Correlation between Ki-67 proliferation index in invasive breast cancer and molecular signatures: EndoPredict and MammaPrint

J. Eduardo Amezcua-Gálvez
Hospital San José Tecnológico de Monterrey Pathology Service
Carlos A. Lopez-Garcia
Hospital San José Tecnológico de Monterrey Pathology Service
Villarreal-Garza Cynthia
Tecnologico de Monterrey, Hospital Zambrano-Hellion, Centro de Cáncer de Mama.
Lopez-Rivera Victor
The University of Texas Health Science Center at Houston
Canavati-Marcos Mauricio
Tecnologico de Monterrey, Hospital Zambrano-Hellion, Centro de Cáncer de Mama.
Santuario-Facio Sandra
Tecnologico de Monterrey, Escuela de Medicina y ciencias de la salud. CITES.
Dono Antonio
The University of Texas Health Science Center at Houston
Paloma del C. Monroig
Houston Methodist
Rocio Ortiz-Lopez
Tecnologico de Monterrey, Escuela de Medicina y ciencias de la salud. CITES.
Gabriela Sofia Gomez-Macias (dra.gabrielagomez@tecsalud.mx)
Hospital San José Tecnológico de Monterrey Pathology Service

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Abstract

Background: The need to identify patients with hormone receptor-positive (HR+) early invasive breast cancer (EIBC) who could benefit from adjuvant chemotherapy has been enhanced with the development of molecular signature tests. However, due to their high cost and limited availability alternative low-cost prognostic and predictive tests are used in clinical practice. Here, we sought to evaluate the performance of the proliferation marker Ki-67 to identify these patients and explore its association with molecular signatures and risk stratification markers.

Methods: From our prospectively maintained multicenter breast cancer registry, we identified EIBC HR+ patients tested with EndoPredict or MammaPrint and Ki-67 as part of their routine workup. Patients were categorized into two groups: Group 1 (2016-2018) was evaluated using EndoPredict and Group 2 (2011 to 2018) with MammaPrint. A ≥20% Ki67 cutoff was utilized for identify high proliferative EIBC and a receiver-operative curve area under the curve (AUC) and kappa concordance were utilized to evaluate the performance of Ki-67 compared to molecular signature tests.

Results: In the EndoPredict group, 54/96 patients were considered high-risk by EPclin. 57/96 patients had a Ki-67 ≥20%. However, there was no significant overall concordance between them (59.37%, κ = 0.168, *p*=0.09). In the MammaPrint group, 21/70 patients were considered high-risk. Ki67 ≥20% was present in 36 patients with a significant overall concordance (67.14%, κ 0.35, p<0.001). Additionally, Ki-67 was associated with the Nottingham histological grade (NHG) in both groups.

Conclusion: There is a fair concordance between Ki-67 and MammaPrint risk stratification of HR + EIBC and no concordance with EndoPredict molecular signature. Cost-effectiveness analysis of these tests in developing countries are needed, until then, the use of Ki-67 seems reasonable to aid clinical decision.

Introduction

Invasive breast carcinoma (IBC) is the second most common malignancy worldwide representing 11.6% of cancer cases and has a mortality rate of 6.6% [1]. IBC is comprised of a heterogeneous group of breast malignancies with different clinical, biological, and prognostic characteristics [2].

IBC can be divided into three molecular cancer subtypes: luminal, HER2-enriched, and basal-like. Sørlie et al. [3] further divided the luminal subtype into luminal A and B. This is particularly relevant in EIBC, as hormonal therapy is usually sufficient for luminal A tumors. Meanwhile, luminal B tumors benefit from more aggressive therapeutics including chemotherapy regimens [4–6].

MammaPrint (Agendia Inc., Irvine, CA, USA) evaluates the expression of 70 genes, which mostly have known biological functions implicated in tumor progression and metastasis [7, 8]. EndoPredict (Myriad Genetics Inc., Salt Lake City, UT, USA) is a 12-gene signature test (8 cancer-related genes, 3 normalization genes, and 1 control gene) that was designed to add clinic-pathological factors like tumor size and nodal status to the EPclin score [9, 10]. The prognostic performances of these two gene molecular signatures

panels have level I evidence [11]. Importantly, they are independent of other well-known prognostic tumor parameters such as tumor size, histological grade, and nodal status. The principal implication of both molecular signature tests in clinical management is the selection of patients that are unlikely to benefit from conventional chemotherapy regimens [10, 12].

Despite the importance of molecular signature tests on patient management, their cost limits their routine utilization. As a result, conventional immunohistochemistry (IHC) has been explored as an alternative to these tests [13–15]. It has been proposed that the Ki-67 proliferative index could be utilized in addition to the estrogen receptor (ER), progesterone receptor (PR), and HER2 receptor to discriminate between luminal A and B subtypes [16, 17].

High values of the Ki-67 proliferative index have been associated with benefit from chemotherapy regimens in IBC [6, 18]. However, establishing Ki-67 cut-offs for stratifying patient prognosis has proven to be a difficult task due to a lack of assessment standardization [19] and the changing recommendations of the St. Gallen consensus through time [4, 20]. The latest recommendation (2015) suggests the median Ki-67 laboratory value, as the cut-off for proliferative tumors [6].

In this study, we evaluate the Ki-67 proliferation index as an alternative to molecular signature tests to identify high-risk recurrence in HR + EIBC patients.

