Original Study

21-Gene Recurrence Score Testing in HER2-positive Patients

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Abstract

Introduction: The 21-gene recurrence score (RS) has been extensively studied and validated in patients with estrogen receptor-positive (ER^+), human epidermal growth factor 2 (HER2)-negative breast cancer; however, RS testing is not routinely performed in patients with HER2-positive ($HER2^+$) disease. We sought to determine patterns of RS testing, to characterize RS distributions, and to determine the impact of RS results on clinical decision-making for patients with ER^+, $HER2^+$ breast cancer. **Materials and Methods:** Using the Surveillance and Epidemiology End Results program database, we identified women with ER^+, $HER2^+$ breast cancer. We stratified patients using TAILORx RS cutoffs and evaluated treatment characteristics across patients. Multivariable logistic regression was performed to determine factors associated with RS testing and receipt of a high-risk RS. **Results:** Overall, 5% of patients with ER^+, $HER2^+$, early stage breast cancer underwent RS testing. The distribution of RS testing by TAILORx cutoffs were: high-risk, 17%; intermediate-risk, 49%; and low-risk, 34%. Chemotherapy utilization among those not tested was 66%. Among those tested, utilization was significantly associated with RS results: 67% of high-risk, 30% of intermediate-risk, and 19% of low-risk patients received chemotherapy. Progesterone receptor-negative status, larger tumor size, and high tumor grade were significantly associated with high-risk RS. **Conclusions:** RS testing is used sparingly among patients with $HER2^+$ early-stage breast cancer; however, test results appear to impact clinician's decision-making on chemotherapy use.

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Introduction

The 21-gene recurrence score (RS) assay (Oncotype DX; Genomic Health Inc, Redwood City, CA) is a quantitative reverse-transcriptase polymerase chain reaction assay to assess the expression of 21 cancer-related genes in patients with early stage breast cancer. The RS has been extensively validated as a tool to measure risk of distant recurrence and the benefit of adjuvant chemotherapy in lymph node (LN)-negative and LN-positive patients with estrogen receptor-positive (ER^+), human epidermal growth factor receptor-2-negative ($HER2^-$) breast cancer.[1-7] Currently, RS testing is recommended by the National Comprehensive Cancer Network (NCCN) guidelines for patients with LN-negative, ER^+, $HER2^-$ breast cancer.[8]

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HER2 is a growth factor receptor gene that is amplified in 25% to 30% of breast cancer cases.[9] Overexpression of HER2 has been shown to impact tumor biology, causing a more aggressive disease as manifest by worse disease-free and overall survival.[9-12] Owing to the aggressive nature of HER2-positive ($HER2^+$) breast cancer, guidelines recommend the addition of adjuvant chemotherapy and targeted HER2 therapy with trastuzumab.[8] The initial validation studies of RS testing were performed in patients with $HER2^+$ and $HER2^-$ disease[7]; however, all subsequent studies have excluded patients with $HER2^+$ disease, likely owing to their worse prognosis. We sought to evaluate rates of RS testing, to define the distribution of RS, and to determine the impact of RS results on clinical decision-making for patients with ER^+, $HER2^+$ breast cancer.

Patients and Methods

An augmented version of the Surveillance, Epidemiology, and End Results (SEER) program database was used to identify women with ER^+, $HER2^+$, stage I and II carcinoma of the breast from 2010 to 2013, with linked data on RS testing. The SEER program database is an aggregate of 18 distinct geographic cancer registries that represents 28% of the United States population.[13] Women with ER-negative (ER^-) or unknown status were excluded. The

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study was exempt from institutional review board review at the University of Minnesota as only deidentified data was used.

Statistical Analysis

We characterized patients based on demographic, disease, and treatment characteristics. HER2$^+$ patients were stratified by receipt of RS testing and by score into high-, intermediate- and low-risk scores by the TAILORx (Trial Assigning Individualized Options for Treatment) RS cutoffs. Based on the TAILORx trial, these cutoffs are: low-risk score (< 11), intermediate-risk score (11-25), and high-risk score (> 25).[14] We performed multivariable logistic regression to determine factors associated with RS testing and with receipt of a high-risk score. All statistical analyses were completed using SAS software, version 9.3 (SAS Institute, Cary, NC).

