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Ductal carcinoma in situ and invasive breast cancer: diagnostic accuracy and prognosis

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Chapter 6

Prognostic value of residual disease after neoadjuvant therapy in HER2-positive breast cancer evaluated by Residual Cancer Burden, Neoadjuvant Response Index & Neo-Bioscore

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ABSTRACT

Purpose

In breast cancer, pathologic complete response (pCR) to neoadjuvant systemic therapy (NST) is associated with favorable long-term outcome. Trastuzumab-emtansine as additional adjuvant therapy improves recurrence-free survival of patients with HER2-positive breast cancer without pCR, but it is uncertain whether all patients without pCR need additional therapy. We evaluated the prognostic value of residual disease after trastuzumab-based NST in patients with HER2-positive breast cancer using Residual Cancer Burden (RCB), Neoadjuvant Response Index (NRI) and Neo-Bioscore.

Experimental Design

We included patients with stage II or III HER2-positive breast cancer, treated with trastuzumab-based NST and surgery at the Netherlands Cancer Institute between 2004 and 2016. RCB, NRI and Neo-Bioscore were determined. Primary endpoint was 5-year recurrence-free interval (RFI). A 3% difference compared with the pCR-group was considered acceptable as noninferiority margin on the 5-year RFI estimate, based on a proportional hazards model, and its lower 95% confidence boundary.

Results

A total of 283 women were included. Median follow-up was 67 months (interquartile range 44–100). A total of 157 patients (56%) with pCR (breast and axilla) had a 5-year RFI of 92% (95%CI, 88–97); patients without pCR had a 5-year RFI of 80% (95%CI, 72–88). Patients with an RCB=1 (N=40, 15%), an NRI-score between 0.75–0.99 (N=30, 11%), or a Neo-Bioscore of 0 to 1 (without pCR; N=28, 11%) have a 5-year RFI that falls within a pre-defined noninferiority margin of 3% compared to patients with pCR.

Conclusions

The RCB, NRI, and Neo-Bioscore can identify patients with HER2-positive breast cancer patients with minimal residual disease (i.e., RCB=1, NRI \geq 0.75 or Neo-Bioscore=0–1) after NST who have similar 5-year RFI compared with patients with pCR.

Introduction

Neoadjuvant systemic therapy (NST) is increasingly used in patients with high-risk breast cancer, in particular in case of HER2-positive disease. NST increases rates of breast-conserving surgery and enables response monitoring during therapy. In addition, the pathological response after therapy is increasingly recognized as prognostic indicator to guide further treatment. The recently published Katherine-study used non-pathologic complete response (non-pCR) to select patients for additional adjuvant therapy with the trastuzumab drug-conjugate trastuzumab-emtansine (T-DM1). T-DM1 reduced the relative risk of recurrence of invasive breast cancer or death with 50% and the risk of distant recurrence with 40% compared with trastuzumab alone(1). The effect was consistent in all subgroups. HR for patients with ypT0, ypT1a, ypT1b, ypT1mic, ypTis, ypT1 or ypT1c, ypT2, and ypT3 were 0.66 (95% confidence interval (CI), 0.44-1.00), 0.34 (95% CI, 0.19-0.62), 0.50 (95% CI, 0.31-0.82), and 0.40 (95% CI, 0.18-0.88), respectively. As patients' recurrence risk is related to the extent of residual disease after NST, adjuvant therapy might be adapted according to an individual patients' risk.

A pCR is associated with favorable long-term outcome, in particular for triple-negative and HER2-positive breast cancer (2-5). However, with the binary outcome of pCR, valuable response information is lost. Therefore, other response indices that quantify the amount of residual disease were developed including the Residual Cancer Burden (RCB), Neoadjuvant Response Index (NRI), and Neo-Bioscore. The RCB uses the diameter of residual disease, percentage of vital tumor cells, and diameter of largest involved lymph node to quantify residual disease (6). The quantification of residual disease based on the RCB is prognostic for long-term survival after neoadjuvant chemotherapy in hormone receptor-positive/HER2-negative, HER2-positive, and triple-negative subgroups of breast cancer (6). The NRI is a semicontinuous score between 0 and 1 in which the extent of downstaging of the primary tumor and involved axillary lymph nodes is measured (7). It has been shown to better reflect efficacy of NST than the binary pCR classification in breast cancer. Its value to predict recurrence-free survival was validated in an independent cohort of patients with triple negative breast cancer (7,8). The Neo-Bioscore uses clinical stage, pathological stage after NST, estrogen receptor (ER) and HER2-status, and nuclear grade to create seven response categories (9,10). The final score provides a more refined stratification for disease-specific survival than pretreatment clinical stage or final pathologic stage alone across breast cancer subtypes (9,10).

We compared the RCB, NRI, and Neo-Bioscore and established the long-term prognosis based on various categories of residual disease in HER2-positive breast cancer.

