Predictors of successful neoadjuvant treatment in HER2-positive breast cancer

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Abstract. The prognosis of local or locally advanced human epidermal growth factor receptor 2 (HER2)-positive breast cancer after a complete response from neoadjuvant systemic treatment (NAT) is excellent. However, some of the patients succumb to their disease, so novel predictive factors to identify these patients at risk are needed. Retrospective data from 119 patients treated at the Helsinki University Hospital Comprehensive Cancer Centre (Helsinki, Finland) were collected. All patients had *in situ* hybridization-confirmed HER2-positive breast cancer and underwent NAT with a curative intention. The primary tumours were relatively large, most patients had cytologically confirmed lymph node metastases and the treatments used were current regimens. A total of 63 (52.1%) patients had a pathological complete response (pCR) to neoadjuvant therapy. Achieving pCR predicted longer disease-free survival (DFS; P=0.0083) but not overall survival (P=0.061). The patients with a pCR had an estimated DFS rate of 96.8% at 5 years, compared with only 59.7% of the patients with non-pCR. Radiological complete response (CR) was associated with pCR (P=0.00033), although imaging yielded 30.4% false-negative and 36.9% false-positive results. The association between the radiological CR and pCR was more obvious in oestrogen receptor-negative tumours. Moderate (compared with strong) immunohistochemical HER2 expression predicted a lower chance of pCR (P=0.0078) and worse breast cancer-specific survival (P=0.0015). In conclusion, pCR after NAT served as an important prognostic factor in women with high-risk HER2-positive breast cancer. The patients with only moderate immunohistochemical HER2 expression had a lower chance of reaching a pCR, as well as a shorter breast cancer-specific survival.

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Introduction

Neoadjuvant therapy (NAT) is an increasingly used treatment modality for human epidermal growth factor receptor 2 (HER2)-positive early-stage breast cancer (1). It has several advantages: it allows for downstaging the tumour size and provides access to an important prognostic factor, pathological complete response (pCR), defined as no invasive cancer left in the breast or axillary lymph nodes. Achieving pCR increases the incidence of favourable survival outcomes (2-6), whereas patients who do not obtain pCR benefit from the escalation of adjuvant treatment, such as trastuzumab emtansine in the HER2-positive subtype (7).

Discovering novel predictive factors and the identification of patients who do not benefit from conventional NAT are important topics of research. HER2-positive and hormone receptor negative breast cancers respond better to NAT on average (2,6). The use of targeted agents against HER2-positive cancer improves treatment results even further: a meta-analysis by Pathak *et al* (8) including 491 patients in five randomised, controlled trials concluded that the addition of trastuzumab more than doubled the achieved pCR rates (Risk Ratio (RR) 2.20; 95% confidence interval (CI) 1.62-2.99) compared to other targeted therapies. Dual-HER2-blockade seems to be even more effective in this regard. Meta-analyses have shown that combining trastuzumab with either lapatinib or pertuzumab increases pCR rates clinically meaningfully in breast and axillary lymph nodes, independent of the concurrent chemotherapy used (9,10).

In the present observational study, we systematically collected the clinical data of patients with HER2-positive breast cancer receiving modern NAT in a single centre in Finland. Since some of the patients still have poor prognoses after NAT, we aimed to identify the factors contributing to a higher probability of obtaining pCR and a longer survival.

Materials and methods

Patients and data. The clinical data was collected retrospectively from patients with HER2-positive breast cancer receiving NAT in Helsinki University Hospital Comprehensive Cancer Centre, Finland, during 2005-2022. The patient cohort was collected from the Helsinki University Hospital database using the free-text search term 'neoadj*' along with a pathologist's diagnosis of HER2-positivity. This cohort was

manually processed to ensure they met the eligibility criteria (i.e., HER2-positive breast cancer patients receiving NAT with a curative intention). The decision to use NAT for each patient was discussed in a multidisciplinary meeting at the Helsinki University Hospital. Patients with distant metastases were excluded, but lymph node metastases above the collarbone level or in the internal mammary chain were accepted in select cases if the patient had been evaluated as being suitable for NAT with a potentially curative intention.