Results

We identified 17,101 patients with HER2$^+$, ER$^+$, stage I/II breast cancer in the SEER database from 2010 to 2013. Of those, 59% of patients were age 55 or older, 78% of patients were white, and 85% of patients had private insurance. Most tumors were smaller than 2 cm (53%), LN-negative (65%), progesterone receptor-positive (PR$^+$) (72%), and grade I/II (49%). The cohort was equally distributed between stage I and stage II disease (Table 1).

Of the 17,101 patients, 1081 (5%) patients underwent RS testing. Factors associated with a decreased odds of RS testing were younger patient age, other race (compared with non-Hispanic white), more recent year of diagnosis (2012-2013 compared with 2010-2011), Medicaid insurance coverage (compared with private), positive or unknown LN status (compared with negative), PR-negative (PR$^-$) or unknown status (compared with PR$^+$), larger tumor size, and grade III tumors (compared with grade I/II) (Table 2).

The distribution of RS results by TAILORx cutoffs were: high risk, 17%; intermediate risk, 49%; and low risk, 34%. Factors significantly associated with high-risk RS were PR$^-$ status, tumor size greater than 2 cm, and grade III tumors (Table 3).

Chemotherapy utilization was evaluated by RS score. Sixty-four percent of the entire cohort received chemotherapy. Among those who underwent RS testing, 67% of high-risk, 30% of intermediate-risk, and 19% of low-risk RS patients received chemotherapy. Conversely, 66% of patients who were not tested received chemotherapy.

Discussion

The 21-gene RS test is validated in patients with ER$^+$, HER2$^-$ early stage breast cancer. Using a large population-based dataset, we found that RS testing was being performed in 5% of patients with HER2$^+$ disease. Furthermore, in this subset of HER2$^+$, ER$^+$ patients, we found that increased age led to an increased odds of RS testing, whereas PR$^-$ status and high-grade tumors were less likely to be tested. We also found that PR$^-$ status, tumor size greater than 2 cm, and grade III tumors were significantly associated with an increased odds of a high-risk TAILORx score. Chemotherapy

Table 1 Patient and Tumor Characteristics for ER$^+$, HER2$^+$ Breast Cancer From 2010 to 2013

Total n = 17,101	n	%
Age, y		
18-54	7010	41
55-64	4571	27
≥65	5520	32
Year of diagnosis		
2010-2011	8166	48
2012-2013	8935	52
Race		
Non-Hispanic White	13,278	78
Black	1888	11
Other	1935	11
Insurance status		
Insured-private	14,560	85
Medicaid	1893	11
Uninsured/unknown	648	4
Size, cm		
< 2	9055	53
2-5	6715	39
> 5	580	3
Missing	751	4
Negative	11,048	65
Positive	4359	25
Unknown	1694	10
Progesterone receptor status		
Positive	12,353	72.24
Negative	4665	27
Unknown	83	0
Stage		
I	8168	48
II	8192	48
Unknown	741	4
Grade		
I & II	8422	49
III	7862	46
Unknown	817	5
Receipt of chemotherapy		
No/unknown	6144	36
Yes	10,957	64
Recurrence score risk group		
High (>25)	245	1
Intermediate (11 to 25)	392	2
Low (<11)	444	3
Not tested	16,020	94

Abbreviations: ER$^+$ = estrogen receptor-positive; HER2$^+$ = human epidermal growth factor receptor 2-positive.

Table 2 Factors Associated With 21-Gene Recurrence Score Testing in ER$^+$, HER2$^+$, Stage I & II Breast Cancer From 2010 to 2013