Statement of translational relevance

The prognostic value of minimal residual disease after neo-adjuvant breast cancer treatment has become increasingly important as it can aid decision making for additional adjuvant therapy. Non-pathological complete response was used as selection criterion in the recently published Katherine study that showed improved long-term outcome with adjuvant T-DM1; however, offering T-DM1 to all patients with non-pathological complete response may result in significant overtreatment if patients with minimal residual disease have a similarly good prognosis as those with no residual disease. In this study we used Residual Cancer Burden, Neoadjuvant Response Index and Neo-Bioscore to evaluate prognostic value of residual diseases after trastuzumab-based neoadjuvant therapy. We think that using these response indices could help to decide if patients need additional systemic therapy and therefore should (after validation) be incorporated in clinical practice.

Materials and Methods

Patients and data collection

All patients with primary invasive noninflammatory HER2-positive stage II or III breast cancer who received trastuzumab-based neoadjuvant therapy at the Netherlands Cancer Institute between November 2004 and February 2016 were included. Patients with bilateral breast cancer, those who did not undergo surgery for other reasons than inoperability, patients with progressive disease prior to surgery, and those with prior breast cancer <25 years ago were excluded.

Patients were identified from the Netherlands Cancer Institute's tumor registry. Patient, tumor, and treatment characteristics were extracted from the medical records. All patients received one full year of trastuzumab according to Dutch national guidelines, unless precluded by toxicity. Adjuvant chemotherapy in case of non-pCR was not in our institute's guideline. Endocrine therapy was given for 5 to 10 years adjuvantly according to up-to-date guidelines. HER2 positivity was defined as a score of 3+ by immunohistochemistry or gene amplification by *in situ* hybridization (11,12). ER and progesterone receptor (PR) positivity was defined as nuclear staining of $\geq 10\%$ based on European and Dutch guidelines (12,13). Clinical and pathologic staging was based on the tumor-node-metastasis (TNM) classification, American Joint Committee on Cancer (AJCC) stage 6th and 7th based on year of diagnosis. According to these guidelines, the presence of isolated tumor cells (<0.2mm) in the lymph nodes was classified as pN0.

Clinical nodal staging was based on all available information from imaging and results of the sentinel node procedure. The subclassification of a positive nodal stage in N1, N2, or N3 was based on the number and localization of positive lymph nodes, similar to the pathologic nodal staging system of the TNM classification. This adapted counting of positive lymph nodes based on radiology results was used because we could not distill from the patients' records whether the palpable lymph nodes were movable or fixed, and it may better reflect current practice.

This study was approved by the Review Board of the Netherlands Cancer Institute and conducted in accordance with the Declaration of Helsinki.

Response indices

pCR

Pathologic responses were assessed by breast pathologists at the Netherlands Cancer Institute and extracted from original reports. pCR was defined as no residual invasive tumor in breast and axilla (ypT0/is, ypN0).

RCB

The RCB quantifies the extent of residual disease after NST for patients into four categories. RCB=0 is equal to pCR for breast and axilla, RCB=1 indicates minimal residual disease, RCB=2 indicates intermediate residual disease and RCB=3 extensive residual disease (9). To calculate RCB scores, all surgical specimens (breast and axilla tissue) of patients without pCR were reviewed and scored (MvS) as described previously (6). In case of uncertainty of extent of residual disease slides were discussed with another breast cancer specialized pathologist (JW).

NRI

The NRI is a score between 0 and 1 and uses a ratio of pre-NST and post-NST information to classify patients. A score of 1 represents pCR in breast and axilla and a score of 0 indicates no downstaging (or progression). The NRI calculation was based on original pathology reports as described previously (7). In brief, the NRI is the sum of a breast and a nodal response score divided by the maximum achievable score, which is based on the clinical tumor and nodal stage. For our analysis we used a slightly adapted version of the nodal response score (described above) to make it more suitable for current practice. The exact calculation and adapted allocation of points are summarized in Supplementary Table S1.

Neo-Bioscore

The Neo-Bioscore was calculated for each patient based on information from the medical records according to the previous reported staging system, with the exception that clinical nodal staging was performed as described above (9,10). The Neo-Bioscore gives points for higher clinical stages (higher than IIB), higher

pathologic stages (II and III), ER-negativity, grade 3 and HER2-negativity. A higher score represents more unfavorable prognostic characteristics. The maximum Neo-Bioscore in HER2-positive patients is 6, as none receives a point for HER2 negativity. Please note that a score of 0 does not represent pCR.

Statistical analyses

Descriptive statistics were used for baseline and surgery characteristics. For all patients and for the subgroup of patients without a pCR the median NRI was calculated.

Recurrence-free interval (RFI) was calculated as time from breast cancer diagnosis until locoregional or distant recurrence or death due to breast cancer, whichever came first (14). Patients without distant metastases at last follow-up or death due to other or unknown causes were censored at the corresponding dates. Breast-cancer-specific survival (BCSS) was defined as date of diagnosis until date of death due to breast cancer. Patients alive at last follow-up or who died due to other or unknown causes were censored at the respective dates. Database cutoff was set on October 2, 2018.