The pathological characteristics of the tumours (Table I) were determined before the onset of NAT by core needle biopsy samples of the tumour, or, if not available, by fine needle aspiration samples of the lymph nodes (8 patients; 7.6%). HER2-positivity was assessed by immunohistochemistry (IHC), and positive results were confirmed in all cases using chromogenic *in situ* hybridization (CISH) according to the American Society of Clinical Oncology/College of American Pathologists guidelines (11). Two patients with negative IHC but positive CISH were also included. The HER2 status had to be positive preoperatively to be suitable for the study. Postoperative histopathological characteristics were determined either from the residual tumour samples or the lymph nodes, if no breast tumour was available.

Evaluation of responses to NAT. The responses to NAT were evaluated by using ultrasound, magnetic resonance imaging, or, in rare cases, computed tomography. Radiological CR was defined as the disappearance of clearly enhanced lesions before surgery. Pathological response was assessed from the surgical breast samples, and pCR was defined as no invasive cancer left in breast or lymph nodes.

Statistical analysis. Statistical analyses were conducted using SPSS version 28.0.0.0 for Mac (IBM Corporation, Armonk, NY, USA). Associations were calculated with Fisher's exact test, except for oestrogen receptor (ER), progesterone receptor (PgR), and Ki-67, which were assessed as continuous factors with a range of 0-100%. For continuous variables, Mann Whitney U test was used when evaluating their association with pCR. Wilcoxon Signed Rank test was used when comparing the expression of ER, PgR and Ki-67 between the pre-NAT and postoperative samples. Clinical and pathological baseline factors were tested against pCR with two-sided χ^2 test, with the exception of histological type, for which Fisher's exact test had to be used. Survival was analysed using the Kaplan-Meier method and the log-rank test. RRs with 95% confidence intervals (CI) were calculated using Cox regression. Disease-free survival (DFS) was defined as the time between surgery and the time of the first local or distant recurrence. Breast cancer-specific survival (BCSS) was defined as the time between diagnosis and confirmed death due to breast cancer. Overall survival (OS) was calculated from the date of surgery to the time of death or the end of the follow-up. P-values < 0.05 were considered significant.

Results

Of the 121 tumours (119 patients, two with bilateral HER2-positive breast cancer) treated with NAT, 63 (52.1%) had pCR. The median age of the patients was 54 years (range 22-81 years) and the median follow-up time was 42.0 months. One hundred patients (82.6%) were diagnosed during 2016-2020. The dataset included one male patient.

Table II summarises the treatment regimens used. The median number of neoadjuvant cycles was seven (range 3-13), and trastuzumab was included in all treatments except for one patient. Only one patient received three chemotherapy courses (treatment discontinued because of radiological CR), while the other patients received at least five cycles of NAT. Mastectomy and axillary lymph node evacuation was the most common type of surgery. 87.6% of the patients were also treated with trastuzumab in the adjuvant setting, 9.9% with some other adjuvant therapy, and 2.5% with no adjuvant therapy at all. Nine (7.6%) patients received trastuzumab emtansine postoperatively. The median number of postoperative trastuzumab cycles was 12 (range 0-17). Most of the patients (96.7%) received postoperative radiotherapy. Sixty-nine (58.0%) patients received adjuvant endocrine treatment.

In the last radiographical assessment during the neoadjuvant treatment, 56 tumours (46.3%) were in CR. Radiological progression was observed in only three (2.5%) of the tumours (in two patients, one with bilateral HER2-positive breast cancer progression). All three progressive tumours had several factors in common: Ki-67 level of 90%, 3-5% ER positivity, PgR negative, and high (3+) IHC HER2-positivity in the core needle biopsy before the initiation of the NAT. One of these patients was a frail, elderly woman who was treated with vinorelbine and trastuzumab, while the other patient received six cycles of a combination of docetaxel, trastuzumab and pertuzumab, followed by three cycles of doxorubicin and cyclophosphamide. In one (0.8%) case, the best response for NAT was categorised as a stable disease. In the postoperative pathological assessment in the patients with no pCR, the median size of all invasive cancer foci was 21 millimetres (range 2-341 millimetres). The median number of removed lymph nodes was 23 (range 12-30) in the patients with no pCR, while the median number of malignant lymph nodes was 2 (range 1-19). The median size of the malignant lymph nodes after surgery was 6 millimetres (range 1-45 millimetres).

