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The impact of Onco*type* DX® recurrence score of paraffin-embedded core biopsy tissues in predicting response to neoadjuvant chemotherapy in women with breast cancer

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Abstract.
BACKGROUND: Onco*type* DX® test is beneficial in predicting recurrence free survival in estrogen receptor positive (ER+) breast cancer. Ability of the assay to predict response to neoadjuvant chemotherapy (NCT) is less well-studied.
OBJECTIVE: We hypothesize a positive association between the Onco*type* DX® recurrence score (RS) and the percentage tumor response (%TR) after NCT.
METHODS: Pre-therapy RS was measured on core biopsies from 60 patients with ER+, HER2− invasive breast cancer (IBC) who then received NCT. Pre-therapy tumor size was measured using imaging. %TR, partial response (PR; >50%), pathologic complete response (pCR) and breast conserving surgery (BCS) rates were measured.
RESULTS: Median RS was 20 (2–69). Median %TR was 42 (0–97)%. PR was observed in 43% of patients. There was no association between %TR and pre-NCT tumor size, age, Nottingham score or nodal status ($p > 0.05$). No statistically significant association with %TR was seen with RS as a categorical or continuous variable ($p = 0.21$ and 0.7, respectively). Response to NCT improved as ER ($p = 0.02$) by RT-PCR decreased. Lower ER expression by IHC correlated with response ($p = 0.03$).
CONCLUSIONS: In patients with ER+ IBC receiving NCT, RS did not predict response to NCT using %TR. The benefit of the assay prior to NCT requires further study.

Keywords: Estrogen positive, 21 gene assay, tumor volume reduction, breast cancer, neoadjuvant, response

1. Introduction

Breast cancer is the second most common cancer among women in the US with an estimated 232,340 women being diagnosed with invasive breast cancer in 2013 [23]. Of those, between 30–40% will be diagnosed with a locally advanced breast cancer [4,23]. The most difficult challenge for surgical oncologists in the treatment of locally advanced breast cancer is operative planning.

Neoadjuvant chemotherapy (NCT) is standard of care for patients with locally advanced breast cancer. Although no study has clearly shown an increase in disease-free or overall survival compared with conventional adjuvant chemotherapy for all patients with breast cancer, in the patient population with locally advanced disease, this approach has advantages over adjuvant chemotherapy. An inoperable tumor

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may become operable or become amenable to breast-conserving surgery; thereby increasing the percentage of patients who can avoid mastectomy [11,21,27]. It allows the chance to monitor response to therapy, which has prognostic implications [25]. It also allows time for additional testing, such as genetic testing, which may affect eventual local therapy [15].

Patients with locally advanced triple negative or HER2 positive tumors are ideal candidates for NCT. They will generally receive adjuvant chemotherapy, so its use in the neoadjuvant setting only enhances the benefit of systemic therapy by expanding options for locoregional therapy [27]. In contrast, patients with luminal tumors (estrogen and/or progesterone receptor positive, HER2 negative) may not require adjuvant chemotherapy and thus the benefit of preoperative systemic therapy in this population is limited [10].

Several prognostic markers can be used to guide the decision to recommend chemotherapy to a patient with a luminal breast cancer. Both immunohistochemistry [26] and several genomic assays can define the risk of recurrence using endocrine therapy alone, and some can also predict benefit of adjuvant chemotherapy [12,18]. The most widely used genomic assay in the US is the Onco*type* DX® Breast Cancer Assay. It is designed to quantify the expression of a specific group of 21 genes (5 genes are reference genes) related to cell receptors, invasion and proliferation. The Recurrence Score, calculated using the Onco*type* DX® test, has been clinically validated as a predictor of the likelihood of distant recurrence in patients with node negative, estrogen receptor positive (ER+) breast cancer who were treated with adjuvant tamoxifen [1,17]. The Recurrence Score has also been shown to predict the likelihood of adjuvant chemotherapy benefit in the same patient population [18]. Patients with high results receive the largest benefit from the addition of chemotherapy while patients with low results receive little or no benefit from adjuvant chemotherapy.

The ability of such prognostic markers in predicting response to *neo*adjuvant chemotherapy, however, remains in question. Literature on the topic varies according to the assay used. While some support the use of assays in this capacity [2,7,8,14,16,22,24], others question their validity in predicting response to NCT [20]. Few studies concentrate only on ER+ patients and the ability to predict NCT response in this subset [7,14].

