

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

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Abstract. Identifying patients with hormone receptor-positive (HR+) early invasive breast cancer (EIBC) who benefit from adjuvant chemotherapy has improved with molecular signature tests. However, due to high cost and limited availability, alternative tests are used. The present study 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. From the San José TecSalud Hospital in Monterrey México, patients with HR+ EIBC as tested with EndoPredict or MammaPrint and Ki-67 index were identified. They were categorized into two groups: Group 1 (June 2016-August 2018) was evaluated using EndoPredict and Group 2 (June 2016-August 2018) with MammaPrint. A $\geq 20\%$ Ki67 index cutoff was utilized to identify highly proliferative EIBC and an area under the

receiver-operating characteristic curve and κ concordance were utilized to evaluate the performance of Ki-67 index compared to molecular signature tests. In the EndoPredict group, 54/96 patients were considered high-risk based on their EPclin score, while 57/96 patients had Ki-67 index $\geq 20\%$. However, there was no significant overall concordance between them (59.37%, $\kappa=0.168$, $P=0.09$), while the given risk of distant recurrence given in percentage by EPclin had a positive association with the Ki67 index ($P=0.04$). In the MammaPrint group, 21/70 patients were considered high-risk and 36/70 patients presented with a Ki-67 index $\geq 20\%$ with a significant overall concordance (67.14%, $\kappa=0.35$, $P<0.001$). In addition, high Ki-67 index was associated with the Nottingham histological grade in both groups. In conclusion, there was a concordance between Ki-67 and MammaPrint risk stratification of HR+ EIBC and no concordance with the EndoPredict molecular signature, but a positive association with the given percentage of recurrence and the median Ki-67 index as the cutoff at our center. Cost-effectiveness analyses of these tests in developing countries are required; until then, the use of Ki-67 appears reasonable to aid clinical decisions, together with the other established clinicopathological variables.

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Abbreviations: IBC, invasive breast cancer; NHG, Nottingham histological grade; HR+, hormone receptor-positive; CI, confidence interval; IHC, immunohistochemistry; PR, progesterone receptor; QALY, quality-adjusted life year; LVI, lymphovascular invasion

Key words: Ki-67, EndoPredict, MammaPrint, early breast cancer

Introduction

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

IBC may be divided into three molecular cancer subtypes: Luminal, HER2-enriched and basal-like. Sørli *et al* (3)

further divided the luminal subtype into luminal A and B. This is particularly relevant in early IBC (EIBC), as hormonal therapy is usually sufficient for luminal A tumors. By contrast, luminal B tumors benefit from more aggressive therapeutics, including chemotherapy regimens (4-6).

MammaPrint (Agendia, Inc.) evaluates the expression of 70 genes, which mostly have known biological functions implicated in tumor progression and metastasis (7,8). EndoPredict (Myriad Genetics, Inc.) is a 12-gene signature test (8 cancer-related genes, 3 normalization genes and 1 control gene) that was designed to add clinicopathological factors such as tumor size and nodal status to obtain the so-called EPclin score, and an estimated risk for distant recurrence at 10 years (9,10). The prognostic performances of these two gene molecular signature panels have level I evidence in pre- and postmenopausal females (11). Of note, they are independent of other well-known prognostic tumor parameters, including 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 in 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 may 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 proliferative cell marker Ki-67 have been associated with a benefit from chemotherapy regimens in IBC (6,18). However, establishing Ki-67 index cut-offs for stratifying patient prognosis has proven to be a difficult task due to the lack of assessment standardization (19); this has been acknowledged by the St. Gallen consensus with changes in recommendations through time (4,20), the latest of which from 2015 suggests the median Ki-67 index internal laboratory value as the cut-off for highly proliferative tumors (6).

In the present study, the Ki-67 index was evaluated as an alternative to molecular signature tests to identify high risk of recurrence in patients with hormone receptor (HR)+ EIBC.

Materials and methods

Patients. Using the breast cancer registry of San José TecSalud Hospital (Monterrey, México), a retrospective review was performed to identify patients with HR+ EIBC who were tested with molecular signature tests and the Ki-67 index. The cohort was divided according to the molecular signature test utilized. In the EndoPredict cohort, patients were tested between June 2016 and August 2018. This group comprised premenopausal females with HR+ EIBC, HER2 negative, T1-T2, N0-N1 and M0 (21). In the MammaPrint cohort, patients were evaluated from June 2016 to August 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 histological grade (NHG) and lymphovascular invasion (LVI) data were recorded.

IHC. In biopsies of the tumor samples, ER, PR, HER-2 and Ki-67 were analyzed with a Ventana BenchMark GX autostainer (Hoffmann-La Roche, Ltd.) using the internal validated protocol. Paraffin slides were deparaffinized using two changes of xylene for 10 min each and hydrated through an alcohol gradient and distilled water (2 changes 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 and rinsed in tris-buffered saline with Tween-20 for 5 min. Slides were then rinsed with 3% hydrogen peroxide, followed by a rinse with a wash buffer and covered with 300 μ l of protein block (Protein block X0909; Dako; Agilent Technologies, Inc.) for 5 min. Slides were treated with the following antibodies for 16 min at 36°C: Anti-estrogen receptor (clone SP1) rabbit monoclonal primary antibody (cat. no. 790-4324; prediluted concentration, 1 μ g/ml), anti-progesterone receptor rabbit monoclonal primary antibody (clone 1E2; cat. no. 790-2223; prediluted concentration, 1 μ g/ml), anti-HER-2/neu (clone 4B5; cat. no. 790-100; prediluted concentration, 6 μ g/ml) and anti-Ki-67 rabbit monoclonal primary antibody (clone 30-9; cat. no. 790-4286; prediluted concentration, 2 μ g/ml; all from Hoffmann-La Roche, Ltd). Slides were then rinsed with wash buffer and incubated with the secondary reagent, Dako Envision HRP-labeled polymer anti-rabbit (cat. no. M3648; dilution, 1:50; Agilent Technologies, Inc.) min at room temperature for 60 min. Subsequently, diaminobenzidine was applied for 10 min at 36°C and the slides were rinsed with distilled water. Counterstaining was performed with hematoxylin for 3 min and slides were washed in tap water. Slides were then blued in ammonia water, rinsed in tap water, dehydrated in an alcohol gradient (95% ethanol, 100% ethanol), cleared in xylene (two changes) and mounted with coverslips for examination with a microscope. All slides included an external positive tissue control.