Radiological CR was associated with pCR (P=0.00033). Even so, 30.4% of the patients with radiological CR still had invasive disease in the pathological assessment, and 36.9% of the patients with pCR had no radiological CR. The association between the radiological CR and pCR was more obvious in ER-negative tumours (ER expression below 10%; P=0.0030) than in ER-positive tumours (P=0.043).

Next, we analysed how clinical and pathological baseline factors associated with pCR (compared with no pCR). The patients with high (3+) IHC expression of HER2 had a higher chance of achieving pCR than those with moderate (2+) HER2 IHC expression (pCR rates 57.6 and 25%, respectively; Fisher's exact test P=0.0078). Other categorical variables, grade, menopausal status, histological type, or the presence of extracapsular extension in lymph node metastasis, did not predict the possibility of pCR.

From the continuous variables, which were analysed with the Mann-Whitney U test, both low baseline ER expression (P=0.0011) and low baseline PgR expression (P=0.00087) were associated with a pCR (vs. no pCR). Other continuous variables, age at diagnosis, or Ki-67 expression were not associated with achieving pCR.

Table I. Tumour characteristics before the initiation of NAT and in postoperative samples.

Variables	Before NAT, n (%)	Postoperative, n (%)	P-value
Histology			0.00026
Invasive ductal carcinoma	92 (76.0)	35 (28.9)	
Invasive lobular carcinoma	4 (3.3)	3 (2.5)	
Other invasive carcinoma	9 (7.4)	10 (8.3)	
DCIS, no invasive carcinoma	0 (0.0)	12 (9.9)	
No DCIS, no invasive carcinoma	0 (0.0)	61 (50.4)	
Missing/unavailable/pCR	16 (13.2)	0 (0.0)	
Histopathological grade			>0.99
Grade 1	0 (0.0)	5 (4.1)	
Grade 2	14 (11.6)	21 (17.4)	
Grade 3	75 (62.0)	24 (19.8)	
Missing/unavailable/pCR	32 (26.4)	71 (58.7)	
ER expression, %			0.76
0-9	55 (45.5)	13 (10.7)	
10-29	5 (4.1)	3 (2.5)	
30-59	7 (5.8)	0 (0.0)	
>59	54 (44.6)	31 (25.6)	
Missing/unavailable/pCR	0 (0.0)	74 (61.2)	
PgR expression, %			0.21
0-9	85 (70.2)	34 (28.1)	
10-29	11 (9.1)	6 (5.0)	
30-59	10 (8.3)	1 (0.8)	
>59	14 (11.6)	6 (5.0)	
Missing/unavailable/pCR	1 (0.8)	74 (61.2)	
HER2 immunohistochemistry			0.0080
Negative	2 (1.7)	4 (3.3)	
1+	0 (0.0)	3 (2.5)	
2+	20 (16.5)	23 (19.0)	
3+	99 (81.8)	16 (13.2)	
Missing/unavailable/pCR	0 (0.0)	75 (62.0)	
Ki-67 expression, %			0.001
<10	0 (0.0)	12 (9.9)	
10-20	13 (10.7)	10 (8.3)	
21-30	22 (18.2)	6 (5.0)	
>30	82 (67.8)	18 (14.9)	
Missing/unavailable/pCR	4 (3.3)	75 (62.0)	
Preoperative lymph node cytology			
Malignant	93 (76.9)		
Non-malignant	3 (2.5)		
Missing/unavailable	25 (21.7)		
Extracapsular lymph node extension in metastatic lymph nodes			
Present		13 (10.7)	
Not present		22 (18.2)	
Missing/unavailable/pCR		86 (71.1)	

The P-value column shows the result of the statistical testing between the pre-NAT and postoperative samples. The patients with missing values were not included in the analysis. ER, PgR and Ki-67 were assessed as continuous factors in statistical analyses and the presented P-values for these variables are from the Wilcoxon Signed Rank test. There were 47 paired samples available for ER analysis, 46 for PgR analysis and 44 for Ki-67 analysis. The large proportion of cases with missing data for extracapsular lymph node extension is related to the absence of cancer cells in lymph nodes. Extracapsular lymph node extension is not evaluable from the cytological (preoperative) samples. DCIS, ductal carcinoma *in situ*; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; NAT, neoadjuvant therapy; pCR, pathological complete response; PgR, progesterone receptor.