Follow-up time was calculated with the reverse Kaplan-Meier method. Cox proportional hazards models were used to provide hazard ratios (HR) and estimate the RFI-probabilities at five years with their corresponding 95% confidence interval (CI). In order to allow for nonlinearity of their effects, the NRI and Neo-Bioscore were entered as a continuous variable with a restricted cubic-spline transformation. Four knots were chosen so that the resulting model would have approximately 10 events per degree of freedom. For the NRI however it was not possible to place 4 knots so 3 were placed instead. It was not possible to place even 3 knots in a meaningful way for the RCB, because it has only 4 categories. Therefore, a quadratic polynomial model was used instead of a spline-curve for RCB.

A 3% difference in RFI was defined as noninferiority margin. The 3% margin is internationally used in treatment decisions whether to add chemotherapy (15). The cutoff of the NRI score was chosen such that the 5-year RFI-estimate at the cutoff and the lower bound of the 95% CI were within a margin of 3% from the estimate and lower 95% CI bound of the pCR group. For the RCB and Neo-Bioscore we used the predefined categories (6,9,10). The number of patients identified in this way as a percentage of the non-pCR patients, was compared across the three methods with Fisher exact test.

P-values <0.05 were considered statistically significant; all tests were two sided. Statistical analyses were performed using R version 3.5.2.

Results

Patients

We identified 303 patients who were treated with neoadjuvant trastuzumab-based therapy between November 2004 and February 2016, at The Netherlands Cancer Institute. Of them, 283 met the inclusion criteria and were included in the analyses. Figure 1 summarizes numbers and reasons for exclusion. Baseline characteristics, treatment regimens, and surgery are summarized in Table 1.

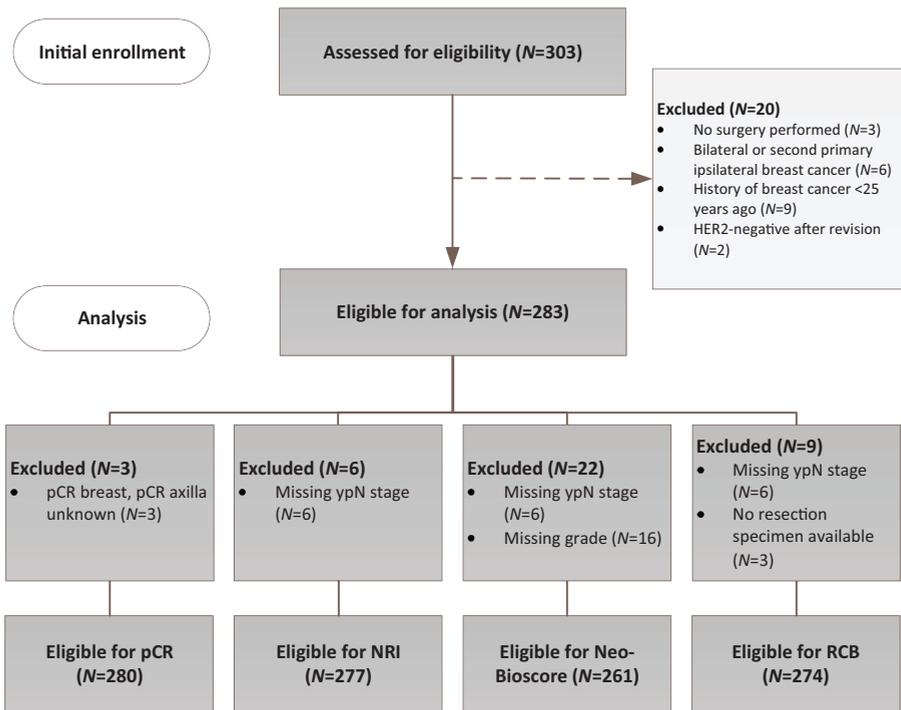


Figure 1. Consort flow diagram of included and excluded patients for analysis. *N*, number of patients; pCR, pathologic complete response; NRI, neoadjuvant response index; RCB, residual cancer burden; ypN, pathological nodal stage.

Table 1. Patient and treatment characteristics (N=283)

		N	(%)
Age	Median age in years (range)	48	(24-82)
Clinical tumor stage	TX	1	(<1)
	T1	32	(11)
	T2	178	(63)
	T3	70	(25)
	T4	2	(1)
Clinical nodal stage	N0	75	(27)
	N1mi	2	(1)
	N1	123	(43)
	N2	36	(13)
	N3	47	(17)
Clinical stage	IIA	77	(27)
	IIB	87	(31)
	IIIA	70	(25)
	IIIB	2	(1)
	IIIC	47	(17)
ER-status	Negative	135	(48)
	Positive	150	(52)
PR-status	Negative	194	(69)
	Positive	88	(31)
Tumor grade	1-2	127	(45)
	3	140	(49)
	Unknown	16	(6)
Histology	Ductal	261	(92)
	Lobular	13	(5)
	Other	9	(3)
<i>Neoadjuvant therapy regimen</i>			
Taxane-based	PTCb	176	(62)
	PTCb-Ptz	40	(14)
	PTCb → Vinorelbine/T	2	(<1)
Anthracycline/taxane	PTCb → FECT	13	(5)
	AC → PTCb	8	(3)
	AC → PT	2	(1)
	EC → PT	1	(<1)
	AC → PTCb-Ptz	1	(<1)
	FECT-Ptz → PTCb-Ptz	39	(14)
	Other	Vinorelbine/T	1
Neoadjuvant pertuzumab	No	203	(72)
	Yes	80	(28)
<i>Surgical treatment</i>			
Type of breast surgery	Breast conserving surgery	166	(59)
	Mastectomy (directly or later)	116	(41)
	No breast surgery ^a	1	(<1)
Axillary node dissection	No	142	(50)
	Yes	141	(50)

Table 1. Continued.