ER and PR were considered positive if >1% of the neoplastic cells exhibited a nuclear stain according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines (23). Low PR was defined as <20% of nuclear-positive tumor cells (24). HER2 was also evaluated according to the ASCO/CAP guidelines (25). Ki-67 index was evaluated using a hot spots method, performed in the area with the highest number of positive nuclei. A total of three high-power fields using a magnification of x400 including a hot spot were examined, as proposed by the International Ki-67 in Breast Cancer Working group (26,27). A cut-off of 20% was used as suggested by the St. Gallen consensus (6).

Molecular signature tests. The EndoPredict (Myriad Genetics, Inc.) and MammaPrint (Agendia, Inc.) molecular signature tests were performed in a validated laboratory. An EpClin index of ≥ 3.3 was considered to indicate high risk of recurrence. For the MammaPrint® (Agendia, Inc.) assay a risk category of recurrence was assigned to each case (LR or HR), along with the molecular subgroup (using Blueprint assay).

Statistical analysis. An unpaired t-test, Fisher's exact and Mann-Whitney U test were used for comparison between patients with high and low Ki-67 index. Spearman rank-order correlation analysis for ordinal and continuous variables. Odds

Table I. Patient characteristics by test type (n=166).

Parameter	Total	EndoPredict (n=96)	MammaPrint (n=70)	P-value
Age, years	45 (40-51)	43 (39-46.5)	51 (43-67)	<0.0001
Tumor size, mm	20 (13-26.5)	22 (15-30)	15.5 (12-25)	0.0127
TNM pathological stage %				0.009
IA	77 (46)	38	39	
IB	3 (2)	2	1	
IIA	60 (36)	34	26	
IIB	26 (16)	22	4	
Histological subtype, %				0.19
IBC/NST	149 (90)	89	60	
Lobular	10 (6.02)	2	8	
Mucinous	3 (1.8)	3	0	
Mixed	4 (1.2)	2	2	
Histological grade (Nottingham), %				0.673
G1	19 (11)	11	8	
G2	123 (74)	72	51	
G3	22 (13)	11	11	
Lymphovascular invasion, %				0.009
Yes	101 (61)	50	51	
No	65 (39)	46	19	
Estrogen receptor-positive tumors	166	96	70	>0.999
Estrogen receptor expression, %	90 (80-100)	98.5 (90-100)	90 (80-100)	<0.0001
Positive progesterone receptor tumors	158	91	67	>0.999
Progesterone receptor expression, %	80 (60-95)	90 (70-100)	80 (50-90)	0.0064
Ki-67 expression, %	20 (10-30)	20 (10-30)	20 (10-30)	0.2512
High recurrence risk, %	75 (45)	54 (56.25)	21 (30)	0.001

Values are expressed as the median (interquartile range) or n.

ratios were determined to evaluate the association between Ki-57 and molecular signature tests. Receiver-operating characteristic curve (ROC) analysis was performed for analysis of the risk of recurrence given by MammaPrint or EndoPredict and the Ki-67 index. Validation/association analysis was performed to test sensitivity and specificity. The Kappa coefficient was determined to evaluate the concordance between the Ki-67 index and the molecular signature tests. $P < 0.05$ was considered to indicate statistical significance. GraphPad Prism 9.0.1 (GraphPad Software, Inc.) was used for statistical analysis and graphics.

Results

Patient characteristics. The clinicopathological characteristics of the two cohorts are listed in Table I. In the MammaPrint cohort, the patients were older, had smaller tumors and a lower stage compared with those in the EndoPredict cohort. The proportion of patients with a high recurrence risk was higher in the EndoPredict cohort (56.25 vs. 30.00%, $P < 0.001$). The median (interquartile range) Ki-67 index for the two cohorts in patients with high and low recurrence risk was 30 (10-35) and 15 (10-25), respectively ($P < 0.001$). In the ROC curve analysis for the performance of Ki-67 index in the identification of

all patients at high risk of recurrence, the accuracy was 65% ($P = 0.001$; Fig. 1). In the ROC curve analysis for the performance of Ki-67 index in the identification of patients from the EndoPredict and MammaPrint cohort at high risk of recurrence, the accuracy was 60% ($P = 0.110$) and 70% ($P = 0.002$) (Figs. 2 and 3, respectively).

Ki-67 index as a surrogate marker for EndoPredict for recurrence risk. A total of 96 patients were included in the EndoPredict cohort and their clinicopathological characteristics are listed in Table II. The median age was 43 years (range, 25-55 years). The median tumor size was 22 mm (range, 5-50 mm). Nodal status was negative (pN0) in 69 patients (71.9%). IBC of no special type (IBC/NST) was diagnosed in 89 patients (92.7%), while 72 (76.59%) had grade 2 NHG. LVI was present in 51 tumors (72.85%). All 96 patients (100%) were ER+ and 91 (94.8%) were PR+.

