Original article

Selection of neoadjuvant treatment based on the 21-GENE test results in luminal breast cancer

Serafin Morales Murillo a,*, Ariadna Gasol Cudos a, Joel Veas Rodriguez a, Carles Canosa Morales b, Jordi Melé Olive b, Felip Vilardell Vilellas c, Douglas Rene Sanchez Guzman c, Edelmiro Iglesias Martinez b, Antonieta Salud Salvia a

a Oncology Department of Hospital, Arnau de Vilanova de Lleida, Avenida Rovira Roure 80, 25198, Lleida, Spain
b Breast Unit of Hospital, Arnau de Vilanova de Lleida, Spain
c Pathology Unit of Hospital, Arnau de Vilanova de Lleida, Spain

Abstract
Neoadjuvant chemotherapy (NAC) is an optimal option in early breast cancer, but in ER-positive/HER2-negative (luminal) is still controversial, although a survival benefit has recently been observed when a histological response by Symmans’ method type 0 or I is achieved. The 21-gene Oncotype DX Breast Recurrence Score® assay (Oncotype DX®) is a validated test to assess the survival benefit of adjuvant chemotherapy in these patients but its role in the neoadjuvant setting is less established. We analyzed the results of the Oncotype DX® test in a cohort of 122 consecutive patients selected to receive NAC based on classical clinicopathological parameters and the correlation between the Oncotype DX® results and the pathological response assessed by Symmans’ method. Median age was 56.5 (range 31–84) years. Initial tumor size was T1 (<20 mm) in 46 patients (37.7%), 57 (46.7%) had a T2 tumor (20–50 mm), and 19 (15.6%) had a tumor size more than 50 mm. 59 (48.4%) had axillary node involvement. The median expression estrogen and progesteron receptors by immunohistochemistry was 280 and 120 respectively and median Ki67 index was 28%. The Recurrence Score (RS) results were <11 in 21 patients (17.2%) patients, RS 11 to 25 in 58 (47.5%), and RS >25 in 43 (35.2%). Considering the Oncotype DX test results, neoadjuvant chemotherapy was administered to 60 patients (49%), 11 (9%) received adjuvant chemotherapy and 51 (42%) no chemotherapy. Testing with the assay has therefore led to 42% fewer chemotherapy treatments. Among 60 patients receiving NAC, pathologic response was achieved for 5 patients (8.3%) with RCB-0 and 15 RCB-1 (25%). We did not find any pathological response RCB-0 and RCB-I in the 20 patients who received NAC and had a Recurrence Score result <21 for the premenopausal group, or a RS result <25 for the postmenopausal group. For patients with highest Recurrence Score results (RS > 21 or 25 according to menopausal status) it was 12% (5/40) RCB-0 and 40% (16/40) RCB-I. Conclusions: The Oncotype DX test could be a useful tool to select patients candidates for neoadjuvant chemotherapy in luminal breast cancer. Neoadjuvant chemotherapy could be avoided in 42% of patients. We found a correlation between Recurrence Score results and pathological response with 14% of RCB-0 and a total of 47% of significant pathological response type RCB-0 and RCB-I in patients with highest Recurrence Score results. Interestingly, patients with a Recurrence Score result inferior to 32 did not get any histological response type 0 and only 5% RCB-I.

© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Neoadjuvant chemotherapy (NAC) is considered an optimal choice in early breast cancer, especially in HER2-positive and triple negative phenotypes due to its higher pathological complete response (pCR) and its significant benefit in survival, but remains controversial in HR-positive and an HER2-negative (luminal)
Patients had at least 18 years of age; an ECOG performance status of 0 or 1; and ipsilateral axillary lymph nodes evaluated by imaging (MRI or ultrasound) within 6 weeks prior to registration. If indicated for abnormal lymph nodes, fine needle aspirate (FNA) or core needle biopsy was performed. All patients were evaluated by a multidisciplinary team that recommended neoadjuvant chemotherapy.

2. Methods

2.1. Patient eligibility

This prospective single-center study enrolled female patients with HR+ (defined as > 10% tumor staining by immunohistochemistry [IHC]), HER2-negative (according to ASCO/CAP guidelines [15]) invasive breast cancers, with a tumor size $\geq 2$ cm. Patients had at least 18 years of age; an ECOG performance status of 0 or 1; and ipsilateral axillary lymph nodes evaluated by imaging (MRI or ultrasound) within 6 weeks prior to registration. If indicated for abnormal lymph nodes, fine needle aspirate (FNA) or core needle biopsy was performed. All patients were evaluated by a multidisciplinary team that recommended neoadjuvant chemotherapy.

