Predictability of 21-Gene Recurrence Score Assay by Using Pathological and Immunohistochemical Parameters in Breast Cancer

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ABSTRACT

BACKGROUND: Oncotype Dx is used to predict the long-term recurrence risk in patients with estrogen receptor (ER)-positive and human epidermal growth factor receptor 2 (HER2)-negative invasive breast cancer (BC). This study aimed at establishing a correlation between clinicopathological parameters and recurrence score (RS), subsequently improving predictability and ultimately justifying the use of the multigene assay.

MATERIALS AND METHODS: A retrospective analysis of the pathology and clinical data of 114 female patients with BC who had Oncotype Dx testing between 2012 and 2019. The pathological parameters included are tumor cell type, tumor grade, pathological stage, and mitotic index (MI). The expression of ER, progesterone receptor (PR), HER2, and Ki67 was assessed by immunohistochemistry. A univariate and multivariate linear regression analysis was performed to assess the correlation between these parameters and the RS.

RESULTS: In univariate analysis, age (<40 years), higher tumor grade, and low PR expression were significantly associated with higher RS ($P = .02$; <.001; and <.001, respectively). Both MI and Ki67 were also strongly correlated with an increase in the RS with a P value of .01 (Spearman correlation 0.34 and 0.33). In multivariate linear regression analysis, age, MI, and Ki67 lost their significance, but both higher grade and PR remained significantly associated with a higher RS along with the tumor stage ($P < .001$; <.001; and .04, respectively).

CONCLUSIONS: Tumor grade and PR immunohistochemical expression are the main predictors of RS in our study population. Other clinicopathological features were not significant predictors of change in RS in multivariate analysis.

KEYWORDS: Breast cancer, Oncotype DX, clinicopathological parameters, risk score, recurrence risk, immunohistochemical expression

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Introduction

Breast cancer (BC) is a leading cause of mortality among women worldwide with an estimated 40 000 deaths in a year in the United States.[1] In the last decade, the mortality rate has substantially decreased due to an early diagnosis and adjuvant therapies.[2] It has been a global standard to report the hormone receptor status of the BC because of its influence on the management decisions. Estrogen negative tumors are more aggressive and more likely to be treated with chemotherapy, compared with the estrogen-positive tumors which are relatively less aggressive and are almost always treated with antiestrogen therapy.[3] Studies have shown that about half of the BC cases would turn out to be estrogen receptor (ER) positive and node negative.[4,5] Some of these patients would also need adjuvant chemotherapy to improve their 5-year recurrence-free survival,[6] and their identification is therefore pivotal. Recent advances in the fields of molecular pathology and genomics have enhanced our ability to identify these cases.

Oncotype Dx is a commercially available multigene assay (Genomic Health, Inc, Redwood City, CA, USA) that uses a quantitative reverse transcription polymerase chain reaction (RT-PCR) technique involving 21 genes. It determines the long-term recurrence risk (RR) for patients with ER-positive and human epidermal growth factor receptor 2 (HER2)-negative BCs and can therefore make the benefit of adjuvant chemotherapy more predictable. It provides a numeric recurrence score (RS) with a range of 0 to 100 and divided into low (0-17), intermediate (18-30), and high (≥31) scores bearing an average RR of 6.8%, 14.3%, and 30.5%, respectively.[7] However, more recently, the range of 3 RS categories has been shifted a

bit based on the Trial Assigning Individualized Options for Treatment (Rx) (TAILORx) data with age being a factor.[8] Of the set of 21 genes, 16 correspond to cancer genes and the remaining 5 are reference genes. The 16 cancer genes are further subdivided into 5 genes from the proliferation group (Ki67, STK15, Survivin, CCNB1, and MYBL2), 5 genes from the ER group (ER, PGR, BCL2, and SCUBE2), 2 genes from the HER2neu group (HER2 and GRB7), 2 genes from the invasion group (MMP11 and CTSL2), and 1 gene from GTSM1.[9] The remaining 5 reference genes include GAPDH, B-Actin, RPLPO, GUS, and TFRC genes.[9] While calculating the RS from RT-PCR results, it uses a formula which gives the highest weight to the genes from the proliferation, ER, and HER2 groups. Four of the 16 cancer genes (Ki67, ER, PGR, and HER2) of the Oncotype Dx assay are routinely measured as proteins at the expression level by using immunohistochemistry on the tumor tissue sections.

