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Original Article

Impact of the 21-Gene Recurrence Score Assay on Treatment Decisions and Cost in Patients with Node-Positive Breast Cancer: A Multicenter Study in Quebec

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Abstract

Background: The 21-gene Breast Recurrence Score (RS) assay, "the assay", has led to a paradigm shift for patients with hormone receptor-positive, node-negative early breast cancer and is emerging as an important tool to assist physician-patient decisions in foregoing chemotherapy in node-positive patients. We wanted to better understand the impact of the RS assay in node-positive patients upon physician treatment decisions and treatment cost in Quebec, Canada.

Patients and Methods: We conducted a multicenter, prospective observational trial for Estrogen/Progesterone Receptor (ER/PR)- positive, Human Epidermal Growth Factor Receptor 2 (HER2)-negative breast cancer patients with 1-3 positive lymph nodes. Physicians completed a questionnaire indicating treatment choice prior to and post availability of RS results. The primary endpoint was change in the physician's recommendation for chemotherapy prior to and post assay results. Secondary endpoints included change in physician's expressed level of confidence, and changes in estimated cost of recommended treatments prior to and post assay results.

Results: For the entire cohort, physician recommendation for chemotherapy was reduced by an absolute 67.1% by knowledge of the RS assay result ($P < .0001$). Physician recommendation of chemotherapy was decreased by 75.9% for patients RS result <14 ($P < .0001$); and 67.5% for patients with RS result 14-25 ($P < .0001$). Changes in treatment recommendations were associated with an overall reduction in cost by 73.7% per patient, and after incorporating the cost of the RS test, a cost benefit of $823 CAN at 6-month follow-up.

Conclusion: Altogether, we established that the assay led to a two-third reduction in the use of chemotherapy, and was a cost-effective approach for hormone receptor-positive, node-positive breast cancer.

Implications for Practice

This prospective, multicenter study evaluated the impact of the Recurrence Score (RS) assay on physician treatment decisions and cost in patients with hormone receptor-positive, node-positive breast cancer in Quebec, the second largest province of Canada. This study is unique in that the investigators analyzed physician treatment recommendations using RS cutpoints from 3 landmark studies, including the RxPONDER trial. Since the patient cohort was very similar to the RxPONDER trial, the two-third reduction in chemotherapy recommendation and three-fourth reduction in cost is highly relevant, and has potential to have important implications on long-term outcomes.

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Introduction

Although lymph-node positivity has been considered the strongest prognostic biomarker,[1] genomic analysis of the primary tumor has become an important tool in assisting patient-physician decisions regarding the need for adjuvant chemotherapy in early estrogen receptor (ER)-positive breast cancer patients.[2-4] In particular, the Oncotype DX Breast Recurrence Score (RS) assay ("the assay"), a 21-gene reverse transcription-polymerase chain reaction test, has assisted in the selection of node-negative and node-positive patients that can either benefit or forego chemotherapy.[5-7]

Amongst node-negative patients, the RS assay result was first evaluated retrospectively in 2 National Surgical Adjuvant Breast and Bowel Project (NSABP) trials using 3 risk categories, low risk (RS < 18), intermediate risk (RS, 18-30), and high risk (RS > 30).[5,8] In the tamoxifen-only arm of the NSABP B-14 trial, patients in the low-risk group demonstrated a much lower rate of distant recurrence, while patients with high-risk tumors demonstrated a large benefit when treated with chemotherapy in the NSABP-B20 trial. In the prospective randomized Trial Assigning Individualized Options for Treatment (TAILORx), patients with RS 11-25 received either endocrine or chemo-endocrine therapy, and demonstrated similar freedom from recurrence of breast cancer at distant or locoregional site (92.2% and 92.9%, respectively), and overall survival (OS) (93.9% and 93.8%, respectively) with a follow-up of 9 years.[6] However, for patients ≤50 years, the freedom from recurrence of breast cancer at a distant or locoregional site for patients receiving endocrine vs chemo-endocrine therapy was 89.6% and 93.0%, respectively for patients with RS 16-20, and 82.0% and 90.7%, respectively, for patients with RS 21-25. Overall, this landmark study demonstrated that up to 85% of patients with early breast cancer could forego chemotherapy.

In addition to node-negative disease, several retrospective studies evaluated the prognostic and/or predictive potential of the RS result in exclusively ER-positive node-positive patients.[9-15] In the Southwest Oncology Group (SWOG)-8814 study,[11] a phase III trial for postmenopausal women, although no benefit was observed for the low or intermediate RS groups, amongst the high-risk RS group, a 12% and 17% improvement in the chemo-endocrine arm vs endocrine arms for 10-year disease-free survival (DFS) and OS was observed, respectively. In the hormone receptor-positive cohort of the NSABP-B28 trial, in which node-positive patients were treated with chemotherapy,[12-14] there was a strong correlation of RS groups with distant recurrence, for both early and late events. The RS result was also associated in a statistically significant manner with DFS, distant recurrence-free interval, OS, and breast cancer-specific survival (BCSS), both in univariate and multivariate analysis.

Using the RS cutpoints of the TAILORx study,[6] a review of the Surveillance, Epidemiology, and End Results (SEER) database identified 19.6% of the patients to be low-risk (RS < 11), 65.7% intermediate-risk (RS 11-25), and 14.7% high risk (RS > 25).[16] With a median follow-up of 57 months, the RS risk-groups and pathologic stage groups were shown to be independent prognostic factors for BCSS and OS after multivariate analysis. The West German Study Group Plan B trial[17,18] prospectively integrated the RS result in patients with <4 positive nodes to omit chemotherapy if RS ≤ 11 or randomized to receive anthracycline-based chemotherapy versus anthracycline-free chemotherapy if RS ≥ 12. Among the patients with RS ≤ 11 who received endocrine therapy, the 5-year DFS and OS was 94% and 99%, respectively. In chemotherapy-treated patients, the DFS and OS was 94% and 97% if RS 12-25, and 84% and 93% if RS > 25, respectively. Interestingly, the study demonstrated the non-inferiority of an anthracycline containing vs anthracycline-free regimen in a genomically intermediate and high-risk cohort.

The SWOG S1007 clinical trial RX for Positive Node, Endocrine Responsive breast cancer (RxPONDER) was a prospective phase III randomized controlled trial that compared the efficacy of endocrine therapy vs chemo-endocrine therapy in hormone receptor-positive HER2-negative patients with 1-3 positive lymph nodes with an RS < 25.[7] Post-menopausal women did not demonstrate any benefit with chemo-endocrine therapy in comparison to endocrine therapy, with a 5-year invasive DFS at 91.3% vs 91.9%. For premenopausal women, the 5-year invasive DFS was 93.9% for chemo-endocrine therapy, in comparison to 89.0% for endocrine therapy alone (P = .002). Using the pre-specified cutpoints, for 5-year invasive DFS, pre-menopausal women demonstrated a trend for improvement with chemo-endocrine therapy with RS 0-13 (3.9% benefit, P = .06), and a 5.8% improvement with RS 14-25 (P = .02).

