Prospective study of the impact of the Prosigna assay on adjuvant clinical decision-making in unselected patients with estrogen receptor-positive, HER2-negative, node-negative early-stage breast cancer.

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ABSTRACT

Purpose: Improved understanding of risk of recurrence (ROR) is needed to reduce cases of recurrence and more effectively treat breast cancer patients. The purpose of this study was to examine how a gene-expression profile (GEP), identified by Prosigna, influences physician adjuvant treatment selection for early breast cancer (EBC) and the effects of this influence on optimizing adjuvant treatment recommendations in clinical practice.

Methods: A prospective, observational, multicenter study was carried out in 15 hospitals across Spain. Participating medical oncologists completed pre-, post-, and follow-up questionnaires recording their treatment recommendations and confidence in these recommendations, before and after knowing the patient's ROR. Patients completed questionnaires on decision-making, anxiety, and health status.

Results: Between June 2013 and January 2014, 217 patients enrolled and a final 200 were included in the study. Patients were postmenopausal, estrogen receptor-positive, human epidermal growth hormone factor negative, and node-negative with either stage 1 or stage 2 tumors. After receiving the GEP results, treatment recommendations were changed for 40 patients (20%). The confidence of medical oncologists in their treatment recommendations increased in 41.6% and decreased in 6.5% of total cases. Patients reported lower anxiety after physicians made treatment recommendations based on the GEP results ($p < 0.05$).

Conclusions: GEP results influenced the treatment decisions of medical oncologists and their confidence in adjuvant therapy selection. Patients' anxiety about the selected adjuvant therapy decreased with use of the GEP.

Keywords: *Prosigna, PAM50, intrinsic subtypes, genomic, adjuvant, breast cancer.*

INTRODUCTION

Breast cancer occurs in 1.7 million women worldwide per year.[1] Approximately 60 percent of patients are diagnosed with early stage disease.[2] Clinicopathological evaluation is recommended to facilitate treatment decisions, such as whether to undergo adjuvant hormonal therapy alone or in combination with adjuvant chemotherapy. Gene-expression profiling (GEP) is recommended in clinical guidelines to assess the risk of distant recurrence and response to chemotherapy.[3] The Prosigna Breast Cancer Assay (NanoString Technologies® Inc., Seattle, WA), which uses the PAM50 gene signature, is a newer standardized test that measures the expression levels of 50 classifier genes from formalin-fixed paraffin-embedded (FFPE) breast tumor samples. It provides intrinsic subtype classification based on the fundamental biology of an individual patient's tumor. Prosigna's clinically validated prognostic risk of recurrence (ROR) score categorizes patients as low, intermediate or high risk and predicts the probability of distant recurrence over 10 years.[4, 5] In the TransATAC study, a trial comparing anastrozole to tamoxifen as adjuvant therapy for early stage breast cancer, Prosigna provided more prognostic information than the Oncotype DX multi-gene-expression assay and categorized fewer patients as intermediate risk.[4] The difference in prognostic performance was particularly pronounced in the clinically relevant subset of patients with estrogen receptor (ER)-positive, human epidermal receptor negative (HER2-) disease. The Prosigna ROR score has also been shown to provide clinically meaningful prognostic information about the risk of late distant recurrence in patients with ER-positive tumors after 5 years of endocrine therapy.[6, 7]

The objective of this study was to examine whether Prosigna influences adjuvant treatment selection, beyond standard immunohistochemistry (IHC) testing and standard

clinicopathological variables, and whether it may be used by oncologists to optimize adjuvant treatment recommendations in clinical practice. The study also sought to assess whether Prosigna could be performed reliably in hospital laboratories and to compare measures of reliability with those obtained using IHC.

PATIENTS AND METHODS

Study design

This was a prospective, observational, multicenter study carried out in 15 hospitals across Spain affiliated with the Spanish Breast Cancer Group (GEICAM). The primary objective of the study was to characterize the impact of Prosigna on adjuvant therapy decision recommendations made by medical oncologists. Secondary study objectives were to elicit information on (1) the confidence of medical oncologists in their treatment recommendation before and after Prosigna, according to cancer recurrence risk, (2) the rate of chemotherapy-related adverse events, and (3) patients' decisional conflict status, anxiety levels, and functional status before and after test results. Additionally, the analytical performance of the assay, when deployed as a decentralized test, was analyzed. The study was approved by all institutions' ethical review boards.