From the EndoPredict cohort, 42 patients (43.8%) were classified as low-risk according to EPclin and 54 as high-risk. The median Ki-67 index in the low-risk group was 19%, while it was 25% in the high-risk group ($P = 0.10$, Fig. 4). No significant association was indicated between Ki-67 index with a cutoff at 20% and EPclin risk category (high vs. low) ($\chi^2 = 2.07$, $P = 0.14$; Fig. 5). However, when analyzed by the estimated risk

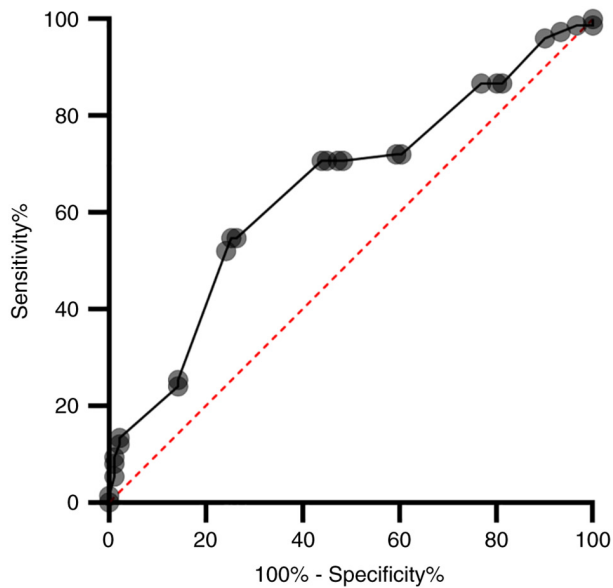


Figure 1. Receiver-operating characteristic curve for the performance of Ki-67 expression in identifying all patients at high risk of recurrence with an area under the curve of 0.6476 (95% CI, 0.5624-0.7328) ($P=0.0011$). Cutoff value for Ki-67 index, 20%.

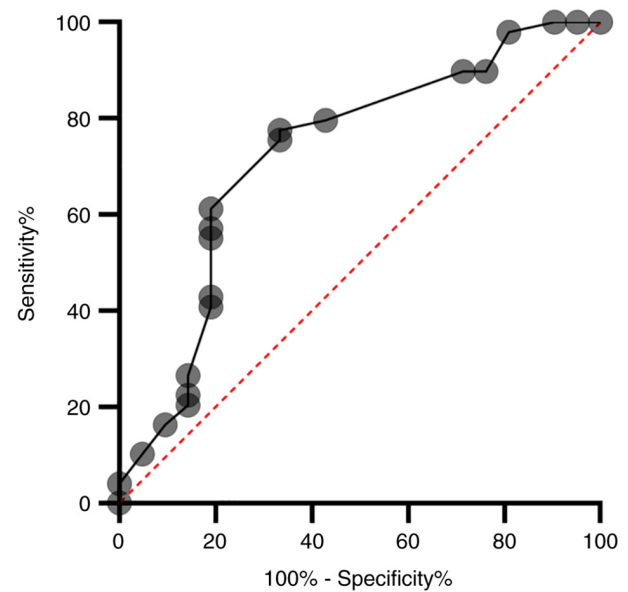


Figure 3. Receiver-operating characteristic curve for the performance of Ki-67 expression in identifying patients at high risk of recurrence according to MammaPrint with an area under the curve 0.7259 (95% CI, 0.5872-0.8647) ($P=0.0029$). Cutoff value for Ki-67 index, 20%.

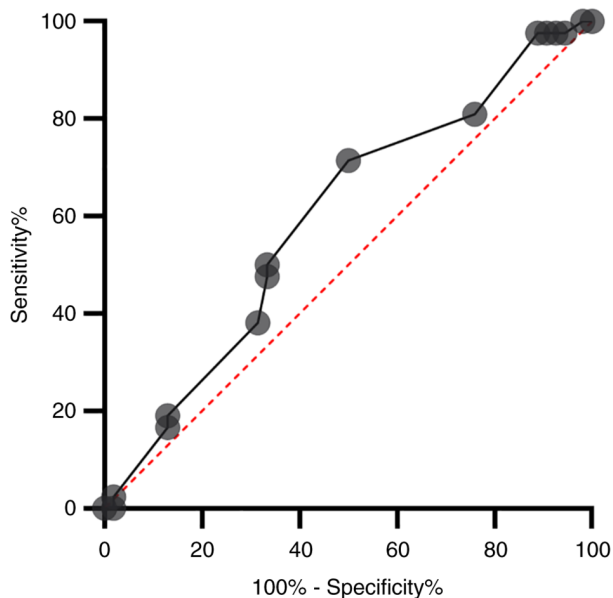


Figure 2. Receiver-operating characteristic curve for the performance of Ki-67 expression in identifying patients at high risk of recurrence according to EndoPredict with an area under the curve of 0.5952 (95% CI, 0.4808-0.7096) ($P=0.1106$). Cutoff value for Ki-67 index, 20%.

for distant recurrence a statistically significant correlation was observed ($r^2=0.2255$, $P=0.04$; Fig. 6). Of the 42 low-risk patients, 50% had low-risk Ki-67 index levels, while from the 52 high-risk patients, 18 had low-risk Ki-67 index expression, resulting in an overall concordance of 59.37% ($\kappa=0.168$; 95% CI, 0.030-0.360; $P=0.09$). The association analysis [sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)] of Ki-67 index ($\geq 20\%$) to predict the risk group demonstrated a low performance (Table III). The presence of LVI was associated with a Ki-67 index $\geq 20\%$.