Since the prognostic and predictive value of the RS in node-positive patients was demonstrated in several retrospective studies and one prospective study (prior to the reporting of RxPONDER study results), we wanted to determine the utility of the assay in Quebec, the second largest province in Canada. The aim of our study was to evaluate the clinical utility and economic impact of the assay among patients with hormone receptor positive, node-positive breast cancer. Therefore, we conducted a prospective multicenter study to determine the impact of the assay upon physician treatment decisions and overall cost.

Methods

Patients and Eligibility Criteria

The study is part of a larger protocol called NeaOnq, which evaluates the impact of the assay result on changes in physician treatment decisions in 2 distinct patient populations for neoadjuvant and adjuvant chemotherapy. We are reporting the results of the first component for the adjuvant setting which includes patients who have undergone surgical treatment for breast cancer with adequate evaluation of lymph node status by a sentinel lymph node biopsy or full axillary dissection with histologically proven T1-T3 disease and 1-3 positive lymph nodes. Tumor size was obtained from the surgical pathology report. Tumors were either ER or progesterone receptor (PR)-positive, defined as >10% tumor staining by immunohistochemistry (IHC) and Human Epidermal Growth Factor Receptor 2 (HER2)-negative, defined by either IHC or fluorescence in situ hybridization (FISH). As per the 2018 ASCO/CAP guidelines, HER2-negative tumors were defined either by an IHC score of 0 or 1+. If IHC was equivocal or 2+, FISH was then conducted and HER2 considered negative if HER2/CEP17 ratio < 2.0 or HER2 copy number < 4.0. Multicentric or multifocal tumors were allowed with a maximum of 2 foci, for which both were ER/PR positive and HER2 negative, wherein tissue samples from both foci were submitted for the assay. Patients needed to be ≥18 years of

age, with an adequate performance status (ECOG ≤ 1), and no contraindication for treatment with systemic chemotherapy in addition to anti-hormonal therapy. Physicians were to include those patients for whom the benefit of chemotherapy was uncertain and would consider a change in treatment recommendation.

Exclusion criteria included male patients, clinical or radiologic evidence of metastatic disease, synchronous or metachronous contralateral invasive breast cancer, and any radiation therapy, chemotherapy, anti-hormonal therapy and/or targeted therapy for the currently diagnosed breast cancer prior to registration. Patients were also excluded if there was a history of ipsilateral invasive breast cancer, ipsilateral DCIS (ductal carcinoma in situ) if treated with radiation therapy, history of non-breast malignancies, except for in situ cancers treated only by local excision, or basal or squamous cell carcinomas of the skin within 5 years prior to registration.

Trial Design

This trial was designed as a prospective, multicenter observational study under the McPeak Sirois Group, consisting of 5 hospital centers in Quebec. Institutional ethics approval was obtained at each of the 5 sites including: the CHUM (Centre hospitalier de l'Université de Montréal), CHUQ (Centre hospitalier universitaire de Québec), Hôpital Maisonneuve-Rosement, Jewish General Hospital, and CUSM (Centre Universitaire de Santé McGill). Informed consent was obtained for all patients.

Patients had an initial consultation with their oncologist in which treatment options were discussed. The physician then completed a baseline pre-assay questionnaire (Supplementary Material), indicating their recommended treatment based upon the available clinicopathological data. Following the completion of this questionnaire, the tissue block(s) were sent for assay testing.

The physician discussed the assay results with the patient and completed a separate post-assay questionnaire (Supplementary Material). The choice of adjuvant chemotherapy or anti-hormonal therapy was at the discretion of the treating physician. The treatment recommended to the patient was recorded. Furthermore, the actual treatment administered (prescribed) was also recorded to take into consideration patient's choice of treatment. In addition, information on anticipated additional supportive growth factor therapy was collected to estimate the total cost associated with chemotherapy pre- and post-assay results. Follow-up was conducted 6 months after the start of adjuvant treatment to determine if the prescribed therapy was still being administered to the patient (Supplementary Material). Patients were considered to have received anti-hormonal therapy if they received either tamoxifen, an aromatase inhibitor, or ovarian suppression via surgical or chemical approaches.

Outcomes

The primary endpoint of the study is the evaluation of the impact of the assay result on changes between physicians' recommendations for adjuvant chemotherapy prior to and post-assay, and actual treatment delivered after receipt of the assay result. Secondary endpoints include evaluation of changes in physicians' expressed level of confidence in their treatment recommendations, evaluation of changes in specific recommended chemotherapy or hormonal therapeutic regimens (including ovarian suppression therapy), evaluation of changes in intended use of supportive therapies including growth factor therapy, and assessment of the economic impact associated with the use of the assay result.

Cost Analysis

Drug costs were obtained from the CHUM Oncology Pharmacy (Centre Integré de Cancérologie du CHUM), which are comparable to other Quebec sites. The different chemotherapy regimens and use of growth factor supportive therapy proposed by the physician pre- and post-assay were captured from the physician questionnaire. The cost of anti-emetics and pre-medications was calculated for each chemotherapy regimen. The total cost of chemotherapy comprised the cost of the chemotherapy drugs themselves, growth factor supportive therapy, and anti-emetics, and pre-medications. Mean hourly rates of nursing assistants, nurses, pharmacists, and technicians were obtained from their respective Quebec collective agreements. The cost of blood tests for each chemotherapy regimen was also calculated, including salaries for nursing assistants for blood procurement and blood test analysis (based on hematology and biochemistry laboratory rates at CHUM). Pharmacy costs were derived from the allocated times for each regimen from Cancer Care Ontario,[19] which is representative of the practice in CHUM and Quebec. Here, the salaries were divided into 60% pharmacist and 40% technician. The salaries for nurses were reflective of time needed for infusion of each treatment and patient counselling throughout the treatment. Physician fees were obtained from the RAMQ (Regie de l'assurance maladie de Quebec), using the mean for medical and surgical oncologists fee per service for a new consultation and oncological follow-up visits. Total cost also incorporated the cost of the RS test and cost savings represented the difference between the pre-RS assay cost and either the total cost post-RS assay recommendation or at the 6-month follow-up time point.

Sample Size

Sample size is based on the McNemar test comparing the pre-assay proportion of patients with recommended chemo-hormonal therapy vs the post-assay proportion actually receiving chemo-hormonal therapy.

Assuming a net reduction in chemotherapy use of 28% and the proportion of discordant pairs (patients with discordance between the recommended therapy prior to ordering the assay and the actual therapy received after the assay results are received) not to exceed 70%, a sample size of 70 patients was calculated to have more than 80% power, to detect a difference of 28% with a 0.05 2-sided significance level.