Patient population

A group of postmenopausal women with histologically proven early stage breast cancer with T1 or T2 tumors (< 5 cm), negative lymph nodes (N0) and absence of metastasis were eligible for this study. Eligibility criteria also included: Eastern Cooperative Oncology Group score of 0 or 1, ER-positive, and HER2- by IHC and in-situ hybridization (ISH), and no contraindications for receiving adjuvant chemotherapy.

Participants provided written consent and were able to complete patient reported outcome surveys without assistance. To minimize selection bias, all consecutively seen postmenopausal women meeting the inclusion criteria were screened and asked to take part in the study by the participating medical oncologists.

Tumor sample assessments

FFPE surgical specimens were reviewed and prepared in tumor sections for Prosigna analysis in a GEICAM central pathology laboratory (Fundación Jiménez Díaz, Madrid, Spain) according to the manufacturer specifications.[8] RNA was extracted and tested with Prosigna on the NanoString nCounter® Analysis System in a central laboratory (IiSGM Translational Oncology laboratory, Madrid, Spain) following the manufacturer guidelines.[8] Patients were classified according to the intrinsic subtype (Luminal A, Luminal B, HER2-enriched, basal-like) and ROR risk group (low risk, 0-40; intermediate risk, 41-60; and high risk, 61-100).[4, 9, 10] A second set of FFPE tumor sections was subsequently analyzed with Prosigna in an independent replication laboratory (VHIO Translational Genomics Group, Barcelona, Spain) to assess concordance with the central laboratory.

For IHC-subtyping of ER, progesterone receptor (PR), HER2 and antigen Ki67 (Ki67) samples, retrospective assessment was done in a third set of FFPE tumor sections in the central pathology laboratory, following the American Society of Clinical Oncology and the College of American Pathologists guidelines.[11-13] ER and PR were analyzed by IHC using anti-ERα specificity protein 1 clone antibody and anti-PR (1E2) clone antibody (Ventana Medical System-Roche, Tucson, AZ, USA), respectively; HER2 status was determined by IHC using Herceptest (Dako, Glostrup, Denmark) and

confirmed by fluorescence ISH (FISH) using Pathvysion (Abbott Molecular, IL, USA) when indicated; and Ki67 was assessed by IHC using anti-Ki-67 MIB1 clone antibody (Dako, Glostrup, Denmark). Prosigna subtypes were compared to IHC intrinsic subtypes following the St. Gallen 2013 criteria: Luminal A (ER+, HER2-, PR > 20%, Ki67 < 20%); Luminal B (Luminal B1: ER+, HER2-, Ki67 ≥ 20% and/or PR ≤ 20%; Luminal B2: ER+, HER2+, any PR, any Ki67); HER2 positive (ER-, PR-, HER2+); and Triple Negative (ER-, PR-, HER2-)[9, 14].

Physician questionnaires

Participating medical oncologists completed a pre-test questionnaire recording their initial treatment recommendation, based upon standard clinical and pathological treatment variables as well as the IHC results, and their confidence in this recommendation before knowing the patient's ROR and intrinsic tumor subtype. After receiving the test results and at a 6-month follow up, oncologists stated their final treatment recommendation and their confidence in their decision.

Patient questionnaires

Participating patients completed three standardized questionnaires at pre- and post-assessment of Prosigna: the Decisional Conflict Scale (DCS)[15] to assess perceived level of decisional conflict; the State-Trait Anxiety Inventory (STAI)[16] to differentiate between temporary "state anxiety", long-lasting "trait anxiety," and depression; and the Functional Assessment of Cancer Therapy-General, version 4 (FACT-G v.4)[17] to assess patients' quality-of-life and health status over time.