Material And Methods

From our prospectively maintained multicenter breast cancer registry, we performed a retrospective review to identify HR + EIBC patients who were tested with molecular signature tests and Ki-67 proliferative index. We divided the cohort depending on the molecular signature test utilized. In the EndoPredict (Myriad Genetics, Inc., Salt Lake City, UT, USA) cohort patients were tested between June 2016 to August 2018. This group was comprised of premenopausal women with HR + EIBC, HER2 negative, T1-T2, N0-N1, M0 [21]. In the MammaPrint (Agendia, Inc., Irvine, CA, USA) cohort patients were evaluated from 2011 to 2018. This group included patients with HR + EIBC, HER2 negative, T1-T2 and operable T3, and N0-N1 tumors according to previously utilized criteria [22]. For both cohorts: age, tumor size, TNM stage, histological subtype, Nottingham combined histologic grade (NHG), and lymphovascular invasion (LVI) data were recorded.

Immunohistochemistry

ER, PR, HER-2, and Ki-67 IHC analysis were performed with a Ventana BenchMarck GX autostainer (F. Hoffmann-La Roche Ltd. USA), using the internal validated protocol. Paraffin slides were deparaffinized in two changes of xylene for 10 minutes each and hydrated through graded alcohol and distilled water (2 changes rinsed of 100% ethanol, 2 changes of 95% ethanol, 2 changes of distilled water). Heat-induced epitope retrieval with citrate buffer was performed. Slides were then cooled and rinsed with distilled water, rinsed in tris buffered saline with tween for 5 minutes. Slides were then rinsed with 3% hydrogen peroxide, followed by a rinse with a wash buffer and covered with 300µl of protein block for 5 minutes. Slides were

treated with the following antibodies to ER (Clone SP1) (F. Hoffmann-La Roche Ltd. USA), PR (Clone 1E2) (F. Hoffmann-La Roche Ltd. USA), HER2/Neu (Clone 4B5) (F. Hoffmann-La Roche Ltd. USA), and Ki-67 (Clone 30 – 9) (F. Hoffmann-La Roche Ltd. USA). Slides were then rinsed with wash buffer, and the secondary reagent Dako Envision (Agilent, USA) labeled polymer HRP anti-Rabbit was applied. After the secondary reagent, diaminobenzidine was applied for 10 minutes, and the slides were rinsed with distilled water. Counterstaining was done with hematoxylin for 3 minutes, and slides were washed in tap water. Slides were then blued in ammonia water, rinsed in tap water, dehydrated in graded alcohol (95% ethanol, 100% ethanol), cleared in xylene (two changes), and coverslipped for microscopic examination. All slides included a external positive tissue control.

ER and PR were considered positive if > 1% of the neoplastic cells presented a nuclear stain according to ASCO/CAP guidelines [24]. HER2 was reported according to ASCO/CAP guidelines [24]. Ki-67 was evaluated as the percentage of nuclear staining cells as proposed by the International Ki-67 in Breast Cancer Working Group [22]. A cut-off of 20% was used as suggested by the St. Gallen consensus [6].

Molecular signature tests

The EndoPredict (Myriad Genetics, Inc., Salt Lake City, UT, USA) and MammaPrint (Agendia, Inc., Irvine, CA, USA) molecular signature tests were performed in a validated laboratory. An EpClin index of ≥ 3.3 was considered as high-risk of recurrence. For the MammaPrint (Agendia, Inc., Irvine, CA, USA), a BluePrint assay for categorization by molecular subgroup was reported. In addition, a, together with tlow-risk or high-risk category for recurrence was assigned.

Statistical analysis

Unpaired t-test and Chi-square were used for the comparison between high and low Ki-67 patients. Spearman rank-order correlation analysis was performed for the ordinal and continuous variables. Odds Ratio (OR) was performed to evaluate the association between Ki-67 and the molecular signatures tests. The Kappa coefficient was performed to evaluate the concordance between Ki-67 and the molecular signature tests. A p-value ≤ 0.05 was considered statistically significant. GraphPad Prism 9.0.1 (GraphPad, La Jolla, CA, USA) was used for statistical analysis and graphics.

Results

The clinicopathological characteristics of the two cohorts are listed in Table 1. In the MammaPrint cohort the patient were older, had smaller tumors and lower stage compared to EndoPredict. The proportion of patients with a high recurrence risk were higher in EndoPredict (56.25% vs 30% $p < 0.001$). Median Ki-67 proliferation index for the two cohorts in patients with high and low recurrence risk was IQR 30 [10–35] and 15 [10–25], respectively ($p < 0.001$). In the Receiver-operator characteristic (ROC) curve for the performance of Ki-67 expression in the identification of patients at high risk of recurrence the accuracy was 65% ($p = 0.001$), Fig. 1.