2.2. Study design

The primary objective of this prospectively designed study, was to assess the distribution of Recurrence Score results in pretreatment biopsies from early breast cancer patients who were candidates for neoadjuvant chemotherapy primarily because of tumor size and/or biological criteria such as high Ki67 index, always evaluated in an multidisciplinary team. The secondary objective was to evaluate the distribution of the Recurrence Score results in patients with and without pCR according to National Surgical Adjuvant Breast Project (NSABP) criteria, or histological response based in Symmans’ criteria [3].

Tissue blocks from the biopsies were sent to the Genomic Health laboratory (Clinical Laboratory Improvement Amendments certified) for Oncotype DX testing according to standard procedures [9].

Treatment was assigned based on the Recurrence Score (RS) result by study protocol: patients with RS $< 11$ were to undergo initial surgery; patients with RS $\geq 25$ in postmenopausal patients or RS $> 20$ in premenopausal patients were to receive NAC; patients with midrange RS 11 to 25 were assigned mainly to initial surgery.

2.3. Statistical analysis

Descriptive analyses included frequencies and percentages for categorical variables and means, medians, and ranges for continuous variables. The Recurrence Score results were analyzed as a continuous variable and in Recurrence Score results groups, considering the cut-offs of 11 and 20 for women of 50 years of age or younger and of 11 and 25 for more than 50 years according to the results to the TAILORx trial [16]. Fisher’s exact test and Student’s t-test were performed to compare distribution of Recurrence Score results according to pCR and breast preservation.

Univariate and multivariate logistic regression models were performed to investigate the associated factors with pCR. We studied concordance between IHC analysis and RT-PCR for ER, PR, and HER2 status. Statistical analysis was performed with SAS software 9.3 (SAS Institute, Inc., Cary, NC, http://www.sas.com).

3. Results

Between January 2016 and September 2019, 122 consecutive patients were included with locally or advanced breast cancer, considered candidates to receive neoadjuvant chemotherapy (NAC) to achieve a survival benefit, based on clinical variables such as initial tumor size or lymph node involvement. Patients were evaluated by a multidisciplinary team and an Oncotype DX test was performed to select treatment based on the Recurrence Score result according with the outcome of the prospective TAILORx trial that demonstrated an absence of chemotherapy benefit overall in patients with a Recurrence Score 11 to 25 [16]. The mean age was 56.5 (range 31–84) years. The distribution of Recurrence Score results was RS $< 11$ in 21 (17.2%) patients, RS 11–25 in 58 (47.5%), and RS $> 25$ in 43 (35.2%). Initial tumor size was T1 (< 20 mm) in 46 (37.7%), 57 (46.7%) had a T2 tumor (20–50 mm), and 19 (15.6%) had a tumor size more than 50 mm. In addition, 59 (48.4%) had axillary node involvement assessed clinically as N1 by ultrasounds and confirmed histologically. The expression of classical biological variables was: median estrogen and progesterone receptors by immunohistochemistry 280 and 120 respectively and the median Ki67 index was 28%. General characteristics of the cohort are shown in Table 1.

After receiving results of the Oncotype DX test, 60 patients (49%)...
received NAC, 11 (9%) adjuvant chemotherapy (after surgery for axillary involvement) and 51 (42%) did not receive any chemotherapy. Therefore, the Oncotype DX test has avoided a total of 42% of chemotherapy treatment in a cohort of patients with initial indication of chemotherapy by multidisciplinary team according to axillary involvement and 51 (42%) did not receive any chemotherapy. Twelve (10%) received NAC, 11 (9%) adjuvant chemotherapy (after surgery for axillary involvement) and 51 (42%) did not receive any chemotherapy. Therefore, the Oncotype DX test has avoided a total of 42% of chemotherapy treatment in a cohort of patients with initial indication of chemotherapy by multidisciplinary team according to axillary involvement and 51 (42%) did not receive any chemotherapy. Twelve (10%) received NAC, 11 (9%) adjuvant chemotherapy (after surgery for axillary involvement) and 51 (42%) did not receive any chemotherapy.

A ROC curve was designed to establish a correlation within the RS and pathological response. We found a statistically significant correlation with an area under the curve of 0.763 (CI 95% 0.643 - 0.882). ROC curve is shown in Fig. 4.