		N	(%)
<i>Adjuvant treatment</i>			
Adjuvant 1 year of trastuzumab-therapy completed	No	10 ^b	(4)
	Yes	273	(96)
Adjuvant endocrine therapy in case of ER-positive tumor	No	3 ^c	(2)
	Yes	147	(98)

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; PTCb, paclitaxel, trastuzumab, carboplatin; Ptz, pertuzumab; FEC, 5-fluorouracil, epirubicin, cyclophosphamide; AC, doxorubicin, cyclophosphamide; PT, paclitaxel, trastuzumab; EC, epirubicin, cyclophosphamide.

^a One patient had an occult breast cancer

^b Five patients discontinued adjuvant trastuzumab treatment because of toxicity during neoadjuvant or adjuvant trastuzumab treatment, three patients declined adjuvant trastuzumab treatment, two patients did not start with adjuvant trastuzumab treatment for unknown reasons.

^c Three patients declined endocrine therapy.

Response indices and 5-year RFI per category

The median follow-up was 67 months (interquartile range (IQR) = 44–101). In total, there were 37 patients (13%) who experienced an RFI-event: 5 patients had a locoregional recurrence and 32 patients had distant metastases as first RFI-event. The 5-year RFI for all patients was 87% (95% CI, 82–91).

One-hundred and fifty-seven patients (56%) achieved pCR. The pCR rate was significantly higher in ER-negative compared with ER-positive tumors (74% versus 40%, $P < 0.001$). The 5-year RFI was 92% (95% CI, 88–97) for patients with pCR and 80% (95% CI, 72–88) for patients without pCR. As we defined the noninferiority margin as a maximum of 3% decrease in RFI, the extra patients with residual disease should have a 5-year RFI of minimum 89%, with a 95% lower bound CI of at least 85%.

One-hundred and sixty-one patients (59%) were classified as RCB=0. In the group with residual disease, 40 patients (15%) were classified as RCB=1, 61 (22%) as RCB=2, and 12 (4%) as RCB=3 (Fig. 2A). RCB was significant for RFI prognosis ($p < 0.0001$) when modeled with a polynomial shape, although the test for nonlinearity was not significant ($p = 0.18$). Relative hazard rates per RCB score and estimated 5-year RFI per class with corresponding 95% CI are shown in Fig. 3A and B and Supplementary Table S2. As can be distilled from the table, patients with an RCB=1 ($N = 40$), 35% of patients without pCR meet the noninferiority margin of 89% 5-year RFI, and thus have a similar good prognosis as the pCR patients.