Statistical Analysis

Patients and tumor characteristics were summarized using descriptive statistics as follows: categorical variables were summarized as counts and percentages, and continuous variables as medians and ranges. All statistical tests were conducted at a 2-sided α level of 0.05. Statistical analysis was done using SAS version 9.4.

Pre-assay treatment recommendation, post-assay recommendation, and treatment prescribed post-assay was analyzed using a generalized estimating equations (GEE) model

that accounted for the correlated nature of the assessments. The binary variable therapy (anti-hormonal therapy only or chemo-hormonal therapy) was the dependent variable and timing (pre-assay recommendation, post-assay recommendation, post-assay prescription) was the independent variable. Under this model, odds ratios (odds of recommending/prescribing chemo-hormonal therapy post-assay/odds of recommending chemo-hormonal therapy pre-assay) were calculated and were presented with 95% CI. For patients who were <50 years or pre-menopausal, the GEE model could not be used because there were no subjects who were recommended to receive anti-hormonal therapy pre-assay. Here, the McNemar exact test was used to calculate the odds ratio and *P*-value.

Physician confidence in treatment was also analyzed using a GEE model, with the ordinal variable physician confidence (somewhat not confident, neutral, somewhat confident, strongly confident) as the dependent variable and timing (pre-assay or post-assay recommendation) as the independent variable. Odds ratio (odds of a higher level of confidence post-assay/odds of a higher level of confidence pre-assay) was calculated and presented with 95% CI. Finally, usage of growth factor was analyzed similarly with a GEE model, with the nominal variable usage of growth factor (yes, no, unknown) as the dependent variable and timing (pre-assay recommendation, post-assay recommendation, or 6 months) as the independent variable. Again, odds ratios (odds of recommending growth factor post-assay/odds of recommending growth factor pre-assay; odds of using growth factor at 6 months/odds of recommending growth factor pre-assay) were calculated and presented with 95% CI.

Results

Seventy-one patients were enrolled in the study between March 2018 and September 2019 as described in Supplementary Fig. S1. One patient withdrew consent, leaving 70 patients eligible for Oncotype DX testing and evaluation for study outcomes. Patients were enrolled at each of the 5 hospital centers which are part of the McPeak Sirois Group of Quebec.

All patients had an ECOG performance status of 0. None of the patients demonstrated clinical or radiologic evidence of metastatic disease at registration. Clinicopathological characteristics are summarized in Table 1. Eighty-one percent of the patients were ≥50 years and 77.1% of the patients were post-menopausal. The median tumor size was 1.9 cm, with 65.7% of the tumors ≤ 2 cm. All patient tumors were ER-positive and HER2-negative, while <10% were PR-negative. Sixty-four percent of patients had 1 positive lymph node, 25.7% had 2 positive lymph nodes, and 10.0% of patients had 3 positive lymph nodes. Among the 9 patients (12.9%) with multicentric and multifocal tumors, second tumor (tumor 2) size was available for 7 patients, of which median tumor size was 0.9 cm and 71.4% demonstrated the absence of lymphovascular invasion (5/7 evaluable) (Supplementary Table S1).

We categorized the RS result of tumor 1 using 3 different cutpoint thresholds: as per prespecified cutpoints of the RxPONDER trial[7,20] (0-13, 14-25, >25), TAILORx trial (0-10, 11-25, >25),[10] and Paik et al's cutpoints (0-18, 18-30, >30)[5] (Table 2, Supplementary Table S2). Forty-one percent (*N* = 29) of the cohort had a RS result 0-13, 52.9% (*N* = 37) had a RS result 14-25, and 5.7% (*N* = 4) had a RS result 26-100. RS results for tumor 2 were available for 3 patients, but did not change physician recommendation any differently from tumor 1. Therefore, no further analysis was performed on tumor 2.

Table 1. Clinico-pathological characteristics of tumor 1 and patient cohort.

Variable	No. of patients (%)	Median (range)
Age (years)		61 (38– 82)
<50	13 (18.6)	
50–59	18 (25.7)	
60–69	21 (30.0)	
≥70	18 (25.7)	
Menopausal status		
Pre-menopausal	16 (22.9)	
Post-menopausal	54 (77.1)	
Histology subtype		
Ductal	59 (84.3)	
Lobular	7 (10.0)	
Mixed	4 (5.7)	
Tumor size (cm)		1.9 (0.6–5.0)
≤2	46 (65.7)	
>2	24 (34.3)	
Tumor grade		
1	15 (21.4)	
2	43 (61.4)	
3	12 (17.1)	
Lymphovascular invasion		
Yes	38 (54.3)	
No	30 (42.9)	
NA	2 (2.9)	
ER status		
Positive	70 (100.0)	
Negative	0 (0.0)	
PR status		
Positive	64 (91.4)	
Negative	6 (8.6)	
HER2 status		
Positive	0 (0.0)	
Negative	70 (100.0)	
Ki67 positivity		
0-10%	5 (7.1)	
11-20%	5 (7.1)	
≥21%	4 (5.7)	
Not done	56 (80.0)	
Multicentric or multifocal tumor		
Yes	9 (12.9)	
No	46 (65.7)	
NA	15 (21.4)	
N stage		
N1mic	21 (30.0)	
N1	49 (70.0)	
No. of positive lymph nodes		
1	45 (64.3)	
2	18 (25.7)	
3	7 (10.0)	

Table 2. Variables categorized as per Recurrence Score result with RxPONDER trial[7] cutpoints.

Variable	RS categories		
	0-13	14-25	26-100
Overall population	29 (41.4)	37 (52.9)	4 (5.7)
Age (years)			
<50	3 (23.1)	9 (69.2)	1(7.7)
≥50	26 (45.6)	28 (49.1)	3 (5.3)
Menopausal status			
Pre-menopausal	3 (18.9)	12 (75.0)	1 (6.3)
Post-menopausal	26 (48.1)	25 (46.3)	3(5.6)
Tumor size (cm)			
≤2	20 (43.5)	23 (50.0)	3 (6.5)
>2	9 (37.5)	14 (58.3)	1 (4.2)
Tumor grade			
1	8 (53.3)	7 (46.7)	0 (0.0)
2	19 (44.2)	23 (53.5)	1 (2.3)
3	2 (16.7)	7 (58.3)	3 (25.0)
Lymphovascular invasion			
Yes	16 (42.1)	20 (52.6)	2 (5.3)
No	12 (40.0)	16 (53.3)	2 (6.7)
PR status			
Positive	29 (45.3)	33 (51.6)	2 (3.1)
Negative	0 (0.0)	4 (66.7)	2 (33.3)
No. of positive lymph nodes			
1	19 (42.2)	24 (53.3)	2 (4.4)
2 or 3	10 (40.0)	13 (52.0)	2 (8.0)