Table II. Oncological and surgical treatments received by the patients in the study cohort.

Treatment	Value
Neoadjuvant therapy, n (%)	
Docetaxel + trastuzumab + pertuzumab followed by anthracycline + cyclophosphamide	39 (32.2)
Docetaxel + trastuzumab followed by anthracycline + cyclophosphamide	2 (1.7)
Trastuzumab + taxane (no anthracycline)	28 (23.1)
Trastuzumab + pertuzumab + taxane (no anthracycline)	23 (19.0)
Other trastuzumab-containing treatment	28 (23.1)
Other non-trastuzumab-containing treatment	1 (0.8)
Median number of NAT cycles (range)	7 (3-13)
Type of breast surgery, n (%)	
Resection + axillary lymph node evacuation	32 (26.4)
Mastectomy + axillary lymph node evacuation	75 (62.0)
Resection + sentinel lymph node biopsy	9 (7.4)
Mastectomy + sentinel lymph node biopsy	5 (4.1)
Adjuvant therapy, n (%)	
None	3 (2.5)
Trastuzumab	106 (87.6)
Other	12 (9.9)
Median postoperative trastuzumab cycles (range)	12 (0-17)
Postoperative radiotherapy, n (%)	
Yes	117 (96.7)
No	4 (3.3)
Endocrine adjuvant therapy[a], n (%)	
None	2 (2.9)
Tamoxifen	13 (18.8)
Aromatase inhibitor	31 (44.9)
LHRH analogue + tamoxifen	11 (15.9)
LHRH analogue + aromatase inhibitor	5 (7.2)
Other	7 (10.1)

[a]Only patients with oestrogen receptor expression >0% (n=69) were included. LHRH, luteinizing hormone-releasing hormone; NAT, neoadjuvant therapy.

Postoperative samples had a lower Ki-67 expression (P=0.001), and lower immunohistochemical HER2-positivity (P=0.0080) than preoperative samples (Table I). In postoperative analysis, HER2 status as defined by ISH assay changed from positive to negative in 13.2% of the evaluable cases (n=38), and similarly in both of the HER2 IHC 2+ and 3+ groups.

In the whole study population, the DFS rate at five years was 71.8%, OS was 86.6%, and BCSS was 91.8%, respectively. The patients with pCR had a longer DFS than those with invasive cancer remaining after surgery (survival at five years 96.8 and 59.7%, respectively; log-rank P=0.0083; Cox regression RR 0.17; 95% CI 0.039-0.76) (Fig. 1). OS was not significantly longer in the patients with pCR after NAT (log-rank P=0.061; Cox regression RR 0.31; 95% CI 0.084-1.1). In BCSS, no difference was observed between the pCR and non-pCR groups (log-rank P=0.21; Cox regression RR 0.37; 95% CI 0.072-1.9). Radiological CR was not associated with any of the survival endpoints.

The patients who had tumours with high (3+) HER2 immunostaining preoperatively had longer BCSS than those with moderate HER2+ immunostaining (log-rank P=0.0015; Cox regression RR 0.14; 95% CI 0.035-0.58) (Fig. 2). A trend of shorter DFS and OS was seen in the group with moderate HER2 immunostaining compared with strong staining (for DFS log-rank P=0.062; Cox regression RR 0.38; 95% CI 0.13-1.1 and for OS log-rank P=0.057; Cox regression RR 0.34; 95% CI 0.10-1.1, respectively). Preoperative ER, PgR or Ki-67 expression, grade, or the presence of extracapsular extension in lymph node metastases did not predict survival. Multivariate analyses were considered unreliable due to the low number of events and were thus not performed.