Ki-67 index as a surrogate marker for MammaPrint for recurrence risk. The clinicopathological characteristics of the MammaPrint cohort are presented in Table IV. The median age was 51 years (range, 33-77 years). The median tumor size was 15.7 mm (range, 4-52 mm). The nodal status was pN0 in 60 patients (85.71%). Furthermore, 60 patients (85.71%) had an IBC/NST histologic type, 51 (72.85%) had grade 2 NHG and 50 cases (72.85%) presented with LVI. All cases were ER+ and 67 (95.71%) were PR+, all were Luminal by Blueprint, and 21 were high-risk and 49 low-risk according to MammaPrint.

The overall median Ki-67 index was 20%. Furthermore, the median Ki-67 index in the low-risk group was 15% and that in the high-risk group was 30% ($P=0.002$; Fig. 2). The analysis indicated a significant association between Ki-67 index and MammaPrint with a $\chi^2=8.85$ ($P=0.0021$; Fig. 5). Of the 49 low-risk patients, 30 had a Ki-67 index $<20\%$. Furthermore, only 4 of the 21 high-risk patients had a Ki-67 index $<20\%$ (Fig. 3). The kappa coefficient demonstrated a fair concordance between Ki-67 index and MammaPrint, with an overall concordance of 67.14% ($\kappa=0.35$; 95% CI, 0.15-0.55; $P=0.001$). The predictive accuracy analysis revealed good sensitivity and NPV to predict the risk group. However, the specificity and PPV were low (Table III). In addition, the Ki-67 index was significantly associated with the NHG and the histological type.

Discussion

Efforts have been made to match the molecular signature tests with clinicopathological characteristics. The ASCO/CAP associations have published guidelines for the 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

Table II. Clinicopathological characteristics of the EndoPredict cohort according to Ki-67 expression (n=96).

Parameter	Total	Ki-67 <20% (n=39)	Ki-67 ≥20% (n=57)	P-value
Age, years	43 (39-46.5)	44 (39-47)	43 (38-46)	0.43 ^a
Tumor size, mm	22 (15-30)	21 (13-25)	24 (15-30)	0.11 ^a
Nodal stage				0.82 ^b
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)				>0.99 ^b
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				0.44 ^b
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)			0.43 ^b
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)	
Lymphovascular invasion				0.01 ^b
Yes	50 (52.98)	14 (14.58)	36 (37.5)	
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 ^a
Positive progesterone receptor	91 (94.79)			
Progesterone receptor ≤20%	11 (11.45)			
% expression (media)	69.16±28.77	66.41±29.73	70.44±28.24	0.50 ^a
EPclin score				0.14 ^b
Low risk	42 (43.75)	21 (21.87)	21 (21.87)	
High risk	54 (56.25)	18 (18.75)	36 (37.5)	
Recurrence ^c , %	15.13±15.86	11.56±10.03	18.25±18.44	0.04 ^a

^aMann-Withney U test and ^bFisher's exact test. ^cEstimated of risk for distant recurrence at 10 years. Nonparametric variables are expressed as n (%) or the median (interquartile range), parametric variables are expressed as mean ± standard deviation. 6 patients had missing size in mm and 2 had missing histological grade. Mixed carcinomas were as follows: IBC/NST-lobular and IBC/NST-micropapillary (n=1). IBC/NST, invasive ductal carcinomas/no special type; EPclin score, EndoPredict score; AJCC, American Joint Committee on Cancer.

is not ideal. Even with the standardization of the technique, there is a 30-40% discrepancy between IHC and multigene expression assays, with a substantial impact on treatment decisions (28).

While evaluating molecular signatures, it is important to note the relationship between Ki-67 index and the multitude of genes tested. The Oncotype Dx gene test is a well-known reverse transcription PCR assay of 21 genes usually implemented to calculate the recurrence score of ER-positive breast cancers (29,30); one of the genes assessed is the marker of proliferation Ki-67 (MKI67), which is probably why the Oncotype Dx assay is one of the molecular signatures with a robust correlation with Ki-67 index (31). On the other hand, it

should be taken into consideration that neither EndoPredict nor MammaPrint include the MKI67 gene as part of their analysis; however, they maintain a relationship with Ki-67 significance as a proliferation marker through other proliferation-associated genes. Bertucci *et al* (32) provided a comprehensive evaluation of the expression of genes that may be encountered in patients stratified as high-risk by EndoPredict. The group of genes that exhibited upregulation were involved in cell processes such as mitotic cell cycle, proliferation and DNA replication and division. Conversely, the ones that displayed downregulation included genes associated with anti-apoptosis, cell-matrix adhesion and cell cycle arrest, among others (32). The study concluded that the upregulated genes demonstrated

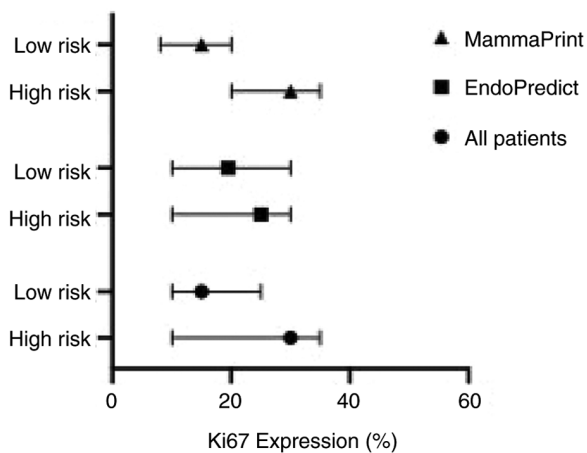


Figure 4. Forest plot for the Ki-67 expression index in the two groups and all patients. The median (interquartile range) (%) were as follows: EndoPredict: 19.5 (10-30) vs. 25 (10-30), Low vs. High, $P=0.1062$; MammaPrint: 15 (8-20) vs. 30 (20-35), Low vs. High, $P=0.002$, all patients: 15 (10-25) vs. 30 (10-35), Low vs. High, $P=0.009$.

