RESEARCH ARTICLE

Predictive Value of IHC4 Score for Pathological Response to Neoadjuvant Chemotherapy in Hormone Receptor-Positive Breast Cancer

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Abstract

Purpose: This study aimed to explore the value of IHC4 in predicting pathological response after neoadjuvant chemotherapy in patients with hormonal receptor (HR)-positive breast cancer (BC). Materials and Methods: In this retrospective exploratory study, data for 68 HR-positive BC patients who received neoadjuvant chemotherapy were recorded. IHC4 scores were calculated based on estrogen receptors/progesterone receptors, Ki-67 and HER2 status. Logistic and ordinal regression analyses in addition to likelihood ratio test were used to explore associations of IHC4 scores and other clinico-pathological parameters with pathological complete response (pCR) and pathological stage. Results: Taking the 25th percentile as the cut-off, a lower IHC4 score was associated with an increased probability of pCR (low; 52.9% vs. High; 21.6%, OR=4.1, 95% CI= 1.28-13.16, p=0.018) and a lower pathological stage (OR =3.9, 95% CI=1.34-11.33, p=0.012). When the IHC4 score was treated as a continuous variable, a lower score was again associated with an increased probability of pCR (OR=1.010, 95% CI=1.001-1.018, p=0.025) and lower pathological stage (OR=1.009, 95% CI= 1.002-1.017, P=0.008). Lower clinical stage was associated with a better pCR rate that was of borderline significance (P=0.056). When clinical stage and IHC4 score were incorporated together in a logistic model, the likelihood ratio test gave a P-value of 0.004 after removal of the IHC4 score and 0.011 after removal of the stage, indicating a more significant predictive value of the IHC4 score for pCR. Conclusions: This study suggests that the IHC4 score can predict pathological response to neoadjuvant chemotherapy in HR-positive BC patients. This finding now needs to be validated in a larger cohort of patients.

Keywords: IHC4 score - neoadjuvant chemotherapy - pathological response - hormonal receptors - breast cancer

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Introduction

Neoadjuvant chemotherapy is usually utilized in inoperable breast cancer (BC) patients to allow for surgical resection and to facilitate breast conservative surgery in borderline candidates (Aapro 2011). However, compelling evidence displayed improved long term survival with the achievement of pathological complete response (pCR) (Cortazar et al., 2014). This raised a critical need for predictors of response to neoadjuvant chemotherapy to optimize therapeutic outcome. The Oncotype DX recurrence score, based on a 21-gene signature, has been proven to estimate the risk of recurrence in early hormone receptors (HR)-positive BC patients treated with adjuvant hormonal therapy (Dowsett et al., 2010). It has also been shown to predict response to neoadjuvant chemotherapy. In Patients treated with neoadjuvant docetaxel, those with a high recurrence score were more likely to have pCR (Chang et al., 2008). In addition, pCR was linked with higher expression of proliferation-related genes and lower expression of estrogen receptor (ER)-related genes (Gianni et al., 2005). However, the cost and complexity of gene-based assays highlight the need for developing more simplified predictive tools.

Immune-histochemistry (IHC)-4 score was developed based on the assessment of four key proteins in breast cancer including ER, progesterone receptors (PR), HER2 and Ki-67 (Cuzick et al., 2011). In the adjuvant setting of ER-positive patients, IHC4 score was found to provide prognostic information similar to that provided by Oncotype DX-recurrence score (Cuzick et al., 2011). This score was further validated in the cohort of

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patients involved in the tamoxifen exemestene adjuvant multinational (TEAM) study (Christiansen et al., 2012). However, the value of IHC4 score as a predictor of response to neoadjuvant chemotherapy is yet to be explored.

The present study aimed to explore the value of IHC4 score in predicting pathological response to neoadjuvant chemotherapy in HR-positive BC patients. This score may provide a simple non-costly approach to predict the benefit of neoadjuvant chemotherapy in this group of BC patients.

Materials and Methods

Study population

We screened female patients with histologically confirmed BC who received neoadjuvant chemotherapy and presented to three institutes in Saudi Arabia from September 2012 to September 2013. We included patients with locally advanced disease or those who required downsizing to be eligible for conservative breast surgery. We selected patients who have received at least four cycles of neoadjuvant chemotherapy; anthracycline-based, taxane-based or both. The type of chemotherapy was at the discretion of the treating physician. Patients must have available data of ER, PR, HER2 and Ki-67 at diagnostic biopsies and only those with HR-positive phenotype were included.