Discussion

This is the first study to show that patients with only moderate immunohistochemical HER2 expression, irrespective of positive ISH status, had a lower chance of reaching pCR and

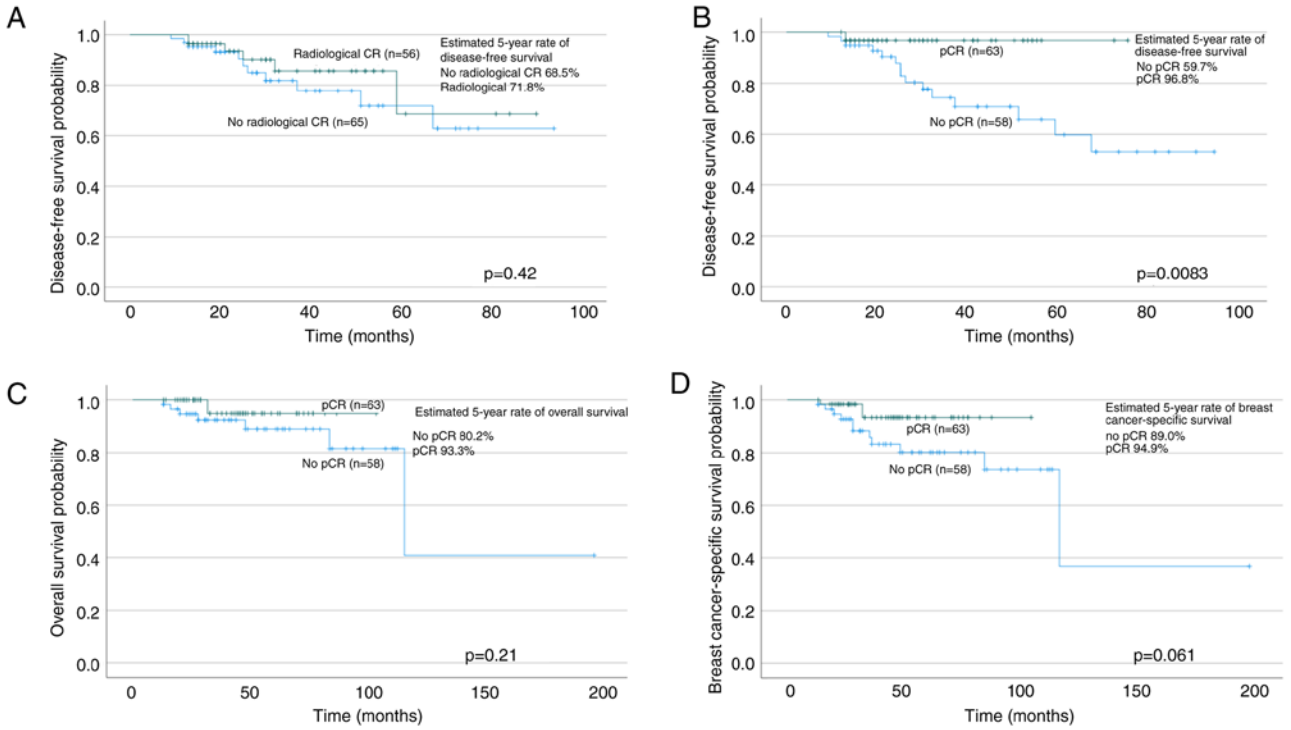

Figure 1. Kaplan-Meier curves of survival probability. (A) Achieving radiological CR for neoadjuvant treatment was not associated with disease-free survival. (B) pCR predicted longer disease-free survival compared with that of patients without this response. Pathological complete response did not predict statistically significantly (C) overall survival or (D) breast cancer-specific survival. CR, complete response; pCR, pathological complete response.