Our study evaluates the ability of the 21-gene assay (Onco*type* DX recurrence score®) to predict benefit of NCT in patients with ER+ breast cancer. We hypothesize that a positive association exists between the Recurrence Score from pre-therapy percutaneous large core needle biopsy (CNB) and the percentage tumor response (%TR) after neoadjuvant chemotherapy.

2. Methods

2.1. Patient selection

After receiving IRB approval for the study, clinical and pathologic data was collected retrospectively for 71 patients with ER+/HER2− invasive ductal carcinoma of the breast diagnosed with CNB and treated between 2011 and 2012. All patients were females, $\geqslant$18 years of age with no prior history of any cancer, including breast cancer. Patients were diagnosed with T1-3 N0-1 M0 tumor in which the tumor size prevented primary surgical management (either mastectomy or lumpectomy). Pre-treatment paraffin-embedded tissue samples were sent to Genomic Health, Inc. for Onco*type* DX® testing, which was performed following standard procedures while blinded to clinical and pathology data [17].

2.2. Systemic therapy

Patients received 24 weeks of one of the two standard neoadjuvant chemotherapy regimens that consisted of the following agents: doxorubicin, cyclophosphamide and a taxane. These were administered per NCCN guidelines [5]. Following completion of chemotherapy and further imaging, the patient and her physician decided if mastectomy, breast conserving surgery (BCS) or total mastectomy would be appropriate.

2.3. Measurement of response

Tumor response to chemotherapy was defined as (1) relative change in tumor size (mm), as measured by MRI and US, at baseline and after neo-adjuvant chemotherapy, (2) clinical (partial) response (PR) defined as at least 50% reduction in area on imaging, (3) pathologic complete response (pCR) defined as complete absence of viable invasive tumor cells on pathologic examination, including surgical margins and lymph nodes. A clinically significant endpoint was defined as the ability of the patient to undergo BCS.

Percentage tumor size reduction (%TR) was based on pre-therapy size (largest dimension) and detailed

pathology evaluation of the resection specimen. The pre-therapy tumor size was abstracted from clinical charts. Modality was selected in the following preferential order: MRI, ultrasound, mammogram, physical examination maximum dimension (uni-dimensional measurement). The post-therapy tumor size was defined as the product of: maximum dimension of tumor-bed (or area of fibrosis) × percentage cellularity (compared with pre-therapy biopsy) of the tumor-bed (or area of fibrosis) by microscopic exam. %TR was calculated as the difference between the pre and post-therapy tumor size divided by pre-therapy tumor size, multiplied by 100 (available at http://path.upmc.edu/onlineTools/ptvr.html).

2.4. Statistical considerations

The clinical and demographics characteristics of the study sample were summarized using descriptive statistics. The association between Recurrence Score and both clinical and pathologic response to neoadjuvant chemotherapy were assessed. Differences in continuous variables were analyzed using the Mann–Whitney U test. Categorical variables were analyzed using Chi-square tests. Linear regression models were used to examine if the changes in largest tumor dimension (mm) and/or %TR were associated with the Recurrence Score at baseline (i.e. core biopsy). Additionally other explorative analyses were conducted using the tumor volume (mm^3), instead of uni-dimensional tumor size (in mm), and using the ratio of the difference in pre- and post-treatment tumor measurements. Logistic regression was used to examine, by odds ratio (OR), the association between the Recurrence Score and the binary (i.e. presence, absence) response variables: clinical response and pathologic response. We examined the effect of Recurrence Score modeling it both as a continuous variable result as well as the 3 categories (low, intermediate and high risk). All hypotheses were tested with an α of 0.05. All statistical tests were performed using SAS/STAT® version 9.3 (Copyright © 2002–2010. SAS Institute, Inc., Cary, NC).

3. Results

Out of the total of 71 core biopsy samples submitted to Genomic Health for testing, two samples failed RNA extraction and the remaining 69 patient samples were processed by RT-PCR. There were no RT-PCR failures. 9 samples were identified as HER2+ by RT-PCR, and excluded from analysis of Recurrence Score versus tumor response, leaving 60 patients evaluable for that analysis. Patients who were HER2+ by RT-PCR remained in the study for purpose of comparison of clinicopathologic factors with tumor response.