Study design and procedures

In this retrospective exploratory study, clinico-pathological data was collected including pre-chemotherapy tumour size and lymph node (LN) status as assessed at baseline mammograms/ breast ultrasound. Clinical stage before starting chemotherapy was recorded according to the TNM staging system of the American Joint Committee on Cancer (AJCC), 7th edition. Tumour phenotype (ER, PR, HER2 status) and Ki-67 were recorded from reports of diagnostic biopsies taken before starting chemotherapy. HR- positivity was defined as ER and/or PR-positive tumour status and IHC4 score was calculated as stated below. Treatment data was recorded including type and number of chemotherapy cycles. Pathological tumour size and nodal status were recorded from pathological reports of definitive breast surgery. We defined pCR as the absence of any invasive carcinoma or carcinoma in situ in the breast or axillary LNs at the time of definitive breast surgery. Partial response (PR) and stable disease (SD) were defined according to Sataloff classification where PR corresponds to presence of evidence of therapeutic effect (<50% or >50%) while SD shows no evidence of therapeutic effect. Approvals from the institutional review board of contributing institutions were obtained before starting the study procedures.

Calculation of IHC4 score

As reported by Cuzick et al., 2011, ER was quantified using the H-score, which was defined as the percentage of cells staining weakly plus two times the percentage of cells staining moderately plus three times the percentage of cells staining strongly. The variable ER10 was obtained by dividing the H-score by 30 to obtain a variable with a range of 0-10. PR was scored as the percentage of cells staining positive with a cut-off of 10%. PR10 was obtained by dividing this percentage by 10. HER2 status was assessed by IHC in addition to FISH confirmation in cases with (2+) by IHC. Tumours that were (3+) by IHC or (2+) with FISH-positive testing were considered HER2 positive. Ki67 was recorded according to the percentage of positively staining malignant cells. Manual reading of Ki67 was utilized and Ki67 values were divided by 2.5 for correction of higher values compared to computer-aided reading method that was used in generating the below equation. IHC4 score was then calculated as follows (Cuzick et al., 2011):

$$IHC4=94.7 \times \{-0.100\ ER10 - 0.079\ PR10+0.586\ HER2 + 0.240 \ln (1+10 \times Ki\ 67)\}$$

Statistical analysis

The data was analyzed using STATA version 11.0. Numeric data was presented as median as well as mean values ± standard deviation (SD). Categorical variables were presented as percentages. A logistic and an ordinal regression models were constructed using the pCR and the pathological stage after surgery as the dependent variables respectively. The following parameters were entered into each model as explanatory variables in a univariate manner: absolute value of the IHC4 score, clinical stage at diagnosis, other clinico-pathological parameters (such as age, menopausal status at diagnosis and multicentricity), type and number of chemotherapy cycles (<6 *vs*. 6-8). In addition, categorical explanatory variables were created by setting two cut-off values to divide the IHC4 scores at the median and the 25% percentile. The latter variables were used to study the association of pCR and pathological stage after surgery with IHC4 score categories. In addition, the test of trend was used to assess the relation of IHC4 score with pathological stage. For the 1st dependent variable (pCR), a multivariate model was constructed that included all factors with significant association in univariate analysis. A likelihood ratio test was performed to illustrate the impact of removing each variable from the multivariate model. A two-sided alpha level of <0.05 was considered significant for all comparisons.

Results

We screened 241 BC patients who received neoadjuvant chemotherapy in the contributing institutes within the above specified period. Data of ER/PR, HER2 status at diagnostic biopsies was available for all patients while results of Ki67 were available in 101 patients and only 68 of them were HR-positive. Among those 68 patients, infiltrating duct carcinoma was the predominant pathological type (91.2%), 25% were younger than 40 years and 63.2% were premenopausal at diagnosis. T4 tumours and clinical stage III were found in 19.1% and 63.2% of patients respectively. Only 3 patients with stage I disease who initially refused surgery were included. They have agreed later to go for definitive surgery after receiving primary chemotherapy course. Noteworthy, two thirds of our patients (67.6%) had luminal B subtype

with either Ki67 expressed in >14% of tumour cells or ER+/HER2+ irrespective of Ki67 level. The majority of patients received both anthracycline and taxane-based chemotherapy regimens (85.3%) (3-4 cycles of anthracycline-based followed by 3-4 cycles of taxane-based chemotherapy) (Table 1). All HER2-positive patients received trastuzumab combined with taxane-based chemotherapy.