Before the advent of Oncotype Dx analysis, the determination of the aggressive nature of the tumor and decision of adjuvant chemotherapy were based on the common clinicopathological features (tumor cell type, tumor grade, mitotic score, and tumor size) and above-mentioned immunohistochemical parameters. In light of the importance of these parameters and the weight given to the proliferation, ER, and HER2 groups in the multigene assay, it has been speculated that the clinicopathological parameters and immunohistochemical assessments may also be used to predict the RS.[10] The use of the adjuvant chemotherapy also comes along with risks of toxicity and risk for complications and adverse effects. Therefore, its use must be justified, and Oncotype Dx assay is a big revolution in this regard. Contrarily the economic burden on health care providers to perform this assay on all patients with BC can limit its use even in patients who would benefit from it.

This study aimed to assess the correlation between clinicopathological parameters, immunohistochemical assessment, and Oncotype Dx RS, to better select the patients for the assay and subsequently better predict RR and ultimately justify the use of multigene assay and use of adjuvant chemotherapy among patients who would actually benefit from it most. This will also lead to easing the economic burden by preventing an unnecessary ordering of expensive testing. The secondary aim of the study was to assess the correlation between quantitative expression by Oncotype Dx and the semi-quantification results of the proliferation and hormonal markers by immunohistochemistry.

Materials and Methods

Study sample

It is a cross-sectional study involving retrospective analysis of the pathology reports from 114 female patients with BC (mean age ± SD = 52.2 ± 9.3 years) by nonprobability consecutive sampling. Clinicopathological characteristics are given in Table 1. All patients included were positive for ER and negative for HER2 and underwent Oncotype Dx testing. Most of the patients were older than 40 years, postmenopausal, and having ductal cell type. Patients were diagnosed with BC between 2012 and 2019 at a tertiary care hospital and an affiliated center in Riyadh, Saudi Arabia. The study was approved by the local research ethics committee.

Oncotype Dx testing

The tumor sections for all 114 patients were sent for Oncotype Dx multigene testing to Genomic Health, Inc, Redwood City, CA, USA. The reports predicting the RR were received given as continuous variables as well as stratified into low- (0-17), intermediate- (18-30), and high-risk (⩾31) groups. Mean RS of our patients was 16.8 ± 8.2, with 65.8%, 25.4%, and 8.8% patients in low-, intermediate-, and high-risk categories, respectively (Table 1).

Pathological parameters

Formalin-fixed, paraffin-embedded, hematoxylin and eosin–stained tumor sections were used for histologic assessment by qualified pathologists. The pathological parameters included histologic cell type of tumor (lobular or ductal), histological tumor grade (1, 2, and 3), tumor pathological stage, and mitotic score. Tumor grade was assessed by Nottingham criteria, and mitosis was counted per 10 high-power fields. The mitotic index (MI) was used as a continuous variable, while tumor grade, tumor stage, and overall stage (TNM) were categorized as 1, 2, and 3, and the lymph node status was dichotomized into 2 groups, negative (0 nodes) and positive (metastasis in 1-3 axillary lymph nodes).