Table 1
Patient characteristics by test type

Table 1.				
	All N = 166	EndoPredict N = 96	MammaPrint N = 70	*P*
Age, median [IQR]	45[40–51]	43 [39-46.5]	51 [43–67]	< 0.0001
Tumor size, median [IQR]	20 [13-26.5]	22 [15–30]	15.5 [12–25]	0.0127
Pathological stage TNM %				0.009
IA	77 (46)	38	39	
IB	3 (2)	2	1	
IIA	60 (36)	34	26	
IIB	26 (16)	22	4	
Histological subtype, %				0.023
IBC/NST	149 (90)	89	60	
Lobular	4 (2)	2	2	
Mixed	3 (2)	3	0	
Histological grade (Nottingham), %				0.673
G1	19 (11)	11	8	
G2	123 (74)	72	51	
G3	22 (13)	11	11	
LVI, %	101 (61)	50	51	…
Estrogen progesterone receptor tumors	166	96	70	…
Estrogen receptor, percentage expression, median [IQR] (%)	90 [80–100]	98.5 [90–100]	90 [80–100]	< 0.0001
Positive progesterone receptor tumors	158	91	67	–
Progesterone receptor, percentage expression, median [IQR] (%)	80 [60–95]	90 [70–100]	80 [50–90]	0.0064
Ki-67 expression, median [IQR]	20 [10–30]	20 [10–30]	20 [10–30]	0.2512
High recurrence risk, %	75 (45)	54 (56.25%)	21 (30%)	0.001

Ki-67 as a surrogate marker of EndoPredict for recurrence risk

Clinical and pathological characteristics.

A total of 96 patients were included in this cohort, the clinicopathological characteristics are listed in Table 2. The median age was 43 years (range 25–55). The median tumor size was 22 mm (range 5–50 mm). Nodal status was negative (pN0) in 69 (71.9%) patients. IBC of no especial type (IBC/NST) was diagnosed in 89 (92.7%) patients, while 72 (76.59%) had a grade 2 NHG. LVI was present in 51 (72.85%) of tumors. All 96 (100%) patients were ER + and 91 (94.8%) were PR+.

Table 2
Clinicopathological characteristics of the EndoPredict (EPclin) cohort.

Table 2.					
	Total (96)	Ki 67 < 20 (N = 39)	Ki 67 > = 20 (N = 57)	*p* value	
Age	42.59 (±)	42.9 (± 6.17)	42.39 (± 4.84)	0.65	T test
Tumor size (mm)	22.27 (±) □	20.37 (± 9.08)	23.67 (± 10.19)	0.12	T test
Nodal stage				0.82	Chi-square
N0	69 (71.87)	29 (30.20)	40 (41.66)		
N1	25 (26.04)	10 (10.41)	15 (15.62)		
N1mi	2 (2.08)	0 (0)	2 (2.08)		
Pathological stage TNM (AJCC)				0.83	Chi-square
IA	38 (39.58)	15 (15.62)	23 (23.95)		
IB	2 (2.12)	1 (1.04)	1 (1.04)		
IIA	34 (35.41)	16 (16.66)	18 (18.75)		
IIB	22 (22.91)	7 (17.7)	15 (15.62)		
Histological subtype				0.39	Chi-square
IBC/NST	89 (92.70)	35 (36.45)	54 (56.25)		
Lobular	2 (2.04)	1 (1.04)	1 (1.04)		
Mucinous	3 (3.12)	2 (2.04)	1 (1.04)		
Mixed	2 (2.04)	1 (1.04)	1 (1.04)		
Nottingham histological grade	94 (100) □			0.03	Chi-square
G1	11 (11.70)	6 (6.38)	5 (3.21)		
G2	72 (76.59)	32 (34.04)	40 (42.44)		
G3	11 (11.70)	1 (1.06)	10 (10.63)		
LVI				0.01	Fisher´s exact test
Yes	50 (52.98)	14 (14.58)	36 (37.5)		

Table 2.					
No	46 (47.91)	25 (26.04)	21 (21.87)		
Positive estrogen receptor	96 (100)				
% expression (media)	83.24 (± 17.81)	80.38 (± 21.1)	84.56 (± 15.13)	0.26	T test
Positive progesterone receptor	91 (94.79)				
Porgestere receptor < = 20%	11 (11.45)				
% expression (media)	69.16 (± 28.77)	66.41 (± 29.73)	70.44 (± 28.24)	0.5	T test
EPclin score				0.14	Fisher´s exact test
Low risk	42 (43.75)	21 (21.87)	21 (21.87)		
High risk	54 (56.25)	18 (18.75)	36 (37.5)		
% of recurrence	15.13 (± 15.86)	11.56 (± 10.03)	18.25 (± 18.44)	0.04	T test
All variables are expressed in total of patients with the percentages or standard deviation in parenthesis.					
IDC/NST = Invasive ductal carcinomas / no special type, LVI = Lymphovascular invasion.					
6 patient had missed size in mm and 2 had missed histological grade. Mixed carcinomas were (1 IBC/NST– lobular and 1 IBC/NST – micropapillary).					

Forty-two (43.8%) patients were classified as low-risk according to EPclin, and 54 as high-risk. The median Ki-67 in the low-risk group was 19% vs 25% in the high-risk group ($p = 0.10$), Fig. 2. No association was found between EPclin and the 20% Ki-67 cutoff ($\chi^2 = 2.07$, $p = 0.14$). However, when stratified by percentage of recurrence, a statistically significant association was observed ($r^2 = 0.2255$, $p = 0.05$), Fig. 3. Of the 42 low-risk patients, 50% had a low-risk Ki-67, while from the 52 high-risk patients, 18 had a low-risk Ki-67. Showing an overall concordance of 59.37% ($\kappa = 0.168$, 0.030–0.360 CI 95% $p = 0.09$). The association analysis (sensibility, specificity,positive predictive value (PPV), and negative predictive value (NPV)) of Ki-67 (≥ 20%) to predict the risk group demonstrated a low performance (Table 3). A higher NHG and presence of LVI were associated with a Ki-67 ≥ 20%.

Table 3
Correlation analysis of Ki67 with EndoPredict (EPclin) and MammaPrint.

Table 4.