Table 1. Characteristics of Study Population

Parameters	n (%)
Age at diagnosis	
≤40	16 (23.5)
> 40	52 (76.5)
Menopause	
Premenopausal	43 (63.2)
Postmenopausal	25 (36.8)
Pathology	
Infiltrating duct carcinoma	62 (91.2)
Lobular carcinoma	6 (8.8)
Grade	
1	4 (5.9)
2	44 (64.7)
3	20 (29.4)
Multicentric tumours	
Yes	14 (20.6)
No	54 (79.4)
Lymphovascular invasion	
Yes	30 (44.1)
No	38 (55.9)
Clinical stage	
I	3 (4.4)
II	22 (32.4)
IIIA	28 (41.2)
IIIB	15 (22.0)
Tumour size before chemotherapy	
T1	5 (7.4)
T2	23 (33.8)
T3	27 (39.7)
T4	13 (19.1)
Progesteron receptors	
Positive	57 (83.8)
Negative	11 (16.2)
Ki-67 expression	
≤14%	22 (32.4)
>14%	46 (67.6)
Parameters	n (%)
HER2	
Negative	54 (79.4)
Positive	14 (20.6)
Type of chemotherapy	
Anthracycline and taxan	58 (85.3)
Anthracycline or taxan	10 (14.7)
No of chemotherapy cycles	
<6	9 (13.2)
6-8	59 (86.8)
Response to chemotherapy	
Complete response	20 (29.4)
Partial response	31 (45.6)
Stable disease	14 (20.6)
Progressive disease	3 (4.4)
Pathological stage	
0	20 (29.4)
I	8 (11.8)
II	18 (26.5)
IIIA	11 (16.2)
IIIB	11 (16.2)

In our cohort, pCR was found in 29.4% of patients while PR, SD and progressive disease were encountered in 45.6%, 20.6% and 4.4% of patients respectively. Pathological stages I and II were found in 11.8% and 26.5% of patients respectively (Table 1). The distribution of IHC4 score in the study group is shown in (Figure 1) showing relatively symmetrical distribution (skewness = -0.138) and similarity to the Gaussian distribution (kurtosis = -0.567). The median value and the 25th percentile of IHC4 score were (-10.79) and (-58.28) respectively, while the mean value ± SD was (-10.68±67.77). When the 25th percentile was utilized as the cut-off, lower IHC4 score was associated with an increased probability of having pCR (low; 52.9% *vs*. High; 21.6%, OR=4.1, 95% CI= 1.28-13.16, p=0.018) (Table 2) as well as lower pathological stage (OR =3.9, 95% CI=1.34-11.33, p=0.012) (Table 3). Likewise, taking the median value as the cut-off, patients with lower IHC4 score were more likely to have pCR compared to those with higher score, however, the difference was not statistically significant (38.2% *vs*. 20.6% respectively, OR=2.4, 95% CI=0.81-7.04, p=0.115) (Table 2) and were more likely to have lower pathological stage (OR=3.18, 95% CI=1.31-7.71,

Table 2. Relation of IHC4 Score with Pathological Complete Response (pCR)

	pCR n (%)	No pCR n (%)	OR[a] (95%CI[b])	P
IHC4 (25th percentile)				
Low (n=17)	9 (52.9)	8 (47.1)	4.1 (1.28- 13.16)	0.018
High (n=51)	11 (21.6)	40 (78.4)		
IHC4 (median)				
Low(n=34)	13 (38.2)	21 (61.8)	2.4(0.81-7.04)	0.115
High(n=34)	7 (20.6)	27(79.4)		
Mean IHC4 score	-40.1	1.58	—	—
± SD[c]	± 78.88	± 59.24		
Median IHC4 score	-47.27	2.13	—	—