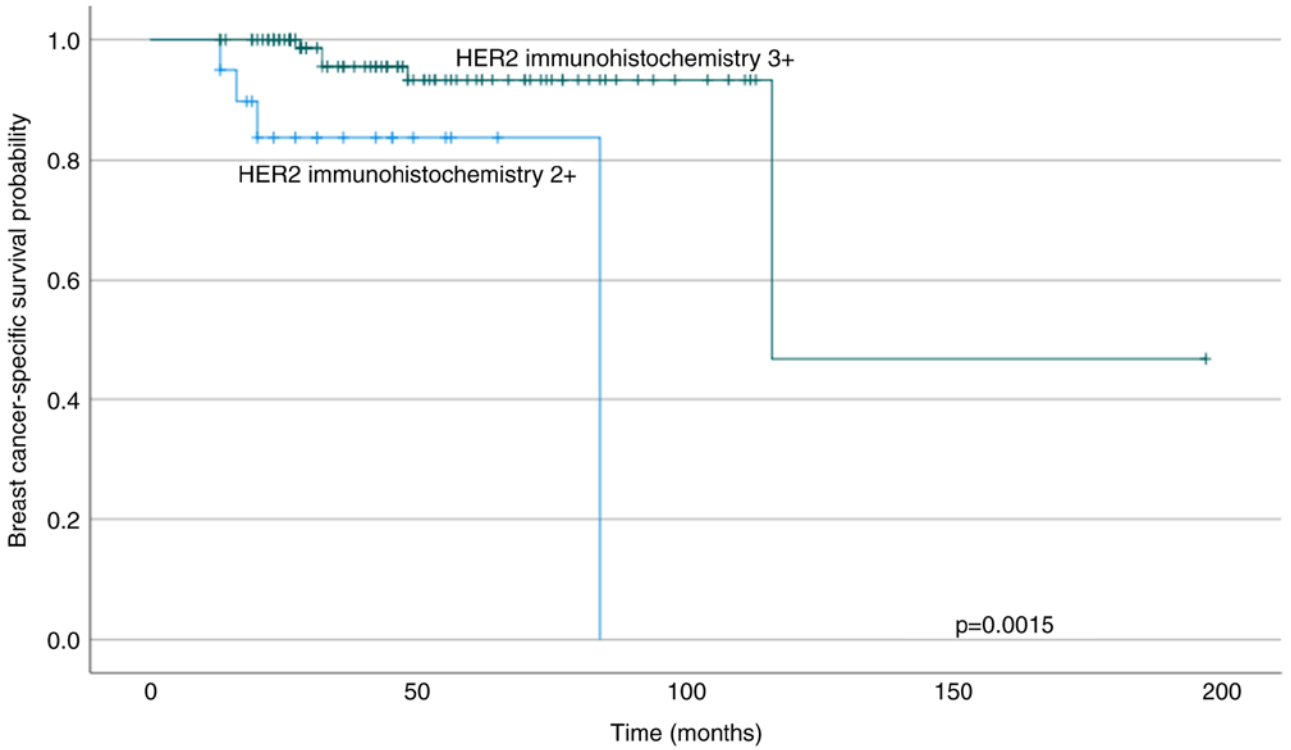

Figure 2. Immunohistochemical HER2 staining of 3+ predicted prolonged breast cancer-specific survival compared with HER2 immunostaining of 2+. HER2, human epidermal growth factor receptor 2.

also experienced worse survival rates. Another main finding was that only a moderate correlation between radiological and pathological CR existed. Finally, this study confirmed an excellent long-term outcome of the patients with HER2-positive breast cancer, in spite of large primary tumours and lymph node-involved diseases.

pCR was achieved in 52.1% of the ISH-confirmed HER2-positive tumours in our cohort, which is in accordance with previous observational evidence. For example, a study with 776 patients reported pCR rates of 51% when pCR was defined as ypT0/is ypN0 (12). Notably, most of our patients had locally advanced cancers with preoperatively large inoperable tumours and/or biopsy-confirmed multiple lymph node metastases. Consequently, in our study, those with residual cancer had a substantial cancer mass in both the breast and axilla.

Radiological CR was moderately associated with pCR, although approximately one-third of the tumours with radiological CR had invasive disease left in the pathological assessment, and more than one third of the tumours with pCR had no radiological CR. For a small number of patients, this could be due to the heterogenous imaging modalities used in evaluating the responses to NAT, as some of the patients' responses were assessed with ultrasonography, as well as the challenges in the evaluation of the contrast medium intensity. The correlation between imaging and the pathological evaluation was notably more accurate in ER-negative tumours. This correlation can result from the higher likelihood of false-negative results if the pCR rate is lower, or if there is non-mass or diffuse enhancement, as often observed in ER-positive tumours (13-15). These results are still consistent with the previous findings estimating the accuracy of MRI in HER2-positive breast cancer, which have shown radiological CR corresponding to a pCR in 70-73% of the patients, with the proportion increasing to 88% in hormone receptor-negative subgroup (16,17).

