients based on NPRI scores, compared to only 15% of patients by using the pCR criteria.

This score has the potential to become the best prognostic tool after neoadjuvant chemotherapy, and to standardized important factors for the reporting of results in this setting. However, the NPRI has to be tested as a clinical tool to guide, and make a difference to, the choice and outcome of adjuvant-therapy in a prospective clinical trial.
Abstract

Purpose: There is a need to identify more sensitive clinico-pathological criteria to assess the response to Neo-ACT and guide subsequent adjuvant-therapy.

Experimental Design: We performed a clinico-pathological assessment of 426 patients who had completed Neo-ACT for locally advanced breast cancer (LABC) with a median follow-up of 72-months. Patients were divided into a training set treated with anthracycline combination chemotherapy (AC, n=172); an internal validation set treated with AC and taxane (n=129); and an external validation set treated with AC with or without taxane (n=125).

Results: A multivariate Cox regression model demonstrated the absence of fibrosis, presence of lympho-vascular invasion, increasing number of lymph node metastases and administration of hormone therapy were significantly associated with short breast-cancer specific survival (BCSS) and disease-free survival (DFS); ps<0.01, whilst reduction of tumour size was associated with DFS (p=0.022). Nottingham Clinico-Pathological Response Indexes (NPRI) were calculated and four prognostic groups (NPRI-PGs) were identified. Patients in prognostic group 2 (NPRI-PG2) for BCSS (66/172; 38.4%) have the same prognosis as those who achieved pCR (NPRI-PG1; 15%). Receiver operating characteristic (ROC) curves indicated that the NPRI outperformed the currently used prognostic factors and adding NPRI improved their performance as a predictor for both BCSS (AUC= 0.88) and DFS (AUC=0.87). Conclusions: The NPRI predicts BCSS and DFS, with a higher sensitivity than pCR. The
NPRI can also improve the sensitivity and specificity of clinico-pathological response as a study end-point, for assessing response to Neo-ACT, and can serve as a valuable tool for the discovery of future predictive molecular markers.
Key Words: Breast Cancer, Response to Neoadjuvant Chemotherapy, Clinico-pathological assessment, NPRI

Running title: NPRI predicts clinical outcome in LABC after Neo-ACT
Introduction

A meta-analysis combining data from over 3,900 patients (1) with locally advanced breast cancer (LABC) demonstrated no difference in overall survival and disease progression between neoadjuvant and adjuvant chemotherapy. Several clinical trials have confirmed the safety and efficacy of neoadjuvant chemotherapy and have established its utility in the management of LABC (2). Response to neoadjuvant chemotherapy is informative about a cancer's biology (3), and provides some indication about the potential response of the tumour to further treatment (4). However, the assessment of response and its impact on survival is yet to be established and there are no universally accepted criteria (5, 6).

Studies have shown that achieving pathological complete response (pCR) after neoadjuvant chemotherapy predicts overall survival, independent of treatment regimen (7-9). However, other trials comparing different neoadjuvant chemotherapy regimens have failed to demonstrate an association between pCR rate and improved outcome (10). Furthermore, pCR is an imperfect surrogate for clinical outcome, because: i) only a small fraction of neoadjuvant chemotherapy patients achieve pCR (3-28%; depends on the definition of pCR) (11), ii) patients who do not achieve pCR may still have a good prognosis whilst patients who achieve pCR can still experience recurrent disease (12), and iii) the presence of residual cancer cells observed after neoadjuvant therapy reflects a wide range of responses from near-pCR to complete resistance (3).

In this study, we identified clinico-pathological criteria that could grade response to...
neoadjuvant chemotherapy and correlate with clinical outcome. We used these criteria to develop a new clinico-pathological response index (the Nottingham Clinico-Pathological Response Index, NPRI).
Materials and Methods

Patients

426 patients with clinical stage IIA-IIIC disease (T1-4, N0-3, and M0) who completed neoadjuvant chemotherapy for LABC were included in this study and divided into 3 cohorts according to treatment centre and regimen:

1) The training cohort (n=172) were diagnosed at Nottingham University Hospitals (NUH) between 1996 and 2011 and treated with standard anthracycline regimens (AC) in the form of 6 cycles of FEC (5-fluorouracil 500 mg/m², epirubicin 75-100 mg/m², cyclophosphamide 500 mg/m², on day 1 of a 21-day cycle). This cohort was used to characterize the NPRIs and to determine cut-off points for prognostic groups (NPRI-PGs) based on BCSS DFS.

2) The internal validation cohort (n=129) included patients who were treated at the same institution (NUH) between 2002 and 2011 and received a taxane in addition to the AC regimens (AC-T) as part of clinical trial protocols.

3) The external validation cohort (n=125) were treated at the Breast Clinical Trials Unit at Mount Hospital (Perth, Western Australia) between 1999 and 2011 and received AC regimens with or without a taxane.

Detailed patient demographics and clinico-pathological characteristics were prospectively assessed and regularly updated (summarized in supplementary online table S1). All patients underwent neoadjuvant chemotherapy,
mastectomy or breast-conserving surgery and axillary dissection, followed by adjuvant radiation therapy. All patients involved in our study received endocrine-therapy for 5 years if >1% of tumour was positive for ER. A number of patients received adjuvant chemotherapy if 1) the tumour is known to be triple negative from base line assessments, 2) The surgical specimen demonstrates three or more involved lymph nodes, 3) The patients showed no response to, or progression on, neo-adjuvant chemotherapy, 4) Significant residual tumour is present in the surgical specimen. The median follow-up time was 70 months for the entire population and all patients gave their informed consent before initiation of therapy. The Nottingham Research Ethics Committee approved this work. The Reporting Recommendations for Tumour Marker Prognostic Studies ( REMARK) criteria were followed throughout this study (13)

Pathological Review

Six authors (IOE, SEP, AHSL, BL, DP and TM A-F) contributed to a comprehensive review of the pathology reports and haematoxylin and eosin stained slides from pair- matched diagnostic core biopsies and surgical resection specimens (breast and regional lymph nodes). All slides were centrally reviewed by (TM A-F). Pathological features were assessed and their evaluation criteria are summarized in supplementary online table (S2). In view of the subjectivity and sub classification of fibrosis (none, mild, moderate, severe), we had cause to simplify classification of fibrosis into present or
absent. Thus absence of fibrosis with or without granulation tissue/necrosis in both the tumour bed and dissected regional lymph nodes was considered as evidence for the absence of any pathological response reaction to chemotherapy. Intra- (kappa; range 0.75 to 0.88, Cohen kappa test) and inter-(kappa; range from 0.70 to 82; using multi-rater kappa tests) observer agreements were moderate for the evaluation of fibrosis. In cases where discordant results were obtained, the slides were re-evaluated by IOE and TM A-F together and a consensus reached. The pCR was defined as the absence of residual invasive carcinoma in both the breast and regional lymph nodes. The number of histologically positive lymph nodes was determined by examination of serial macroscopic sections of each lymph node. On average, 16 breast-blocks and all submitted lymph nodes were examined for each case before a diagnosis of pCR was reached.

Oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) measurements were available for all patients and reassessed according to the most recent American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines (14, 15). The tumour histological grade was assessed according to Nottingham histological system (16). The primary tumour size and lymph node stage at the time of diagnosis were determined by physical examination and imaging, including mammography and sonography. The sonography measurements for primary tumour size at time of diagnosis (baseline size) and the pathological measurements of invasive carcinoma after surgery (final primary tumour size)
were used to calculate the reduction in the primary tumour size. Clinical staging of the BC at the time of diagnosis (clinical-TNM stage) and the pathological staging after neoadjuvant chemotherapy (yp-TNM stage) were determined using the revised American Joint Committee on Cancer (y-AJCC) staging system for BC (17). Pre-treatment Ultrasound assessment of the axilla is routine and if any morphological abnormality is detected an Ultrasound guided biopsy is performed of one of the nodes, even if it is not palpable. No patients in our study had their sentinel lymph node biopsied as part of their baseline diagnosis.

The Miller-Payne system for classification of neoadjuvant chemotherapy

The histological grading system for response of neoadjuvant chemotherapy based on the percentage of reduction in tumour cellularity has been assessed according to the Miller-Payne system as previously described (18).

Clinical-Pathologic Scoring System (CPS) and CPS-Oestrogen receptor-histological grade (CPS-EG) score

Two prognostic scoring systems, CPS and CPS-EG systems, based on clinical TNM stage, yp-TNM stage, oestrogen receptor status, and histological grade were calculated as previously described (19).

Residual cancer burden

Residual cancer burden (RCB) was estimated from routine pathologic sections
of the primary breast tumour site and the regional lymph nodes after completion
of neoadjuvant chemotherapy according to the MD Anderson Cancer Centre
criteria (3). A calculated RCB index for each patient was generated using the
calculation formula at the MD Anderson Cancer Centre's website
(http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3)
(last accessed 19th June 2014).

Detailed statistical methods

Statistical analyses were performed using STATISTICA (Stat Soft Ltd, Tulsa,
USA) and SPSS (version 17, Chicago, USA). Where appropriate, Pearson's
chi-squared; student's t-test and ANOVA tests were used. All tests were two-
sided with a 95% confidence interval (CI) and a p value <0.05 was considered
to be indicative of statistical significance. Survival data including survival time,
disease-free survival (DFS), and development of loco-regional and distant
metastases (DM) were maintained on a prospective basis. BC specific survival
(BCSS) was defined as the number of months from diagnosis to the occurrence
BC-related death. DFS was defined as the number of months from time of
surgery to the occurrence of recurrence or DM relapse. Survival was censored
if the patient was still alive, lost to follow-up, or died from other causes.
Cumulative survival probabilities and 5-year BCSS and DFS were estimated
using the univariate Cox models and the Kaplan-Meier plot method where
appropriate, and differences between survival rates were tested for significance
using the log-rank test.
Development and calculation of NPRI scores

After the definition of factors associated with BCSS and DFS, multivariate Cox proportional hazards models (with backward stepwise exclusion of factors, using a criterion of $p<0.05$ for retention of factors in the model) were used to identify which factors were independently associated with clinical outcomes. The statistical significance of the model was assessed based on the likelihood ratio test. The proportional hazards assumption was tested using both standard log-log plots and by generating Kaplan-Meier survival estimate curves, and observing that the curves did not intersect with each other. Hazard ratios (HRs) for death risks and relapse and 95% confidence intervals were calculated from the Cox proportional hazards analysis. Subsequently, NPRI scores for both BCSS and DFS were calculated using the summations of p-coefficient values of the factors/measurements retained in the final model after controlling for both hormonal and chemotherapies.

Determination of NPRI cut-off

Thresholds were determined to define four NPRI prognostic groups (NPRI-PGs) with distinct prognoses: NPRI-PG1 included those with no traces of residual disease (i.e. those who achieved pCR), NPRI-PG2 included those with good response (near-pCR) and NPRI-PG3 and NPRI-PG4 those with moderate and the minimal responses respectively. To determine the first cut-off point (between NPRI-PG4 and other NPRI score groups), a multivariate Cox
regression model was used based on cut-off points selected between the 5% and the 95% quartiles of the NPRI score distribution. The optimal cut-off point was selected as the quartile that maximized the profile log-likelihood of this model. A second cut-off point (between NPRI-PG2 and NPRI-PG3) was determined similarly by maximizing the profile log-likelihood of a Cox model that included all clinical covariates and the first dichotomous PRI-score factor (i.e. NPRI-PG4 versus NPRI-PG2/3).

**Clinical impact of NPRI and model discrimination**

To assess the potential clinical impact of the NPRI, multivariable analyses using the Cox proportional hazards model were performed with the NPRI score as a continuous variable, while controlling for both the neoadjuvant and adjuvant chemotherapy regimens.

Receiver operating characteristic (ROC) curves were generated to compare NPRI performance as a prognostic tool and the other currently used prognostic models. Logistic fit of low versus high survival category by cumulative hazard (the product of the hazard ratios of each incorporated variable) was performed. Area under the curve (AUC) values was calculated from ROC curves. An AUC of 0.8 or above was considered a good classifier.

Model discrimination was evaluated based on Harrell's concordance index (c index), which is a generalized area under the receiver operating curve (AUC) for censored observations and is equal to the probability of concordance.
between the predicted probability of relapse and the relapse outcome (20). The c index was adjusted for bias using bootstrap resampling with 300 replications. The CI for the c index was obtained based on approximate normality using the variance estimate of the unadjusted index.