[a]OR, odds ratio; [b]CI, confidence interval; [c]SD, standard deviation

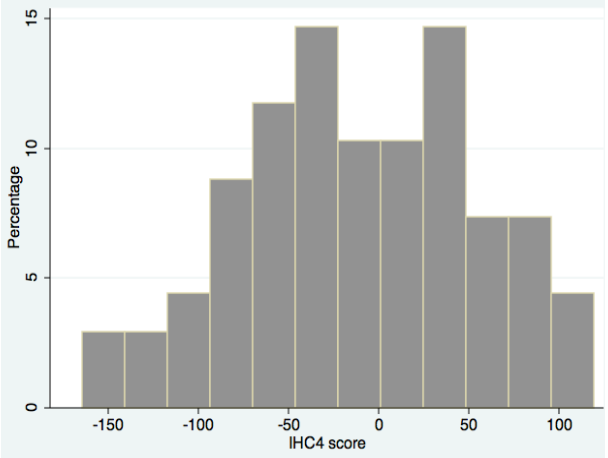

Figure 1. Histogram of IHC4 Score Distribution in the Study Group

Table 3. Relation of IHC4 Score with Pathological Stage After Surgery

	Pathological stage						
	0 n (%)	I n (%)	II n (%)	IIIA n (%)	IIIB n (%)	OR[a] (95%CI[b])	p- value
IHC4 (25th percentile)							
Low (n=17)	9 (52.9)	3(17.6)	3(17.6)	1(5.9)	1(5.9)	3.9	
High (n=51)	11 (21.6)	5(9.8)	15(29.4)	10(19.6)	10(19.6)	(1.34-11.33)	0.012
IHC4 (median)							
Low (n=34)	13 (38.2)	7(20.6)	6(17.6)	5(14.7)	3(8.8)	3.18	
High (n=34)	7 (20.6)	1(2.9)	12(35.3)	6(17.6)	8(23.6)	(1.31-7.71)	0.01
Mean IHC4 score ± SD[c]	-40.1 ± 78.88	-28.08 ±63.88	11.74 ± 57.93	-20.05 ± 48.02	28.17 ± 58.69	___	___
Median IHC4 score	-47.27	-43.33	30.09	-11.32	21.49	___	___

[a]OR, odds ratio; [b]CI, confidence interval; [c]SD, standard deviation

p=0.010) (Table 3).

Noteworthy, the median and mean IHC4 scores were much lower in patients who achieved pCR compared to those with no pCR (median; -47.27 *vs*. 2.13, mean± SD; -40.1 ± 78.88 *vs*. 1.58 ± 59.24, respectively) (table 2). Likewise, patients with pathological stage I had a much lower IHC4 score compared to those who had stage IIIB post chemotherapy (median; -43.33 *vs*. 21.49, mean± SD; -28.09 ±63.89 *vs*. 28.17±58.69 respectively) (table 3). When IHC4 score was treated as a continuous variable, lower score was significantly associated with an increased probability of pCR (OR=1.01, 95% CI=1.001-1.018, p=0.025) and lower pathological stage (OR=1.009, 95% CI= 1.002-1.017, P=0.008). By doing test of trend, IHC4 score was significantly correlated with the pathological stage, p=0.034.

Lower clinical stage was associated with a better pCR rate that was of borderline significance (p=0.056), while no significant associations were found between other parameters and pCR rate. Accordingly, the clinical stage and the IHCR score were incorporated in a bivariate logistic model. Using the likelihood ratio test, removal of IHC4 score from the bivariate model gave a chi square value (LR-χ^2) of 8.19, p=0.004. After removal of clinical stage from the model, LR-χ^2 was 11.1, p=0.011 indicating a more powerful predictive value of IHC4 score for pCR.

Discussion

In current clinical practice, neoadjuvant chemotherapy is commonly used for the treatment of breast cancer patients (Telli 2013). In a pooled analysis of data of 12 trials including almost 12,000 patients, achievement of pCR was associated with long term survival benefit in those with HER2-positive, triple negative BC in addition to patients with luminal-B disease (Cortazar et al., 2014). In addition, patients with lower pathological stage after neoadjuvant chemotherapy were associated with a better survival outcome (Kim et al., 2013). Despite the low pCR rates following neoadjuvant chemotherapy in HR-positive breast cancer (Telli, 2013), certain HR-positive patients still have appreciated benefit from adjuvant chemotherapy (Dowsett et al., 2010). This data raises the need to explore new predictive tools to optimize the use of neoadjuvant chemotherapy in HR-positive patients to select patients who are more likely to benefit from it.