4. Discussion

To date, the most common use of the Oncotype DX test was to guide adjuvant chemotherapy treatment decisions [16,17]. Retrospective and prospective studies have supported its clinical utility, and its use has been standardized and included in all international guidelines [18,19]. In the larger series, Surveillance, Epidemiology, and End Results (SEER), with more than 105,000 patients, the proportion of patients with Recurrence Score >25 is lower than what we observed, around 15% for N0 and 13% for >1500 N1 patients, probably because the patients tested were of better prognosis overall. In fact, in this series, most tumors had tumoral grade I or II, half had tumor size less than 2 cm and only a 16% of initial nodal involvement [20]. These observations contrast with our outcomes in a selected population with higher clinical risk, we found a significantly greater proportion of patients with Recurrence Score >25, around 35%. It is very interesting that despite the high clinical risk of this population considered candidates for treatment with NAC, 65% of patients had a Recurrence Score <25 suggesting no benefit of chemotherapy treatment. Overall we avoided chemotherapy in a total of 42% of these patients. Fig. 4.

In the WSG-plan B study that included high clinical risk patients [21] with a proportion of 41% of node-positive patients and only 5% of tumoral grade I, the proportion of Recurrence Score >25 was 21% and interestingly similar in node-positive and negative-patients. We included node-positive patients (48%) in the study reported hereby because in our institution we use the Oncotype DX test to guide adjuvant chemotherapy decisions in patients with 1–3 node involvement based on the collective evidence for this test in this population with an excellent survival in patients with low Recurrence Score results without chemotherapy treatment [22]. We observed that, ultimately, 9% (11 of 122) of patients received adjuvant chemotherapy because more than 3 positive nodes were identified after surgical removal of the tumour. Importantly, all cases that had no initial axillary lymph node involvement, did not have axillary involvement after surgical resection following NAC. Half of the patients had an initial axillary node involvement, 32% in the patients who did not received NAC and 63% in the NAC group. The axillary node response were validated with the RCB symandy’s method. The identification of axillary involvement prior to administering NAC can be a suboptimal especially when it comes to identifying the number of nodes affected. Generally, patients with more than 3 positive nodes would receive chemotherapy treatment due to their higher risk of relapse [23] and the test has not been validated for this patient population. Therefore, the presence of an
axillary involvement can be a limitation for the selection of NAC guided by the Oncotype DX test, however, a 9% proportion of adjuvant chemotherapy, may be acceptable, especially considering the significant reduction in overall chemotherapy use for 42% of the patients.

The correlation between residual tumor size after NAC and survival has been demonstrated in multiple studies. Although in the triple negative and HER2 phenotypes this effect seems more significant, it has also been demonstrated in the luminal subtype [1]. Results from a large meta-analysis of 5100 breast cancer patients, funded by the Department of Defense, the National Institutes of Health (NIH), the Cancer Prevention Research Institute of Texas, and the Breast Cancer Research Foundation, presented recently in SABCS 2020 demonstrated that RCB after NAC is an accurate long-

### Table 2
Recurrence Score Results, treatment received and histological response to NAC.

| MULTIDISCIPLINARY TEAM TREATMENT RECOMMENDATION AFTER ONCOTYPE DX TESTING: TOTAL 122 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| PREMENOPAUSAL PATIENTS: n = 45 | POSTMENOPAUSAL PATIENTS: n = 77 |
| Recurrence Score <11 n = 8 | Recurrence Score 11–20 n = 14 | Recurrence Score >20 n = 23 | Recurrence Score <11 n = 13 | Recurrence Score 11–25 n = 44 | Recurrence Score >25 n = 20 |
| No CT 6 | 9 | 2 | 9 | 24 | 1 |
| Adjuvant CT 2 | 0 | 0 | 4 | 5 | 0 |
| Neoadjuvant 0 | 5 | 21 | 0 | 15 | 19 |
| RCB TYPE 0-I N/A 0% | 9/21 42.9% | N/A 6.7% | 10/19 52.6% |

Fig. 1. Final treatment received based on the Oncotype DX test results.
We found 9% of pCR in the overall series that is similar to most studies [7], but in patients with Recurrence Score > 25 this percentage increases to 14% and in patients with a Recurrence Score > 32 the pCR was 22%. It is remarkable that there was no patient with a Recurrence Score lower than 32 that achieved a pCR.

We also found a significant correlation between residual size and Recurrence Score results (pearson 0.714 P<0.027). The grouped response type RCB-0 and –RCB-I is also used as a predictor of survival and thus the percentage of patients with significant histological response can be increased. In recent studies this type of response was achieved around 20% [24], in our study we found a 20% in the global series but in patients with high RS this response was increased to 40%.