We determined the impact of the assay by evaluating physician recommendation of chemotherapy pre- and post-assay (Table 3). For all patients, we identified an absolute reduction in chemotherapy recommendation of 67.1%, from pre-assay (90.0%) to post-assay (22.9%), odd's ratio (OR) 0.03; 95% CI, 0.01-0.08; $P < .0001$. We also evaluated the change in chemotherapy recommendation in several patient subpopulations. Interestingly, we determined that the absolute reduction in chemotherapy was 38.5% for patients <50 years ($P = .06$), and 43.8% ($P = .02$) in pre-menopausal women. Among the pre-menopausal patients who were switched to endocrine therapy alone, 42.9% of patients (3/7) were recommended an aromatase inhibitor with ovarian suppression. Physician recommendation for chemotherapy was reduced by 74% for both patients ≥50 years and postmenopausal women ($P < .0001$ for both groups). There was a noteworthy decrease in physician recommendation for chemotherapy, irrespective of the context of one positive lymph node, by 73.3% (OR 0.02; 95% CI 0.01-0.07; $P < .0001$), or 2 or 3 positive lymph nodes, by 56.0% (OR 0.06; 95% CI 0.02-0.23; $P < .0001$).

We also evaluated the change in physician recommendation using different RS cutpoints. The change in physician recommendation was observed to a similar extent with RS < 18: 77.1% (OR 0.02; 95% CI 0.01-0.05; $P < .001$), and with RS < 14: 75.9% (OR 0.01; 95% CI 0.00-0.08; $P < .0001$). However, we observed somewhat different proportions of changes in physician recommendations with different intermediate RS cutpoints. The reduction in physician recommendation for chemotherapy for RS 14-25 was 67.6% (OR 0.01; 95% CI, 0.00-0.09; $P < .0001$; in comparison to RS 18-30 was 47.6% (OR 0.05; 95% CI, 0.01-0.34; $P = .003$).

In addition to the post-assay physician recommendation, we also evaluated: treatments that were actually prescribed—to take into account patient preference (post-assay prescription, Supplementary Table S3), and which treatments were being administered at a 6-month follow-up (6-month post, Supplementary Table S4). Overall, there was a trend toward less use of chemotherapy by the 6-month follow-up (Fig. 1). This was observed in all patients, with a 67.1% ($P < .0001$) reduction in chemotherapy recommendation post-assay, 72.9% (OR 0.02; 95% CI, 0.01-0.06; $P < .0001$) reduction in chemotherapy at the time of prescription, and 75.7% reduction in use of chemotherapy at 6-month follow-up in comparison to pre-assay recommendation.

Similarly, although a smaller subgroup, the post-assay recommendation for chemotherapy for patients <50 years was decreased by 38.5%, but at the time of prescription decreased by 53.8%. Furthermore, amongst patients with 2 or 3 positive lymph nodes, the post-assay recommendation resulted in an absolute reduction of chemotherapy by 56.0% ($P < .0001$), whereas the post-assay prescription demonstrated a reduction in chemotherapy by 68.0% (OR 0.03; 95% CI 0.01-0.14; $P < .0001$). In an analogous manner, for patients with a RS 14-25, the post-assay physician recommendation resulted in a 67.6% decrease in chemotherapy ($P < .0001$), in comparison to 75.7% (OR 0.01; 95% CI 0.00-0.06, $P < .0001$) at the time of prescription. To summarize, there was a decrease in the use of chemotherapy at post-assay prescription in comparison to post-assay recommendation by 15.3% for patients <50 years, 12.0% for patients with 2/3 positive lymph nodes, and 8.1% for patients with RS 14-25.

We further evaluated the patients individually for whom there was a discordance in the treatment prescribed vs treatment received at 6-month follow-up (Supplementary Table S5). In total, there were 5 patients who refused chemotherapy. The mean age of these patients was 56.8 (range, 38-82), and the mean number of lymph nodes was 1.8 (range, 1-3). Interestingly, 3 of these patients had a RS ≤18, and one of these patients explained the reasoning for refusal was due to the minimal benefits of survival that were outweighed by the potential side effects. Therefore, age of patient or the number of positive lymph nodes did not seem to influence adherence to prescription of chemotherapy.

We also evaluated the impact of the RS result upon physician confidence in treatment administration (Fig. 2, Supplementary Table S6). Figure 2A demonstrates that pre-assay, 31.4% of physicians were neutral, 55.7% of physicians were somewhat confident, and only 10.0% of physicians were strongly confident of their treatment recommendation. In contrast, post-assay, the level of confidence of most physicians increased, with 30.0% of physicians being somewhat confident and 67.1% of physicians being strongly confident. Overall, there was a 68.6% increase in confidence secondary to RS results (OR 18.3; 95% CI 7.90-42.28; $P < .0001$). We further dissected the change in physician confidence based on RS categories. Confidence levels increased the most, by 75.9% when RS < 14 (OR 25.0; 95% CI 7.82-111.13; $P < .0001$) (Fig. 2B), and to a lesser extent with RS 14-25, by 59.5% (OR 11.4; 95% CI 3.72-34.61; $P < .0001$) (Fig. 2C). Physician confidence also increased by 100% when RS >25 (Fig. 2D), but there were too few patients to determine statistical significance.

Table 3. Pre-assay versus post-assay physician recommendation.