	Total (70)	Ki 67 < 20 (N = 34)	Ki 67 > = 20 (N = 36)	p value	
Age	52.79 (± 11.08)	55.21 (± 9.76)	50.5 (± 11.9)	0.08	T test
Tumor size (mm)	18.66 (± 10.02)	18.68 (± 11.33)	18.64 (± 8.77)	0.99	T test
Nodal stage				0.07	Chi-square
N0	60 (85.71)	26 (37.14)	34 (48.57)		
N1	10 (14.28)	8 (11.42)	2 (2.85)		
Pathological stage TNM (AJCC)				0.44	Chi-square
IA	39 (55.71)	18 (25.71)	21 (30)		
IB	1 (1.42)	1(1.42)	0 (0)		
IIA	26 (37.14)	11(15.71)	15 (21.42)		
IIB	4 (5.71)	4(5.71)	0 (0)		
Histological subtype				0.03	Chi-square
IBC/NST	60 (85.71)	26 (37.14)	34 (48.57)		
Lobular	8 (11.42)	6 (8.57)	2 (2.85)		
Mixed	2 (2.85)	2 (2.85)	0 (0)		
Histological grade (Nottingham)				0.01	Chi-square
G1	8 (11.42)	6 (8.57)	2 (2.85)		
G2	51 (72.85)	26 (37.14)	25 (35.71)		
G3	11(15.71)	2 (2.85)	9 (12.85)		
LVI				0.79	Fisher´s exact test
Yes	51 (72.85)	24 (34.28)	27 (38.57)		
No	19 (27.14)	10 (14.28)	9 (12.85)		
Positive estrogen receptor	70 (100)				

Table 4.					
% expression (media)	92 (± 12.73)	93.97 (± 7.96)	90.33 (± 15.92)	0.24	T test
Positive progesterone receptor	67 (95.71)				
Porgestere receptor < = 20%	6 (8.57)				
% expression (media)	78.22 (± 28.08)	84.53 (± 26.17)	72.28 (± 28.88)	0.07	T test
MammaPrint				0.002	Fisher´s exact test
Low risk	49 (70%)	30 (42.85)	19 (27.14)		
High risk	21 (30%)	4 (5.71)	17 (24.28)		
All variables are expressed in total of patients with the percentages or standard deviation in parenthesis.					
IBC/NST = Invasive breast carcinoma / no special type, LVI = Lymphovascular invasion.					

Table 3. Correlation analysis of Ki67 with EndoPredict (EPclin) and MammaPrint.

	Ki67 and Epclin	Ki67 and MammaPrint
Sensitivity	0.54 (0.37–0.70 CI95%)	0.88 (0.73.0.97 CI95%)
Specificity	0.63 (0.49–0.76 CI95%)	0.47 (0.30–0.65 CI95%)
PPV	0.50 (0.34–0.66 CI95%)	0.61 (0.46–0.75 CI95%)
NPV	0.67 (0.53–0.79 CI95%)	0.81 (0.58–0.95 CI95%)
Kappa	0.168 (-0.03-0.36 CI 95%)	0.35 (0.15–0.55 CI95%)
* PPV = positive predictive value, NPV = negative predictive value.		

Ki-67 as a surrogate marker of MammaPrint for recurrence risk

Clinical and pathological characteristics

The clinicopathological characteristics of this cohort are presented in **Table 4**. The median age was 51 years (range 33–77). Median tumor size was 15.7 mm (range 4–52 mm). Nodal status was pN0 in 60 (85.71%). Sixty (85.71%) patients had an IBC/NST histologic type, 51(72.85%) had a grade 2 NHG, and 50 cases (72.85%) presented LVI. All cases were ER + and 67 (95.71%) were PR+, all were Luminal by BluePrint, 21 were high-risk and 49 low-risk by MammaPrint.

Table 4. Clinicopathological characteristics of the MammaPrint cohort.

	Total (70)	Ki 67 < 20 (N = 34)	Ki 67 > = 20 (N = 36)	p value	
Age	52.79 (± 11.08)	55.21 (± 9.76)	50.5 (± 11.9)	0.08	T test
Tumor size (mm)	18.66 (± 10.02)	18.68 (± 11.33)	18.64 (± 8.77)	0.99	T test
Nodal stage				0.07	Chi-square
N0	60 (85.71)	26 (37.14)	34 (48.57)		
N1	10 (14.28)	8 (11.42)	2 (2.85)		
Pathological stage TNM (AJCC)				0.44	Chi-square
IA	39 (55.71)	18 (25.71)	21 (30)		
IB	1 (1.42)	1(1.42)	0 (0)		
IIA	26 (37.14)	11(15.71)	15 (21.42)		
IIB	4 (5.71)	4(5.71)	0 (0)		
Histological subtype				0.03	Chi-square
IBC/NST	60 (85.71)	26 (37.14)	34 (48.57)		
Lobular	8 (11.42)	6 (8.57)	2 (2.85)		
Mixed	2 (2.85)	2 (2.85)	0 (0)		
Histological grade (Nottingham)				0.01	Chi-square
G1	8 (11.42)	6 (8.57)	2 (2.85)		
G2	51 (72.85)	26 (37.14)	25 (35.71)		
G3	11(15.71)	2 (2.85)	9 (12.85)		
LVI				0.79	Fisher´s exact test
Yes	51 (72.85)	24 (34.28)	27 (38.57)		
No	19 (27.14)	10 (14.28)	9 (12.85)		
Positive estrogen receptor	70 (100)				
% expression (media)	92 (± 12.73)	93.97 (± 7.96)	90.33 (± 15.92)	0.24	T test

Table 4. Clinicopathological characteristics of the MammaPrint cohort.					
Positive progesterone receptor	67 (95.71)				
Porgestere receptor < = 20%	6 (8.57)				
% expression (media)	78.22 (± 28.08)	84.53 (± 26.17)	72.28 (± 28.88)	0.07	T test
MammaPrint				0.002	Fisher´s exact test
Low risk	49 (70%)	30 (42.85)	19 (27.14)		
High risk	21 (30%)	4 (5.71)	17 (24.28)		
All variables are expressed in total of patients with the percentages or standard deviation in parenthesis.					
IBC/NST = Invasive breast carcinoma / no special type, LVI = Lymphovascular invasion.					