Pathological response rates have been associated with higher expression of the proliferation gene group from the 21 gene assay in earlier studies [25]. Yardley et al. [26] showed an interesting correlation between achievement of pCR and Recurrence Score results with no pCR achieved in the Recurrence Score groups RS 0–30 and 26% in the Recurrence Score >30. More recently Pease et al. [27] found in an important retrospective study with 890 patients that a high Recurrence Score result (RS > 30) was associated with an increased pathologic complete response rate, with 10% of such patients achieving pCR, compared to 14% of patients with RS > 25 in our series. Similarly Kantor et al. [28] found a 7.8% pCR rate in the RS > 25 group in a similar retrospective study of the national cancer database in the US. It is important to note the concordance of low pathological response found in both studies in patients with lower Recurrence Score results (RS 0–25), suggesting these patients will not benefit from NAC despite having clinical criteria of NAC. Pivot et al. [29] showed a similar effect in patients selected for NAC primarily based on large tumor size, in that patients with low RS results, with minimal if any expected long-term clinical benefit to NAC, were unlikely to achieve pCR.

The rate of pathological response in this study for patients with higher Recurrence Score results treated with NAC is consistent with observations from studies that assigned NAC to patients with higher scores. Bear et al. [30] found a 14% pCR rate in the group of patients with a RS > 25, while patients with lower RS did not receive chemotherapy treatment. We also consider important the quantification of the response according to the residual cancer burden, which impacts survival benefit. So far, few studies have assessed this type of response, although it is important for the selection of patients for NAC. In our study there is a very interesting

![Fig. 2. Distribution of Recurrence Score results in patients with respect to final tumor size after NAC.](image)

![Fig. 3. Probability of pathologic response RCB type 0–1 as a function of Recurrence Score results.](image)
correlation between the final size and the Recurrence Score result that shows the importance of the Oncotype DX test in the histological response. This correlation was not observed with any of the other variables assessed.

5. Conclusion

Selecting NAC based on results from the Oncotype DX test allows application of this treatment with more accuracy, and avoids chemotherapy in nearly half of patients previously selected to NAC based on clinical common parameters. We found only 35% of patients had a high Recurrence Score (>25) that would suggest a clear long-term benefit of chemotherapy treatment.

The pathological response was strongly correlated with the Recurrence Score results in the quantitative model with 14% of RCB-0 in patients with highest Recurrence Score results and conversely, patients with a RCB-0 below 20 did not obtain any RCB-0. By grouping the pathological response in RCB-0 and RCB-1 to enrich the survival benefits, we found a total of 47% response (19 of 40) in patients with high Recurrence Score results (RS > 21, premenopausal; RS > 25, postmenopausal) in contrast to only 5% (1 of 20) in the groups of lower Recurrence Score results.

This prospective study confirms the findings of several earlier studies of RS performed on archived tumors from patients treated with neoadjuvant chemotherapy. Overall, the findings suggest that a sizable proportion of patients with locally advanced ER+HER2-breast cancer do not benefit from NAC, and the Oncotype DX test substantially reduces over-treatment of such patients.

Table 3

| Variable             | RCB 0–1 Univariate | RCB 0–1 Multivariate |
|----------------------|--------------------|----------------------|
| Tumor size (mm)      | OR 1.04 (CI 95% 0.991–1.041) | Non Significant |
| Node (N+)            | OR 0.812 (CI 95% 0.336–1.964) | Non Significant |
| Age                  | OR 1.014 (CI 95% 0.978–1.052) | Non Significant |
| Estrogen R           | OR 1.008 (CI 95% 1.000–1.017) | Non Significant |
| Progesterone R       | OR 1.004 (CI 95% 1.000–1.009) | Non Significant |
| Ki67                 | OR 0.97 (CI 95% 0.94–0.99) | Non Significant |
| RS                   | OR 0.94 (CI 95% 0.90–0.975) | OR 0.946 (CI 95% 0.901–0.993) |

Fig. 4. Curve COR with the correlation to RS score with pathological response (RCB type 0–1).