Variable	Pre-assay recommendation		Post-assay recommendation		Absolute reduction in chemotherapy No. of patients (%)	Odds ratio[a] (95% CI)	*P*-value[a]
	Anti-hormonal therapy No. of patients (%)	Chemo-hormonal therapy No. of patients (%)	Anti-hormonal therapy No. of patients (%)	Chemo-hormonal therapy No. of patients (%)			
All	7 (10.0)	63 (90.0)	54 (77.1)	16 (22.9)	**47 (67.1)**	0.03 [0.01-0.08]	<.0001
Age (years)							
<50	0 (0.0)	13 (100.0)	5 (38.5)	8 (61.5)	**5 (38.5)**	0.00 [0.00-1.09][b]	.06[b]
≥50	7 (12.3)	50 (87.7)	49 (86.0)	8 (14.0)	**42 (73.7)**	0.02 [0.01-0.06]	<.0001
Menopausal status							
Pre-menopausal	0 (0.0)	16 (100.0)	7 (43.8)	9 (56.3)	**7 (43.8)**	0.00 [0.00-0.69][b]	.02[b]
Post-menopausal	7 (13.0)	47 (87.0)	47 (87.0)	7 (13.0)	**40 (74.1)**	0.02 [0.01-0.06]	<.0001
No. of positive lymph nodes							
1	4 (8.9)	41 (91.1)	37 (82.2)	8 (17.8)	**33 (73.3)**	0.02 [0.01-0.07]	<.0001
2 or 3	3 (12.0)	22 (88.0)	17 (68.0)	8 (32.0)	**14 (56.0)**	0.06 [0.02-0.23]	<.0001
RS results based on Paik et al.'s[5] cutpoints							
<18	6 (12.5)	42 (87.5)	43 (89.6)	5 (10.4)	**37 (77.1)**	0.02 [0.01-0.05]	<.0001
18–30	1 (4.8)	20 (95.2)	11 (52.4)	10 (47.6)	**10 (47.6)**	0.05 [0.01-0.34]	.003
>30	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	**(0.0)**	NA*	NA*
RS results based on TAILORx[6] cutpoints							
<11	4 (22.2)	14 (77.8)	17 (94.4)	1 (5.6)	**13 (72.2)**	0.02 [0.00-0.15]	.0002
11–25	3 (6.3)	45 (93.8)	37 (77.1)	11 (22.9)	**34 (70.8)**	0.02 [0.01-0.07]	<.0001
>25	0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)	**0 (0.0)**	NA*	NA*
RS results based on RxPONDER[7] cutpoints							
<14	6 (20.7)	23 (79.3)	28 (96.6)	1 (3.4)	**22 (75.9)**	0.01 [0.00-0.08]	<.0001
14–25	1 (2.7)	36 (97.3)	26 (70.3)	11 (29.7)	**25 (67.6)**	0.01 [0.00-0.09]	<.0001
>25	0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)	**0 (0.0)**	NA*	NA*

[a]Odds ratio and *P*-value calculated using the generalized estimating equations (GEEs) model.
[b] Since the GEE model could not be used because there were no subjects who were recommended to receive anti-hormonal therapy pre-assay, McNemar exact test was used to calculate the odds ratio and a *P*-value for patients <50 years and pre-menopausal patients.
*No statistical tests were performed as there were too few subjects.
Abbreviation: NA, not available.

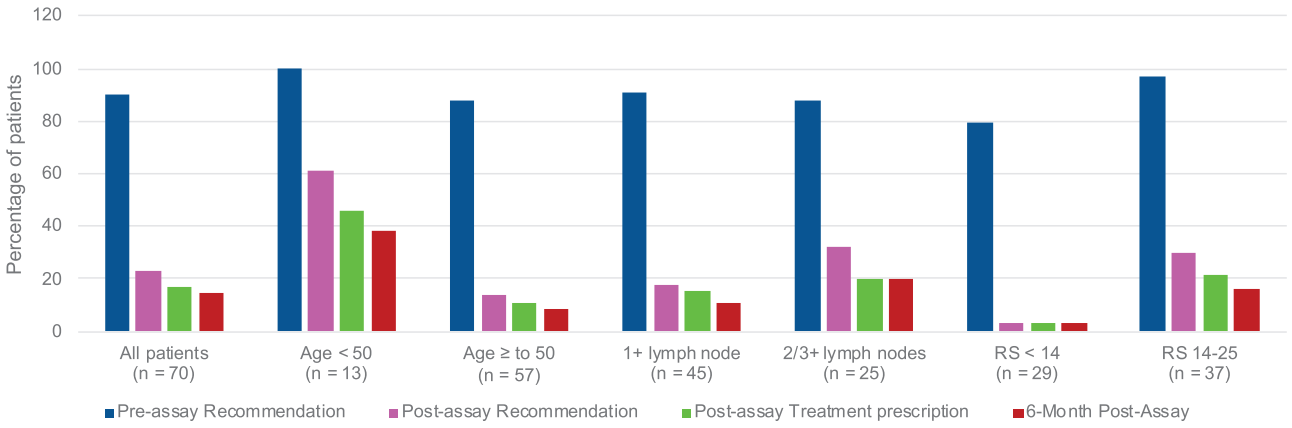

Figure 1. Recommendation, prescription, and use of chemo-hormonal therapy at 6-month follow-up. Physician recommendation of chemo-hormonal therapy was compared prior to ordering assay, and post-receipt of assay. In addition to physician recommendation, proportion of patients that were prescribed chemo-hormonal therapy and actual chemo-hormonal therapy received at 6-month follow-up is also reported.

An additional observed trend of the RS result is that physicians may use this tool to modify the type of chemotherapy recommended, summarized in Table 4, which can have a significant impact upon the duration of treatment and number of hospital visits. Among the patients for whom chemotherapy was still recommended post-assay results, 4/16 (25.0%) of patients were recommended an important change in treatment regimen from 4 cycles of doxorubicin and cyclophosphamide plus 12 cycles of paclitaxel, to 4 cycles of docetaxel and cyclophosphamide. Changing the type of chemotherapy from anthracycline-containing to anthracycline-free regimens can also reduce the toxicity burden in terms of

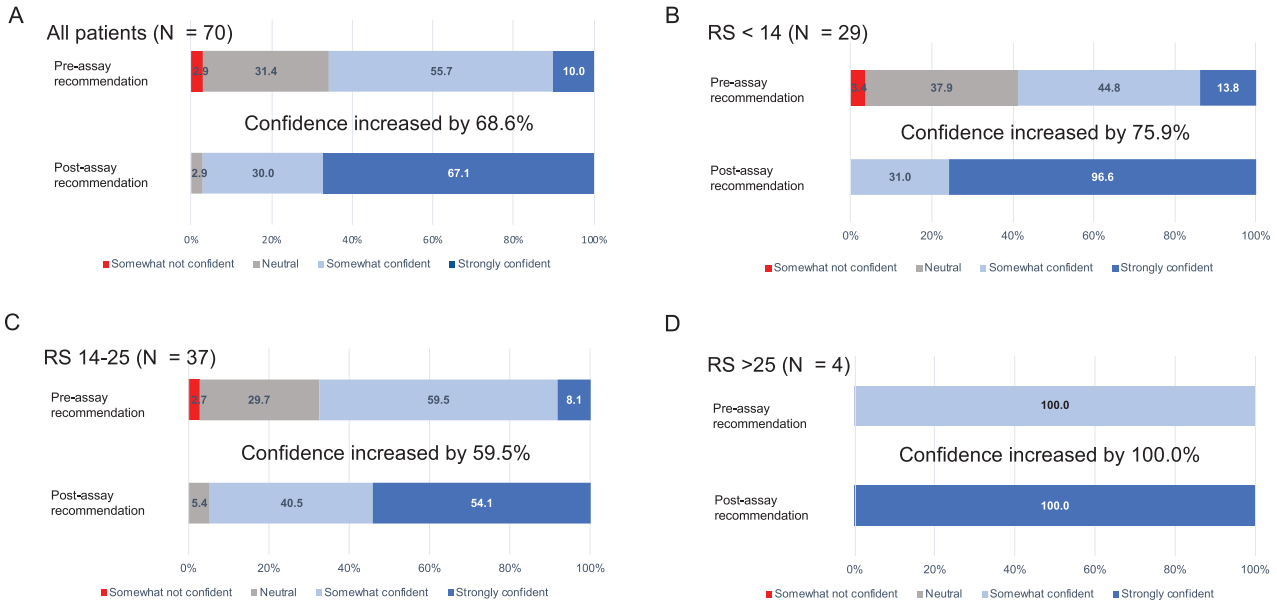

Figure 2. Physician confidence comparing pre-assay and post-assay recommendation. Levels of confidence, scored as somewhat not confident, neutral, somewhat confident, and strongly confident, were recorded by the physician pre- and post-receipt of assay results. Physician confidence was demonstrated in (A) all patients and were grouped as per recurrence score (RS) in (A) RS < 14; (B) RS 14-25; (C) RS > 25.