Immunohistochemical parameters

The expression of the hormonal receptor (ER, progesterone receptor [PR]), HER2, and Ki67 was assessed by immunohistochemistry using monoclonal antibodies, for ER (clone SP1), PR (clone 1E2), HER2 (clone 4B5), and Ki67 (MIBI). Immunostaining of ER and PR of ⩾1.0% nuclear staining of tumor cells is interpreted as positive and <1.0% of tumor cells or no nuclear is negative. Progesterone receptor immunostaining was further stratified into 0 (<1.0% or no staining), 1+ (⩾1.0%-25%), 2+ (>25%-75%), and 3+ (>75%). HER2 immunostaining was categorized into 0, 1+, 2+, and 3+, as per American Society of Clinical Oncology/College of American Pathologists guidelines.[11] Human epidermal growth factor receptor 2 is interpreted as negative when there is either no staining or incomplete faint membrane staining in ⩽10% of tumor cells (0) and when there is incomplete faint membrane staining in >10% of tumor cells (1+). It is interpreted as positive when there is complete intense circumferential membrane staining in >10% of invasive cancer cells (3+). Equivocal interpretation (2+) is used for either incomplete weak/

Table 1. Clinicopathological characteristics of 114 patients with breast cancer.

CHARACTERISTICS	N	%
Age (years)		
⩽40	13	11.4
41+	101	88.6
Menopause		
Pre	51	44.7
Post	63	55.3
Cell type		
Lobular	5	4.4
Ductal	109	95.6
Grade		
1	41	36.0
2	61	53.5
3	12	10.5
T stage		
1	57	50.0
2	53	46.5
3	4	3.5
N stage (nodes)		
Negative (0)	86	75.4
Positive (1-3)	28	24.6
Overall stage (TNM)		
Stage I	50	43.9
Stage II	59	51.8
Stage III	5	4.4
PR status		
0	4	3.5
1+	14	12.3
2+	7	6.1
3+	89	78.1
Oncotype risk category		
Low risk (0-17)	75	65.8
Intermediate risk (18-30)	29	25.4
High risk (⩾31)	10	8.8

Abbreviations: PR, progesterone receptor; TNM, tumor Node Metastasis.

moderate membrane staining in >10% of tumor cells or when ⩽10% of tumor cells show complete, intense, and circumferential membrane staining.[12] Equivocal HER2 stained samples were only included if they come out to be negative with fluorescence in situ hybridization analysis. For Ki67, 2 areas at the edge of the tumor tissue and 1 in the center were focused, and the percentage of positive cells either mild, moderate, or strongly stained were counted. Based on its expression, the proliferation index was given as continuous variables.

Statistical analysis

Univariate and multivariate analyses were performed to assess the correlation between the clinicopathological (age, menopausal status, cell type, tumor grade, MI, tumor stage, and nodal status) and immunohistochemical parameters (PR and Ki67) with the Oncotype Dx scores using SPSS version 20. The risk scores were used both as groups (low, intermediate, and high risk) and as continuous variables. The descriptive statistics were given as mean ± SD for numerical variables and as frequencies and percentages for categorical variables. Mann-Whitney and Kruskal-Wallis tests were used to compare the mean difference across the groups. Linear regression was performed to assess the effect of a risk factor on the RS and summarize in stepwise linear regression. Stepwise linear regression was performed for those predictors whose P value was ⩽.2 in multivariate analysis. A test with a P value of <.05 was considered statistically significant.

Results

All 114 patients with BC underwent Oncotype Dx testing over a period of 7 years. A total of 96% of our cases were invasive ductal carcinoma (N = 109) compared with only 4% of cases with invasive lobular carcinoma (N = 5). Mean RS for the whole sample was 16.8 ± 8.2, and the mean RS of both ductal and lobular cases was almost equal (17.2 ± 9.1 and 17 ± 8.1, respectively). Most of our cases were grade 2 (53.5%), followed by grade 1 (36%) and grade 3 (10.5%). A large number of our cases were tumor stage 1 and 2 (50% and 46.5%) compared with only 3.5% of stage 3 cases. On the other hand, 52% of cases were overall stage 2, compared with 44% with overall stage 1 and only 4.4% cases were overall stage 3. No lymph node involvement was observed in 75.4% cases, whereas only 24.6% of our cases had nodal involvement (1-3 nodes; Table 1).