The overall median Ki-67 proliferation index was 20%. Meanwhile, the low-risk group median Ki-67 was 15%, the high-risk Ki-67 median was 30% ($p = 0.002$), Fig. 2. The univariate analysis showed a significant association between Ki-67 and MammaPrint (Agendia, Inc., Irvine, CA, USA) with a $\chi^2 = 8.85$ ($p = 0.002$). Of the 49 low-risk patients, 30 had a Ki-67 < 20%. Meanwhile, only 4 of the 21 high-risk patients had a Ki-67 < 20% (Fig. 3). The kappa coefficient demonstrated a fair concordance between Ki-67 and MammaPrint, with an overall concordance of 67.14% (κ = 0.35, 0.15–0.55 CI95%, $p = 0.001$). The association analysis revealed a good sensibility and NPV to predict the risk group. However, the specificity and PPV were low, Table 2. Additionally, the Ki-67 proliferation index was significantly correlated with the NHG and the histological type.

Discussion

Efforts have been made to match the molecular signatures tests with clinicopathological characteristics. The ASCO/CAP associations have emitted guidelines for interpretation of HR and HER2 expression by IHC with the intent to reduce the interobserver variability and to achieve a better correlation with the molecular classification. However, the capacity to discriminate between the luminal A and B subtypes by IHC is not ideal. Even with the standardization of the technique, there is a 30–40% discrepancy between IHC and multigene expression assays, with substantial implications in treatment decisions [23].

In our study, the observed range of Ki-67 expression was wide, with 2–70% vs 1–85% in the EndoPredict (Myriad Genetics, Inc., Salt Lake City, UT, USA) cohort and 2–50% vs 3–70% in theMammaPrint (Agendia,

Inc., Irvine, CA, USA) cohort, low-risk and high-risk, respectively. However, the medians were slightly different for the two risk groups.

It is known that the Ki-67 assay has a moderate interobserver variability [24]. The hot-spot vs. the whole-slide analysis of Ki67 has been an area of controversy, with the first being more practical by taking into account the more aggressive biology spot, acknowledging tumor heterogeneity. Thakur et al. evaluated the hot-spot vs. whole-slide Ki-67, identifying a strong correlation between the two methods (r = 0.938) [25]. To reduce the interobserver variability, the International Ki-67 in Breast Cancer Working Group recommends if the stain is homogenous to count at least three randomly selected high-power (×40 objective) fields and if it is heterogenous, three fields in the tumor edge or hot spots, with some exceptions and scoring preferably 1000 cells with 500 at a minimum [26].

In the MammaPrint cohort, a low Ki-67 (< 20%) demonstrated a high sensibility (88%) and could modestly predict patients with low-risk of recurrence (PPV 0.61%, 0.46–0.75 CI 95%). Moreover, the Ki-67 and the molecular test, Mammaprint (Agendia, Inc., Irvine, CA, USA), had an overall concordance of 67.14% and a fair agreement (κ = 0.35 $p = 0.001$). Similar to our results and utilizing the same Ki-67 cutoff, Viale et al. reported a concordance of 71% (69–72 CI95%) between the molecular classification of Luminal IBC and Ki-67 in the EORTC 10041/BIG 3–04 MINDACT trial (κ = 0.35, 0.32–0.37 95% CI) [23]. Additionally, another report demonstrated similar results (κ = 0.35) between Mammaprint and Ki-67 in 65 IBC patients, however, they utilize a different Ki67 cutoff (14%) [27]. Similar to our study, Bösl et al. compared MammaPrint and EndoPredict with Ki-67, achieving a significant correlation with MammaPrint ($p = 0.004$) but not with EPclin score ($p = 0.09$) [28]. Despite this encouraging concordance between Ki-67 and MammaPrint, in the EndoPredict cohort, the Ki-67 overall concordance was low and did not significantly correlate with the EPclin risk category (59.37%, κ = 0.168, $p = 0.09$). This mean that when patients were stratified by Ki-67, 30–40% in each cohort were assigned to other risk categories compared to molecular testing.

In clinical practice, the indication of adjuvant chemotherapy is based on the consideration of multiple variables such as patient age, tumor size, histological type and grade, PR status, and LVI. In our analysis, we did not observe a correlation between PR and Ki-67, EndoPredict, or MammaPrint. It is worth noting that only a small number of patients (11 and 6 patients in each cohort) had a PR expression of < 20%, highlighting the limited value of PR in the luminal classification of EIBC compared to Ki-67. Interestingly, a significant correlation between Ki-67 and NHG was observed in both cohorts (Chi-square EndoPredict $\chi^2 = 4.68$ $p = 0.03$ and MammaPrint $\chi^2 = 6.32$, $p = 0.01$), as has been previously reported [29–31].