Table 4. Impact of Recurrence Score assay on chemotherapy regimens.

Chemotherapy regimen	No. of patients (%)			
	Pre-assay recommendation	Post-assay recommendation	Post-assay prescription	6-month follow-up[a]
AC/ddAC x 4 + weekly paclitaxel × 12	45 (64.3)	5 (7.1)	4 (5.7)	5 (7.2)
ddACx4 + paclitaxel × 4	3 (4.3)	3 (4.3)	3 (4.3)	0 (0)
AC + docetaxel × 4	1 (1.4)	0 (0)	0 (0)	0 (0)
FEC100+ docetaxel × 3	4 (5.7)	0 (0)	0 (0)	1 (1.4)
CMF × 6	1 (1.4)	1 (1.4)	0 (0)	0 (0)
TC × 4/6	8 (11.4)	7 (10.0)	5 (7.1)	4 (5.8)
Taxol × 12	1 (1.4)	0 (0)	0 (0)	0 (0)
No chemotherapy	7 (10.0)	54 (77.1)	58 (82.9)	59 (85.5)

[a]Total number of patients are 69 at 6-month follow-up because 1 patient developed distant metastasis.
Abbreviations: AC, adriamycin (doxorubicin) + cyclophosphamide; dd, dose-dense, FEC, 5-fluorouracil + epirubicin + cyclophosphamide; CMF (cyclophosphamide + methotrexate + 5-fluorouracil); TC, Taxotere (docetaxel) + cyclophosphamide.

cardiotoxicity and secondary malignancies including acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS).[21] Moreover, our physician questionnaire also interrogated the use of growth factor (supportive therapy) prior to assay results, post-assay results, and at 6-month follow-up. Pre-assay, 53.6% of patients were recommended to receive growth factor. Post-assay, there was a 36.2% decrease in growth factor recommendation (OR 0.16; 95% CI 0.07-0.34; $P < .0001$), and at 6-month follow-up there was a 40.6% decrease in growth factor use (OR 0.11; 95% CI 0.05-0.24; $P < .0001$) (Supplementary Table S7).

Based on physician recommendations and prescriptions, we calculated the cost of treatment pre- and post-assay results. All of the patients who were initially recommended endocrine therapy remained with the same recommendation post-RS assay. We evaluated the total and per patient cost of chemotherapy, growth factor supportive therapy, anti-emetics, and pre-medications, in addition to costs associated with blood tests, pharmacists, nurses, and physicians (Table 5, Supplementary Table S8). In comparison to pre-assay physician recommendation, we determined that the average cost per patient decreased by $4551 CAN (73.7%) at the post-assay recommendation, and by $4998 CAN (80.9%) at the 6-month follow-up. After incorporating the cost of the Oncotype DX RS test, there was a cost benefit of $376 CAN per patient at the time of post-assay recommendation, and $823 CAN at 6-month follow-up.

Discussion

Our study evaluated the impact of the assay upon physician treatment recommendation and cost in patients with node-positive hormone receptor-positive breast cancer. We identified an overall reduction in physician recommendation of chemotherapy of 67%. The RS assay result influenced physician recommendations of chemotherapy in contexts

Table 5. Total cost of chemotherapy, blood tests, pharmacy, nurses, and physicians in context of the RS assay.

Cost component	Pre-assay recommendation	Post-assay recommendation	6-month post-assay	Absolute reduction in cost in post- vs pre-assay recommendation (CAN $, %)	Absolute reduction in cost at 6-months post- and pre-assay recommendation (CAN $, %)
Chemotherapy (including growth factor supportive therapy, + anti-emetics/pre-medications)	$277,784	$83,728	$61,117	$194,056 (69.9)	$216,667 (78.0)
Blood tests (including procurement + lab analysis)	$12,231	$2494	$1663	$9,737 (79.6)	$10,568 (86.4)
Pharmacy (including teaching + drug preparation)	$12,615	$2415	$1730	$10,200 (80.6)	$10,885 (86.3)
Nurses (including counseling of adverse effects, + time during treatment)	$104,510	$19,236	$13,123	$85,274 (81.6)	$91,387 (87.4)
Physicians (including new cases + follow-ups)	$25,097	$5783	$3957	$19,314 (77.0)	$21,140 (84.2)
Subtotal	$432,237	$111,241	$81,230	$318,581 (73.7)	$351,007 (81.2)
Average cost/patient[a]	$6175	$1624	$1177	$4551 (73.7)	$4998 (80.9)
Cost of RS test	NA	$4125	$4125		
Total cost per patient	$6175	$5799	$5,352	$376 (6.1)	$823 (13.3)

[a]Average cost/patient was calculated by using the entire cohort of patients, n = 70 for pre- and post-assay recommendation, and n = 69 for 6-month post-assay since 1 patient developed distant metastasis.

that have been commonly considered clinically high risk. Chemotherapy recommendations were reduced by 44% in pre-menopausal patients and by 56% in patients with 2 or 3 positive lymph nodes. We are the first to report a reduction in chemotherapy recommendation in both low-risk or intermediate-risk RS scores, using different cutpoint classifications derived from 3 trials including RxPONDER, TAILORx, and Paik et al.[5-7]

Overall, our study reported a 67% decrease in the use of chemotherapy, which is higher than what previous Canadian studies have reported.[22] In our study, initial physician recommendation for chemotherapy was 90%, proportion of patients with an RS < 18 was 69%, and patient recruitment was completed by September 2019. Physicians reduced their chemotherapy recommendation by 47.6% with RS 18-30, and 70.8% in patients with RS 11-25. Two previous studies from Ontario and British Columbia demonstrated a lower overall reduction in chemotherapy use by 31% and 45%, respectively.[23,24] These studies had a smaller proportion of patients with RS<18 (55-60%) and demonstrated a lower use of initial chemotherapy (65-76%). Interestingly, both studies completed patient recruitment in 2016 and 2017, and also demonstrated a smaller reduction in chemotherapy recommendation with intermediate RS (18-30), between 13% and 37%. Similarly, in an Italian study, where patient enrollment was completed in February 2018, among node-positive patients, chemotherapy recommendation was reduced by 30% in patients with RS 11-17.[25] However, in Mattar et al's study, where patient enrollment was completed almost 1 year after the TAILORx results were released in June 2018, chemotherapy recommendations were reduced from 60%-80% for patients with RS 11-25.[26] Furthermore, the overall reduction in chemotherapy recommendation of 67% in our study was comparable to the magnitude of reduction of 2 recent studies, 66%-74%,[26,27] which is higher than several earlier trials.[23-25,28-30] This is suggestive that although the TAILORx study consisted of node-negative patients, the cutpoints and results from this study probably influenced physician treatment recommendations among node-positive patients as well.