Univariate analysis

The analysis was performed to assess the association between clinicopathological and immunohistochemical parameters and RS (Table 2). A significant association was observed with age (P = .02), with higher RSs among cases <40 years of age. Tumor grade was found to be a strongly significant variable (P <.001), with higher RS for cases with grade 3 tumors. Among immunohistochemical parameters, PR status was observed to be of strong significance (P <.001), with higher RS for 1+ expression followed by 0, 2+, and 3+, respectively. Mitotic index and Ki67 were found to be correlated (P = .01) with a higher RS (Spearman correlation 0.34 and 0.33, respectively) and were

Table 2. Univariate analysis of clinicopathological and immunohistochemical parameters with the recurrence score.

	RECURRENCE SCORE					*P* VALUE[a]
	MEAN	SD	MEDIAN	PERCENTILE 25	PERCENTILE 75	
Age (years)						
⩽40	21.0	6.6	19.0	18.0	27.0	.02
41+	16.3	8.3	15.0	11.0	20.0	
Menopause						
Pre	18.1	8.0	17.0	11.0	23.0	.19
Post	15.8	8.3	15.0	10.0	20.0	
Cell type						
Lobular	17.0	8.1	17.0	13.0	19.0	.90
Ductal	16.8	8.3	16.0	11.0	21.0	
Grade						
1	12.5	5.3	12.0	10.0	17.0	<.001
2	17.4	7.5	17.0	13.0	22.0	
3	28.3	8.4	26.5	23.5	32.5	
Progesterone receptor						
0	22.5	9.5	22.0	14.5	30.5	<.001
1+	25.9	9.0	24.0	19.0	30.0	
2+	17.9	7.4	18.0	13.0	21.0	
3+	15.1	7.1	14.0	10.0	19.0	
T stage						
1	16.5	8.6	16.0	10.0	21.0	.42
2	17.5	7.8	16.0	13.0	22.0	
3	12.0	9.4	11.0	4.0	20.0	
N stage						
No nodes	16.9	8.4	16.0	11.0	22.0	.89
1-3 nodes	16.5	7.7	15.5	11.5	19.5	
Overall stage (TNM)						
Stage I	15.9	8.7	15.0	10.0	19.0	.13
Stage II	18.0	7.7	17.0	13.0	23.0	
Stage III	11.6	7.3	14.0	4.0	16.0	

[a]*P* value based on nonparametric test (Mann-Whitney and Kruskal-Wallis).
Abbreviations: TNM, tumor Node Metastasis.

also found to be correlated with each other ($P=.01$ and Spearman correlation 0.43). No other parameter was found to be significantly associated with RS.

Multivariate linear regression analysis

The linear regression analyses revealed a significant association of tumor grade, stage, and PR status and a trend toward association for nodal involvement (Table 3). Similar to univariate analysis, significant associations were observed between RS and grades 1 and 2 ($P<.001$ and .005, respectively). The mean RS was highest for grade 3 followed by grades 2 and grade 1. PR status was the other parameter that was significant in univariate analysis and retained its significance in linear regression also ($P<.001$). Progesterone receptor expression 1+ had the highest mean RS

Table 3. Linear regression analysis of clinicopathological and immunohistochemical parameters with the recurrence score.

VARIABLE	CATEGORIES	COEFFICIENT	*P* VALUE	95% CONFIDENCE INTERVAL FOR COEFFICIENT	
				LOWER	UPPER
Intercept		12.71	.01	3.04	22.38
Age (years)	⩽40	2.86	.17	−1.27	6.98
	41+ (ref)	0.00			
Menopause	Pre	1.07	.44	−1.69	3.83
	Post (ref)	0.00			
Cell type	Lobular	−0.74	.81	−6.79	5.31
	Ductal (ref)	0.00			
Grade	1	−10.39	<.001	−15.66	−5.13
	2	−6.78	.005	−11.48	−2.07
	3 (ref)	0.00			
Progesterone receptor	0	4.27	.21	−2.46	11.00
	1+	8.82	<.001	4.64	12.99
	2+	3.45	.20	−1.80	8.69
	3+ (ref)	0.00			
T stage	1	9.17	.04	0.48	17.87
	2	6.36	.11	−1.35	14.07
	3 (ref)	0.00			
N stage	No nodes	3.47	.06	−0.26	7.21
	1-3 nodes	0.00			
Overall stage (TNM)	Stage I	−5.25	.27	−14.66	4.16
	Stage II	−1.27	.73	−8.51	5.96
	Stage III (ref)	0.00			
MI		0.37	.22	−0.22	0.95
Ki67		0.05	.38	−0.07	0.18