The differences in the association between Ki-67 to MammaPrint and Ki-67 to EndoPredict could be due to the different patient selection criteria, clinicopathological differences between cohorts, and the acquisition of data from multiple centers, potentially introducing interobserver variability in the Ki-67.

Despite that molecular signature tests are an important tool to identify patients with low-risk of recurrence, the agreement between different tests is far from perfect. Pelaez et al. compared MammaPrint

and EndoPredict, identifying an overall concordance of 72.5%, with a slight improvement using the EPclin score to an overall concordance of 75% [32], Similarly, Bösl et al. reported a concordance of 66% with more patients being placed in the low-risk category with MammaPrint [28].

Financially, the different molecular signatures tests have been evaluated with mixed results depending on the geographic location. A Canadian study found EndoPredict to be cost-effective with a ratio of $36,274 per quality-adjusted life-year (QALY), with a total gain of 379 QALYs/year [33]. Meanwhile, in the United Kingdom (UK), EndoPredict was not identified as cost-effective with a threshold of £20,000/QALY. However, it was if the incremental cost-effectiveness ratio was £26,836/QALY [34]. Moreover, a recent analysis in the UK showed that EndoPredict was cost-effective only if lymph node disease was present (1–3 positive nodes) with £30,000/QALY [35]. On the other hand, MammaPrint in the USA was found to be cost-effective at a ratio of $10,000/QALY [36]. Nevertheless, another study in the UK found that MammaPrint was not cost-effective compared to current clinical practice [35]. Overall, in western European countries and the USA, molecular signature tests are cost-effective. The willingness to pay for QALYs in the healthcare systems of developing countries has yet to be studied. However, the cost of these tests might be onerous to healthcare systems in precarious situations.

Limitations to our study

Some limitations of our study include its retrospective nature and the potential for selection bias. Moreover, the groups tested with the different molecular signature tests were heterogeneous. However, this study represents a multicentric cohort of a large number of EIBC with molecular testing that allowed us the evaluation of the Ki-67 proliferation index to molecular signatures tests.

Conclusion

There is a fair concordance between Ki-67 and MammaPrint risk stratification of HR + early IBC and no concordance with EndoPredict molecular signature. Although there is not perfect molecular signature tests, these are high-value tools for therapy selection in HR + EIBC patients. Cost-effectiveness analysis of these tests in developing countries are needed, until then, the use of Ki-67 seems reasonable to aid clinical decision.

Abbreviations

IBC: Invasive breast cancer

NHG: Nottingham histological grade

HR+: Hormone receptor-positive

CI: coefficient interval

IHC: Immunohistochemistry

PR: Progesterone receptor

UK: United Kingdom

QALY: Quality-adjusted life year

LVI: Lymphovascular invasion

Declarations

Ethics approval and consent to participate

IRB approval was obtained from the Ethics Committee of Research at Tecnológico de Monterrey and the National Bioethics Commission (code id: CONBIOETICA19CE100820130520), and was also granted in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient prior to tumor sample collection.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conception and design, development of methodology, analysis and interpretation of data, and original draft preparation: EG, CL, GGM; Development of methodology, analysis: VL, AD, SS; Acquisition of data and supervision: GGM, CL, CV Analysis and interpretation of data and supervision: CV, MC; Acquisition of data: MC, PCM, RO, MC. All of the authors reviewed **and approved the final manuscript.**

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Authors' information

[1]Pathology department, Hospital San Jose, Tecnologico de Monterrey, Mexico. [2]Breast Cancer Center, Hospital Zambrano-Hellion, Tecnologico de Monterrey, Mexico. [3]School of medicine and health sciences. CITES, Tecnologico de Monterrey, Mexico. [4]Department of Neurology, UTHealth McGovern Medical School, Houston, TX. [5]Department of Pathology and Laboratory Medicine, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX. [6]Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, Texas. [7]Universidad Autonoma de Nuevo Leon. School of medicine.

References

1. Bray, F., et al., *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.* CA Cancer J Clin, 2018. **68**(6): p. 394-424.
2. Perou, C.M., et al., *Molecular portraits of human breast tumours.* Nature, 2000. **406**(6797): p. 747-52.
3. Sorlie, T., et al., *Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications.* Proc Natl Acad Sci U S A, 2001. **98**(19): p. 10869-74.
4. Goldhirsch, A., et al., *Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013.* Ann Oncol, 2013. **24**(9): p. 2206-23.
5. Gluck, S., et al., *Molecular subtyping of early-stage breast cancer identifies a group of patients who do not benefit from neoadjuvant chemotherapy.* Breast Cancer Res Treat, 2013. **139**(3): p. 759-67.
6. Coates, A.S., et al., *Tailoring therapies–improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015.* Ann Oncol, 2015. **26**(8): p. 1533-46.
7. Tian, S., et al., *Biological functions of the genes in the mammaprint breast cancer profile reflect the hallmarks of cancer.* Biomark Insights, 2010. **5**: p. 129-38.
8. van de Vijver, M.J., et al., *A gene-expression signature as a predictor of survival in breast cancer.* N Engl J Med, 2002. **347**(25): p. 1999-2009.
9. Müller, B.M., et al., *The EndoPredict Gene-Expression Assay in Clinical Practice - Performance and Impact on Clinical Decisions.* PLoS One, 2013. **8**(6): p. e68252.
10. Filipits, M., et al., *A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors.* Clin Cancer Res, 2011. **17**(18): p. 6012-20.
11. Duffy, M.J., et al., *Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM).* Eur J Cancer, 2017. **75**: p. 284-298.
12. Weigelt, B., J.S. Reis-Filho, and C. Swanton, *Genomic analyses to select patients for adjuvant chemotherapy: trials and tribulations.* Ann Oncol, 2012. **23 Suppl 10**: p. x211-8.
13. van Steenhoven, J.E.C., et al., *Conventional Pathology Versus Gene Signatures for Assessing Luminal A and B Type Breast Cancers: Results of a Prospective Cohort Study.* Genes (Basel), 2018. **9**(5).