Statistical analyses

All variables and summarized distributions were plotted with means or proportions, standard deviations, and 95% confidence intervals. The primary endpoint of the study was the proportion of adjuvant treatment recommendations that changed [e.g., from hormonal therapy only (HT) to chemotherapy plus hormonal therapy (CHT)] after physicians received Prosigna reports; the test statistic was the Fisher Exact test.

A sample size of 200 patients was calculated in order to have a one-sided 95% lower-limit confidence interval, with a 0.050 distance from the sample proportion to the lower limit when the sample proportion was 0.250 (Clopper-Pearson). The concordance of Prosigna subtypes and ROR scores between central and replication laboratories was determined, and score differences greater than 6.75 were considered to be clinically meaningful.[5] Prosigna subtypes assessed by central laboratory were compared to IHC intrinsic subtypes using the St. Gallen 2013 criteria.[9]

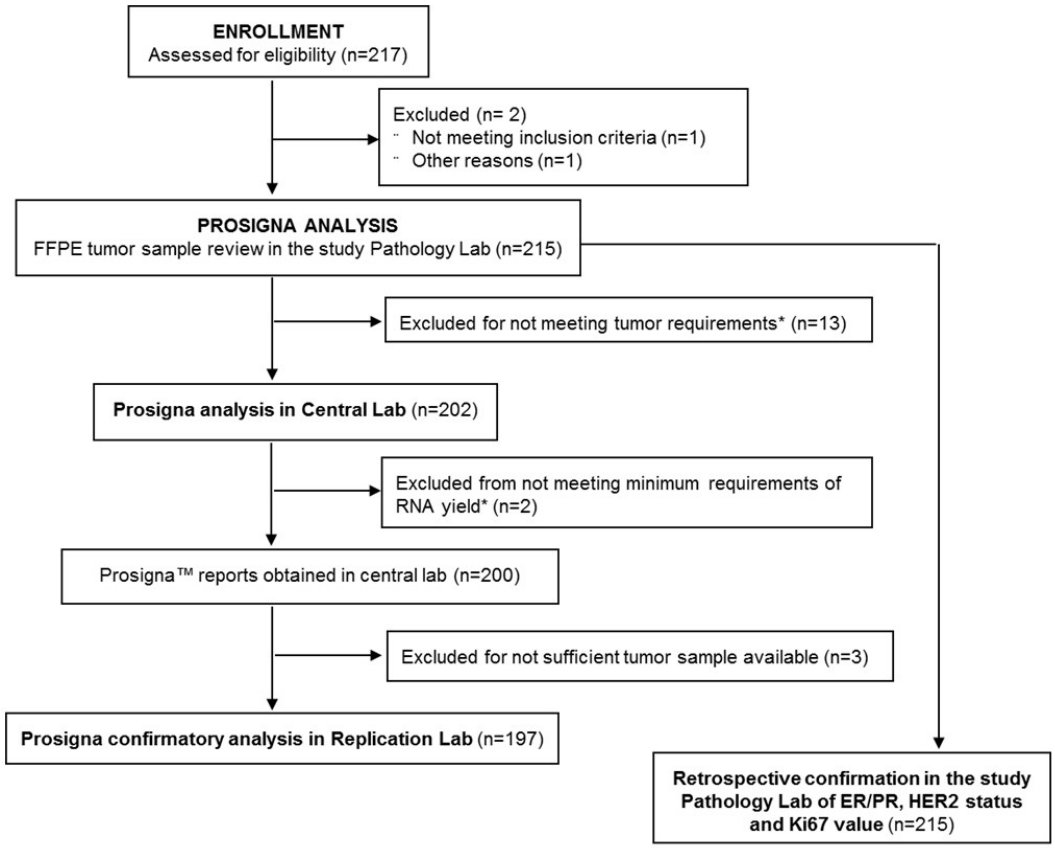
Responses to DCS, STAI, and FACT-G questionnaires administered before and after Prosigna were compared using paired Student's t-tests. Changes in decision conflict, anxiety levels and functional status responses were examined through analysis of variance (ANOVA).