Abbreviations: MI, mitotic index; ref, reference group; TNM, tumor Node Metastasis.
Dependent variable: recurrence score.

followed by 0, 2+, and 3+. Tumor stage was an insignificant parameter in univariate analysis, but tumor stage 1 was significantly associated ($P = .04$) with a higher mean RS in linear regression. Tumor stage 1 was associated with the highest mean RS followed by stages 2 and 3. Another parameter which was not significant in univariate analysis but was observed to show a trend toward significance ($P = .06$) in linear regression analysis was N stage (nodal involvement). Interestingly, the mean RS for cases with negative nodes was marginally higher than that with positive nodes. Contrary to this, the age, which was a significant parameter in univariate analysis, lost its significance in linear regression analysis ($P = .17$). Similarly, MI and Ki67, which were significant parameters in univariate analysis, lost their significance in this analysis ($P = .22$ and .38, respectively).

Stepwise linear regression analysis

Stepwise linear regression was performed for variables that were observed to have a *P* value of ⩽.2 as shown in Table 3. Analyses revealed similar trends of significance for tumor grade and PR status, which were observed in the linear regression model. But tumor stage 1, which was significant earlier, lost its significance ($P = .06$) and stage 2 became significant ($P = .04$).

Table 4. Stepwise linear regression analysis model.

VARIABLE	CATEGORIES	B	*P* VALUE	95% CONFIDENCE INTERVAL	
				LOWER	UPPER
	Intercept	18.9	<.001	11.8	26.1
Grade	1	−13.5	<.001	−17.9	−9.2
	2	−9.3	<.001	−13.4	−5.2
	3 (ref)	0.0			
Progesterone receptor	0	4.1	.22	−2.5	10.7
	1+	8.7	<.001	5.0	12.5
	2+	2.4	.36	−2.7	7.4
	3+ (ref)	0.0			
T stage	1	6.4	.06	−0.2	12.9
	2	6.9	.04	0.3	13.5
	3 (ref)	0.0			

Abbreviation: ref, reference group.
Dependent variable: recurrence score.

Also in these analyses, it was observed that stage 2 had a slightly higher mean RS compared with stage 1 and the lowest mean RS was observed for stage 3 (Table 4).

Discussion

Estrogen receptor–negative BC tumors are more aggressive compared with ER-positive cases. Even though less aggressive, some cases of ER-positive, HER2-negative BC can be associated with greater risk of recurrence, despite receiving hormonal therapy, and therefore must be treated with adjuvant chemotherapy. Identification of this subset of patients should not be missed at all. On the other hand, chemotherapy has its own complications and adverse effects. Therefore, the use of chemotherapy has to be justified in these patients.

Oncotype Dx is one such gene assay that has been reported to be more accurate than clinicopathological parameters, in predicting the risk of recurrence and facilitating the use of chemotherapy.[7] Contrary to this, various studies have reported the role of clinicopathological parameters in an equally successful prediction of cases with high RR,[13,14] and Cuzick et al[15] have reported an even better prediction by these parameters compared with Oncotype Dx. Therefore, the importance of these parameters alone or in combination with Oncotype Dx for a better selection of patients cannot be undermined. A research group has found a decrease in the use of chemotherapy from 25% to 10% in early BC cases which were ER positive and HER2 negative after the advent of Oncotype Dx.[16] Despite facilitating the selection of high-risk patients for adjuvant chemotherapy, the biggest limitation in the use of Oncotype Dx is the high cost (4175$) of the assay,[17] specifically for developing countries and even in the developed world with insurance-based health care system, which in most cases will not cover for the price of this assay. Therefore, the use of such an expensive test needs to be justified and a correct subset of patients should be selected for this assay based on identification of the parameters which indicate toward higher risk of recurrence or poor prognosis in ER-positive cases. This study explored the correlation between clinicopathological parameters, immunohistochemical assessment, and Oncotype Dx RS, to better select the patients for the assay and ultimately justify the use of an expensive multigene assay and use of adjuvant chemotherapy among patients who would actually benefit from it most.

