



Prosigna® breast cancer assay: histopathologic correlation, development, and assessment of size, nodal status, Ki-67 (SiNK™) index for breast cancer prognosis

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Abstract

The Prosigna® assay is a United States Food and Drug Administration (US-FDA) cleared molecular test for prognostic use in hormone receptor-positive stage I/II breast cancer in postmenopausal women. We analyzed histopathologic features of 79 cases with Prosigna® assay results and found a significant correlation between tumor size, grade, and Ki-67 labeling index with Prosigna® score (0–40, 41–60, and 61–100) and Prosigna® risk categories. Since the Prosigna® risk stratification is influenced by lymph node status, we designed an index that included lymph node status and the two most correlated variables (size and Ki-67 labeling index). This was termed the size, nodal, and Ki-67 (SiNK™) index and is calculated as follows: (size in mm) + (pN × 10) + (Ki-67 labeling index). The SiNK™ index was divided into ≤40 and >40 to test its prognostic significance in a well-characterized dataset of 106 ER+/HER2-negative stage I–II invasive breast cancers treated with standard multi-modality therapy with long term follow-up (average 101 months follow-up). Patients with SiNK™ ≤40 showed significantly improved distant recurrence-free survival (96% distant recurrence-free survival in SiNK™ ≤40 compared to 81% in SiNK™ >40; log-rank test *p* value: 0.0027). SiNK™ provides strong prognostic information in ERo+/HER2-negative breast cancers. SiNK™ index is simple to calculate using data from routine pathology reports. This should be further evaluated in larger datasets.

Introduction

Commercial multigene expression assays (such as *Oncotype DX*®, *MammaPrint*®, *EndoPredict*®, *Breast Cancer Index*®, and *Prosigna*®) are now routinely performed for breast cancer management. All of these assays were initially devised for prognostic use, but are now routinely used for making therapy decisions in estrogen receptor (ER) positive breast cancers [1–7]. Low-risk patients are spared chemotherapy, while high-risk patients are offered chemotherapy with the hope of reducing the risk of recurrence and improving survival [8]. This approach seems to have merit, but the principle does not always hold as

there are many patients with a high risk of recurrence whose risk cannot be always minimized with chemotherapy (large tumor size, multiple positive nodes, but low tumor grade with strong hormone receptor expression, and low proliferation). With routine usage, it appears that although these tests have both prognostic and predictive value, they are not interchangeable and some tests are more predictive, and while some others are more prognostic [9–13]. Prosigna® is a second-generation multigene expression assay, which comprises of 50 discriminator genes and 8 controls, popularly known as prediction analysis of microarray 50 (PAM50) gene signature [14]. This test has been cleared by the United States Food and Drug Administration (US-FDA) for prognostic use in hormone receptor-positive early-stage breast cancer in postmenopausal women. The Prosigna® score is derived from the correlation of information obtained from the PAM50 gene expression profiling, as well as proliferation and the pathologic tumor size. The risk of recurrence (ROR) at 10 years is classified as low (<5% risk, scores 0–40), intermediate (~10% risk, scores 41–60), and high (>15%, scores 61–100) in node-negative patients. The ROR is low (~5% risk, scores 0–40) and high (~25%,

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scores 41–100) in node-positive patients [15]. Given the inclusion of tumor size and nodal status in the classification of recurrence risk, Prosigna® test is a strong prognostic assay while the predictive value has not been widely tested. In the past, we have devised and demonstrated simple algorithmic measures using morphologic and immunohistochemical characteristics like Magee Equations™/Magee Decision Algorithm™ to be of significant clinical utility which can be used in lieu of expensive molecular assays like *oncotype* DX [16–18]. To the best of our knowledge, no prior studies have compared the clinical–pathologic and immunohistochemical parameters with the Prosigna® assay. Our current study was undertaken to study histopathologic features that correlate with Prosigna® scores. Subsequently, these features were used to devise a simple-to-use prognostic tool for estimating breast cancer prognosis.