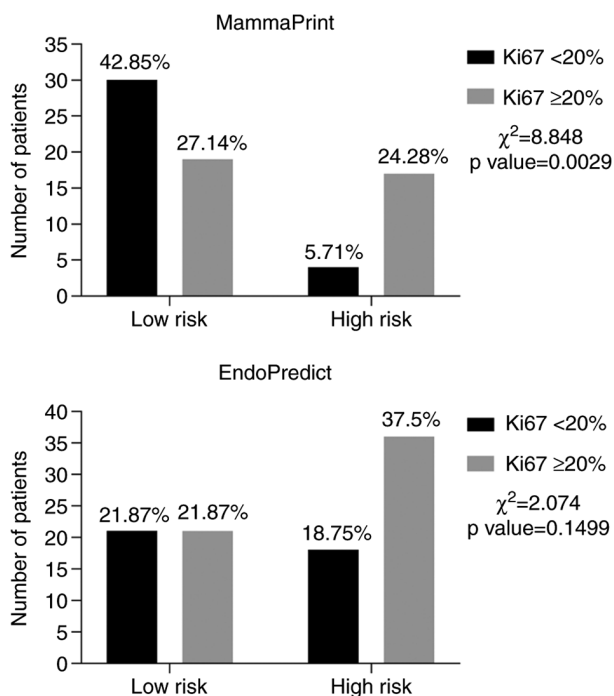


Figure 5. Proportional differences of patients with high and low risk according to MammaPrint and EndoPredict results. The black bars indicate the proportion of the patients with Ki-67 <20% and the gray labels the proportion in patients with Ki-67 ≥20%.

a correlation with proliferation markers, such as Ki-67 (32). When analyzing MammaPrint, Tian *et al* (7) and others (8) were able to elucidate the genes involved in the tumorigenesis of cancerous cells. Their results provided different groups of genes that took part in several phases of the cell cycle, emphasizing the upregulation of genes driving proliferation by evading apoptosis (e.g., BCL2 binding component 3 and egl nine homolog 1, providing self-sufficiency in growth signals [e.g., transforming growth factor beta 3 (TGFB3), insulin-like growth factor binding protein 5 and fibroblast growth factor 18] and insensitivity to anti-growth signals (e.g., TGFB3) (7).

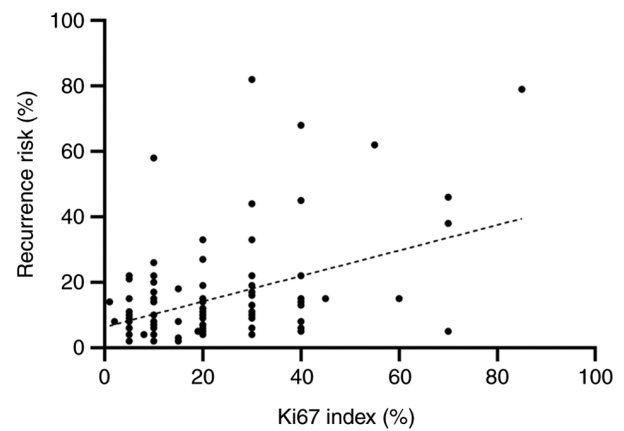


Figure 6. Positive correlation between Ki-67 index and the estimated risk for distant recurrence at 10 years assessed by EPclin in patients with early breast cancer ($r^2=0.2255$, $P=0.04$).

With these findings they were able to establish a connection between MammaPrint and the molecular mechanisms of tumor growth and spread (7). Thus, a possible correlation between a proliferation marker such as Ki-67 in IHC with genes tested in the EndoPredict and MammaPrint molecular signatures was demonstrated.

In the present study, the observed range of Ki-67 index was wide, with 2-70 vs. 1-85% in the EndoPredict cohort and 2-50 vs. 3-70% in the MammaPrint cohort, low-risk and high-risk, respectively. However, the medians were slightly different for the two risk groups. Maranta *et al* (33) explored the distribution of the Ki-67 index in patients with breast cancer at their institution and its association with other risk factors for breast cancer; their median Ki-67 index at 22-26% was similar to that of the cohort of the present study, acknowledging the importance for decision-making of adjuvant therapies; however, they did not involve the use of molecular signatures.

It is known that the Ki-67 assay has a moderate interobserver variability (34). The hot-spot vs. the whole-slide analysis of Ki-67 index 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* (35) evaluated the hot-spot vs. whole-slide Ki-67 index, identifying a strong correlation between the two methods ($r=0.938$). To reduce the interobserver variability, the International Ki-67 in Breast Cancer Working Group recommends, if the staining is homogenous, to count at least three randomly selected high-power fields (objective magnification, x40) and if it is heterogenous, three fields at the tumor edge or hot spots, with certain exceptions and scoring of preferably 1,000 cells with 500 at a minimum (27).

In the MammaPrint cohort, a low Ki-67 index (<20%) demonstrated high sensitivity (88%) and was able to modestly predict patients with a low risk of recurrence (PPV, 0.61%; 95% CI, 0.46-0.75). Furthermore, regarding the agreement of the Ki-67 index and the molecular test, the MammaPrint had an overall concordance of 67.14% and concordance index $\kappa=0.35$ ($P=0.001$), indicating a fair agreement. Similar to the present results and utilizing the same Ki-67 index cutoff, Viale *et al* (28) reported a concordance of 71% (95% CI, 69-72%) between

Table III. Validation and concordance analysis of Ki-67 with EPclin and MammaPrint.