Our study reveals tumor grade and PR status as main predictors of Oncotype Dx RS. Younger age group (<40 years) patients had a higher risk of recurrence in our study population and support the observation that younger patients with BC often require chemotherapy. Sparano et al[8] reported the benefit of chemotherapy in younger age group (<50 years) with an RS of 16 to 25, which redefined the risk ranges in TAILORx data. On the other hand, Thibodeau and Voutsadakis[16] could not establish any association of age with Oncotype RS. Age lost its significance in multivariate linear regression analysis, indicating a minor contribution to the RS. Our finding of a higher RS with grade 3 tumors compared with the grades 1 and 2 is in line with what has been reported earlier.[18,19] Progesterone receptor status was another highly significant parameter with the highest RS associated with 1+ staining. Different studies have reported the negativity of the PR to be inversely correlated with the Oncotype RS.[20,21] Arpino et al[22] have speculated the

absence of PR in ER-positive tumors as a marker for increased proliferation through growth factor signaling and that may be the reason for reported resistance of PR-negative tumors to tamoxifen.[23] Our result supports the previously reported associations of PR status with Oncotype RS. Two other pathological parameters which were found to be positively correlated with RS were MI and Ki67. Both of them are markers for proliferation, and interestingly, they were also strongly correlated with each other in our analysis. Our findings of a positive correlation of MI with RS are in line with those of previously published studies highlighting the role of MI in cell proliferation in patients with BC.[24] Ki67 expression increases as the cell move from G1 to M (mitosis) phase, and this justifies our finding of a strong correlation between Ki67 and MI.[25] Ki67 has been reported to be the most important determinant of Oncotype Dx RS, but its value as the only factor has not been established so far.[26]

Although the univariate analysis in this study strengthened the already published findings related to the predictability of Oncotype Dx RS by clinicopathological parameters, we performed multivariate linear regression analysis to explore the contribution by individual factors. We found that association of only tumor grade and PR status retained significance, and other factors like age, MI, and Ki67, which were associated in univariate analysis, lost their significance. This could be expected as MI is part of the tumor grading process and as Ki67 was strongly correlated with it, they both lost significance, whereas tumor grade retained it. This indicates the significant contribution of tumor grade and PR negativity to high Oncotype RS. Singh et al[19] also showed that in a multivariate analysis, the grade was the only significant factor with a positive correlation with Oncotype RS. In our study, the RS for grade 3 cases was more than 2 times that of grade 1, and there was a remarkable decrease in RS as the grade decreased. Thibodeau and Voutsadakis[16] also reported a similar trend with all grade 3 tumors having a high RS compared with grades 1 and 2. The interesting finding in this study was that although negativity of PR status was in inverse correlation to RS, having 1+ staining was associated with higher RS compared with no staining. This trend was seen both in multivariate linear regression analysis as well as in stepwise linear regression. RS for 2+ and 3+ stained cases was found to be less compared with 1+ stained cases. Similar findings of grade and PR status to be the strongest predictors of RS were reported by Orucevic et al.[27] Studies have proposed to rely on grade and PR status to predict the RR and therefore avoid ordering Oncotype Dx assay in ER-positive, HER2-negative, and node-negative tumors, specifically where the resources are limited.[16] Contrary to this, our study revealed a trend toward association for lymph node involvement, and it was observed that RS for cases with no nodal involvement was much higher compared with those with nodes. This may be because, in our sample, the number of cases with positive nodes was 3 times less than that with negative nodes. Despite this limitation, we propose that the Oncotype Dx assay should be performed in even node-positive cases, for chemotherapy administration and avoiding death or recurrence. Similar to our findings, Hanna et al[28] also reported the RS for node-positive cases to be predominantly low or intermediate. These findings highlight the heterogeneity among the subsets of the node-positive BC cases, and hence, Oncotype RS should be used along with clinicopathological parameters in making a decision regarding adjuvant chemotherapy.[29] Although offering chemotherapy to node-positive cases without Oncotype testing is a norm, studies have reported that RS has a role in predicting the benefit of chemotherapy in postmenopausal women.[30,31] In stepwise linear regression, we observed a significant association of stage 1 and 2 cases with a higher RS compared with stage 3 cases. This finding is contrary to the findings earlier reported where stage 1 was found to be associated with a more favorable clinical outcome compared with stages 2 and 3.[32] This discrepancy could be because of the selection bias leading to only 3.5% of stage 3 cases in our study population.