The percentage of pCR in our study was high compared to previous reports that demonstrated low response rate in HR- positive tumours ranging from 2-10% (Colleoni et al., 2004; Kaufmann et al., 2012). Our cohort has several characteristics that may explain this unexpected high response rate to chemotherapy. Two thirds of patients were premenopausal and the majority received 6-8 cycles of both anthracycline and taxan-based chemotherapy. Two thirds of patients had luminal B subtype which is more likely to respond to chemotherapy (Lonning, 2012).

In the present study involving HR-positive patients, low IHC4 score was associated with better pCR and lower pathological stage after neoadjuvant chemotherapy. Noteworthy, IHC4 score and clinical stage had additive predictive powers for pCR, however, the score was more predictive for pCR which highlights its promising role in this regard. Cuzick et al., (2011) demonstrated improved survival outcome in patients with lower IHC4 score in the adjuvant setting. It is to be noted, however, that lower IHC4 score is linked with higher HR- positivity which is usually associated with favourable prognostic outcome but lower benefit from chemotherapy. Several reports have suggested that ER-negative tumours derive more benefit from neoadjuvant chemotherapy than their ER-positive counterparts (Barrios et al., 2009). In addition, the degree of response to chemotherapy was linked with degree of HR-positivity (Colleoni and Montagna, 2012).Yet, the recent 2012 overview analysis of Early Breast Cancer Trialist Collaborative Group displayed that chemotherapy benefit is independent of ER receptor status (Peto et al., 2012). In view of this, the higher response to neoadjuvant chemotherapy linked with lower IHC4 score among our cohort needs to be taken with caution.

In HR-positive patients, chemotherapy type and intensity seems to be of paramount value. Several reports displayed improved pCR in HR-positive patients with prolonged duration of chemotherapy (Colleoni and Montagna, 2012). In NSABP-B 27 trial, adding docetaxel after 4 cycles of doxorubicin and cyclophoshamide chemotherapy was associated with increased pCR in HR-positive patients (Bear et al., 2003). Furthermore, in a pooled analysis of the German neoadjuvant chemotherapy trials including more than 3000 women, the association of increased number of chemotherapy courses with pCR

was more pronounced in HR-positive compared to HR-negative patients (Minckwitz et al., 2011).

Among HR-positive patients, certain high-risk subgroups seem to derive increased benefit from intensified neoadjuvant chemotherapy such as ER-positive/HER2-positive tumours or ER-positive tumours with a high proliferation rate. Penault et al. (2009) showed improved outcome with the addition of taxanes to 3 cycles of FEC compared to 6 cycles of FEC alone in ER-positive patients with elevated Ki67 >20%. Similarly, in HER2-positive tumours, trastuzumab-containing chemotherapy produced high pCR in the range of 30-40% which is double that reported in HER2-negative patients (Gianni et al., 2010; Untch et al., 2011).

It is to be noted that high Ki-67 expression was predominant among our patients and 20% of our cohort, who were HER2-positive, received trastuzumab-containing chemotherapy. Chemotherapy type and intensity may be linked with this unexpected improved response to neoadjuvant chemotherapy in those patients with lower IHC4 score. Our study including mainly high-risk, HR-positive patients may raise the issue of the interplay between the type of treatment and tumour biology in formulating the overall outcome of HR-positive patients. It seems that more intense chemotherapy may translate the favourable prognosis of lower IHC4 score into better response to neoadjuvant chemotherapy as well.

Many questions are still to be answered. Proper utilization of IHC4 score to stratify patients according to differential response to neoadjuvant chemotherapy, integration of clinical characteristics with this biomarker score and how to apply the score for the individual patient in routine practice, are still open fields that need further work to enhance the utility of this score. In conclusion, lower IHC4 score seems to be associated with improved response to neoadjuvant chemotherapy in a group of predominantly high risk, HR-positive patients. This finding needs to be confirmed in a larger cohort of patients to validate the role of IHC4 score as a predictive tool in this context.

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