Item	Ki-67 and EPclin	Ki-67 and MammaPrint
Sensitivity, %	54 (37-70)	88 (73-97)
Specificity, %	63 (49-76)	47 (30-65)
PPV, %	50 (34-66)	61 (46-75)
NPV, %	67 (53-79)	81 (58-95)
OR	2 (0.87-4.58), P=0.140	6.71 (1.96-23), P=0.001
Kappa	0.168 (-0.03-0.36)	0.35 (0.15-0.55)

Values are provided with 95% CI in brackets. EPclin and MammaPrint were used as the gold standard. EPclin, EndoPredict; PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio.

the molecular classification of Luminal IBC and Ki-67 index in the EORTC 10041/BIG 3-04 MINDACT trial ($\kappa=0.35$; 95% CI, 0.32-0.37). In addition, another study demonstrated comparable results ($\kappa=0.35$) between MammaPrint and Ki-67 index in 65 patients with IBC; however, they utilized a different cutoff for Ki-67 (14%) (36). Similar to the present study, Bösl *et al* (37) compared MammaPrint and EndoPredict with Ki-67 index, achieving a significant correlation with MammaPrint ($P=0.004$) but not with the EPclin score ($P=0.09$). Despite this fair concordance between Ki-67 index and MammaPrint, in the EndoPredict cohort, the Ki-67 index overall concordance was low and did not significantly correlate with the EPclin risk category (59.37%; $\kappa=0.168$; $P=0.09$). This means that when patients were stratified by Ki-67 index, 30-40% in each cohort were assigned to other risk categories compared to molecular testing. The EndoPredict test gave an approximate percentage of recurrence and this continuous variable had a positive correlation with the Ki-67 index ($P=0.04$).

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, LVI, and, at certain institutions, Ki-67 index. In the present analysis, no correlation was observed between PR and Ki-67 index, 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 index. In addition, a significant correlation between Ki-67 index and NHG was observed in both cohorts (EndoPredict $\chi^2=4.68$, $P=0.03$; and MammaPrint $\chi^2=6.32$, $P=0.01$), as has been previously reported (38-40). The proliferative index Ki67 is now also in use for selecting patients who fail to achieve two weeks of Ki-67 index reduction at <10% in the neoadjuvant endocrine therapy setting for the addition of other therapies (34,35).

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

Despite the fact 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-Garcia *et al* (41) compared MammaPrint and EndoPredict and determined an overall concordance of 72.5%, with a slight improvement using the EPclin score to an overall concordance of 75%. Similarly, Bösl *et al* (37) reported a concordance of 66% with more patients being placed in the low-risk category with MammaPrint.

Finally, the different molecular signature tests have been evaluated with mixed results depending on the geographic location. A Canadian study indicated that EndoPredict is cost-effective with a ratio of \$36,274 per quality-adjusted life-year (QALY), with a total gain of 379 QALYs/year (42). Furthermore, in the 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 (43). In addition, a recent analysis in the UK indicated that EndoPredict was cost-effective only if lymph node disease was present (1-3 positive nodes) with £30,000/QALY (44). On the other hand, in the USA, MammaPrint was determined to be cost-effective at a ratio of \$10,000/QALY (45). However, another study from the UK indicated that MammaPrint was not cost-effective compared to current clinical practice (44). Overall, in certain countries such as Canada and the USA, molecular signature tests are cost-effective. The willingness of the healthcare systems of developing countries to pay for QALYs has yet to be evaluated. However, the cost of these tests may be onerous to healthcare systems in precarious situations.

To the best of our knowledge, the present study was the first to evaluate the performance of the proliferative marker Ki-67 index for identification of high-risk patients with HR+ early breast cancer and at the same time explore the association of Ki-67 index with two molecular signatures, MammaPrint and EndoPredict, and risk stratification markers.

Limitations of the present study include its retrospective nature and the potential for selection bias based on the oncologist's selection of high clinical risk patients. Furthermore, the groups assessed with the different molecular signature tests were heterogeneous. However, the present study represents a multicentric cohort of a large number of EIBC with molecular testing that allowed the evaluation of the Ki-67 index compared to molecular signatures tests.

In conclusion, the present study determined a concordance between Ki-67 index and MammaPrint risk stratification

Table IV. Clinicopathological characteristics of the MammaPrint cohort (n=70).

Parameter	Total	Ki-67 <20 (n=34)	Ki-67 ≥20% (n=36)	P-value
Age, years	51 (43-67)	57 (48-61).	47 (41.5-61.5)	0.056 ^a
Tumor size, mm	15.5 (12-25)	15 (10-25)	17 (12.5-25)	0.60 ^a
Nodal stage				0.04 ^b
N0	60 (85.71)	26 (37.14)	34 (48.57)	
N1	10 (14.28)	8 (11.42)	2 (2.85)	
Pathological stage TNM (AJCC)				>0.99 ^b
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				0.04 ^b
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)				0.15 ^b
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)	
Lymphovascular invasion				0.79 ^b
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 ^c
Positive progesterone receptor	67 (95.71)			
Progesterone receptor ≤20%	6 (8.57)			
% expression (media)	78.22±28.08	84.53±26.17	72.28±28.88	0.07 ^c
MammaPrint				0.002 ^b
Low risk	49 (70)	30 (42.85)	19 (27.14)	
High risk	21 (30)	4 (5.71)	17 (24.28)	

^aMann Whitney U test; ^bFisher's exact test; ^ct-test. No parametric variables are expressed as n (%) or the median (interquartile range), parametric variables are expressed as the mean ± standard deviation. IBC/NST, invasive ductal carcinomas/no special type; AJCC, American Joint Committee on Cancer.

of HR+ EIBC and no concordance with the EndoPredict molecular signature, but a positive association with the given percentage of recurrence. Although there is no perfect molecular signature test, these are high-value tools for therapy selection in patients with HR+ EIBC. Cost-effectiveness analysis of these tests in developing countries is required, and until then, the use of Ki-67 index appears reasonable to aid in clinical decision-making together with the other well-known clinicopathological variables.