Fitted polynomial function curves were calculated which summarise the broad relativity between the NPRI value and both 5- and 10-year BCSS and DFS. These were constructed from the raw data by applying median BCSS and DFS of each NPRI-PG against the 5 and 10 year BCSS and DFS for each division. Predictions from the curves were compared with the actual values.

**Predictive accuracy of NPRI compared with other prognostic clinico-pathological factors**

To evaluate whether the NPRI-PGs add new independent prognostic information to current prognostic systems, we performed separate Kaplan-Meier analyses by NPRI prognostic group within each ER status, AJCC stage stratum, CPS, CPS-EG, RCB classes and non-pCR subgroup. The significance of the additional stratification provided by the NPRI was evaluated based on the log-rank test.
Results

Clinico-pathological factors associated with higher risk of death and relapse after FEC chemotherapy in univariate Cox analysis included absence of fibrosis in primary tumour site and regional lymph nodes, presence of lympho-vascular invasion (LVI), increasing number of lymph node metastases and hormonal therapy. These factors maintained significance as independent predictors for both BCSS and DFS after controlling for adjuvant chemotherapy and other covariates by using multivariate Cox proportional hazards models with backward stepwise exclusion (Table 1 and supplementary table S3). The percentage of reduction in primary tumour size showed statistical significant association with DFS only (supplementary table S3, figure S1). None of the covariates exhibited significant deviations from the proportionality assumption or had time-dependent effects (code system used is summarized in supplementary table S4). Subsequently, the summations of p coefficient values produced by the Cox analysis were used to calculate the NPRIs for each patient as follows:

Developing of Nottingham prognostic response index (NPRI) for BCSS

The following formula has been used to calculate NPRI for BCSS:

\[(NPRI_{bcss}) = \text{Fibrosis status (0, 1)} \times 1.618028 + \text{LVI status (0, 1)} \times 1.048666 + \text{number of positive lymph nodes (0-43)} \times 0.063750 + \text{planed hormonal therapy status (0, 1)} \times -1.093202\]
The prognostic value of the NPRI compared with prognostic pathological and clinical factors

We examined the predictive accuracy of the NPRI score compared with other prognostic clinico-pathological factors by performing the Cox proportional hazards univariate and multivariable analyses controlling for neoadjuvant and adjuvant chemotherapy. In the training cohort, patients had almost a three-fold increase in death (HR 2.83; 95% CI, 2.17 to 3.68; p = 1.1x 10^{-14}) for each unit of increase in the NPRI (supplementary table S5). When the NPRI was included in a multivariate Cox regression model (Figures 1-A), the overall predictive power of the model was significantly improved (p = 3.6x10^{-9}), and the NPRI was significantly associated with a two-fold increase of the risk of death (HR 2.14; 95% CI, 1.37 to 3.36; p = 0.001). Using univariate analysis, a similar statistically significant association between NPRI and BCSS was found in both the internal and external validation cohorts (Supplementary table S5). When the Cox proportional hazard multivariable analysis was repeated in the two validation sets controlling for neoadjuvant and adjuvant chemotherapy, the NPRI score outperformed other pathological and clinical covariates and was independently associated with BCSS (Figure 1-B and Figure 2A). All other pathological and clinical covariates failed to show a consistent association with prognosis (Figures 1 and 2). Similar results were confirmed when the three cohorts were combined together (n=426; Figure 2-B).

To assess the contribution of the NPRI toward the prediction of clinical outcome
of neoadjuvant chemotherapy, Cox proportional hazards statistical models containing relevant pathological and other clinical predictors controlling for neoadjuvant and adjuvant chemotherapy were constructed. ROC analyses were performed as follows:

1. NPRI score alone.
2. RCB score alone.
3. pCR vs none pCR
4. c-TNM
5. yp-TNM
6. The Miller-Payne system alone
7. CPS alone
8. CPS-EG alone
9. A prognostic model including RCB score, yp-TNM, c-TNM, histological grade, HER2, ER, pCR status, age, neoadjuvant and adjuvant chemotherapy without NPRI score.
10. The same prognostic model as number 9 with NPRI scores.
AUC values for BCSS confirmed that NPRI (AUC = 0.85) was superior to other prognostic models, and that it could be a good prognostic tool (Figure 3-A and supplementary figure S2-A) which remained consistent when the analysis was repeated for both internal (Figure 3-B and supplementary figure S2-B) and external (Figure 2-C and supplementary figure S2-C) validation cohorts, and after combining the three cohorts together (Figure 3-D and supplementary figures S2-D). NPRI identifies distinct prognostic groups of none-pCR patients.

We identified two cut-off points to assign patients with residual disease (non-pCR; or non NPRI-PG1) after FEC treatment into one of three classes: NPRI-PG2 (good prognosis group), NPRI-PG3 (moderate prognostic group), and NPRI-PG4 (poor prognostic group). The first cut-off point (NPRI-PG4 v NPRI-PG2/3) was selected as the 92th percentile (NPRI, 1.87383) for BCSS. The second cut-off point (NPRI-PG2 v NPRI-PG3) corresponds to the 53.5th percentile for BCSS. The cut-off points defined subgroups of NPRI-PG1 to NPRI-PG4 with increasingly poor prognosis (Figure 4). The cumulative incidence estimate of the overall probability of death within 5 years was 4% for the pCR group (NPRI PG1) and 5% for NPRI-PG2, whereas it was 33% and 71% for NPRI-PG3 and NPRI-PG4, respectively. Applying the NPRI in both the internal and the external independent cohorts defined groups with increasingly poor 5-year prognoses (Figures 4-B&C). The separation of death rates was somewhat smaller in the training set than for the internal validation cohort. To explore this further, we combined the poor prognosis groups together (NPRI-PG4 and NPRI-PG3) and compared the 5-year BCSS with those in the NPRI-
PG1 (pCR patients) and found the separation of both the 5-year death rates were still wider in AC-T cohort vs. FEC-only cohort (12% for BCSS).

The c-index of a prognostic model that included current prognostic factors without adding NPRI score was consistently lower than the c-index generated after adding NPRI to the same model in the training, internal and external validation cohorts, as well as in the pooled data that included all three cohorts (Figure 3).