RESULTS

Patient, tumor characteristics and Prosigna analysis

Two hundred and seventeen patients were enrolled in the study between June 2013 and January 2014 in 15 Spanish sites. Among them, 2 patients were excluded from the study. FFPE tumor samples from 215 patients were reviewed in the central pathology laboratory. Fifteen tumors did not meet the minimum requirements and were not included in the final analysis. Prosigna was successfully performed in 200 samples at the central laboratory and in 197 samples at the replication laboratory. ER, PR, HER2 status and Ki67 values were retrospectively confirmed in the central pathology laboratory (Fig. 1 Patient Flow Diagram).

Fig. 1 Patient flow diagram



**According to specifications of the manufacturer [8]*

Of the 200 postmenopausal, ER-positive, HER2-, node-negative patients included in the study, 162 (81%) patients had T1 tumors, 38 (19%) had T2, 166 (83%) were PR positive and 94 (47%) were Ki67 < 14%. Patients' characteristics categorized by ROR group are described in Table 1.

Table 1 Patient and tumor characteristics

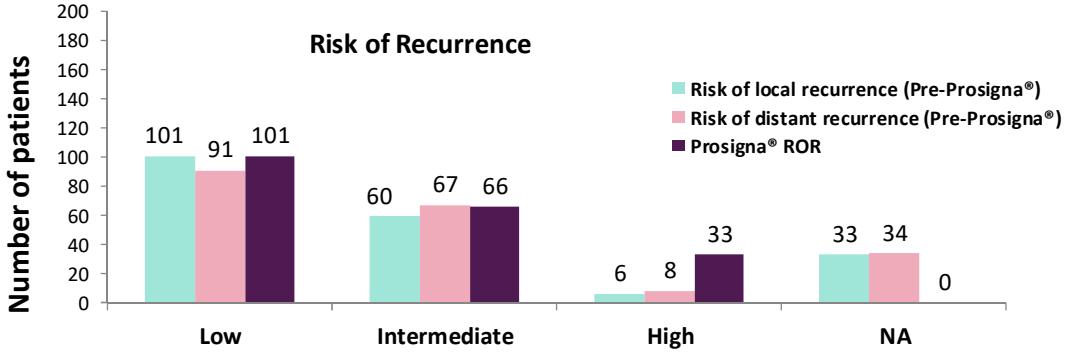
Characteristic	Type	N (%)	ROR low	ROR intermediate	ROR high
			n (%)	n (%)	n (%)
Age (years)	**< 50**	2 (1.0)	2 (2.0)	0 (0.0)	0 (0.0)
	≥ 50	198 (99.0)	99 (98.0)	66 (100.0)	33 (100.0)
Tumor size	**T1**	162 (81.0)	83 (87.1)	56 (84.8)	18 (54.5)
	T2	38 (19.0)	13 (12.9)	10 (15.2)	15 (45.5)
PR (local)	**Positive**	166 (83.0)	85 (84.2)	54 (81.8)	27 (81.8)
	Negative	34 (17.0)	16 (15.8)	12 (18.2)	6 (18.2)
Ki67 (local)	**< 14%**	94 (47.0)	70 (69.3)	18 (27.3)	6 (18.2)
	≥ 14%	96 (48.0)	25 (24.8)	44 (66.6)	27 (81.8)
	Unknown	10 (5.0)	6 (5.9)	4 (6.1)	0 (0.0)
TOTAL		**200**	**101**	**66**	**33**

Abbreviations: ROR, risk of recurrence; PR, progesterone receptor; Ki67, Antigen Ki67.

The most likely tumor subtypes based on classical clinicopathological factors, pre-Prosigna were luminal A for 115 (57.5%) patients and luminal B for 85 (42.5%) patients. Post-Prosigna, intrinsic tumor subtypes of these patients were distributed as follows: 129 luminal A (64.5%), 66 luminal B (33.0%), 3 HER2-enriched (1.5%) and 2 basal-like (1.0%). The concordance at the individual level between subtype classification based on classical clinicopathological factors and Prosigna results was 63.2%.