References

[1] Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTOncRBC pooled analysis. Lancet Lond Engl 2014;384(9938):164–72.
[2] Sheri A, Smith JE, Johnston SR, A'Hern R, Nerurkar A, Jones RL, et al. Residual proliferative cancer burden to predict long-term outcome following neoadjuvant chemotherapy. Ann Oncol Off J Eur Soc Med Oncol 2015;26(1):75–80.
[3] Symmans WF, Wei C, Gould R, Yu X, Zhang Y, Liu M, et al. Long-term prognostic risk after neoadjuvant chemotherapy associated with residual cancer burden and breast cancer subtype. J Clin Oncol Off J Am Soc Clin Oncol 2017;35(10):1049–60.
[4] Fukuda I, Mucida S, Grim J, Ryska A, Hornychova H. Predictive biomarkers in breast cancer: their value in neoadjuvant chemotherapy. Canc Invest 2012;30(9):663–78.
[5] Keam B, Im S-A, Kim H-J, Oh D-Y, Kim JH, Lee S-H, et al. Prognostic impact of clinicopathologic parameters in stage II/III breast cancer treated with neoadjuvant docetaxel and doxorubicin chemotherapy: paradoxical features of the triple negative breast cancer. BMC Canc 2007;7:203.
[6] Fasching PA, Heusinger K, Haeberle L, Niklus M, Hein A, Bayer CM, et al. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Canc 2011;11:486.
[7] Denkert C, Loibl S, Müller BM, Eidtmann H, Schmitt WD, Eiermann W, et al. Ki67 levels as predictive and prognostic parameters in pretherapeutic breast cancer core biopsies: a translational investigation in the neoadjuvant GeparTrio trial. Ann Oncol Off J Eur Soc Med Oncol 2013;24(11):2786–93.
[8] Paik S, Shack S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;350(27):2817–26.
[9] Paik S, Tang G, Shack S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 2006;24(21):3726–34.
[10] Pivot X, Manli L, Chaigneau L, Montcuquet P, Thierry-Vuillemin A, Bazan F, et al. In the era of genomics, should tumor size be reconsidered as a criterion for neoadjuvant chemotherapy? 2015;7.
[11] Soran A, Bhargava R, Johnson R, Ahrendt G, Bonaventura M, Diego E, et al. The impact of Oncotype DX® recurrence score of paraffin-embedded core biopsy tissues in predicting response to neoadjuvant chemotherapy in women with breast cancer. Breast Dis 2016;36(2–3):65–71.
[12] Petkov VI, Miller DP, Howlader N, Gliner N, Howe W, Schussler 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.
[13] Thekkadka RJ, Bhardwaj S, Yadav U, Baranwal A, Peace D, Rogowski W, et al. Predicting response to neoadjuvant chemotherapy in nonmetastatic hormone receptor-positive breast cancer using 21-gene Breast Recurrence Score test. J Clin Oncol 2019;37(15_suppl):e12093.
[14] Parker JS, Mullins M, Cheang MCU, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol Off J Am Soc Clin Oncol 2009;27(8):1160–7.
[15] Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol Off J Am Soc Clin Oncol 2013;31(31):3997–4013.
[16] Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. N Engl J Med 2018;379(2):111–21.
Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, et al. Prospective validation of a 21-gene expression assay in breast cancer. N Engl J Med 2015;373(21):2005.

Harris LN, Ismaila N, McShane LM, Andre F, Collyar DE, Gonzalez-Angulo AM, 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 Off J Am Soc Clin Oncol 2016;34(10):1134–50.

Andre F, Ismaila N, Henry NL, Somerfeld MR, Bast RC, Barlow W, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: ASCO clinical practice guideline update-integration of results from TAILORx. J Clin Oncol Off J Am Soc Clin Oncol 2019;37(22):1956–64.

Zhang L, Hsieh M-C, Petkov V, Yu Q, Chiu Y-W, Wu X-C. Trend and survival benefit of Oncotype DX use among female hormone receptor-positive breast cancer patients in 17 SEER registries, 2004-2015. Breast Canc Res Treat 2020;180(2):491–501.

Nitz U, Gluz O, Christgen M, Kates RE, Clemens M, Malter W, 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 Canc Res Treat 2017;165(3):573–83.

Mamounas EP, Russell CA, Lau A, Turner MP, Albain KS. Clinical relevance of the 21-gene Recurrence Score® assay in treatment decisions for patients with node-positive breast cancer in the genomic era. NPJ Breast Cancer 2018;4:27.

Sestak I, Dowsett M, Zabaglo L, Lopez-Knowles E, Ferree S, Cowens JW, et al. Factors predicting late recurrence for estrogen receptor-positive breast cancer. J Natl Cancer Inst 2013;105(19):1504–11.