	OR	95% CI	
Age, y			
18-54		REF	
55-64	**1.61**	**1.37**	**1.88**
≥ 65	**1.49**	**1.28**	**1.74**
Race			
Non-Hispanic White		REF	
Black	1.04	0.85	1.28
Other	**0.67**	**0.52**	**0.85**
Year of diagnosis			
2010-2011		REF	
2012-2013	**0.66**	**0.58**	**0.74**
Insurance status			
Insured-private		REF	
Medicaid	**0.77**	**0.61**	**0.97**
Uninsured/unknown	0.92	0.64	1.32
Lymph node status			
Negative		REF	
Positive	**0.44**	**0.36**	**0.53**
Unknown	**0.38**	**0.28**	**0.51**
Progesterone receptor status			
Positive		REF	
Negative	**0.42**	**0.35**	**0.50**
Unknown	**0.53**	**0.19**	**1.47**
Size, cm			
< 2		REF	
2-5	**0.81**	**0.71**	**0.94**
> 5	**0.48**	**0.30**	**0.77**
Missing	**0.25**	**0.14**	**0.47**
Grade			
I & II		REF	
III	**0.43**	**0.37**	**0.49**
Unknown	**0.75**	**0.53**	**1.05**

Abbreviations: CI = confidence interval; ER$^+$ = estrogen receptor-positive; HER2$^+$ = human epidermal growth factor receptor 2-positive; OR = odds ratio; REF = reference.
Bold text designates statistical significance.

Table 3 Factors Associated With Receipt of a High-risk RS Score Among Patients With HER2$^+$, ER$^+$ Breast Cancer From 2010 to 2013

	OR	95% CI	
Age, y			
18-54		REF	
55-64	0.91	0.63	1.32
≥ 65	0.99	0.70	1.42
Race			
Non-Hispanic White		REF	
Black	1.04	0.65	1.67
Other	1.52	0.88	2.62
Year of diagnosis			
2010-2011		REF	
2012-2014	1.06	0.80	1.42
Insurance status			
Insured-private		REF	
Medicaid	0.85	0.49	1.48
Uninsured/unknown	0.60	0.21	1.67
Lymph node status			
Negative		REF	
Positive	0.69	0.44	1.09
Unknown	0.89	0.42	1.88
Progesterone receptor status			
Positive		REF	
Negative	**6.33**	**4.21**	**9.51**
Size, cm			
< 2		REF	
≥ 2	**1.39**	**1.03**	**1.89**
Grade			
I & II		REF	
III	**4.85**	**3.54**	**6.65**
Unknown	1.05	0.47	2.37

Abbreviations: CI = confidence interval; ER$^+$ = estrogen receptor-positive; HER2$^+$ = human epidermal growth factor receptor 2-positive; OR = odds ratio; REF = reference.
Bold text designates statistical significance.

utilization was greater in patients with a high-risk score than in patients with a low or intermediate score. However, rates of chemotherapy utilization among patients with a high-risk RS were comparable with rates in patients that were not tested.

RS testing has been extensively validated in patients with early stage, ER$^+$, HER2$^-$ patients. TAILORx, a randomized controlled trial to evaluate the role of adjuvant chemotherapy by RS, found no recurrence or survival benefit to the addition of chemotherapy to endocrine therapy in patients with intermediate or low scores.[14] However, this study and previous studies focused on patients with HER2$^-$ breast cancer. In the initial validation study of RS testing by Paik et all, HER2$^+$ and HER2$^-$ patients were included.[7] The study found a statistically insignificant greater proportion of patients with distant recurrence at 10 years among patients with HER2$^+$ tumors versus those without. However, on multivariate analysis, which included ER, PR, and HER2 status, RS was the only significant predictor of distant recurrence.[7] Subsequent studies of RS have thus only focused on the HER2$^-$ population, and clinically patients with HER2$^+$ breast cancer receive HER2-targeted therapy in addition to chemotherapy.

Current NCCN and American Society of Clinical Oncology guidelines recommend the use of RS testing in select patients with early stage, ER$^+$ HER2$^-$ breast cancer, but not in patients with HER2$^+$ breast cancer.[8,15] As patients with HER2$^+$ tumors have a more aggressive disease and carry a worse prognosis, they are believed to derive a greater benefit from HER2-directed therapy and chemotherapy; therefore, RS testing is not recommended in this cohort. Nonetheless, we saw that approximately 5% of the patients in the United States with ER$^+$, HER2$^+$ breast cancer underwent RS testing. Use of RS testing occurred more frequently in elderly patients, and thus may be an attempt to find patients

with a low or intermediate score in order to forego chemotherapy, as seen by the decreased rates of chemotherapy use in these populations.