Based on physician assessment, 101 (50.5%) patients had a low risk of local recurrence (< 10%), 60 (30.0%) had an intermediate risk of local recurrence (11-20%), and 6 (3.0%) had a high risk of local recurrence (>20%) Information was not available in 33 (16.5%) patients. Similarly, 91 (45.5%), 67 (33.5%), and 8 (4.0%) patients had low, intermediate, and high risk of distant recurrence, respectively, and 34 (17%) patients had no available risk assignment. Prosigna was performed in a central laboratory on 200 patients, classifying them according to risk of recurrence; 101 (50.5%), 66 (33%), and 33 (16.5%) patients had low, intermediate and high ROR, respectively. Patients with an intermediate risk of distant recurrence based on physician assessment were categorized by Prosigna as low (38.8%), intermediate (40.3%) and high (20.0%) risk. The concordance at the individual level between risk classification based on classical clinicopathological factors and Prosigna results was 51.2%. Results are summarized in Fig. 2.

Fig. 2 Risk of Recurrence determined by the oncologist based on clinicopathological information and Prosigna results



Treatment recommendations before and after Prosigna

Prior to assessment with Prosigna, subtyping was based on standard clinicopathological factors in 177 patients (88.5%) and Adjuvant Online in 47 patients (23.5%). Upon reviewing Prosigna results, treatment recommendations were changed in 40 patients (20%). For 22 patients (11%), the initial recommendation was revised from combined CHT to HT and for 18 (9%), from HT to CHT. In an additional 15 patients (7.5%), there was a change in at least one agent in the regimen (Table 2). Among 67 patients with an initial intermediate risk of distant recurrence, 14 (20.9%) received a change in treatment recommendation: 13 from CHT to HT and 1 from HT to CHT. The percentage of patients receiving CHT in the low, intermediate and high risk groups by Prosigna was 3.0%, 36.4% and 84.8%, respectively. In the Prosigna low risk group, treatment recommendations changed in 19 of 101 patients (18.8%), 13 (68.4%) of whom changed from CHT to HT. A total of 22 (33.3%) of the 66 patients in the intermediate risk group had changes made to their treatment plan: 9 (40.9%) from HT to CHT and 9 (40.9%) from CHT to HT. Among the 33 patients with Prosigna high risk, 14 (42.4%) had a change in the treatment recommendation: 9 (64.3%) of them from HT to CHT. Five patients in this high risk group received only HT after Prosigna. Among the 68 patients with ROR values proximal to the cut-points of the risk groups (± 5% from cut-points), 10 (14.7%) changed from CHT to HT (7 of whom were of intermediate-low group) and 6 (8.8%) changed from HT to CHT (5 of whom were of intermediate-high group).

Table 2 Changes in physicians' treatment recommendation pre- and post-Prosigna

Adjuvant Treatment Recommendation	Prosigna Low risk n=101 (50.5%)	Prosigna Intermediate risk n=66 (33.0%)	Prosigna High risk n=33 (16.5%)	Total n=200 (100%)
Adjuvant treatment recommendations before Prosigna				
CHT	16 (15.9)	24 (36.3)	19 (57.5)	59 (29.5)
CT	0 (0.0)	1 (1.5)[1]	0 (0.0)	1 (0.5)[1]
HT	85 (84.2)	41 (61.75)	14 (42.5)	140 (70.0)
Adjuvant treatment recommendations after Prosigna				
CHT	3 (3.0)	24 (36.4)	28 (84.8)	55 (27.5)
CT	0 (0.0)	1 (1.5)[1]	0 (0.0)	1 (0.5)[1]
HT	98 (97.0)	41 (62.1)	5 (15.2)	144 (72.0)
Change in adjuvant treatment recommendation pre- to post- Prosigna				
Change in treatment type[2]				
HT to CHT	0 (0.0)	9 (13.6)	9 (27.3)	18 (9)
CHT to HT	13 (12.9)	9 (13.6)	0 (0.0)	22 (11)
Change in regimen (at least one agent) within treatment type				
HT to HT	6 (5.9)	2 (3.0)	1 (3.0)	9 (4.5)
CHT to CHT	0 (0.0)	2 (3.0)	4 (12.1)	6 (3)
Any change	19 (18.8)	22 (33.3)	14 (42.4)	55 (27.5)

Abbreviations: CHT, chemotherapy and hormonal therapy; CT, chemotherapy; HT, hormone therapy; ROR, risk of recurrence.