Materials and methods

A search for cases with Prosigna® testing requests at our institution resulted in 79 cases. All cases were ER-positive and HER2-negative invasive breast carcinomas diagnosed between late 2014 to early 2018. Clinical and pathologic information was obtained from the clinical and laboratory information systems. The clinical–pathologic features of each case were recorded and analyzed for its association with the Prosigna® scores categories, 0–40, 41–60, and 61–100. The variables found to be significant in univariable analysis were taken to create an index with similar score categories as the Prosigna® test. The concordance between Prosigna® score categories and the resulting index categories (≤ 40 , >40 –60, >60) was evaluated. The resulting index was then used as a binary classifier (≤ 40 and >40) and tested on a separate set of 106 well-characterized ER+/HER2-negative American Joint Committee on Cancer stage I and II invasive breast carcinomas treated with routine multi-modality therapy with long term follow-up (validation set). The database used for validation is from a prior study where ER/PR and HER2 data was taken from pathology reports and Ki-67 was performed and assessed using semiautomated image analysis system as previously described [19]. Distant recurrence-free survival (DRFS) and breast cancer-specific survival (BCSS) for the validation set cases was analyzed via Kaplan–Meier curves with respect to the newly developed index. DRFS was defined as the time from diagnosis to first distant recurrence or the date of last contact. BCSS was defined as the time from diagnosis to death due to breast cancer or the date of last contact. For comparison of means, independent sample *t* tests were performed. Univariable analysis was performed using χ^2 and Fisher exact tests to compare the differences in percentages between groups. A *P* value < 0.05 was considered

Table 1 Univariable analysis of clinical–pathologic features with Prosigna® score categories in the test development cohort ($n = 79$).

	Prosigna 0–40 ($n = 43$)	Prosigna 41–60 ($n = 19$)	Prosigna 61–100 ($n = 17$)	<i>p</i> value ^a
Age (years)				
Mean	63	64	66	0.1537
Median	63	65	66	
Tumor size (mm)				
Mean	17	27	26	0.0374 ^b
Median	14	22	21	
Lymph node				
Positive	11 (26%)	8 (42%)	6 (35%)	0.5301
Negative	32 (74%)	11 (58%)	11 (65%)	
Tumor type				
Ductal	32 (74%)	19 (100%)	14 (82%)	0.7369
Others	11 (26%)	0 (0%)	3 (18%)	
Nottingham grade				
Grade III	2 (5%)	3 (16%)	4 (24%)	0.0485 ^b
Grade I and II	41 (95%)	16 (84%)	13 (76%)	
ER H-score				
Mean	268	271	256	0.3225
Median	290	290	260	
PR H-score				
Mean	166	152	136	0.3300
Median	190	170	135	
Ki-67 index				
Mean	13	23	42	< 0.0001 ^b
Median	10	20	35	

^aComparison between Prosigna 0–40 versus 61–100.

^bStatistically significant.

significant. Log-rank test was used to compare Kaplan–Meier curves. Statistical analysis was performed using Prism GraphPad software, version 8.3.0, San Diego, CA.

Results

Univariable analysis of the clinical–pathologic features of 79 cases with Prosigna® test results showed a strong association with known clinical–pathologic prognostic factors. Table 1 highlights the significant correlation of Prosigna® score categories with tumor size, grade, and Ki-67 labeling index (*p* value: < 0.05).

Although no association was identified between Prosigna® scores and lymph node status, the final Prosigna® risk categories do take into account the tumor lymph node status. Therefore, lymph node status was taken into

Table 2 Comparison of Prosigna® and SiNK™ score categories (3-tiered) in the test development cohort (*n* = 79).

	Prosigna 0–40	Prosigna 41–60	Prosigna 61–100	Total
SiNK ≤ 40	35	6	0	41
SiNK >40–60	6	9	5	20
SiNK >60	2	4	12	18
Total	43	19	17	79

Score categorization concordant in 71% (56 of 79). Two step discordance: 2.5%.

Table 3 Comparison of Prosigna® and SiNK™ score categories (2-tiered) in the test development cohort (*n* = 79).