Using genomic testing to further characterize risk stratification and assist with treatment decisions was similarly studied in the MINDACT (Microarray in Node negative Disease may Avoid ChemoTherapy) trial. The MINDACT study, which evaluated the use of the 70-gene signature test in patients with early stage breast cancer, compared clinical versus genomic risk stratification.[16] Patients with discordant risk stratifications were randomized to treatment by either their clinical or genomic risk stratification.[16] Survival without distant metastases was 94.7% among all high clinical risk but low genomic risk patients who were randomized to no chemotherapy, which was comparable to that of those who received chemotherapy.[16] Although the study included mostly patients with ER^+, $HER2^-$ breast cancer, it did include 501 women with ER^+, $HER2^+$ disease. Of these 501 patients, 211 were found to have low genomic risk by the 70-gene assay. Furthermore, the study found much lower use of chemotherapy when using genomic stratification.[16] Taken together, the use of genomic testing in patients classically deemed high risk may be able to spare some patients adjuvant chemotherapy without detrimental oncologic outcomes; however, these results cannot specifically be extrapolated to the subgroup of ER^+, $HER2^+$ patients.

Among the tested $HER2^+$ patients in our study, 17% received a high-risk score, 49% intermediate-risk, and 34% low-risk by the TAILORx cutoffs. In a review of the SEER database of patients with stage I to III, ER^+ $HER2^-$ patients, Kizy et al reported the RS distributions using TAILORx cutoffs were; high-risk, 14%; intermediate-risk, 60%; and low-risk, 26%.[17] In the TAILORx trial of $HER2^-$ patients, 14% of patients had a high-risk RS, 69% of patients had an intermediate RS, and 17% of patients had a low-risk RS.[14] In our present study of $HER2^+$ patients, the proportion of patients categorized as high-risk was slightly higher than reported in $HER2^-$ patients, but the majority of tested patients were still low and intermediate risk.

We found increased utilization of chemotherapy among patients who had high-risk RS compared to those with a low- or intermediate-risk RS. In fact, most patients with $HER2^+$ breast cancer with either low- or intermediate-risk RS did not receive chemotherapy. Previous studies have shown that clinician's use of chemotherapy is correlated with and impacted by RS.[18-23] Although the use of chemotherapy between those not tested and those who received a high-risk RS was comparable, there was a sizable decrease in chemotherapy use among those with low- and intermediate-risk scores. Thus, despite current guidelines, receipt of a low or intermediate RS score in $HER2^+$ patients appears to correlate with a decreased use of chemotherapy.

We acknowledge some important limitations to the study. Given that SEER is a national database of abstracted clinical information, there is selection bias as to which patients underwent RS testing. As clinicians are selecting which patients to test, the RS distributions may not reflect the true distributions in $HER2^+$ patients. This may in part explain why the RS distributions are similar to those patients with $HER2^-$ breast cancer, as we would expect that the more aggressive tumor biology in $HER2^+$ patients would lead to increased high-risk scores. Further, because SEER only began recording HER2 status in 2010, our study is limited from that date onward. With this short time period, we are unable to determine the implications of RS stratification and chemotherapy use on survival in this cohort. Further, HER2 information is collected as a dichotomous variable from several different data items in SEER, including results of immunohistochemistry, fluorescence in situ hybridization, and 'unknown' or 'other' test results. We are unable to parse out the variation in receptor expression among those who are positive. Additionally, we are unable to obtain data on the use of HER2-directed therapy from the SEER database, and therefore cannot draw any conclusions on if RS testing in this population is impacting use of trastuzumab.

Despite these limitations, this study is the first population-based study to evaluate the use of RS testing among patients with $HER2^+$ breast cancer. We found that 5% of patients with $HER2^+$ breast cancer in the United States underwent RS testing. Among those tested, the distributions of scores appear similar to those with $HER2^-$ disease, and clinicians appear to be forgoing chemotherapy administration more frequently in patients with low or intermediate scores. Further studies on RS testing in patients with $HER2^+$ breast cancer are necessary to determine the validity of the test in this population, characterize long-term outcomes, and help clinicians identify additional patients who may be able to forgo chemotherapy administration without decrements to long-term outcomes.