One of the limitations of our study is sample size, even though a sample size calculation was performed a priori for the overall population. Our study was designed to select a patient cohort with hormone receptor-positive node-positive patients for whom treatment decisions were unclear and could be changed upon receipt of the RS result. This could have led to a selection bias, since only 6% of our population had a RS >25, but is comparable to 11% of patients identified in another node-positive cohort.[31] Nonetheless, the clinicopathological characteristics of our patient population were similar to those in previously reported studies.[10,20,23] Less than one-quarter of our patient population was <50 years or pre-menopausal. Sixty-six percent of our patients had tumors ≤2 cm, 61.4% had tumors of intermediate grade, and 64.3% had one positive lymph node. These characteristics are very similar to the RxPONDER cohort wherein 58.3% of the tumors were ≤2 cm, 64.3% of tumors were of intermediate grade, and 65.3% of patients had one positive lymph node.[7] In our study, 41.4% of patients had a RS result 0-13, and 52.9% had a RS between 14 and 25, which is comparable to the RxPONDER cohort in which 42.8% of the overall population had a RS 0-13, and 57.2% had a RS 14-25. Therefore, with a similar RS distribution, we can extrapolate our results onto the RxPONDER study.

Our study was conducted prior to the reporting of the RxPONDER trial[20] which demonstrated a benefit of chemo-endocrine therapy in pre-menopausal patients. In our cohort, physician recommendation for chemotherapy in

pre-menopausal patients was reduced by 43.8%. Therefore, while the reduction in use of chemotherapy among pre-menopausal women could explain in part the overall larger reduction in chemotherapy in our study, future studies will be required to determine the true benefit of chemo-endocrine therapy versus endocrine therapy plus ovarian suppression in this population.

We conducted a cost analysis which included the immediate costs of chemotherapy, blood tests, and salaries for the time provided by pharmacists, nurses, and physicians. We identified a significant reduction at 6-month follow-up cost, that is, a reduction of about $5000 per patient, with a cost benefit of $832 per patient after incorporation of the cost of the RS test. However, the savings could actually be greater if the cost of managing short- and long-term toxicities were also considered, including febrile neutropenia, hospitalizations, congestive heart failure, leukemia, and neuropathy.[32-34] Furthermore, while we did not conduct a formal cost-effectiveness model analysis, when incorporating cumulative costs and quality-adjusted life years (QALY), other studies also determined the RS result to be a cost-effective tool.[35,36] It is important to note, however, that the selection of our patient cohort may have influenced the magnitude of cost reduction, which could have otherwise been less pronounced among all-comers.[33]

Taken together, we conducted a multicenter prospective study to determine the impact of the RS result upon physician recommendation of chemotherapy in patients with ER-positive breast cancer with 1-3 positive lymph nodes in Quebec. Overall, we identified that physician recommendation was significantly reduced by 67%, which was associated with a cost reduction by 74%. We also demonstrated an important reduction of chemotherapy recommendation among patients with 2 or 3 positive lymph nodes. Our study is unique in that we followed up on physician recommendation, to evaluate treatment prescription and use at 6-month follow-up. Moreover, we showed an improvement in physician confidence on chemotherapy recommendation with use of the Breast Recurrence Score assay. Thus, alongside the 5-year survival results of the RxPONDER trial, our study demonstrates the utility of the Breast Recurrence Score assay among node-positive patients, and the potential of the assay to significantly decrease the use of chemotherapy for such patients.

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Conflict of Interest

Saima Hassan: Exact Sciences (RF, H, SAB), Pfizer (RF, outside the current work); **Rami Younan** Exact Sciences (H, SAB); **Jean-François Boileau:** Exact Sciences (C/A), Genomic Health (RF); **André Robidoux**: Exact Sciences (RF). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board.

Author Contributions

Conception, design, funding acquisition, and study supervision: A.R. Provision of study material/patients: S.H., R.Y., E.P., L.P., B.P., L.S., P.D., C.M., J-F.B., A.R. Collection and/or assembly of data: S.H., R.Y., E.P., L.P., B.P., L.S., P.D., C.M., J-F.B., A.R. Data analysis and interpretation: M.C-B., M-C.G. Manuscript writing: S.H., R.Y., L.P., L.S., J-F.B. Final approval of manuscript: S.H., R.Y., E.P., L.P., B.P., L.S., P.D., C.M., J-F.B.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Saez RA, McGuire WL, Clark GM. Prognostic factors in breast cancer. *Semin Surg Oncol*. 1989;5(2):102-110. https://doi.org/10.1002/ssu.2980050206.
2. Villarreal-Garza C, Ferrigno AS, De la Garza-Ramos C, et al. Clinical utility of genomic signatures in young breast cancer patients: a systematic review. *NPJ Breast Cancer*. 2020;6(1):46.
3. Chia SKL. Clinical application and utility of genomic assays in early-stage breast cancer: key lessons learned to date. *Curr Oncol*. 2018;25(Suppl 1):S125-S130.
4. Bou Zerdan M, Ibrahim M, El Nakib C, et al. Genomic assays in node positive breast cancer patients: a review. *Front Oncol*. 2021;10(3461):1-18.
5. 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(27):2817-2826.
6. 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(2):111-121.
7. Kalinsky K, Barlow WE, Gralow JR, et al. 21-gene assay to inform chemotherapy benefit in node-positive breast cancer. *N Engl J Med*. 2021;385(25):2336-2347.
8. 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(23):3726-3734.
9. 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(11):1829-1834.
10. Goldstein LJ, Gray R, Badve S, et al. Prognostic utility of the 21-gene assay in hormone receptor-positive operable breast cancer compared with classical clinicopathologic features. *J Clin Oncol*. 2008;26(25):4063-4071.