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Availability of data and materials

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

Authors' contributions

Conception and design, development of methodology, analysis and interpretation of data and original draft preparation: EJAG, CALG and GSGM. Development of methodology, analysis: VLR, AD and SSF. Acquisition of data and supervision: GSGM, CALG, CVG and ALL. Analysis and interpretation of data and supervision: CVG and MCM. Acquisition and interpretation of data: MCM, PDCMB and ROL. All of the authors reviewed and approved the final manuscript. The authors GSGM and CALG approve the authenticity of the raw data.

Ethics approval and consent to participate

Institutional review board 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. As the present study was retrospective, informed consent from the subjects was not mandatory; however, our institution requires informed consent for any research project.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 68: 394-424, 2018.
- Perou CM, Sørli T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, *et al*: Molecular portraits of human breast tumours. *Nature* 406: 747-752, 2000.
- Sørli T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, *et al*: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 98: 10869-10874, 2001.
- Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B and Senn HJ; Panel members: 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* 24: 2206-2223, 2013.
- Gluck S, de Snoo F, Peeters J, Stork-Sloots L and Somlo G: Molecular subtyping of early-stage breast cancer identifies a group of patients who do not benefit from neoadjuvant chemotherapy. *Breast Cancer Res Treat* 139: 759-767, 2013.
- Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, Thürlimann B and Senn HJ; Panel Members: 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* 26: 1533-1546, 2015.
- Tian S, Roepman P, Van't Veer LJ, Bernards R, de Snoo F and Glas AM: Biological functions of the genes in the mammaprint breast cancer profile reflect the hallmarks of cancer. *Biomark Insights* 5: 129-138, 2010.
- van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, *et al*: A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 347: 1999-2009, 2002.
- Müller BM, Keil E, Lehmann A, Winzer KJ, Richter-Ehrenstein C, Prinzler J, Bangemann N, Reles A, Stadie S, Schoenegg W, *et al*: The endopredict gene-expression assay in clinical practice-performance and impact on clinical decisions. *PLoS One* 8: e68252, 2013.
- Filipits M, Rudas M, Jakesz R, Dubsy P, Fitzal F, Singer CF, Dietze O, Greil R, Jelen A, Sevelde P, *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* 17: 6012-6020, 2011.
- Duffy MJ, Harbeck N, Nap M, Molina R, Nicolini A, Senkus E and Cardoso F: Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM). *Eur J Cancer* 75: 284-298, 2017.
- Weigelt B, Reis-Filho JS and Swanton C: Genomic analyses to select patients for adjuvant chemotherapy: Trials and tribulations. *Ann Oncol* 23 (Suppl 10): x211-x218, 2012.
- van Steenhoven JEC, Kuijter A, van Diest PJ, van Gorp JM, Straver M, Elias SG, Wesseling J, Rutgers E, Timmer-Bonte JNH, Nieboer P, *et al*: Conventional pathology versus gene signatures for assessing Luminal A and B type breast cancers: Results of a prospective cohort study. *Genes (Basel)* 9: 261, 2018.
- Bustreo S, Osella-Abate S, Cassoni P, Donadio M, Airolidi M, Pedani F, Papotti M, Sapino A and Castellano I: 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* 157: 363-371, 2016.
- Dubsy P, Filipits M, Jakesz R, Rudas M, Singer CF, Greil R, Dietze O, Luisser I, Klug E, Sedivy R, *et al*: EndoPredict improves the prognostic classification derived from common clinical guidelines in ER-positive, HER2-negative early breast cancer. *Ann Oncol* 24: 640-647, 2013.
- Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker JS, *et al*: Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst* 101: 736-750, 2009.
- Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, Zabolgo L, Mallon E, Green AR, Ellis IO, *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* 29: 4273-4278, 2011.
- de Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V, Sotiriou C, Larsimont D, Piccart-Gebhart MJ and Paesmans M: Ki-67 as prognostic marker in early breast cancer: A meta-analysis of published studies involving 12,155 patients. *Br J Cancer* 96: 1504-1513, 2007.
- Focke CM, van Diest PJ and Decker T: St Gallen 2015 subtyping of luminal breast cancers: Impact of different Ki67-based proliferation assessment methods. *Breast Cancer Res Treat* 159: 257-263, 2016.
- Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B and Senn HJ; Panel members: 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* 22: 1736-1747, 2011.
- Villarreal-Garza C, Lopez-Martinez EA, Deneken-Hernandez Z, Maffuz-Aziz A, Muñoz-Lozano JF, Barragan-Carrillo R, Ramos-Elias P, Moreno B, Diaz-Perez H, Peña-Curiel O, *et al*: Change in therapeutic management after the EndoPredict assay in a prospective decision impact study of Mexican premenopausal breast cancer patients. *PLoS One* 15: e0228884, 2020.
- Cardoso F, van't Veer LJ, Bogaerts J, Slaets L, Viale G, Delalogue S, Pierga JY, Brain E, Causseret S, DeLorenzi M, *et al*: 70-Gene signature as an aid to treatment decisions in early-stage breast cancer. *N Engl J Med* 375: 717-729, 2016.
- Allison KH, Hammond ME, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, *et al*: Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. *J Clin Oncol* 38: 1346-1366, 2020.
- Prat A, Cheang MC, Martín M, Parker JS, Carrasco E, Caballero R, Tyldesley S, Gelmon K, Bernard PS, Nielsen TO and Perou CM: Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. *J Clin Oncol* 31: 203-209, 2013.
- Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, *et al*: Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of American pathologists clinical practice guideline focused update. *J Clin Oncol* 36: 2105-2122, 2018.
- Penault-Llorca F and Radošević-Rubin N: Ki67 assessment in breast cancer: An update. *Pathology* 49: 166-171, 2017.
- Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, *et al*: Assessment of Ki67 in breast cancer: Recommendations from the International Ki67 in breast cancer working group. *J Natl Cancer Inst* 103: 1656-1664, 2011.
- Viale G, de Snoo FA, Slaets L, Bogaerts J, van't Veer L, Rutgers EJ, Piccart-Gebhart MJ, Stork-Sloots L, Glas A, Russo L, *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* 167: 123-131, 2018.
- Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, *et al*: A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 351: 2817-2826, 2004.