NPRI prognostic groups stratify clinical outcome of breast cancer molecular sub-groups

Subgroup analysis of the combined patient cohort confirmed that the NPRI is a valid prognostic tool regardless of the molecular classification of BC (Figures 4 and 5). In previous studies, pCR and RCB have been demonstrated to be a much more reliable prognostic factor in hormone receptor negative (HR-negative) disease. In our study NPRI has separated both HR positive and HR negative cohorts into distinct prognostic groups (Figure 4-E & F). Applying the NPRI to the HR positive subgroup demonstrated that 46% of patients have poor clinical outcome despite receiving adjuvant hormone therapy after completing neoadjuvant chemotherapy, whilst 49% of HR negative cancers had an excellent prognosis. Moreover, only 55% of HER2 overexpression BC’s had a favourable outcome despite receiving adjuvant Trastuzumab following neoadjuvant therapy (Figure 5-A). Although patients with triple negative BC did not receive targeted adjuvant therapy after neoadjuvant therapy, our results
demonstrated that 49% of those patients had excellent prognosis (Figure 5-B).

**The NPRI prognostic groups stratify prognoses of c-TNM, yp-TNM, CPS and CPS-EG stages**

Subgroup analyses of the TNM stage at diagnosis also confirmed the prognostic power of the NPRI to separate clinical TNM stage III cancers into distinct BCSS prognostic groups (p=4.5 x 10^{-11}; data not shown). Results demonstrated that 41% of those patients achieved excellent prognosis. In addition we evaluated the contribution of the NPRI prognostic groups to the prognostic power of each post-therapy yp-TNM stage group (Figure 5-C). Regarding BCSS, NPRI classified yp-TNM stage II/III patients into three distinct prognostic subgroups (ps<0.001; Figure 5-C). Also, applying NPRI to yp-AJCC stage 0/I, indicated that 10% of those patients have a less favourable outcome (p=0.00001; data not shown). Therefore, NPRI classification appears to add significant prognostic power compared with post-treatment pathologic y-AJCC stage. Moreover applying NPRI to the recently developed CPS and CPS-EG prognostic systems (that included information on c-TNM, yp-TNM, ER and histological grade) has added prognostic power to each system (Figure 5 D-E). For instance, the NPRI has re-classified patients with either CPS scored 2/3 (n=245) or CPS-EG scored 3/4 (247) into three distinct prognostic subgroups (ps=0.00005 and 0.00001; respectively, Figure 5 D-E).
The NPRI adds significant prognostic power compared with Residual Cancer Burden classes

Applying the NPRI to residual cancer burden (RCB) classes in all three cohorts of patients improved the prognostic stratification of patients who were designated as RCB classes II (n=195; predicted to have a 5-year BCSS 78%) and III (n=110; predicted to have a 5-year BCSS of 63%). Each class has been stratified into 3 distinctive prognostic groups (Figure 5 F-G, ps<0.00001). Applying NPRI to RCB class II split the patients in this class to 2 groups: those who have a 12% worse, and those who have a 45% better prognosis than that predicted (Figure 5-F). Similarly, NPRI has identified that 25% of RCB class III had a worse, and 27% a better, prognosis than that predicted for that class of patients (Figure 5-G).

The Pearson correlation coefficient and fitted polynomial function curves showed that there is an excellent inverse linear correlation between NPRI and BCSS. In figure 5-I, the fitted polynomial function summarises a broad relationship between the NPRI value and median 5- and 10-year survival.

Developing of Nottingham prognostic response index for DFS (NPRI_{DFS})

The following formula has been used to calculate NPRI for DFS:

$$\text{NPRI for DFS (NPRI}_{DFS}) =$$

- Fibrosis status (0, 1) x 1.2830 + LVI status (0, 1) x 0.8431 + number of positive research.
lymph nodes (0-43) x 0.0537 + percentage of reduction of primary tumour size (+100% to -100%) x -0.008162 + planned hormonal therapy status (0, 1) x -0.7521

In the training internal and external validation cohorts, patients had almost a threefold increase in relapse (ps < 0.0001) for each unit of increase in the \( NPRI_{DFS} \) (supplementary table S5). When the \( NPRI_{DFS} \) was included in a multivariate Cox regression model controlling for other prognostic models and neoadjuvant and adjuvant chemotherapy (supplementary S3 A-B and S4), the overall predictive power of the model was significantly improved (p <0.0001) and the \( NPRI_{DFS} \) outperformed other clinico-pathological covariates which failed to show a consistent association with prognosis.

\( NPRI_{DFS} \) identifies distinct DFS prognostic groups of none-pCR patients

The first cut-off point (\( NPRI_{DFS} \)-PG4 v \( NPRI_{DFS} \)-PG2/3) was selected as the 83th percentile (\( NPRI_{DFS} \), 0.91021) for DFS. The second cut-off point (\( NPRI_{DFS} \)-PG2 v \( NPRI_{DFS} \)-PG3) corresponds to the 64.5th percentile (\( NPRI_{DFS} \), 0.522257) DFS. The cut-off points defined subgroups of \( NPRI_{DFS} \)-PG1 to \( NPRI_{DFS} \)-PG4 with increasingly poor prognosis (Figure supplementary S5). Applicability of the \( NPRI_{DFS} \) was evaluated in both the internal and the external independent cohorts and \( NPRI_{DFS} \) defined groups with increasingly poor 5-year prognoses (Figures S5). The c-index of the prognostic model on the internal and external validation cohorts was 0.776 (95% CI, 0.67 to 0.87) and 0.841
(95% CI, 0.76 to 0.92), respectively (Figure S6).
Discussion

Up to 20% of BC patients present with locally advanced disease which is associated with a poor prognosis (21). In those tumours that lack a specific therapeutic target (i.e. ER or HER2), conventional chemotherapy remains the mainstay of systemic therapy. Knowledge of the primary tumour's sensitivity or resistance to neoadjuvant chemotherapy can predict the efficacy of these agents on micro-metastatic disease. A trial of the effectiveness of Adjuvant chemotherapy, following poor response to Neo Adjuvant chemotherapy, is desperately needed. However, at present, there are no effective prognostic tools to guide adjuvant therapy based on response to neoadjuvant chemotherapy. Although the pathological evaluation of tumour response is still the gold standard, the lack of uniform reporting of pathological response remains a problem (22).