11. 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(1):55-65.
12. Mamounas EP, Bryant J, Lembersky B, et al. Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: results from nsabp b-28. *J Clin Oncol*. 2005;23(16):3686-3696.
13. Wolmark N, Mamounas EP, Baehner FL, et al. Prognostic impact of the combination of recurrence score and quantitative estrogen receptor expression (esr1) on predicting late distant recurrence risk in estrogen receptor-positive breast cancer after 5 years of tamoxifen: results from nrg oncology/national surgical adjuvant breast and bowel project b-28 and b-14. *J Clin Oncol*. 2016;34(20):2350-2358.
14. Mamounas EP, Tang G, Paik S, et al. 21-gene recurrence score for prognosis and prediction of taxane benefit after adjuvant chemotherapy plus endocrine therapy: results from nsabp b-28/nrg oncology. *Breast Cancer Res Treat*. 2018;168(1):69-77.
15. Penault-Llorca F, Filleron T, Asselain B, et al. The 21-gene recurrence score® assay predicts distant recurrence in lymph node-positive, hormone receptor-positive, breast cancer patients treated with adjuvant sequential epirubicin- and docetaxel-based or epirubicin-based chemotherapy (pacs-01 trial). *BMC Cancer*. 2018;18(1):526.
16. Wang M, Wu K, Zhang P, et al. The prognostic significance of the oncotype dx recurrence score in t1-2n1m0 estrogen receptor-positive her2-negative breast cancer based on the prognostic stage in the updated ajcc 8th edition. *Ann Surg Oncol*. 2019;26(5):1227-1235.
17. Nitz U, Gluz O, Christgen M, et al. Reducing chemotherapy use in clinically high-risk, genomically low-risk pn0 and pn1 early breast cancer patients: five-year data from the prospective, randomised phase 3 west german study group (wsg) planb trial. *Breast Cancer Res Treat*. 2017;165(3):573-583.
18. Nitz U, Gluz O, Clemens M, et al. West german study planb trial: Adjuvant four cycles of epirubicin and cyclophosphamide plus docetaxel versus six cycles of docetaxel and cyclophosphamide in her2-negative early breast cancer. *J Clin Oncol*. 2019;37(10):799-808.
19. Cancer Care Ontario. Drug formulary. Accessed May 24, 2022. https://www.cancercareontario.ca/en/cancer-treatments/chemotherapy/drug-formulary.
20. Kalinsky K, Barlow WE, Meric-Bernstam F, et al. Abstract gs3-00: first results from a phase iii randomized clinical trial of standard adjuvant endocrine therapy (et) +/- chemotherapy (ct) in patients (pts) with 1-3 positive nodes, hormone receptor-positive (hr+) and her2-negative (her2-) breast cancer (bc) with recurrence score (rs) < 25: Swog s1007 (rxponder). *Cancer Res*. 2021;81(4_suppl):GS3-00-GS03-00.
21. Pfizer Canada. Product monograph: doxorubicin. 2021. Accessed May 22, 2022. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwidi63L6_P3AhWXZ-80KHWPrC-sQFnoECAUQAQ&url=https%3A%2F%2Fwww.pfizer.ca%2Fpm%2Fen%2Fdoxorubicin.pdf&usg=AOvVaw1O-QJblFQD7hqMgCa4PGHLh.
22. Yordanova M, Hassan S. The role of the 21-gene recurrence score assay in hormone receptor-positive, node-positive breast cancer: the canadian experience. *Current Oncol*. 2022;29(3):2008-2020.
23. Torres S, Trudeau M, Gandhi S, et al. Prospective evaluation of the impact of the 21-gene recurrence score assay on adjuvant treatment decisions for women with node-positive breast cancer in ontario, canada. *Oncologist* 2018;23(7):768-775.
24. LeVasseur N, Sun J, Fenton D, et al. Impact of the 21-gene recurrence score assay on the treatment of estrogen receptor-positive, her2-negative, breast cancer patients with 1-3 positive nodes: a prospective clinical utility study. *Clin Breast Cancer*. 2022;22(1):e74-e79.
25. Dieci MV, Guarneri V, Zustovich F, et al. Impact of 21-gene breast cancer assay on treatment decision for patients with t1–t3, n0–n1, estrogen receptor-positive/human epidermal growth receptor 2-negative breast cancer: final results of the prospective multicenter roxane study. *Oncologist* 2019;24(11):1424-1431.
26. Mattar A, Fonseca GR, Romao MBA, et al. Substantial reduction in adjuvant chemotherapy with the use of the 21-gene test to manage early breast cancer in a public hospital in brazil. *JCO Glob Oncol* 2021;7:1003-1011.
27. Thomas D, Maxwell W, Archer C, et al. Abstract ot3-17-03: Impact of the oncotype dx breast cancer assay on treatment decisions in a UK population of patients with oestrogen receptor positive early breast cancer with 1-3 lymph nodes positive who are candidates for chemotherapy, but for whom the benefits are uncertain—interim results. *Cancer Res*. 2020;80(4_suppl):OT3-17-03-OT13-17-03.
28. Cognetti F, Masetti R, Fabi A, et al. Pondx: real-life utilization and decision impact of the 21-gene assay on clinical practice in italy. *NPJ Breast Cancer*. 2021;7(1):1-8.
29. Chin-Lenn L, De Boer RH, Segelov E, et al. The impact and indications for oncotype dx on adjuvant treatment recommendations when third-party funding is unavailable. *Asia Pac J Clin Oncol*. 2018;14(6):410-416.
30. Eiermann W, Rezai M, Kümmel S, et al. The 21-gene recurrence score assay impacts adjuvant therapy recommendations for ER-positive, node-negative and node-positive early breast cancer resulting in a risk-adapted change in chemotherapy use. *Ann Oncol*. 2013;24(3):618-624.
31. Losk K, Freedman RA, Laws A, et al. Oncotype dx testing in node-positive breast cancer strongly impacts chemotherapy use at a comprehensive cancer center. *Breast Cancer Res. Treat*. 2021;185(1):215-227.
32. Masucci L, Torres S, Eisen A, et al. Cost–utility analysis of 21-gene assay for node-positive early breast cancer. *Current Oncol*. 2019;26(5):307-318.
33. Wang S-Y, Dang W, Richman I, et al. Cost-effectiveness analyses of the 21-gene assay in breast cancer: systematic review and critical appraisal. *J Clin Oncol*. 2018;36(16):1619-1627.
34. Paulden M, Franek J, Pham B, et al. Cost-effectiveness of the 21-gene assay for guiding adjuvant chemotherapy decisions in early breast cancer. *Value Health*. 2013;16(5):729-739.
35. Lamond NWD, Skedgel C, Rayson D, et al. Cost-utility of the 21-gene recurrence score assay in node-negative and node-positive breast cancer. *Breast Cancer Res Treat*. 2012;133(3):1115-1123.
36. Berdunov V, Millen S, Paramore A, et al. Cost-effectiveness of the oncotype dx breast recurrence score test in postmenopausal women with node-positive early breast cancer based on the rxponder trial. *J Clin Oncol*. 2021;39(15_suppl):534-534.