	Prosigna ≤ 40	Prosigna > 40	Total
SiNK ≤ 40	35	6	41
SiNK > 40	8	30	38
Total	43	36	79

Score categorization concordant in 82.3% (65 of 79).

consideration along with the two most significantly correlated features (Ki-67 labeling index and tumor size) to create the size, nodal, Ki-67, or the SiNK™ index. SiNK™ index is calculated by adding the tumor size in millimeters to the pathologic nodal status (pN) × 10 and the Ki-67 labeling index. The score can theoretically range from <1 to over 100. Since it is modeled after the Prosigna® test scores, the SiNK™ index was categorized similarly to Prosigna® score categories (≤40, >40–60, >60) and compared to Prosigna® test scores. The comparison is shown in Table 2. The overall concordance between Prosigna® score and SiNK™ index categories was 71% (56 of 79), with 2-step discordance rate of only 2.5% (see Table 2). When both Prosigna® score and SiNK™ index are evaluated using a binary cutoff value (≤40 and >40), the concordance increased to 82% (Table 3). No deaths occurred due to breast cancer in this test development cohort, but four distant recurrences were recorded (average follow-up of 33 months), two in lymph node-positive, and two in lymph node-negative patients. Three patients who recurred had tumors characterized as Prosigna® high risk and one as Prosigna® low risk. However, all recurrences happened in cases with SiNK™ >40 group (4 of 38 or 10.5%) and none in the SiNK™ ≤40 group (0 of 41; *p* value: 0.0491). Therefore, the binary values (≤40 and >40) were used to test the prognostic significance in the validation set. The patient and tumor characteristics of the 106 cases used for validation are provided in Table 4. SiNK™ indices were calculated, 75 patients (71%) had SiNK™ index ≤40 and 31 (29%) had SiNK™ index >40. The average follow-up for these cases was 101 months. Patients with SiNK™ index ≤40

Table 4 Patient and tumor characteristics of the 106 cases used for validation.

Age in years	Mean: 59.9 Median: 58.5 Range: 38–90
Size in mm	Mean: 15.5 Median: 15 Range: 6–45
Grade	I: 40 (38%) II: 56 (44%) III: 25 (18%)
Tumor type	Ductal: 97 (91%) Lobular: 7 (7%) Mixed: 2 (2%)
Lymph node status	Negative: 76 (72%) Positive: 30 (28%)
AJCC anatomic stage	I: 75 (71%) II: 31 (29%)
Ki-67 labeling index	Mean: 17.5 Median: 11 Range: 1–86
PR status	Negative: 9 (8%) Positive: 97 (92%)
Systemic therapy	Chemotherapy only: 2 (2%) Chemo + endocrine therapy: 39 (37%) Endocrine therapy only: 40 (38%) None: 8 (7%) Unknown: 17 (16%)

experienced 4% distant recurrence compared to 19% distant recurrence in the group with SiNK™ index >40. The BCSS was 99% for patients with SiNK™ index ≤40 compared to 97% for the group with SiNK™ >40. DRFS and BCSS assessed by Kaplan–Meier curves are shown in Fig. 1. Significantly improved DRFS was noted in patients with SiNK™ ≤40. The BCSS was excellent for all patients.

The significance of the SiNK™ index was also evaluated separately in the lymph node-negative (*n* = 76) and lymph node-positive (*n* = 30) groups. Statistically significant improved DRFS for SiNK™ ≤40 was noted but BCSS was not significantly different between SiNK™ ≤40 and >40 in the lymph node-negative group (Fig. 2a, b). Although more distant recurrences were noted for patients with SiNK™ >40 in the lymph node-positive group (3 of 15 or 20% in SiNK™ >40 versus 1 of 15 or 7% in SiNK™ <40), the difference in DRFS was not statistically significant (Fig. 2c). The BCSS was not significantly different based on SiNK™ in lymph node-positive patients (Fig. 2d).