In this study, a comprehensive clinico-pathological evaluation of a cohort of patients who received neoadjuvant AC/FEC chemotherapy at a single centre was conducted and the NPRI was developed, which was then validated in two independent cohorts. Patients with higher NPRI scores showed statistically significant associations with shorter DFS and BCSS. The NPRI can categorise patients into four prognostic groups (NPRI-PG1 to PG4) with patients in NPRI-PG2 having the same 5-year outcome as those with pCR (NPRI-PG1), irrespective of the type of neoadjuvant chemotherapy administered, ER status, or the pathological stage of residual disease. The prognostic information described herein represents the most detailed data available on DFS and BCSS outcomes for patients treated with neoadjuvant chemotherapy. The NPRI outperforms other traditional predictors of clinical outcome.
of BC such as residual cancer burden, pCR and the revised yp-TNM stage, with high predictive accuracy in the training cohort and in the two independent validation sets.

The NPRI identifies an important subset of patients with combined insensitivity to chemo- and hormone therapies that may benefit from novel therapy in a trial setting. For example, the NPRI identifies a second good prognostic group (NPRI-PG2) that have responded as well as the cohort with pCR (PG1) and can be spared further multiple cycles of unnecessary (and potentially toxic) treatments. Likewise, HR positive patients in the NPRI-PG3 and PG4 groups had a moderate to poor prognosis despite receiving adjuvant hormone therapy after completing neoadjuvant chemotherapy.

The separation of the 5-year relapse and death rates were somewhat better in patients who received AC+T chemotherapy compared with those who received AC only, indicating some benefit from the addition of a taxane. But this is uncertain due to the lower number of patients in the NPRI-PG4 subgroup. However; after combining NPRI-PG3 and NPRI-PG4 we found the same separation, confirming that those patients might benefit from adding a taxane to anthracycline chemotherapy; in agreement with a recent large meta-analysis study (23). Moreover, the c-index of the prognostic model on the internal and external validation cohorts showed similar sensitivity and specificity.

It has been recommended that the predictive ability of a new score should be evaluated based on whether the score improves an already optimized multivariate model of available risk factors (24). Based on this, a statistical prognostic model, including an NPRI score and known prognostic factors, has shown to be superior to all the prognostic models without the NPRI.
Several studies have attempted to provide criteria for response after neoadjuvant chemotherapy (22) by using clinical, MRI or sonography findings (19, 25) or by bi-dimensional measurement of the primary tumour bed in resection specimens (3, 22). In fact, these systems have incorporated macro-anatomical features of BC [viz. residual tumour size and lymph node status]. The NPRI, with inclusion of lymphovascular invasion, host response and changes in tumour size, highlights also the importance of the tumour micro environment as a predictor for response to chemotherapy.

In agreement with other studies (3, 10, 26) we found that lymph node status after neoadjuvant chemotherapy is still the single most important prognostic factor. However, the increasing use of sentinel lymph node biopsy either before or after neoadjuvant chemotherapy leads to difficulties in evaluating the prognostic importance of lymph node status. With regard to the interpretation of sentinel lymph node status after neoadjuvant chemotherapy, the current data is inconsistent and requires further evaluation (27). However, it is possible that with adequate standardization of the techniques and data from prospective clinical trials, sentinel lymph node status after neoadjuvant chemotherapy could be added to our index for patient selection to reduce surgical morbidity in the good prognostic groups.

In our study, the presence of lympho-vascular invasion after neoadjuvant chemotherapy was an independent predictor of clinical outcomes, in agreement with previous studies (28, 29). It has been shown that tumour emboli in vascular spaces are relatively resistant to treatment when compared to carcinoma invading the stroma (30). It should be noted that the identification of lymphovascular invasion may sometimes be difficult as the residual tumour nests or DCIS may show marked
retraction artefact in the fibrous stroma mimicking invasion (5). Thus, proper tissue fixation and immunohistochemical staining for lymphatic channel markers may be useful to distinguish tissue retraction from lymphatic invasion.

Although residual tumour size has been proposed as a prognostic factor for BC (29, 31), we found the reduction in primary tumour size was more predictive of DFS than actual residual tumour size after neoadjuvant chemotherapy. In fact, using residual tumour size does not discriminate between large contiguous carcinomas (which have shown minimal treatment effect) and microscopic foci scattered in a tumour bed with equally great dimensions (demonstrating significant treatment effect). Many examinations have been proposed to monitor the extent of the residual disease extent during neoadjuvant chemotherapy, such as physical examination, mammography and sonography, but most studies demonstrate modest accuracy when compared with final pathological assessment (32). There is increasing evidence that magnetic resonance imaging (MRI) is an excellent imaging tool with high specificity for both early response monitoring and the assessment of residual disease (33). However, relatively few studies reported direct comparisons between MRI and other cost-effective tests (33). Partridge et al (34) found that MRI tumour volume was more predictive of DFS than tumour diameter, suggesting that volumetric changes measured using MRI may provide a more sensitive assessment of treatment efficacy. Furthermore, changes of metabolic volume measured by functional MRI could reflect early outcomes of neoadjuvant chemotherapy (35). In fact, future incorporation of MRI results into the NPRI score could add another dimension to NPRI for early assessment of response to neoadjuvant chemotherapy.
Limitations of the study and future directions

Although we validated our findings in two independent cohorts and we have shown that the NPRI can supplement existing methods to define pathologic response, the utility of the NPRI requires further validation in larger patient populations. It also requires prospective evaluation to demonstrate its role as a prognostic tool and its potential to select patients for novel systemic therapies following neoadjuvant chemotherapy. In addition, further studies are needed to address inter-observer variability, standardization of NPRI measurements and develop a more objective methodology to quantify such factors as fibrosis, lympho-vascular invasion and changes in tumour size. In particular the effect of new treatment regimens on the components of the index will need to be assessed in more recent cohorts. Further refinement of the scoring system through the addition of new molecular or biologic markers is also needed.

Conclusion

We suggest that the incorporation of the NPRI in assessing clinical outcome following neoadjuvant chemotherapy helps individualisation of systemic treatment in patients with locally advanced BC. In particular, it may identify patients who fail to benefit from standard chemotherapy regimens and in whom treatment with novel therapies is warranted. We believe that this scoring system may provide a standardized approach to reporting the tumour response to neoadjuvant chemotherapy.
Abbreviations

AC = Anthracycline and Cyclophosphamide combination

AC-T = Anthracycline and Cyclophosphamide combination, followed by Taxane

AT = Adjuvant Therapy

AUC = Area Under Curve

BC = Breast Cancer

BCSS = Breast Cancer Specific Survival

CI = Confidence Interval

DCIS = Ductal Carcinoma in Situ

DFS = Disease Free survival

DP = Disease Progression

ER = Oestrogen Receptor

FEC = 5-Fluorouracil (5-FU) 500 mg/m², Epirubicin 75–100 mg/m², Cyclophosphamide 500 mg/m², on day 1 of a 21-day cycle.