Achieving pCR after HER2-positive breast cancer NAT is known to be associated with improved survival, and our DFS results with RR of 0.17 (95% CI 0.039-0.76) were consistent with this previous evidence (2-4,6). The five-year DFS of only 59.7% in the non-pCR group reflects both the nature of large tumours and also the inherent drug resistance of HER2-positive and ER-positive tumours for the available treatments. Both the trend for shorter OS (RR 0.31; 95% CI 0.084-1.1) and BCSS (RR 0.37; 95% CI 0.072-1.9) in the patients with non-pCR were statistically non-significant, which may result from the limited statistical power in our cohort. A recent real-world study by LeVasseur *et al* (18) with a median follow-up of 7.5 years revealed a trend for improved BCSS and OS in patients with HER2-positive cancer obtaining pCR, although the finding did not reach statistical significance. In another retrospective study, combining pertuzumab to a trastuzumab-based neoadjuvant chemotherapy was observed to improve five-year BCSS and OS in early HER2-positive breast cancer, especially in patients younger than 50 years old (19). It is worth noting, that there is no randomised study showing that pertuzumab would improve overall survival when administered as neoadjuvant therapy, but the evidence is relying only applies to improved pCR rates in a phase II study (20). There were only two patients (three breast cancers) with progressive diseases in our material, all with almost absent ER expression, a total lack of PR expression, and a very high proliferation rate. Additionally, one of these patients only received an oncologically suboptimal combination of vinorelbine and trastuzumab due to her fragility.

There is previous observational evidence of strong HER2 IHC staining predicting improved pCR rates. In our material with HER2 status confirmed with ISH in all cases, we also found that the HER2 IHC result of 3+ more than doubled the chances for pCR compared with the IHC result of 2+. In an observational study, IHC 3+ tumours treated with neoadjuvant trastuzumab had pCR rates (ypT0 ypN0) of 46.0%, whereas IHC 2+/FISH-positive tumours had pCR rates of 25.0% (21). Wang *et al* (22) reported pCR rates of 55.1% in IHC 3+ group and only 17.6% on IHC 2+/ISH-positive groups, although ISH was not performed in all IHC 3+ cases. However, to the best of our knowledge, this is the first time when a high IHC score of 3+ has also been associated with improved BCSS. One possible explanation for this is the higher HER2 heterogeneity in HER2 2+ tumours which has been associated with worse outcomes (23-25). Although in the present study, the number of patients with HER2 immunostaining of 2+ was limited (n=20), these results still suggest that treatment intensification strategies should be investigated in this patient population and encourage more intensive surveillance after NAT.

As a single-site, retrospective study, this study contains several inherent limitations. The number of the events was too small to conduct reliable multivariate analysis. At the time of the treatments, trastuzumab emtansine was rarely used as an adjuvant therapy, although it is now routinely used in non-pCR HER2-positive patients in high-income countries and could have improved DFS (but not OS) rates (7). Still, most of the patients received pertuzumab as part of their NAT, and all but one patient were treated with trastuzumab. As the breast imaging interval was not standardised, this variation could have led to the underestimation of the radiological CR rates. Similarly, the residual cancer burden was not standardized in the pathology reports.

We conclude that in this study of patients, most with locally advanced HER2-positive breast cancers and treated with contemporary procedures, pCR after NAT still served as a precise predictor of excellent prognosis. In addition, as a novel finding, the patients with only moderate IHC HER2 expression, irrespective of positive ISH status, seemed to have a lower chance of reaching pCR. Since they also seem to carry a higher risk of breast cancer-related death, novel treatments, such as trastuzumab deruxtecan in the treatment of metastatic HER2-low breast cancer (26), would be needed to improve their prognosis.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PK and JM conceptualized and supervised the study, and oversaw the project administration, resources, software used

and methodology. The data were collected by ENH, who also wrote the original draft. The data were analysed by ENH and PK and interpreted by all authors. All authors participated in the writing and editing process of the manuscript. ENH and PK confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was based on secondary data and did not involve human participants. Therefore, ethics committee approval or patient consent for participation was not required.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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