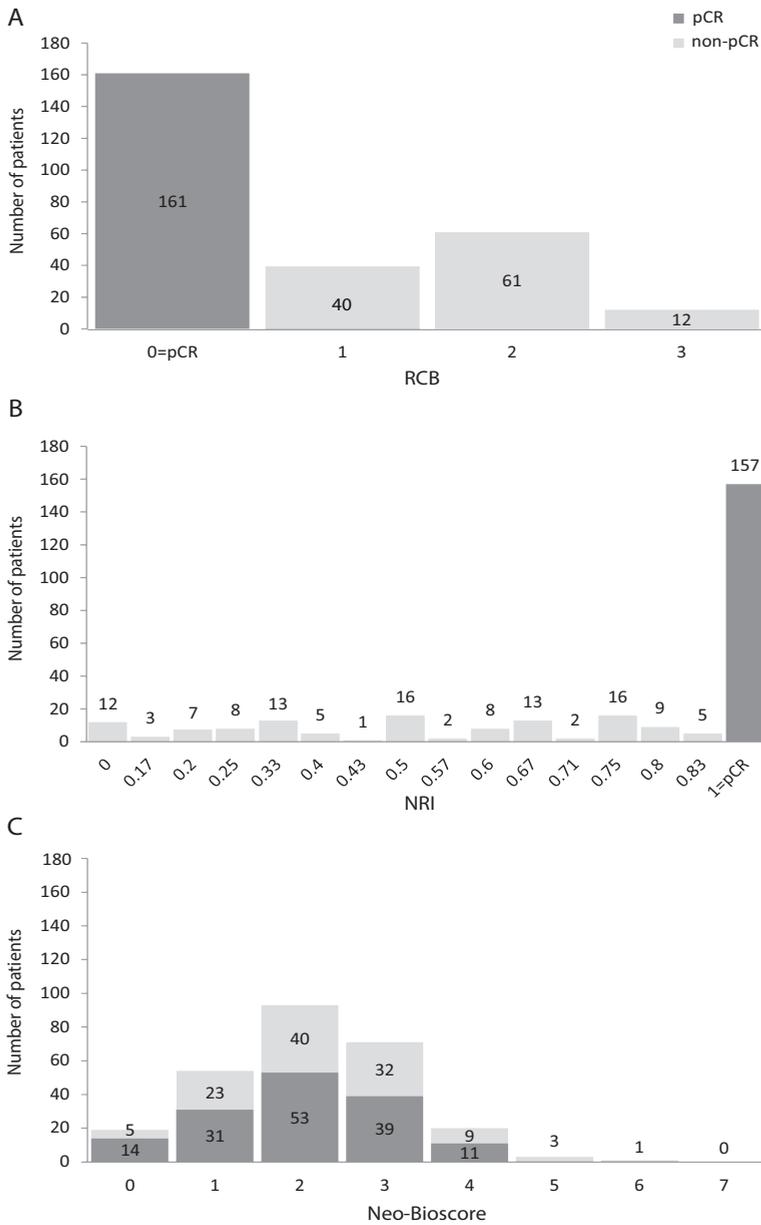


Figure 2. Distribution of patients in the different response indices. A, distribution of RCB scores; B, distribution of NRI scores; C, distribution of Neo-Bioscore scores. Legend: dark grey = pCR, light grey = residual disease. RCB, residual cancer burden; NRI, neoadjuvant response index; pCR, pathological complete response.

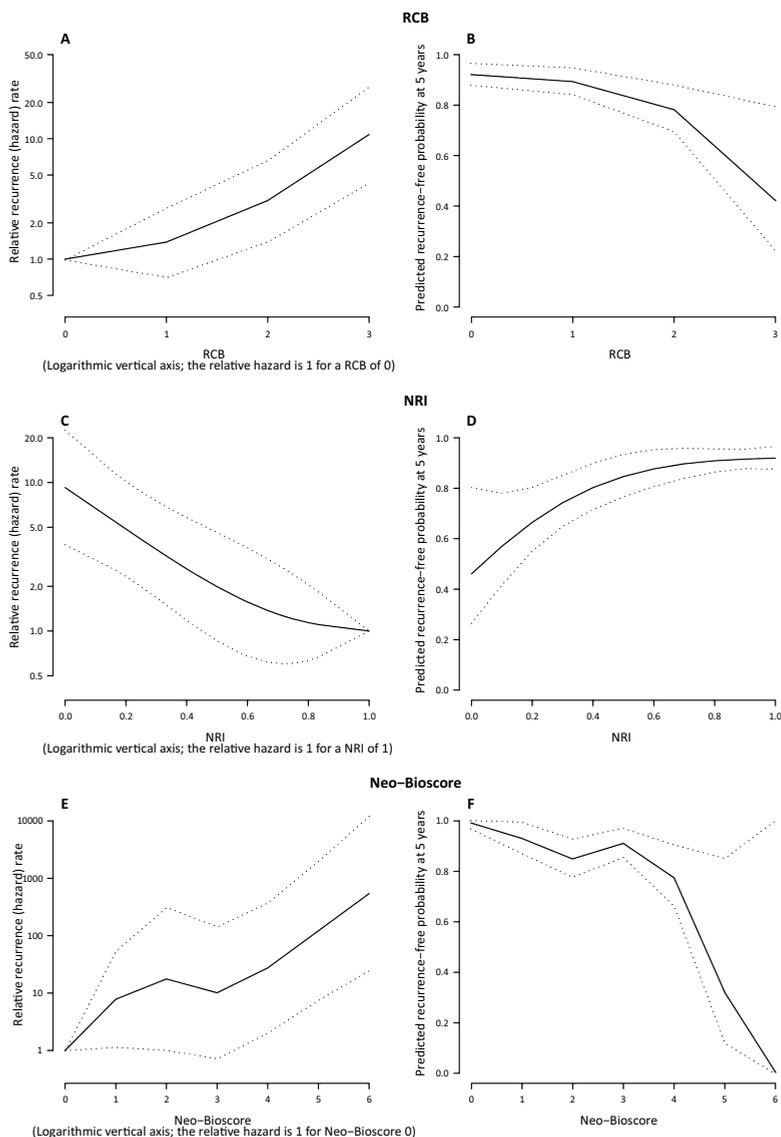


Figure 3. Relative hazard rates for the 5-year RFI prediction for RCB, NRI en Neo-Bioscore A, Relative hazard rate for predicted 5-year recurrence-free-interval per RCB score in a quadratic polynomial model. The relative hazard is 1 for RCB=0; B, Five-year RFI estimates per RCB score. The 95%CI is shown with the dotted lines. C, Relative hazard rate for predicted 5-RFI per NRI score in a proportional hazards model treating NRI as continuous variable. The relative hazard is 1 for NRI=1; D, Five-year RFI estimates per NRI score. The 95%CI is shown with dotted lines. E, Relative hazard rate for predicted 5-year RFI per Neo-Bioscore score in a proportional hazards model treating Neo-Bioscore as continuous variable. F, Five-year RFI estimates per Neo-Bioscore. The 95%CI is shown with dotted lines. RCB, residual cancer burden; NRI, neoadjuvant response index.