Table 1
Clinicopathologic characteristics

	$n = 60$
Age	52 (range 28–86)
Tumor size	48 mm (range 10–230)
Receptor status by IHC	
ER positive	60 (100%)
PR positive	52 (87%)
HER2 positive	0 (0%)
Lymph node status at baseline	
Negative	50 (83%)
Positive	10 (17%)
Nuclear grade*	
1	4 (7%)
2	43 (73%)
3	12 (20%)

*1 patient with missing Nottingham score and grade.

Table 2
Recurrence Score (RS) distribution

Recurrence Score group	*n* (%)
Low risk (<18)	27 (45%)
Intermediate risk (18–30)	10 (17%)
High risk (⩾31)	23 (38%)

3.1. Patient/tumor characteristics

The mean patient age was 52 years (range 28–86). The mean tumor size (mm) was 48 (range 10–230). Of the 60 evaluable samples, 60 (100%) were ER positive and 52 (87%) were PR positive. 50 (83%) patients had no lymph node involvement, while 10 (17%) were clinically and/or pathologically N1 at presentation. The most common nuclear grade was 2 (73%) on the core biopsy (Table 1).

The 21-gene assay calculated by Genomic Health for each of the 60 evaluable specimens, demonstrated a low (<18) in 27 (45%), intermediate (18–30) in 10 (17%) and high (⩾31) Recurrence Score in 23 (38%) specimens (Table 2).

3.2. Response/outcome

All 60 patients completed 24 weeks of neoadjuvant chemotherapy, but none had a pCR. The median %TR was 42% (range 0–97%). A partial clinical response