HER2 = Human Epidermal Receptor 2

inv-CA size = Residual invasive size

LABC = Locally Advanced Breast Cancer

LN = Lymph node

LVI = Lympho-vascular Invasion
Neo-ACT = Neoadjuvant chemotherapy

OS = Overall Survival

NPRI = Nottingham clinico-Pathological Response Index

NUH = Nottingham University Hospitals Trust

PER-BC = Primary Oestrogen Receptor Negative Breast Cancer

pCR = Pathological Complete Response (Primary and Lymph nodes are negative)

pre-PTS = pre-chemo Patient Tumour Size (maximum diameter)

PGs = Prognostic Groups

PTS = Patient Tumour Size (maximum diameter)

RCB = Residual Cancer Burden

RD = Residual Disease

ROC = Receiver Operating Characteristic curves

RT = Residual Tumour

SLN = Sentinel Lymph node

T = Taxane

c-TNM = Clinical TMA stage

yp-TNM = revised pathological TNM stage

% CA = Percentage of overall cancer cellularity

% CIS = Percentage of cancer that is residual intra-ductal carcinoma
% inv-CA = Percentage of invasive component

% cp-PTS-R = Percentage of the clinico-pathological tumour size reduction
Funding

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Authors' Contributions

References


26. Pinder SE, Provenzano E, Earl H, Ellis IO. Laboratory handling and histology reporting of breast specimens from patients who have received neoadjuvant chemotherapy. Histopathology. 2007;50:409-17.


### Table (1): Univariate and multivariate backward step-wise analysis for factors associated with breast cancer specific survival (BCSS) in the training cohort.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age continuous</td>
<td>1.04</td>
<td>1.01</td>
</tr>
<tr>
<td>Hormonal therapy</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Adjuvant Chemotherapy</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Presenting tumour size mm</td>
<td>continuous</td>
<td>1.01</td>
</tr>
<tr>
<td>Presenting grade</td>
<td>low/intermediate</td>
<td>1</td>
</tr>
<tr>
<td>Tumour type types</td>
<td>IDC-NST</td>
<td>1</td>
</tr>
<tr>
<td>ER expression</td>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>HER2 status</td>
<td>Normal</td>
<td>1</td>
</tr>
<tr>
<td>PR expression</td>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>Triple negative phenotype</td>
<td>No</td>
<td>1</td>
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<tr>
<td>Clinical TNM (c-TNM)</td>
<td>Stage II</td>
<td>1</td>
</tr>
<tr>
<td>Pathological TNM (yp TNM)</td>
<td>Stage 0/I</td>
<td>1</td>
</tr>
<tr>
<td>Number of positive lymph nodes</td>
<td>continuous</td>
<td>1.01</td>
</tr>
<tr>
<td>Residual inv CA size</td>
<td>continuous</td>
<td>0.99</td>
</tr>
<tr>
<td>Percentage of tumour size reduction</td>
<td>continuous</td>
<td>0.99</td>
</tr>
<tr>
<td>Percentage of cellularity of residual inv CA</td>
<td>continuous</td>
<td>0.99</td>
</tr>
<tr>
<td>Percentage of reduction in cellularity of inv CA (Miller-Payne system)</td>
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<td>Extension and distribution of Inv</td>
<td>No cell/scattered</td>
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<tr>
<td>Fibrosis</td>
<td>Yes</td>
<td>4.28</td>
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<tr>
<td>LVI</td>
<td>Yes</td>
<td>3.26</td>
</tr>
<tr>
<td>Ductal carcinoma</td>
<td>Yes</td>
<td>1.67</td>
</tr>
</tbody>
</table>

IDC-NS; invasive ductal carcinoma-no special type, ER; oestrogen receptor, PR; progesterone receptor, HER2; human epidermal receptor 2, inv-CA; invasive carcinoma, LVI; Lymphovascular invasion
Figure legends

**Figure 1: A-B.** Multivariable Cox proportional hazards regression analyses for breast cancer specific survival (BCSS; **left panel**) and corresponding forest plots (**Right panel**). Comparison of Nottingham clinico-pathological response index (NPRI) score (as continuous variable) with known prognostic clinico-pathological factors including: residual cancer burden (RCB) score, Clinical-Pathologic Scoring System (CPS) and CPS-Oestrogen receptor-histological grade (CPS-EG) score, histological grade based on Nottingham grading system (1/2 vs. 3), HR (hormone receptors) expression status (negative vs. positive), HER2 (human epidermal receptor 2) overexpression/amplification status (overexpression/amplification vs. no overexpression/amplification), chemotherapy therapy (if applicable) and age at diagnosis in the training (A), and internal validation (B) cohorts. Solid squares represent the hazard ratio (HR) of recurrence and open-ended horizontal lines represent the 95% confidence intervals (CIs). All p values were calculated using Cox proportional hazards analysis and p < 0.05 was considered as statistical significant p value. AC: Anthracycline, T: Taxane, AC-T: Anthracycline and Taxane.

**Figure 2: A-B.** Multivariable Cox proportional hazards regression analyses for breast cancer specific survival (BCSS; **left panel**) and corresponding forest plots (**Right panel**). Comparison of Nottingham clinico-pathological response index (NPRI) score (as continuous variable) with known prognostic clinico-pathological factors including: residual cancer burden (RCB) score, Clinical-Pathologic Scoring System (CPS) and CPS-Oestrogen receptor-histological grade (CPS-EG) score, histological grade based on Nottingham grading system (1/2 vs. 3), HR (hormone receptors) expression status (negative vs. positive), HER2 (human epidermal receptor 2) overexpression/amplification status (overexpression/amplification vs. no overexpression/amplification), chemotherapy therapy (if applicable) and age at diagnosis in the external validation cohort (A), and data after combined the three cohorts (B). Solid squares represent the hazard ratio (HR) of recurrence and open-ended horizontal lines represent the 95% confidence intervals (CIs). All p values were calculated using Cox proportional hazards analysis and p < 0.05 was considered as statistical significant p value. AC: Anthracycline, T: Taxane, AC-T: Anthracycline and Taxane.