The median NRI was 1.00 (IQR= 0.60-1.00) in all patients and 0.50 (IQR =0.31-0.75) in the subgroup of patients who did not achieve pCR. The distribution of all NRI scores is shown in Fig. 2B. NRI was significant for RFI prognosis ($p<0.0001$) when modeled with a restricted cubic spline with 3 knots, although the test for nonlinearity was not significant ($p=0.30$). The relative hazard rates per NRI score are shown in Fig. 3C. Five-year RFI predictions per NRI score are shown in Fig. 3D and Supplementary Table S3. For NRI scores ≥ 0.75 -0.99 the corresponding 5-year RFI is higher than 90% (95% CI, 85-96; Supplementary Table S3) and meet the noninferiority margin. As a result, 30 patients without pCR (25% of patients without pCR) were identified by NRI with similar good prognosis as pCR, this was not significantly different from the number identified by the RCB ($p=0.09$).

The distribution of the Neo-Bioscore in the overall cohort is summarized in Fig. 2C. Neo-Bioscore was significant for RFI prognosis ($p<0.0001$) when modeled with a spline curve with 4 knots, and the test for nonlinearity was significant ($p=0.008$). Relative hazard rates per Neo-Bioscore score are shown in Fig. 3E. Five-year RFI-estimates with 95% CI per score are given in Fig. 3F and Supplementary Table S4. Patients with Neo-Bioscores of 0 or 1 have a higher estimated 5-year RFI compared with patients achieving pCR; 99% (95% CI, 97-100) and 93% (95% CI, 87-99), respectively. These categories jointly comprise 72 patients, of whom 45 patients achieved pCR and 28 patients did not. As a result 28 patients (10%) without pCR were identified by Neo-Bioscore with similar good prognosis as pCR (not significantly different from the RCB, $p=0.11$).

Overlap of patients classified in the categories that meets the noninferiority margin by each response measure is shown in Supplementary Fig. S1. The difference as a percentage of non-pCR patients was not significantly different across the 3 methods ($p=0.14$).

Discussion

We evaluated the prognostic value of residual disease using the RCB, NRI, and Neo-Bioscore in a HER2-positive breast cancer patient cohort to select a subgroup with minimal residual disease after NST but similar long-term outcome as patients achieving pCR. Indeed, the RCB, NRI and Neo-Bioscore were all able to identify a group of patients within the 3% noninferiority margin of the 5-year RFI as the pCR-group, that is 92% (95% CI, 88-97).

Our findings underline the clinical importance of response indices that accurately predict long-term outcome of patients after neoadjuvant systemic therapy. An adequate neoadjuvant response measure serves at least two purposes. First, a response index with demonstrated prognostic value may aid selecting patients

for more, less or no additional adjuvant therapy. Second, the more accurate the magnitude of response can be assessed, the better we can evaluate the true effect of new treatments in neoadjuvant trials. We showed that all response measures give more prognostic information than the binary pCR index and thereby select a subgroup that could be considered similar to patients achieving pCR.

In our cohort, the 5-year RFI for all patients was 87%, patients who achieved pCR had an estimated 5-year RFI of 92%. This is comparable with 5-year follow-up data in studies evaluating trastuzumab in HER2-positive breast cancer. In the BCIRG-006 study 5-year disease-free survival of the two groups of patients who received trastuzumab was 82% and 84% (16). Recurrence-free survival of the subgroup with HER2-positive breast cancer, who achieved pCR according to Symmans and colleagues (17) was 95%. In the NeoSphere study, all treatment groups combined, 5-year disease-free survival was 85% for patients achieving pCR (18). However, these 5-year survival data leave room for improvement in the treatment of HER2-positive breast cancer. The recently published Katherine-study for patients who did not achieve pCR showed an improvement in invasive-disease-free survival and distant-recurrence-free survival for patients who received T-DM1 compared with patients who received trastuzumab-monotherapy adjuvantly (HR 0.50; 95% CI, 0.39-0.64). Overall survival results were not mature yet. The improvement was seen regardless of the size of the residual tumor, with some suggestion of a stronger effect in case of more extensive residual disease (1). Masuda and colleagues (19) showed benefit in overall survival from adjuvant capecitabine therapy after NST in patients with triple negative breast cancer who did not achieve pCR. Two trials are currently evaluating the effect of adjuvant chemotherapy in patients not achieving pCR after NST; one adds capecitabine (NCT03684863) in patients with HER2-positive breast cancer and the other is open for all breast cancer subtypes and adds eribulin (NCT01401959). Aside from optional additional adjuvant chemotherapy, all patients with HER2-positive breast cancer receive a total of 1-year trastuzumab, partly adjuvant. The optimal duration of adjuvant trastuzumab is questioned and three studies evaluated noninferiority of 6 months trastuzumab to the arbitrary set 12 months that is considered standard. Studies that compared 6 versus 12 months adjuvant trastuzumab all showed similar survival curves for shorter or longer duration, although noninferiority was not shown in all studies (20–22). Patients with an excellent prognosis based on a pCR or near pCR may be suitable candidates to further pursue a strategy to reduce the duration of trastuzumab-treatment adjuvantly. In order to select patients for additional adjuvant therapy and optimize the balance between improving outcome and forego overtreatment, adequate response measures are crucial.