Discussion

In this genomic/molecular era, multigene commercial assays are increasingly used for breast cancer management. The five commonly available assays include the 12 gene

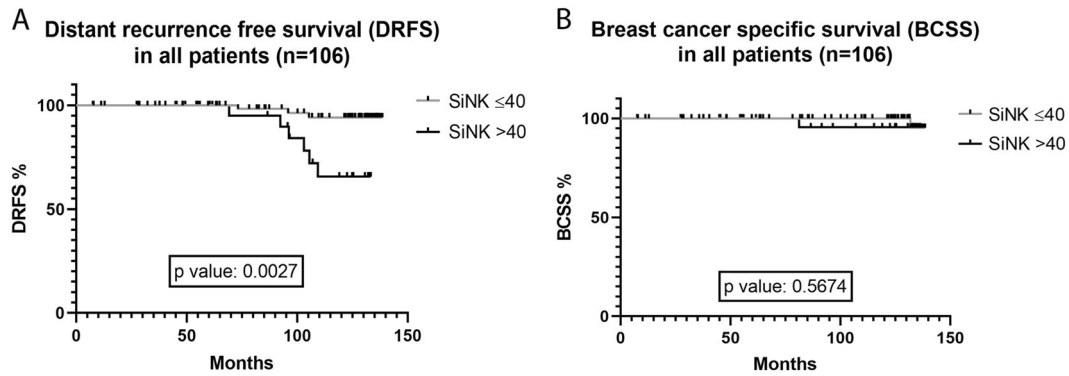


Fig. 1 Kaplan–Meier survival curves for all patients in the validation cohort. Distant recurrence-free survival (a) and breast cancer-specific survival (b).

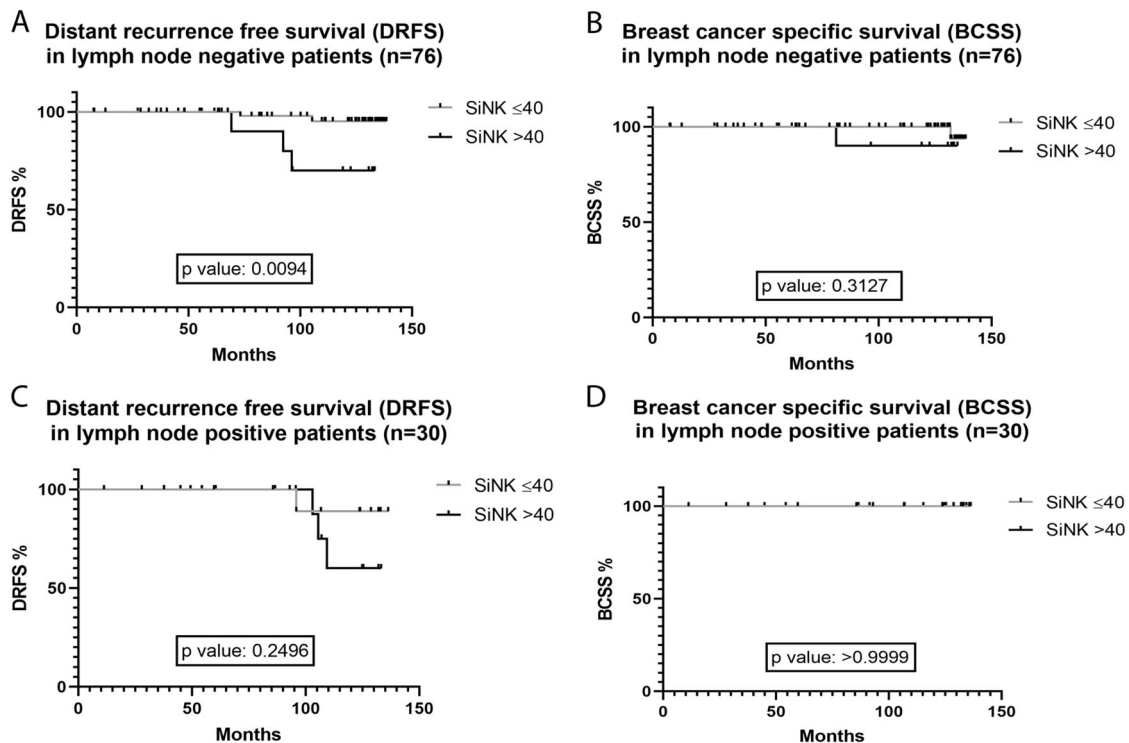


Fig. 2 Kaplan–Meier survival curves for patients in the validation cohort based on lymph node status. Lymph node-negative patients: distant recurrence-free survival (a) and breast cancer-specific survival (b).