Strengths of our study are a moderate sample size, a strong statistical analysis where the univariate analysis was followed by simple linear regression as well as stepwise linear regression to substantially explore major predictors of RS. Further, we included node-positive cases as well, and their comparison to node-negative cases revealed some interesting results which were discussed earlier. Although the confidence interval for association with RS for any given variable is extremely wide and can impact the interpretation of the results, however, given the data and results, the multivariate analysis is strengthening our conclusions. On the other hand, limitations of this study include retrospective nature without the possibility for follow-up data, inability to perform survival analysis, and the selection bias by clinicians at inclusion.

In conclusion, BC tumor grade and PR immunohistochemical expression are the main predictors of Oncotype Dx RS in our study population. Other clinicopathological features were not significant predictors of change in RS in multivariate analysis. This study further supports the value of requesting Oncotype Dx testing in node-positive cases. Our findings facilitate better patient selection for the gene assay, which is important given the economic burden of testing.

Author Contributions

All authors have substantially contributed to conception, data acquisition, analysis and interpretation, drafting of the manuscript and have given final approval of the submitted version.

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REFERENCES

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014;64:9-29.
2. Gianni L, Baselga J, Eiermann W, et al. Phase III trial evaluating the addition of paclitaxel to doxorubicin followed by cyclophosphamide, methotrexate, and