[1]*One patient was not scheduled for HT pre and post Prosigna but had received CHT when assessed in the 6 months follow-up.*

[2] *The association between changes in physicians' treatment recommendations and Prosigna risk groups was analyzed by Fisher exact test ($p < 0.001$).*

The Prosigna risk group was significantly associated with the likelihood of change in treatment recommendations ($p < 0.001$, Fisher exact test) (Table 2). In addition, a significant association was found between the continuous ROR score and the probability of changing to a stronger treatment (HT to CHT); as the ROR score increased, the probability for changing to a stronger treatment increased [OR, 1.08 95% CI (1.04 – 1.12), $p < 0.001$]. There was no association between the ROR score and the probability to change to a weaker treatment (CHT to HT) [OR 0.99 95% CI (0.96 – 1.01), $p = 0.368$].

Medical oncologists' confidence in treatment recommendation before and after knowledge of Prosigna results

A total of 185 cases were evaluated with the medical oncologists' confidence in their before and after the knowledge of ROR and subtype data. Results are summarized in Table 3. The confidence of medical oncologists in their treatment recommendation increased in 41.6% and decreased in 6.5% of cases.

Table 3 Change in physician confidence with knowledge of Prosigna

	No change in confidence n (%)	Increased confidence n (%)	Decreased confidence n (%)
Confidence with prognosis (subtype, risk of recurrence), n=185	101 (54.6)	74 (40.0)	10 (5.4)
Confidence with intended treatment (optimal for the patient), n=185	96 (51.9)	77 (41.6)	12 (6.5)

Patient-reported outcomes

Patients reported lower state anxiety after physicians' treatment recommendations based on Prosigna results ($p < 0.01$). In a subgroup analysis, only low risk patients decreased state anxiety ($p < 0.01$). No significant changes in trait anxiety, decisional conflict or functional status were observed (Supplementary Table 1). However, there was a statistically significant association between changes in state and changes in trait anxiety for patients in the ROR groups ($p < 0.01$) (Supplementary Table 2).

Prosigna tumor subtyping and ROR concordance between central and replication laboratories

Subtype results obtained by the central and replication laboratories were concordant in 190 samples (95%) (Kappa = 0.89) (Supplementary Table 3). Prosigna subtype was discrepant in 10 patients (3 luminal A to B, 6 luminal B to A and 1 basal-like to HER2-enriched). Nine of them were classified as intermediate risk and 1 as low risk, according to central laboratory results. Only 4 risk group discrepancies resulted from tests carried out in the replication laboratory (intermediate to low risk) (Supplementary Table 4).

Risk groups were concordant in 89% of samples between central and replication laboratories (Kappa=0.81) (Supplementary Table 4). Risk categorization by the central and replication laboratories was discrepant in 22 patients (4 low to intermediate risk, 8 intermediate to low risk, 1 intermediate to high risk, and 8 high to intermediate risk). Fifty percent of the patients with discrepant risk group results demonstrated an ROR difference greater than 6.75. No samples were discordant from low-to-high risk.

Concordance between centrally determined IHC and Prosigna intrinsic subtypes

Intrinsic subtypes defined by IHC, according to St. Gallen 2013 criteria, in a central laboratory agreed with Prosigna in 51.6% of luminal A patients and 75.4% of luminal B patients (Table 4). The concordance of luminal A or B (HER2-enriched included) compared to IHC-luminal A or B between the central IHC subtypes and Prosigna intrinsic subtypes was 60% (Kappa=0.2365, only fair agreement).