**Figure 3:** Receiver operating characteristic (ROC) analysis of Nottingham clinico-pathological response index (NPRI) score and other clinico-pathological covariates were performed for predicting breast cancer specific survival in the training (A), internal validation (B), external validation (C) and combined three cohorts (D). The area under the curve (AUC) was calculated for ROC curves, and sensitivity and specificity was calculated to assess the performance of residual cancer burden (RCB) alone (1), NPRI alone (2), and * a statistical prognostic model that was constructed based on multivariable Cox proportional hazards incorporating known clinico-pathological prognostic variables including: pathological complete response (pCR), RCB score, presenting clinical TNM (Tumour, Node and Metastases) stage, revised pathological TNM stage (yp-TNM) stage, histological grade based on Nottingham grading system, ER (oestrogen receptor) expression status, HER2 (human epidermal receptor-2) status, and age at diagnosis (3). ** ROC analysis was also performed for the aforementioned prognostic model after incorporating the NPRI score (4). Dashed grey lines indicate the 45º angle tangent line marked at a point.
that provides best discrimination between true positives and false positives, assuming that false positives and false negatives have similar costs. AC: Anthracycline, T: Taxane, AC-T: Anthracycline and Taxane.

**Figure 4:** Kaplan Meier curves and lifetime table showing breast cancer specific survival in the training (A), internal validation (B), external validation (C) and combined three cohorts (D) cohorts, hormone receptor (HR; E) positive and HR negative cases (F) stratified according to Nottingham clinicopathological response index prognostic groups (NPRI-PGs). See text for details.

**Figure 5:** A-I. A-G Kaplan Meier curves showing breast cancer specific survival (BCSS) of HER2 overexpression/amplification (A) and triple negative breast cancer (B) patients, stratified according to NPRI prognostic groups (NPRI-PGs). C. Kaplan Meier curves showing BCSS of revised pathological TNM stage II/III (yp-TNM stage II/III patients stratified according to NPRI-PGs. D and E Kaplan Meier curves showing BCSS of Clinical-Pathologic Scoring System (CPS) score 2-3 (D) and CPS-Oestrogen receptor-histological grade (CPS-EG) score 3-4 (E). F and G Kaplan Meier curves showing BCSS of residual cancer burden (RCB) class II (F) and class III (G) patients stratified according to NPRI-PGs. See text for details. AC: Anthracycline, T: Taxane, AC-T: Anthracycline and Taxane. I: Fitted polynomial function curves and equations for BCSS summarises a broad relationship between the Nottingham clinicopathological response index (NPRI) value and median 5 (dashed line) and 10 (solid line) year survivals.
<table>
<thead>
<tr>
<th>Multivariable risk factor</th>
<th>BCSS at 5 years</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 95% CI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower Upper</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy (Adjuvant)</td>
<td>1.534 0.327 7.201</td>
<td>0.587</td>
</tr>
<tr>
<td>Age (continuous)</td>
<td>2.008 0.931 4.331</td>
<td>0.076</td>
</tr>
<tr>
<td>Histological grade (High)</td>
<td>1.236 0.846 1.805</td>
<td>0.273</td>
</tr>
<tr>
<td>HER2 (overexpression)</td>
<td>0.352 0.119 1.044</td>
<td>0.060</td>
</tr>
<tr>
<td>HR (negative expression)</td>
<td>1.657 0.777 3.537</td>
<td>0.192</td>
</tr>
<tr>
<td>CPS score (continuous)</td>
<td>1.319 0.725 2.400</td>
<td>0.365</td>
</tr>
<tr>
<td>RCB score (continuous)</td>
<td>.903 0.516 1.579</td>
<td>0.720</td>
</tr>
<tr>
<td>NPRI score (continuous)</td>
<td>3.965 2.082 7.550</td>
<td>0.00003</td>
</tr>
</tbody>
</table>

**A) Training cohort (Anthracycline)**

**B) Internal validation cohort (Anthracycline + Taxane)**
### A) External validation cohort (Anthraclines +/- Taxane;)

<table>
<thead>
<tr>
<th>Multivariable risk factor</th>
<th>BCSS at 5 years</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Chemotherapy (neo-adjuvant)</td>
<td>1.897</td>
<td>0.647</td>
</tr>
<tr>
<td>Age (continuous)</td>
<td>1.135</td>
<td>0.682</td>
</tr>
<tr>
<td>Histological grade (High)</td>
<td>1.632</td>
<td>0.976</td>
</tr>
<tr>
<td>HER2 (overexpression)</td>
<td>0.66</td>
<td>0.164</td>
</tr>
<tr>
<td>HR (negative expression)</td>
<td>0.425</td>
<td>0.159</td>
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<tr>
<td>CPS score (continuous)</td>
<td>0.852</td>
<td>0.451</td>
</tr>
<tr>
<td>RCB score (continuous)</td>
<td>1.670</td>
<td>0.934</td>
</tr>
<tr>
<td>NPRI score (continuous)</td>
<td>4.410</td>
<td>1.748</td>
</tr>
</tbody>
</table>

### B) Pool of the three cohorts (n=426)

<table>
<thead>
<tr>
<th>Multivariable risk factor</th>
<th>BCSS at 5 years</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Chemotherapy (neo-adjuvant)</td>
<td>1.444</td>
<td>1.084</td>
</tr>
<tr>
<td>Age (continuous)</td>
<td>1.36</td>
<td>1.056</td>
</tr>
<tr>
<td>Histological grade (High)</td>
<td>1.632</td>
<td>0.976</td>
</tr>
<tr>
<td>HER2 (overexpression)</td>
<td>1.253</td>
<td>1.001</td>
</tr>
<tr>
<td>HR (negative expression)</td>
<td>0.622</td>
<td>0.348</td>
</tr>
<tr>
<td>CPS score (continuous)</td>
<td>1.444</td>
<td>0.885</td>
</tr>
<tr>
<td>RCB score (continuous)</td>
<td>1.542</td>
<td>1.156</td>
</tr>
<tr>
<td>NPRI score (continuous)</td>
<td>2.526</td>
<td>1.767</td>
</tr>
</tbody>
</table>

*Note: The values are hazard ratios (HR) with 95% confidence intervals (95% CI).*
Figure 3

A) Training cohort (AC/FEC)  
- RCB score alone  
  AUC = 0.711  
  95% CI: 0.612 - 0.810  
  p = 0.0003