fluorouracil, as adjuvant or primary systemic therapy: European Cooperative Trial in Operable Breast Cancer. *J Clin Oncol*. 2009;27:2474-2481.
3. Klein ME, Dabbs DJ, Shuai Y, et al. Prediction of the Oncotype DX recurrence score: use of pathology-generated equations derived by linear regression analysis. *Mod Pathol*. 2013;26:658-664.
4. Dunnwald LK, Rossing MA, Li CI. Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. *Breast Cancer Res*. 2007;9:R6.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67:7-30.
6. Fisher B, Jeong JH, Bryant J, et al. Treatment of lymph-node-negative, oestrogen-receptor-positive breast cancer: long-term findings from National Surgical Adjuvant Breast and Bowel Project randomised clinical trials. *Lancet*. 2004;364:858-868.
7. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004;351:2817-2826.
8. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. *N Engl J Med*. 2018;379:111-121.
9. McVeigh TP, Kerin MJ. Clinical use of the Oncotype DX genomic test to guide treatment decisions for patients with invasive breast cancer. *Breast Cancer (Dove Med Press)*. 2017;9:393-400.
10. Flanagan MB, Dabbs DJ, Brufsky AM, Beriwal S, Bhargava R. Histopathologic variables predict Oncotype DX recurrence score. *Mod Pathol*. 2008;21:1255-1261.
11. Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. *J Clin Oncol*. 2020;38:1346-1366.
12. Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice guideline focused update. *Arch Pathol Lab Med*. 2018;142:1364-1382.
13. Allison KH, Kandalaft PL, Sitlani CM, Dintzis SM, Gown AM. Routine pathologic parameters can predict Oncotype DX recurrence scores in subsets of ER positive patients: who does not always need testing? *Breast Cancer Res Treat*. 2012;131:413-424.
14. O'Connor SM, Beriwal S, Dabbs DJ, Bhargava R. Concordance between semiquantitative immunohistochemical assay and Oncotype DX RT-PCR assay for estrogen and progesterone receptors. *Appl Immunohistochem Mol Morphol*. 2010;18:268-272.
15. Cuzick J, Dowsett M, Pineda S, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. *J Clin Oncol*. 2011;29:4273-4278.
16. Thibodeau S, Voutsadakis IA. Prediction of Oncotype Dx recurrence score using clinical parameters: a comparison of available tools and a simple predictor based on grade and progesterone receptor. *Hematol Oncol Stem Cell Ther*. 2019;12:89-96.
17. Chandler Y, Schechter CB, Jayasekera J, et al. Cost effectiveness of gene expression profile testing in community practice. *J Clin Oncol*. 2018;36:554-562.
18. Gwin K, Pinto M, Tavassoli FA. Complementary value of the Ki-67 proliferation index to the Oncotype DX recurrence score. *Int J Surg Pathol*. 2009;17:303-310.
19. Singh K, He X, Kalife ET, Ehdaivand S, Wang Y, Sung CJ. Relationship of histologic grade and histologic subtype with Oncotype Dx recurrence score; retrospective review of 863 breast cancer Oncotype Dx results. *Breast Cancer Res Treat*. 2018;168:29-34.
20. Clark BZ, Dabbs DJ, Cooper KL, Bhargava R. Impact of progesterone receptor semiquantitative immunohistochemical result on Oncotype DX recurrence score: a quality assurance study of 1074 cases. *Appl Immunohistochem Mol Morphol*. 2013;21:287-291.
21. Tang P, Wang J, Hicks DG, et al. A lower Allred score for progesterone receptor is strongly associated with a higher recurrence score of 21-gene assay in breast cancer. *Cancer Invest*. 2010;28:978-982.
22. Arpino G, Weiss H, Lee AV, et al. Estrogen receptor-positive, progesterone receptor-negative breast cancer: association with growth factor receptor expression and tamoxifen resistance. *J Natl Cancer Inst*. 2005;97:1254-1261.
23. Moon YW, Park S, Sohn JH, et al. Clinical significance of progesterone receptor and HER2 status in estrogen receptor-positive, operable breast cancer with adjuvant tamoxifen. *J Cancer Res Clin Oncol*. 2011;137:1123-1130.
24. Zbytek B, Cohen C, Wang J, Page A, Williams DJ, Adams AL. Nottingham-defined mitotic score: comparison with visual and image cytometric phosphohistone H3 labeling indices and correlation with Oncotype DX recurrence score. *Appl Immunohistochem Mol Morphol*. 2013;21:48-53.
25. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol*. 2005;23:7212-7220.
26. Sahebjam S, Aloyz R, Pilavdzic D, et al. Ki 67 is a major, but not the sole determinant of Oncotype Dx recurrence score. *Br J Cancer*. 2011;105:1342-1345.
27. Orucevic A, Bell JL, McNabb AP, Heidel RE. Oncotype DX breast cancer recurrence score can be predicted with a novel nomogram using clinicopathologic data. *Breast Cancer Res Treat*. 2017;163:51-61.
28. Hanna MG, Bleiweiss IJ, Nayak A, Jaffer S. Correlation of Oncotype DX recurrence score with histomorphology and immunohistochemistry in over 500 patients. *Int J Breast Cancer*. 2017;2017:1257078.
29. Giuliano AE, Hawes D, Ballman KV, et al. Association of occult metastases in sentinel lymph nodes and bone marrow with survival among women with early-stage invasive breast cancer. *JAMA*. 2011;306:385-393.
30. Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol*. 2010;11:55-65.
31. Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol*. 2010;28:1829-1834.
32. Vissio E, Metovic J, Osella-Abate S, et al. Integration of Ki-67 index into AJCC 2018 staging provides additional prognostic information in breast tumours candidate for genomic profiling. *Br J Cancer*. 2020;122:382-387.