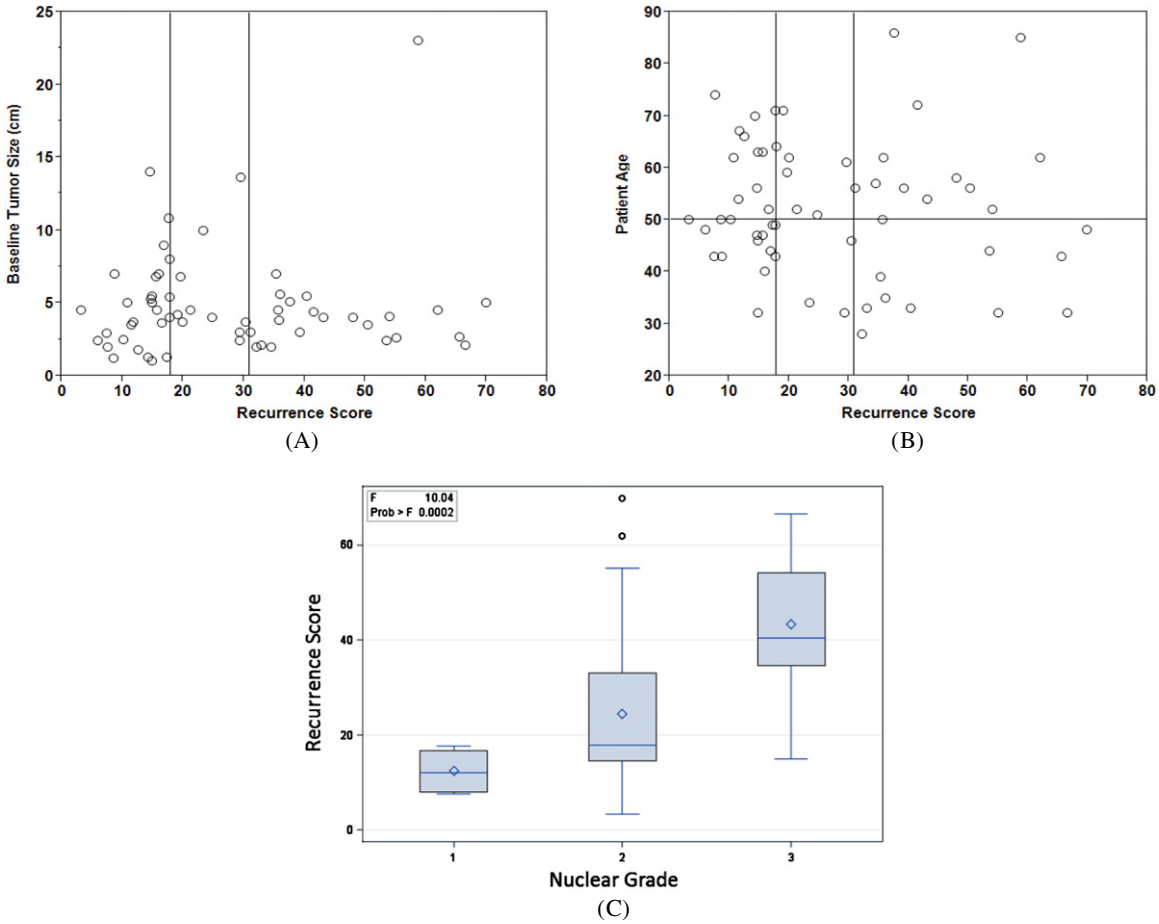

Fig. 1. Recurrence Score versus clinicopathologic features at presentation. (A) (Baseline tumor size) Spearman correlation = 0.05 (95% $CI = -0.21, 0.30$; $p = 0.61$); regression $r^2 = 0.04$, $p = 0.62$. (B) (Age) Spearman correlation = −0.10 (95% $CI = -0.35, 0.16$; $p = 0.50$); regression $r^2 = 0.08$, $p = 0.50$. (C) (Nuclear grade) Spearman correlation = 0.51 (95% $CI = 0.29, 0.68$; $P < 0.001$). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/BD-150199)

(⩾50% reduction in largest tumor dimension) was observed in 26 (43%) patients. No patients had disease progression. 17 (28%) patients underwent BCS and 43 (72%) underwent mastectomy.

3.3. Recurrence score and baseline characteristics

Recurrence Score was not significantly correlated with tumor size at presentation or patient age but it was correlated with nuclear grade on core biopsy (Fig. 1).

3.4. Recurrence score and outcome

Recurrence Score also did not correlate with %TR ($p = 0.07$) when adjusting for pre-treatment size, or when the covariate adjustment was removed from the model ($p = 0.06$) (Fig. 2).

Neither nodal status ($p = 0.79$), patient age ($p = 0.99$), baseline tumor size ($p = 0.38$), nor nuclear grade ($p = 0.18$) were associated with continuous %TR. The mean %TR in the high risk Recurrence Score group was not significantly greater than in the intermediate risk group (Table 3).

Lower values of ER by RT-PCR were associated with increased reduction in tumor size ($p = 0.02$). PR by RT-PCR was not associated with reduction in tumor size ($p = 0.32$). Lower ER expression by IHC correlated with improved response ($p = 0.03$). However, PR and HER2 by IHC did not correlate with response ($p = 0.09$ and $p = 0.37$, respectively). Interestingly, lower values of HER2 by RT-PCR were also associated with increased reduction in tumor size ($p = 0.007$).

Table 3
Percent tumor volume reduction and clinical response in tumor size by RS Group

RS group	% Tumor volume reduction*	Clinical response**
	Mean ± SD	Response/total (%)
Low risk (<18)	35.1 ± 26	10/27 = 37%
Intermediate risk (18–30)	54.4 ± 32	6/10 = 60%
High risk (⩾31)	45.6 ± 37	11/23 = 48%

*$p = 0.21$ and **$p = 0.43$ for comparison among 3 RS groups.

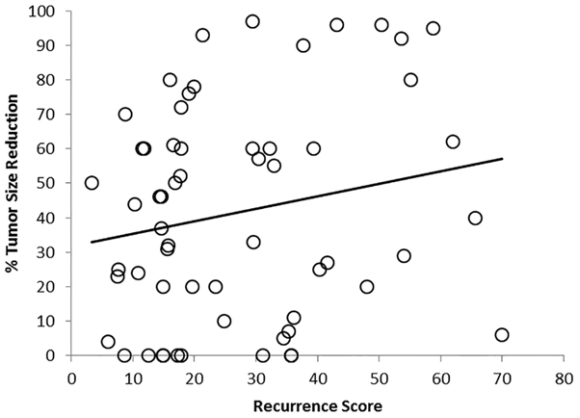

Fig. 2. Percent tumor response versus recurrence score. Regression $r^2 = 0.06$, $p = 0.06$.