Table 4 Prosigna subtypes across IHC subtypes (St. Gallen 2013 guidelines)

IHC Subtype St. Gallen 2013	Prosigna Subtype				
	Luminal A n (%)	Luminal B n (%)	HER2-Enriched n (%)	Basal n (%)	Total
IHC-Luminal A	64 (51.6)	15 (24.6)	0 (0.0)	0 (0.0)	79
IHC-Luminal B	60 (48.4)	46 (75.4)	2 (66.7)	2 (100.0)	110
IHC-Triple negative	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	1
Total	124	61	3	2	190

Abbreviations: IHC, immunohistochemistry testing, HER2-, human epidermal receptor negative.

DISCUSSION

Over the past decade, several multi-gene expression assays—including Oncotype Dx[18], Mammaprint[19], EndoPredict[20, 21], and Prosigna[4]—have been progressively incorporated into the clinical decision-making process. This is the first study to test Prosigna's influence on treatment recommendations for adjuvant therapy in postmenopausal patients with HER2-, node-negative breast cancer in a clinical setting.

Among postmenopausal breast cancer patients with ER-positive tumors, the addition of adjuvant chemotherapy to adjuvant endocrine therapy alone has less than a 5% increase on the otherwise predicted 15-year survival period.[23] This marginal survival benefit means that 20 patients will need to be treated with chemotherapy in order to prevent a single death. The absolute gain in survival from the addition of adjuvant chemotherapy depends on the absolute risk of recurrence with endocrine therapy alone; therefore, accurate tools for establishing patient prognosis on endocrine therapy alone are crucial.

Conventional clinicopathological parameters used in the assessment of ER-positive postmenopausal patients are suboptimal predictors of both relapse risk and chemotherapeutic benefit. In particular, immunohistochemical tests are characterized by several sources of variability (e.g., different time of fixation and type of fixatives, different antibodies, variation in techniques of deparaffinization, antigen retrieval, and antibody staining, etc.) due to the non-automatized nature of the techniques. In addition, they offer only semi-quantitative results, establish somewhat artificial cut-off points of positivity, and depend largely on the skills of pathologists and technicians.[24]

Several studies have shown that the modern multi-gene-expression assays provide prognostic information beyond that of classical clinicopathological parameters and can influence the decision-making process for adjuvant therapy (Mammaprint[19, 25, 26], Oncotype Dx[18, 27, 28], Prosigna[4, 20, 29-31], EndoPredict[21, 32, 33]). In our study, the investigators changed adjuvant treatment recommendations in 20% of patients after knowing the results of Prosigna. This study was designed with consecutive enrollment of patients according to protocol, not taking into consideration the investigators' perception of risk of relapse or confidence regarding therapeutic decisions made prior to obtaining Prosigna results. As a result, approximately half of the patients enrolled in the study were in the low risk group, according to both the Prosigna ROR score and the investigator´s perception before Prosigna; only 33 patients (16.5%) included in the study were in the high-risk group.

Prosigna also influenced the confidence of medical oncologists in their treatment recommendation. After receipt of the Prosigna results, physician confidence increased in 41.6% of cases and decreased in 6.5%. Overall, considering the high proportion of patients with clear clinicopathological low-risk included in the study, the Prosigna results reinforced the confidence of clinicians on the accuracy of adjuvant therapy selected for the patients.

In addition, Prosigna had a significant influence on patient's anxiety about the recommended adjuvant therapy. After receiving the Prosigna results, patients reported significantly lower levels of anxiety regarding their treatment selection ($p < 0.05$).

In our study, the Prosigna results showed 95% concordance between the central and replication laboratories, confirming its analytical validity. We found that the intrinsic subtype classification based on IHC or ISH, as suggested by the St. Gallen Consensus Conference [9], is not an adequate proxy for the real genomic subtypes as determined by Prosigna and previously shown by other studies.[12, 34] Twenty-five percent of patients classified as luminal A by IHC or ISH were accurately re-identified as luminal B by Prosigna and should be considered for adjuvant chemotherapy. In addition, 48% of the patients with tumors classified as luminal B by IHC/ISH were re-identified as luminal A by Prosigna and could be spared chemotherapy.

Our study supports the clinical validity and clinical utility of Prosigna in real-world settings. Physicians' knowledge of Prosigna results increased their confidence in prognosis and influenced adjuvant therapy recommendations as a result.

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