Clinical Practice Points

- The role of 21-gene RS testing in patients with $HER2^+$ breast cancer is currently unknown.
- The RS distributions of patients with $HER2^+$ disease was found to be similar to those with $HER2^-$ tumors; however, a greater proportion had high-risk scores.
- Most patients who had low or intermediate RSs in this study did not receive chemotherapy.
- Future studies on RS testing in patients with $HER2^+$ breast cancer are needed to determine whether this test is appropriate for risk stratification for chemotherapy recommendations in this population.

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Disclosure

Dr Tuttle declares that he holds a position on the advisory board of Genomic Health, Inc. The other authors have stated that they have no conflicts of interest.

References

1. 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.
2. 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; 28:1829-34.
3. Habel LA, Shak S, Jacobs MK, et al. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. *Breast Cancer Res* 2006; 8:R25.
4. Toi M, Iwata H, Yamanaka T, et al. Clinical significance of the 21-gene signature (Oncotype DX) in hormone receptor-positive early stage primary breast cancer in the Japanese population. *Cancer* 2010; 116:3112-8.
5. Mamounas EP, Tang G, Fisher B, et al. Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: results from NSABP B-14 and NSABP B-20. *J Clin Oncol* 2010; 28:1677-83.
6. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006; 24:3726-34.
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; 351:2817-26.
8. Gradishar WJ, Anderson BO, Balassanian R, et al. Breast Cancer, Version 4.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2018; 16:310-20.
9. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989; 244:707-12.
10. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987; 235:177-82.
11. Ravdin PM, Chamness GC. The c-erbB-2 proto-oncogene as a prognostic and predictive marker in breast cancer: a paradigm for the development of other macromolecular markers—a review. *Gene* 1995; 159:19-27.
12. Seshadri R, Firgaira FA, Horsfall DJ, McCaul K, Setlur V, Kitchen P. Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. The South Australian Breast Cancer Study Group. *J Clin Oncol* 1993; 11:1936-42.
13. Noone AM, Howlader N, Krapcho M, eds. SEER Cancer Statistics Review, 1975-2015. Bethesda, MD: National Cancer Institute. Available at: https://seer.cancer.gov/csr/1975_2015/, based on November 2017 SEER data submission, posted to the SEER web site, April 2018.
14. 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; 379:111-21.
15. Harris LN, Ismaila N, McShane LM, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2016; 34:1134-50.
16. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene signature as an aid to treatment decisions in early-stage breast cancer. *N Engl J Med* 2016; 375:717-29.
17. Kizy S, Huang JL, Marmor S, et al. Distribution of 21-gene recurrence scores among breast cancer histologic subtypes. *Arch Pathol Lab Med* 2018; 142:735-41.
18. Peethambaram PP, Hoskin TL, Day CN, Goetz MP, Habermann EB, Boughey JC. Use of 21-gene recurrence score assay to individualize adjuvant chemotherapy recommendations in $ER^{+}/HER2^{-}$ node positive breast cancer-A National Cancer Database study. *NPJ Breast Cancer* 2017; 3:41.
19. Hull LE, Lynch JA, Berse BB, et al. Clinical impact of 21-gene recurrence score test within the Veterans Health Administration: utilization and receipt of guideline-concordant care. *Clin Breast Cancer* 2018; 18:135-43.
20. Loncaster J, Armstrong A, Howell S, et al. Impact of Oncotype DX breast Recurrence Score testing on adjuvant chemotherapy use in early breast cancer: real world experience in Greater Manchester, UK. *Eur J Surg Oncol* 2017; 43:931-7.
21. Enewold L, Geiger AM, Zujewski J, Harlan LC. Oncotype Dx assay and breast cancer in the United States: usage and concordance with chemotherapy. *Breast Cancer Res Treat* 2015; 151:149-56.
22. Petkov VI, Miller DP, Howlader N, et al. Breast-cancer-specific mortality in patients treated based on the 21-gene assay: a SEER population-based study. *NPJ Breast Cancer* 2016; 2:16017.
23. Siegelmann-Danieli N, Silverman B, Zick A, Beit-Or A, Katzir I, Porath A. The impact of the Oncotype DX Recurrence Score on treatment decisions and clinical outcomes in patients with early breast cancer: the Maccabi Healthcare Services experience with a unified testing policy. *Ecancermedicalscience* 2013; 7:380.