14. Bustreo, S., et al., *Optimal Ki67 cut-off for luminal breast cancer prognostic evaluation: a large case series study with a long-term follow-up.* Breast Cancer Res Treat, 2016. **157**(2): p. 363-371.
15. Dubsky, P., et al., *EndoPredict improves the prognostic classification derived from common clinical guidelines in ER-positive, HER2-negative early breast cancer.* Ann Oncol, 2013. **24**(3): p. 640-7.
16. Cheang, M.C., et al., *Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer.* J Natl Cancer Inst, 2009. **101**(10): p. 736-50.
17. Cuzick, J., et al., *Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer.* J Clin Oncol, 2011. **29**(32): p. 4273-8.
18. de Azambuja, E., et al., *Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients.* Br J Cancer, 2007. **96**(10): p. 1504-13.
19. Focke, C.M., P.J. van Diest, and T. Decker, *St Gallen 2015 subtyping of luminal breast cancers: impact of different Ki67-based proliferation assessment methods.* Breast Cancer Res Treat, 2016. **159**(2): p. 257-63.
20. Goldhirsch, A., et al., *Strategies for subtypes–dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011.* Ann Oncol, 2011. **22**(8): p. 1736-47.
21. Villarreal-Garza, C., et al., *Change in therapeutic management after the EndoPredict assay in a prospective decision impact study of Mexican premenopausal breast cancer patients.* PLoS One, 2020. **15**(3): p. e0228884.
22. Cardoso, F., et al., *70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer.* N Engl J Med, 2016. **375**(8): p. 717-29.
23. Viale, G., et al., *Immunohistochemical versus molecular (BluePrint and MammaPrint) subtyping of breast carcinoma. Outcome results from the EORTC 10041/BIG 3-04 MINDACT trial.* Breast Cancer Res Treat, 2018. **167**(1): p. 123-131.
24. Gluz, O., et al., *West German Study Group Phase III PlanB Trial: First Prospective Outcome Data for the 21-Gene Recurrence Score Assay and Concordance of Prognostic Markers by Central and Local Pathology Assessment.* J Clin Oncol, 2016. **34**(20): p. 2341-9.
25. Thakur, S.S., et al., *The use of automated Ki67 analysis to predict Oncotype DX risk-of-recurrence categories in early-stage breast cancer.* PLoS One, 2018. **13**(1): p. e0188983.
26. Dowsett, M., et al., *Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group.* J Natl Cancer Inst, 2011. **103**(22): p. 1656-64.
27. Nguyen, B., et al., *Comparison of molecular subtyping with BluePrint, MammaPrint, and TargetPrint to local clinical subtyping in breast cancer patients.* Ann Surg Oncol, 2012. **19**(10): p. 3257-63.
28. Bosl, A., et al., *MammaPrint versus EndoPredict: Poor correlation in disease recurrence risk classification of hormone receptor positive breast cancer.* PLoS One, 2017. **12**(8): p. e0183458.

29. Trihia, H., et al., *Ki-67 expression in breast carcinoma: its association with grading systems, clinical parameters, and other prognostic factors–a surrogate marker?* Cancer, 2003. **97**(5): p. 1321-31.
30. Marwah, N., et al., *Correlation of proliferative index with various clinicopathologic prognostic parameters in primary breast carcinoma: A study from North India.* J Cancer Res Ther, 2018. **14**(3): p. 537-542.
31. Shokouh, T.Z., A. Ezatollah, and P. Barand, *Interrelationships Between Ki67, HER2/neu, p53, ER, and PR Status and Their Associations With Tumor Grade and Lymph Node Involvement in Breast Carcinoma Subtypes: Retrospective-Observational Analytical Study.* Medicine (Baltimore), 2015. **94**(32): p. e1359.
32. Pelaez-Garcia, A., et al., *Comparison of risk classification between EndoPredict and MammaPrint in ER-positive/HER2-negative primary invasive breast cancer.* PLoS One, 2017. **12**(9): p. e0183452.
33. Hannouf, M.B., et al., *Cost-effectiveness analysis of multigene expression profiling assays to guide adjuvant therapy decisions in women with invasive early-stage breast cancer.* Pharmacogenomics J, 2020. **20**(1): p. 27-46.
34. Sebastian Hinde, C.T., Shirley May, Lucy Matthews, Amy Arbon, Lesley Fallowfield, David Bloomfield., *The cost-effectiveness of EndoPredict to inform adjuvant chemotherapy decisions in early breast cancer.* Health Policy and Technology, 2019. **8**(1): p. 75-83.
35. Harnan, S., et al., *Tumour profiling tests to guide adjuvant chemotherapy decisions in early breast cancer: a systematic review and economic analysis.* Health Technol Assess, 2019. **23**(30): p. 1-328.
36. Chen, E., K.B. Tong, and J.L. Malin, *Cost-effectiveness of 70-gene MammaPrint signature in node-negative breast cancer.* Am J Manag Care, 2010. **16**(12): p. e333-42.