There was no significant association between partial clinical response and patient age ($p = 0.54$), baseline tumor size ($p = 0.78$), nuclear grade ($p = 0.96$), or nodal status ($p = 0.82$). The continuous Recurrence Score was not significantly associated with the odds of response ($p = 0.15$). There was no trend in response by low, intermediate or high risk category ($p = 0.43$). Due to a lack of association between both the continuous or categorical Recurrence Score and response, no attempt was made to identify an optimal score threshold for predicting response. There was a statistically non-significant inverse relationship between continuous ER by RT-PCR and the odds of response ($p = 0.08$, OR = 0.72 per CT, 95% CI = 0.49, 1.05). There was no significant difference in the rate of BCS between responders ($7/26 = 27\%$) and non-responders ($10/34 = 29\%$) ($p = 0.83$).

4. Discussion

The Onco*type* DX® 21-gene assay is well described as an accurate method of assessing risk of recurrence in patients with ER+ invasive breast cancer [9,17]. In addition, it is a prediction tool in determining the benefit of chemotherapy in the adjuvant setting for both node negative and node positive patients [1,18]. However, the ability of this assay to predict the benefit of chemotherapy in the neoadjuvant setting remains controversial [7,14].

In our study, we found no statistically significant association between the Recurrence Score and %TR with NCT. These results reflect the findings of Mina et al. who found that the Recurrence Score did not predict pCR in patients undergoing NCT [14]. In contrast, Gianni et al. determined that Recurrence Score did, in fact, predict for pCR [7]. A finding common to many studies is the ability of lower ER expression by RT-PCR to predict tumor volume reduction and/or pCR [7,8,16].

It is important to note that no statistically significant associations were demonstrated between baseline clinical and pathology factors and %TR with NCT. However, the non-significant increase ($p = 0.07$) in %TR as Recurrence Score increases suggests that with a larger cohort, Recurrence Score may be more predictive than standard patient and tumor characteristics at predicting benefit of NCT. This study and others suggest that ER expression is superior to standard clinicopathologic factors at predicting response.

Regardless of the ability of RS to predict response to neoadjuvant chemotherapy, it is well established that the 21-gene assay provides important predictive and prognostic information and affects the decision on chemotherapy given [1,18]. If an Onco*type* DX® recurrence score predicts recurrence free survival in the adjuvant setting, giving chemotherapy in the neoadjuvant setting should not change that benefit [19]. Thus it can be used as an important decision tool when trying to decide between primary surgery, neoadjuvant chemotherapy or even neoadjuvant endocrine therapy.

In our study, 55% of patients had an intermediate or high RS. This population would most likely receive adjuvant CT, and in this group of patients the mean %TR was 54.4% and 45.6%, respectively. Therefore, one may suggest that RS from a core biopsy not only predicts for overall CT benefit and prognostic information for recurrence (regardless of order in which it is given) but may also provide an indication of patients who are most likely to see a 50% tumor reduction. On the other hand, patients with a low RS on their original core biopsy may derive little survival or tumor downstaging benefit from NCT. In our study 45% of the patients had a low RS, resulting in an average 35% TR. In this group of patients, awaiting a RS on the final surgical specimen, along with other pathologic data, may guide adjuvant therapy decisions better. Alternately, one might consider neoadjuvant endocrine therapy for this population [6].

There are several limitations to our study. Fewer than half of the planned 130 evaluable patients were available for analysis (due to low use of NCT for patients with ER+, HER2− tumors institutionally); which may have affected our ability to prove statistical significance. This is a small, single institution study, and there were two different NCT regimens administered. Interestingly, no pCRs were observed among the 60 patients evaluable for this analysis, making this endpoint impossible to study. Our institutional average pCR in the ER/PR+ population is 7%, reflecting national data [13]. There were also no instances of disease progression during NCT. Finally, special methods developed by Magee-Womens Hospital of UPMC for measuring reduction in tumor size were employed [3]. It is possible that other methods (e.g., RECIST criteria) of tumor measurement and response may yield different results.

In our cohort of patients with ER+ invasive breast cancer, where NCT was recommended for tumor downstaging, Recurrence Score did not successfully identify patients who would respond to NCT as measured by %TR. While there was a trend toward better response with higher scores, the result was nonsignificant, but this may be a function of our underpowered sample size. Lower ER expression, as measured by RT-PCR and IHC, was predictive of an improved response to NCT, which confirms the findings of other investigators. Further study into using this genomic assay to predict long-term recurrence free survival benefit from preoperative neoadjuvant chemotherapy or endocrine therapy may better delineate the benefit of the assay's use in the preoperative setting.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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