Lymph node-positive patients: distant recurrence-free survival (c) and breast cancer-specific survival (d).

assay (EndoPredict®), 21 gene (OncotypeDX®), 70 gene (MammaPrint®), 2–5 gene (HOXB13/IL-17BR or H/I index® and Breast Cancer Index®) or 58 gene assay (Prosigna®) [1–8]. The intended use is prognostic (in fact the FDA approval for MammaPrint® and Prosigna® is for prognostic use only, others are not FDA cleared), but these tests are frequently used for predictive/therapeutic purposes. Generally, such use may be acceptable as tumors that are at high ROR have more biologically aggressive features and high proliferation that can be targeted with drugs that inhibit the cell cycle (i.e., chemotherapy). In fact, the majority of

these assays are influenced by tumor cell proliferation [20]. However, some tumors that present at an anatomically advanced stage but lack increased proliferation and such tumors are at increased ROR but this risk cannot always be minimized by the use of chemotherapy. Therefore, these multigene assays are now coupled with clinical parameters (such as using EndoPredict® clinical score or EPclin over just the EP score) to provide a more accurate assessment of prognosis [21]. Prosigna® assay is a bit different from the rest as it accounts for the tumor size (≤ 2 cm or more) in Prosigna® score calculation and then the Prosigna® risk is

defined based on the lymph node status. The Prosigna® test is based on the 50 gene classifier algorithm previously named as PAM50 [14, 22]. The Prosigna® score is calculated using coefficients from a Cox model that includes the Pearson correlation of a 46-gene subset of the 50 genes to each PAM50 centroid (prototypical gene expression profiles of each of the four PAM50 molecular subtypes), a proliferation score, and tumor size [7]. The score is adjusted to a 0–100 scale and risk categories are defined based on lymph node status (low, intermediate, and high risk in lymph node-negative; low and high risk in lymph node-positive). Although the Prosigna® test result is also heavily influenced by the proliferation gene score, the inclusion of tumor size, and lymph node status in defining risk makes it a robust prognostic assay. However, Prosigna® and other molecular tests are expensive with the cost of over \$4000 per test. We also believe that similar prognostic and predictive information can be derived from routine histopathology and immunohistochemistry testing of breast cancer but similar to multigene assays, the pathologic variables also need to be combined into one index to provide more succinct information to the oncologist [16, 17, 23, 24].

Our primary goal of this study was to compare the histopathologic features of the tumors that were tested by the Prosigna® assay ($n = 79$) and identify the pathologic variables that correlate best with the Prosigna® score. The secondary goal was to attempt combining these significant pathologic variables into one index that could provide an alternative to Prosigna® score. We found that a high Prosigna® score correlated with larger tumor size, higher tumor grade, and higher Ki-67 labeling index. These histopathologic features are known to have strong prognostic value [25–28]. Although the high Prosigna® score cases showed slightly lower ER and PR H-scores, the differences were not statistically significant in this cohort—this is in contrast to the histopathologic correlation that is usually seen with other molecular assays. This may be due to only a small number of cases with high Prosigna® score or reflects the difference in genes that comprise Prosigna® and other molecular assays. In fact, a few studies that have directly compared different molecular tests have shown significant variability in risk classification on an individual case and it is likely that each molecular assay correlates with different morpho-immunohistologic features [10, 12].

Due to the significant correlation of Prosigna® test results with the known prognostic features, we created an index modeled on Prosigna® scores and tested its prognostic significance on a set of well-characterized ER+/HER2-negative stage I and II invasive breast cancers ($n = 106$). This SiNK™ index is easy to calculate and provides useful prognostic information that can be obtained using only tumor size, lymph node status, and Ki-67 labeling index.