30. Sahebjam S, Aloyz R, Pilavdzic D, Brisson ML, Ferrario C, Bouganim N, Cohen V, Miller WH Jr and Panasci LC: Ki 67 is a major, but not the sole determinant of Oncotype Dx recurrence score. *Br J Cancer* 105: 1342-1345, 2011.
31. Ahmed W, Malik MFA, Saeed M and Haq F: Copy number profiling of Oncotype DX genes reveals association with survival of breast cancer patients. *Mol Biol Rep* 45: 2185-2192, 2018.
32. Bertucci F, Finetti P, Viens P and Birnbaum D: EndoPredict predicts for the response to neoadjuvant chemotherapy in ER-positive, HER2-negative breast cancer. *Cancer Lett* 355: 70-75, 2014.
33. Maranta AF, Broder S, Fritzsche C, Knauer M, Thürlimann B, Jochum W and Ruhstaller T: Do YOU know the Ki-67 index of your breast cancer patients? Knowledge of your institution's Ki-67 index distribution and its robustness is essential for decision-making in early breast cancer. *Breast* 51: 120-126, 2020.
34. Gluz O, Nitz UA, Christgen M, Kates RE, Shak S, Clemens M, Kraemer S, Aktas B, Kuemmel S, Reimer T, *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* 34: 2341-2349, 2016.
35. Thakur SS, Li H, Chan AMY, Tudor R, Bigras G, Morris D, Enwere EK and Yang H: The use of automated Ki67 analysis to predict Oncotype DX risk-of-recurrence categories in early-stage breast cancer. *PLoS One* 13: e0188983, 2018.
36. Nguyen B, Cusumano PG, Deck K, Kerlin D, Garcia AA, Barone JL, Rivera E, Yao K, de Snoo FA, van den Akker J, *et al*: Comparison of molecular subtyping with BluePrint, MammaPrint, and TargetPrint to local clinical subtyping in breast cancer patients. *Ann Surg Oncol* 19: 3257-3263, 2012.
37. Bösl A, Spitzmüller A, Jasarevic Z, Rauch S, Jäger S and Offner F: MammaPrint versus EndoPredict: Poor correlation in disease recurrence risk classification of hormone receptor positive breast cancer. *PLoS One* 12: e0183458, 2017.
38. Trihia H, Murray S, Price K, Gelber RD, Golouh R, Goldhirsch A, Coates AS, Collins J, Castiglione-Gertsch M, Gusterson BA, *et al*: Ki-67 expression in breast carcinoma: Its association with grading systems, clinical parameters, and other prognostic factors-a surrogate marker? *Cancer* 97: 1321-1331, 2003.
39. Marwah N, Batra A, Marwah S, Gupta V, Shakya S and Sen R: Correlation of proliferative index with various clinicopathologic prognostic parameters in primary breast carcinoma: A study from North India. *J Cancer Res Ther* 14: 537-542, 2018.
40. Shokouh TZ, Ezatollah A and Barand P: 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)* 94: e1359, 2015.
41. Pelaez-Garcia A, Yébenes L, Berjón A, Angulo A, Zamora P, Sánchez-Méndez JI, Espinosa E, Redondo A, Heredia-Soto V, Mendiola M, *et al*: Comparison of risk classification between EndoPredict and MammaPrint in ER-positive/HER2-negative primary invasive breast cancer. *PLoS One* 12: e0183452, 2017.
42. Hannouf MB, Zaric GS, Blanchette P, Brezden-Masley C, Paulden M, McCabe C, Raphael J and Brackstone M: Cost-effectiveness analysis of multigene expression profiling assays to guide adjuvant therapy decisions in women with invasive early-stage breast cancer. *Pharmacogenomics J* 20: 27-46, 2020.
43. Hinde S, Theriou C, May S, Matthews L, Arbon A, Fallowfield L, Bloomfield D: The cost-effectiveness of EndoPredict to inform adjuvant chemotherapy decisions in early breast cancer. *Health Policy and Technology* 8: 75-83, 2019.
44. Harnan S, Tappenden P, Cooper K, Stevens J, Bessey A, Rafia R, Ward S, Wong R, Stein RC and Brown J: Tumour profiling tests to guide adjuvant chemotherapy decisions in early breast cancer: A systematic review and economic analysis. *Health Technol Assess* 23: 1-328, 2019.
45. Chen E, Tong KB and Malin JL: Cost-effectiveness of 70-gene MammaPrint signature in node-negative breast cancer. *Am J Manag Care* 16: e333-e342, 2010.



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