The RCB, NRI and Neo-Bioscore definitions of minimal residual disease do not identify the exact same patient populations. Discrepancies appear due to unequal

weighing of tumor features, including lymph node status, ER, HER2 and grade. The RCB grants relatively high value to positive lymph nodes compared with NRI and Neo-Bioscore. Neo-Bioscore uses ER/HER2-status and tumor grade additional to downstage calculation, whereas NRI purely uses downstaging. To the best of our knowledge it is not known whether downstaging or extent of residual disease is more important in terms of prognosis. Bianchini and colleagues analyzed PAM50 scores at baseline and in residual disease after neoadjuvant treatment in the NeoSphere study (22) and showed that PAM50 scores at surgery are more informative for prognosis than baseline scores. They also noticed an increase in Luminal A subtype and a decrease in Luminal B and HER2-enriched subtypes at surgery compared to baseline, reflecting the dynamic modulation of tumors to evolve or select a clone under pressure of therapy (23). One could argue that based on the dynamic modulation of tumors under pressure of treatment (23), the extent of residual is more informative for prognosis than the extent of downstaging. In contrast, downstaging may better reflect treatment effectiveness, when evaluating new treatments.

In daily clinical practice, local preference decides which evaluation method is used. To our knowledge, guidelines do not determine how NST should be evaluated. To calculate the NRI, no additional information is needed to the standard TNM classification. This makes this response index easy to incorporate in clinical practice. As we showed, use of different methods could give different prognostic information for individual patients. It is important to be aware of that when used in clinical setting. Additionally, the number of patients with non-pCR that is re-classified as low risk may depend on the method. In our cohort, the RCB seemed to identify most patients without pCR (35%) who meet the noninferiority margin, although the difference with the NRI (25%) was not statistically significant ($p=0.09$).

Although we were able to answer the clinically relevant question about the prognostic value of minimal residual disease after NST in HER2-positive breast cancer, our study has some limitations. First, our cohort is too small to draw conclusions from subgroups of patients with ER-positive versus ER-negative disease. Some ER-positive tumors might derive more benefit from the adjuvant endocrine therapy, which is reflected in the 5-year RFI but not in the response score. Small numbers also precluded subgroup analysis of patients treated with both trastuzumab and pertuzumab as neoadjuvant therapy separately. However, we think that the type of therapy needed to accomplish tumor downstaging is less important than the fact that it is accomplished.

Second, we used information from pathology reports to calculate the NRI and Neo-Bioscore and reviewed surgical specimens from the non-pCR group to score the RCB. This resulted in four patients classified as RCB=0 without pCR in the original pathology report. Furthermore, the RCB is sometimes hard to assess retrospectively, especially when the macroscopic information is incomplete. Consequently, the

scores contain a level of uncertainty. However the 5-year RFI-estimates per RCB group correspond well with previously described results (17), and therefore we think our results are reliable. Third, nonlinearity of the association of 5-year RFI with Neo-Bioscore was significant in our cohort, but not with RCB or NRI. However, it seems unreasonable to assume that these associations are linear, which is why we modeled them with a nonlinear shape nevertheless. In fact, it seems reasonable to believe that with 37 events, the test for nonlinearity had low power. Therefore, the shape of the curve is somewhat uncertain and that is why we defined our noninferiority criterion in terms of the lower bound of the 95% CI. Ideally, our results should be validated in an independent cohort. Actually, conclusive proof of non-inferiority requires a randomized trial.

Despite the limitations, we think that our study reflects daily clinical practice, which makes these response indices suitable to use in clinical practice and make these outcomes relevant.

To conclude, the RCB, NRI, and Neo-Bioscore are able to select a group of HER2-positive breast-cancer patients with minimal residual disease that have a similar good prognosis as patients with pCR. These patients may not benefit from adjuvant therapy with T-DM1, trastuzumab, pertuzumab, neratinib or additional chemotherapy. Validation of our outcomes is needed before these response measures can be incorporated into clinical practice and help to identify which patients may or may not benefit from additional adjuvant systemic therapy.