However, it is difficult to make an exact comparison of the SiNK™ index in this study tested on heterogeneously treated patients with distant recurrence risk defined by Prosigna® score derived from clinical trial data. Prosigna® test is currently intended for use on stage I and II postmenopausal breast cancer patients with up to three positive lymph nodes. The clinical trial data has shown that the risk of distant recurrence at 10 years based on Prosigna® score is <10% with low-risk and >20% for high-risk assuming patients receive 5 years of endocrine therapy [15, 22]. In comparison, the distant recurrence rate in the current study was 4% in patients with SiNK™ ≤ 40 and 19% in patients with SiNK™ > 40 with an average follow-up of 101 months. This suggests a comparable prognostic value of the SiNK™ index. SiNK™ is particularly useful in estimating prognosis for stage I and II, lymph node-negative patients. Because the SiNK index includes tumor size and lymph node status, it correlates with tumor stage, such that almost all stage III patients generally have SiNK™ index > 40 . Therefore, we did not include stage III patients in the validation cohort. In contrast to individual parameters that are hard to factor in for estimating prognosis, the SiNK™ index can provide one cohesive variable for breast cancer management. Moreover, it is simple to calculate based on the pathology report and can be used for counseling patients during office visits without waiting for additional testing.

The current study is the first to correlate histopathologic findings with Prosigna® test and reports on a novel index, it does have some limitations. First, the number of events was low despite long term follow-up, which is partly due to successful breast cancer treatments and partly due to small sample size. Secondly, SiNK™ index contains a variable (Ki-67) for which standardization is lacking and many pathologists and clinicians still do not routinely use it in clinical practice [29]. Several pre-analytical and analytical factors (cold ischemic time, time in formalin, different clones, detection systems, etc.), intra-observer, and interlaboratory variability can alter Ki-67 measurement which can impact the SiNK™ index. In routine practice, Ki-67 labeling index should be used as a continuous score rather than a hard cut off and should be combined along with other pathology variables for clinical management. With most laboratories in the United States adhering to proper fixation guidelines for breast biomarkers [30, 31], and with limited number of clones used for clinical testing, interobserver variability remains the most important source of variability in Ki-67 measurement [32]. For Ki-67 evaluation, the pathologists at our institution have often used a more pragmatic approach rather than actual counting of 500 or 1000 tumor cells. We first estimate the Ki-67 labeling index on the entire tumor. If the estimate falls below 10 or above 50, then estimate stands as the final Ki-67 labeling index. If the estimate is

between 10 and 50, then 50–100 cells are counted in representative areas based on the pathologist's discretion to arrive at the labeling index. This approach seems to have worked, with Ki-67 being an important component of a multivariable model (i.e., Magee Equations™) that can estimate Oncotype DX® score and predict benefit from chemotherapy in the neoadjuvant setting [16, 18]. It is possible that signal counting software (free online software expected to be made available by Ki-67 international working group) may make the task easier and more accurate when the estimated Ki-67 index is between 10 and 50%. Although some variability is expected with the SiNK™ index based on Ki-67 score, the two other components in the SiNK™ index (tumor size and nodal status) are more consistently measured and the combined index can be a strong prognostic factor provided a more consistent approach is taken for Ki-67 measurement [33–35].

Based on our findings in this study, we believe that SiNK™ provides long term prognostic information in stage I or II ER+/HER2-negative breast cancers. The patients with higher SiNK™ index may need more intensive treatment to improve recurrence-free survival. However, it is to be noted that worse prognosis does not always mandate the use of chemotherapy, and certainly, our current study did not evaluate if chemotherapy use will benefit patients with a higher SiNK™ index. Depending on individual tumor features, higher SiNK™ index patients may be treated upfront with chemotherapy, extended endocrine therapy, or a combination of endocrine therapy with cyclin-dependent kinase 4/6 inhibitors in the future [36, 37]. It is, however, important to note that the patients in the validation cohort of the current study were heterogeneously treated, but SiNK™ index was still able to predict prognosis, suggesting that SiNK™ identifies inherent tumor features present at diagnosis, that does not appear to be impacted with standard therapy.

In summary, Prosigna® test scores correlate with known prognostic factors. The SiNK™ index derived from this correlation provides a cost-effective alternative that can provide useful prognostic information in ER+/HER2-negative tumors. However, this study needs to be further verified in a larger cohort of patients for potential wider applicability.

Compliance with ethical standards

Conflict of interest DJD is an independent contractor breast pathologist at PreludeDx (Laguna Hills, CA). RB is an ad hoc advisor to Eli Lilly & Company, Indianapolis, IN.

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