Figures

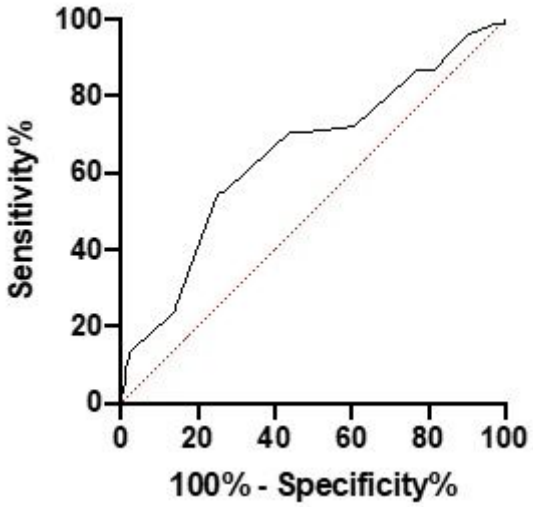

Figure 1

Receiver-operator characteristic curve for the performance of Ki67 expression in identifying patients at high risk of recurrence with AUC 0.6476 [95% CI, 0.5624-0.7328] (P=0.0011).

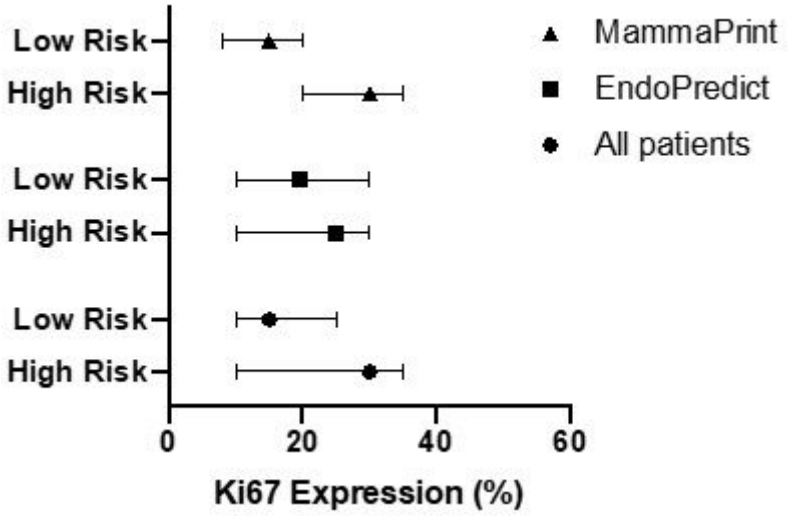

Figure 2

EndoPredict: 19.5[10-30] vs 25[10-30], Low vs High, P=0.1062 MammaPrint: 15[8-20] vs 30[20-35], Low vs High, P=0.002

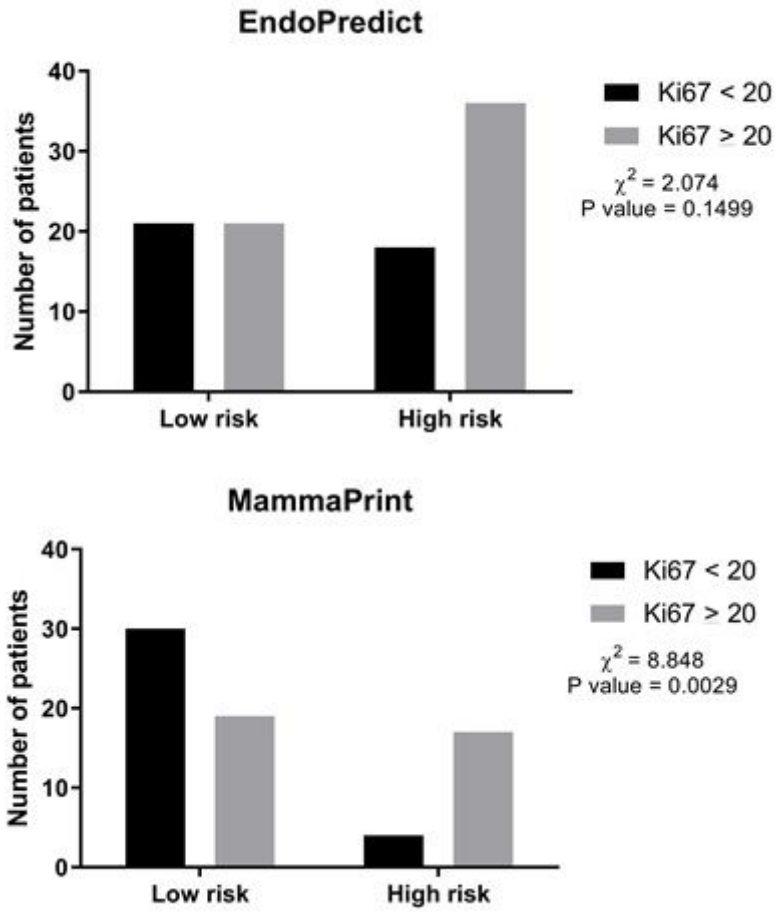

Figure 3

Patient proportional differences with high and low risk in the MammaPrint and EndoPredict results. The black label shows the proportion of the patients with a Ki67 <20% and the gray label shows the proportion in patients with a Ki67 >20%.