Additional information

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Supplementary files

Supplementary Table S1. NRI calculation

NRI calculation				
NRI^a	$= \frac{(a - b + c) + (d - e)}{(a + 1) + d}$ = Breast response score + Nodal response score Sum of maximal achievable response score			
Breast response score				
a	Clinical T stage (cT)			
b	Pathological T stage (ypT) ^b			
c	Response based on pathology			
	Assignment of points: no pCR = 0 points near pCR ^c (<0.5cm) = 1 point pCR = 2 points			
Nodal response score				
d	Clinical N stage (cN)			
e	Pathological N stage (ypN)			
Detailed scoring system for nodal response score				
Baseline clinical stage	d	Post-therapy pathological stage	e	d-e
cN0, clinically negative or FNA/SNP negative	0	ypN0/no ALND/no SNP	0	0
cN1 ^d , on imaging	1	ypN1(mi) (≤3 micro/macrometastases)	0	1
(≤3 LNNs) and FNA/SNP+		ypN0	1	0
cN2 ^d , on imaging	2	ypN1(mi) (≤3 micro/macrometastases)	0	2
(4-9 LNNs) and FNA/SNP+		ypN0	1	1
		ypN2 (4-9 macrometastases)	2	0
cN3 ^d , on imaging	3	ypN0	0	3
(≥10 LNNs and/or infra-/ supraclavicular/ parasternal) and FNA/SNP+		ypN1(mi) (≤3 micro/macrometastases)	1	2
		ypN2 (4-9 macrometastases)	2	1
		ypN3 (≥10 metastases and/or supra-/infraclavicular)	3	0

^a In case of progressive disease NRI=0^b cannot be <1^c The presence of only isolated tumor cells (<0.2mm) in the lymph nodes was classified as ypN0.^d In case of cN1-3 without pathological nodal staging after neoadjuvant therapy (ypNx), NRI cannot be calculated

Supplementary Table S2. RCB-scores and 5-year RFI-estimates

RCB	Number of patients (%)		5-year RFI	(95% CI)	
0	161	(59)	92%	(88-97)	
1	40	(15)	90%	(85-95)	<i>Non-inferiority</i>
2	61	(22)	79%	(70-89)	<i>margin</i>
3	12	(4)	43%	(23-80)	

Estimated from a proportional hazards model using RCB in a quadratic polynomial model.

Supplementary Table S3. NRI scores and 5-year RFI-estimates

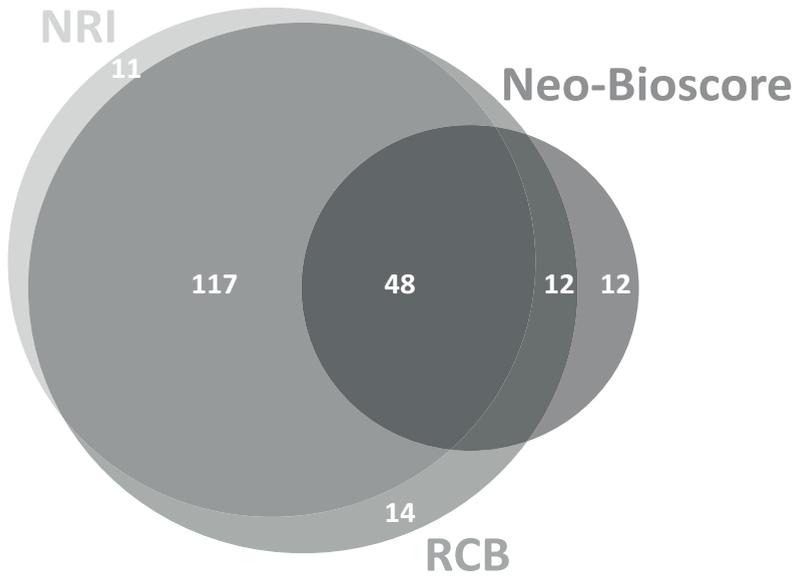
NRI	Number of patients (%)		5-year RFI	(95% CI)	
0	12	(4)	46%	(26-80)	
0.16	3	(1)	64%	(51-79)	
0.20	7	(3)	67%	(55-80)	
0.25	8	(3)	71%	(61-83)	
0.33	13	(5)	77%	(67-87)	
0.40	5	(2)	80%	(72-90)	
0.43	1	(<1)	82%	(73-91)	
0.50	16	(6)	85%	(77-94)	
0.57	2	(1)	87%	(80-95)	
0.60	8	(3)	88%	(81-95)	
0.67	13	(5)	89%	(83-96)	
0.71	2	(1)	90%	(84-96)	<i>Non-inferiority</i>
0.75	16	(6)	90%	(85-96)	<i>margin</i>
0.8	9	(3)	91%	(86-96)	
0.83	5	(2)	91%	(87-96)	
1	157	(57)	92%	(88-97)	

Estimated from a proportional hazards model treating NRI as continuous variable.

Supplementary Table S4. Neo-Bioscore scores and 5-year RFI-estimates

Neo-Bioscore	Number of patients (%)		5-year RFI	(95% CI)	
0	19	(7)	99%	(97-100)	
1	54	(21)	93%	(87-99)	<i>Non-inferiority</i>
2	93	(36)	85%	(78-93)	<i>margin</i>
3	71	(27)	91%	(85-97)	
4	20	(8)	77%	(66-91)	
5	3	(1)	32%	(12-85)	
6	1	(<1)	0.6%	(0-1)	
7	n.a.		n.a.	n.a.	

Estimated from a proportional hazards model treating Neo-Bioscore as continuous variable.



Supplementary Figure S1. Overlap and discrepancies in patients with 5-year estimated RFI $\geq 89\%$ (non-inferiority margin lower bound of 95% CI $\geq 85\%$)
NRI, neoadjuvant response index; RCB, residual cancer burden