B) Internal validation cohort (AC/FEC+T)  
- RCB score alone  
  AUC = 0.657  
  95% CI: 0.532 - 0.783  
  p = 0.012

C) External validation cohort (AC/FEC+/-T)  
- RCB score alone  
  AUC = 0.724  
  95% CI: 0.606 - 0.842  
  p = 0.0019

D) Combined cohorts (n=426)  
- RCB score alone  
  AUC = 0.731  
  95% CI: 0.668 - 0.794  
  p = 1.0 \times 10^{-9}

NPRI score alone  
- AUC = 0.854  
  95% CI: 0.777 - 0.931  
  p = 0.000000001

Prognostic model* alone  
- AUC = 0.837  
  95% CI: 0.762 - 0.911  
  p = 0.000000008

Prognostic model** + NPRI score  
- AUC = 0.877  
  95% CI: 0.809 - 0.944  
  p = 0.000000001

Prognostic model* alone  
- AUC = 0.829  
  95% CI: 0.740 - 0.918  
  p = 1.6 \times 10^{-6}

Prognostic model** + NPRI score  
- AUC = 0.863  
  95% CI: 0.781 - 0.946  
  p = 9.4 \times 10^{-8}

Prognostic model* alone  
- AUC = 0.791  
  95% CI: 0.674 - 0.908  
  p = 5.6 \times 10^{-5}

Prognostic model** + NPRI score  
- AUC = 0.91  
  95% CI: 0.848 - 0.971  
  p = 6.7 \times 10^{-6}

Prognostic model* alone  
- AUC = 0.805  
  95% CI: 0.742 - 0.867  
  p = 7.2 \times 10^{-12}

Prognostic model** + NPRI score  
- AUC = 0.884  
  95% CI: 0.819 - 0.948  
  p = 1.0 \times 10^{-7}
### A) Training whole patients

NPRI-PG1 (pCR)  
NPRI-PG2  
NPRI-PG3  
NPRI-PG4  
*p = 3.0 \times 10^{-16}*

| NPRI-PG1 | 26 25 22 19 15 14 12 12 10 08 06 04 04 03 01 01 01 |
| NPRI-PG2 | 66 64 60 46 38 25 21 20 16 15 10 08 06 03 00 00 00 |
| NPRI-PG3 | 67 57 43 32 27 24 18 16 13 08 07 04 01 01 01 01 00 |
| NPRI-PG4 | 13 10 04 02 02 01 00 00 00 00 00 00 00 00 00 00 00 |

### B) Internal validation cohort

NPRI-PG1 (pCR)  
NPRI-PG2  
NPRI-PG3  
NPRI-PG4  
*p = 1.1 \times 10^{-8}*

| NPRI-PG1 | 28 24 19 16 11 09 06 02 02 01 01 00 00 00 00 00 00 |
| NPRI-PG2 | 62 50 40 32 22 15 07 01 01 00 00 00 00 00 00 00 00 |
| NPRI-PG3 | 33 30 21 17 13 09 04 01 01 01 00 00 00 00 00 00 00 |
| NPRI-PG4 | 06 03 03 02 02 01 00 00 00 00 00 00 00 00 00 00 00 |

### C) External validation cohort

NPRI-PG1 (pCR)  
NPRI-PG2  
NPRI-PG3  
NPRI-PG4  
*p = 3.0 \times 10^{-5}*

| NPRI-PG1 | 22 22 20 18 15 12 09 05 04 03 02 00 00 00 00 00 00 |
| NPRI-PG2 | 17 17 16 15 14 13 09 07 05 03 03 01 00 00 00 00 00 |
| NPRI-PG3 | 54 54 50 44 38 31 17 10 08 07 03 01 01 00 00 00 00 |
| NPRI-PG4 | 32 31 26 20 14 08 04 03 01 00 00 00 00 00 00 00 00 |

### D) Combined three cohorts (n=426)

NPRI-PG1 (pCR; n=76)  
NPRI-PG2 (n=145)  
NPRI-PG3 (n=154)  
NPRI-PG4 (n=51)  
*p = 1.0 \times 10^{-17}*

### E) HR positive (n=252)

NPRI-PG1 (pCR; n=27)  
NPRI-PG2 (n=109)  
NPRI-PG3 (n=85)  
NPRI-PG4 (n=31)  
*p = 0.001*

### F) HR negative (n=174)

NPRI-PG1 (pCR; n=49)  
NPRI-PG2 (n=36)  
NPRI-PG3 (n=69)  
NPRI-PG4 (n=20)  
*p = 1.2 \times 10^{-20}*

---

Figure 4
A) HER2 overexpression (n=101)
NPRI-PG1 (pCR; n=23)
NPRI-PG2 (n=33)
NPRI-PG3 (n=36)
NPRI-PG4 (n=9)
p = 0.00003

B) Triple negative (n=129)
NPRI-PG2 (n=35)
NPRI-PG1 (pCR; n=28)
NPRI-PG3 (n=51)
NPRI-PG4 (n=15)
p = 1.4x10^-16

C) yp-TNM stage II/III (n=282)
NPRI-PG2 (n=90)
NPRI-PG3 (n=142)
NPRI-PG4 (n=50)
p = 1.0x10^-8

D) CSP score 2/3 (n=247)
NPRI-PG2 (n=769)
NPRI-PG1 (n=18)
NPRI-PG3 (n=113)
NPRI-PG4 (n=38)
p = 0.000005

E) CSP-EG score 3/4 (n=208)
NPRI-PG1 (n=29)
NPRI-PG2 (n=61)
NPRI-PG3 (n=114)
NPRI-PG4 (n=43)
p = 0.00000005

F) RCB class II (n=195)
NPRI-PG2 (n=88)
NPRI-PG3 (n=84)
NPRI-PG4 (n=23)
p = 0.00007

G) RCB class III (n=110)
NPRI-PG2 (n=30)
NPRI-PG3 (n=53)
NPRI-PG4 (n=27)
p = 0.000001

H) Breast cancer specific survival
5-year-BCSS: (y=-0.0424x^2-19.441x + 81.8, R^2=1)
10-year-BCSS: (y=-0.2774x+ 71.376, R^2 = 0.9771)
Nottingham Clinico-Pathological Response Index (NPRI) after neoadjuvant chemotherapy (Neo-ACT) accurately predicts clinical outcome in locally advanced breast cancer

Tarek M A Abdel-Fatah, Graham Ball, Andrew HS Lee, et al.

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