A retrospective validation of CanAssist Breast in European early-stage breast cancer patient cohort

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ABSTRACT

CanAssist Breast (CAB), a prognostic test uses immunohistochemistry (IHC) approach coupled with artificial intelligence-based machine learning algorithm for prognosis of early-stage hormone-receptor positive, HER2/neu negative breast cancer patients. It was developed and validated in an Indian cohort. Here we report the first blinded validation of CAB in a multi-country European patient cohort. FFPE tumor samples from 864 patients were obtained from-Spain, Italy, Austria, and Germany. IHC was performed on these samples, followed by recurrence risk score prediction. The outcomes were obtained from medical records. The performance of CAB was analyzed by hazard ratios (HR) and Kaplan Meier curves. CAB stratified European cohort (n = 864) into distinct low- and high-risk groups for recurrence (P < 0.0001) with HR of 3.32 (1.85-5.93) like that of mixed (India, USA, and Europe) (n = 1974), 3.43 (2.34-4.93) and Indian cohort (n = 925), 3.09 (1.83-5.21). CAB provided significant prognostic information (P < 0.0001) in women aged < 50 (HR: 4.42 (1.58-12.3), P < 0.0001) and >50 years (HR: 2.93 (1.44-5.96), P = 0.0002). CAB had an HR of 2.57 (1.26-5.26), P = 0.01 in women with N1 disease. CAB stratified significantly higher proportions (77%) as low-risk over IHC4 (55%) (P < 0.0001) in women aged < 50 and 82% of IHC4 intermediate-risk patients were stratified as low-risk by CAB. Accurate risk stratification of European patients by CAB coupled with its similar performance in Indian patients shows that CAB is robust and functions independent of ethnic differences. CAB can potentially prevent overtreatment in a greater number of patients compared to IHC4 demonstrating its usefulness for adjuvant systemic therapy planning in European breast cancer patients.

1. Introduction

The research in breast cancer prognostication has yielded prognostic tests empowering clinicians and patients plan optimum adjuvant therapy. The importance of prognostic tests, especially the multi-gene tests, is evidenced by their inclusion in international guidelines [1]. Cost-effective analysis entailing use of multigene tests showed savings to patients and insurance companies [2–5]. Thus, use of prognostic tests has a key role in planning treatment for hormone receptor positive, HER2/neu negative early-stage breast cancer patients.

Despite their utility, altered performance with respect to risk stratification or clinical outcomes among women of different races/ethnicities has been observed [6–10]. Post-hoc analysis of TAILORx trial has shown inferior outcomes in non-Caucasian women compared to White/Hispanic despite diagnosis at uniform stage with no disparities in the expression of hormone receptors, recurrence score or clinical care [6]. Notably, another large retrospective cohort analysis using SEER Oncotype DX database showed Black women have higher breast cancer specific mortality compared to White and other races within the same RS category [7]. MammaPrint risk stratification showed striking differences in low-high proportions in Asians compared to Caucasians [8,9]. Similarly, Breast Cancer Test (BCT), developed on Korean patients had lower
agreement in risk categories with Oncotype DX in South East-Asian cohort, particularly in young (<50 years) and in women with node-positive tumors [10]. These data undeniably suggest the role of possible inherent yet unknown factors contributing to the different outcomes in women of different races/ethnicities. These findings point to the missing determinants of clinical outcomes like tumor biology in these prognostic tests, which limits their universal applicability. An ideal prognostic test must perform independent of racial/ethnic differences to have global adaptability.

CanAssist Breast (CAB) is an immunohistochemistry (IHC) based test developed [11] and is till date validated on patients primarily of Indian ethnic origin [12,13]. This test uses a machine learning algorithm; assesses the expression of five biomarkers (CD44, ABCC4, ABCC11, N-Cadherin, pan-Cadherin) involved in tumor biology namely metastasis, drug resistance, stemness and arrives at a score predictive of distant recurrence, along with 3 clinical parameters-tumor size, grade and node status. [12], CAB has been used by more than 2000 women in South Asia prospectively to make ideal choice of chemotherapy over the last 5 years [14].

Ethnicity impacts breast cancer outcomes. This has been confirmed by various biomarker driven tests. Hence it is prudent to validate a test in various ethnic subjects for global adaptability.

With this rationale, we assessed performance of CAB in European cohort. This is the first retrospective validatory study of CAB in a European cohort. This report illustrates accurate performance of CAB in patients of different ethnicities, various age groups, node status and compares CAB with IHC4 and MammaPrint.

2. Methods

2.1. Ethics approval

This retrospective study uses archived FFPE tumor samples, conducted with the approval of Bangalore Ethics Committee (ECR/87/Indt/KA/2013) and in accordance with Declaration of Helsinki. Samples from Spain (VHIO), and Austria (Medical University of Innsbruck) were obtained after IRB approvals of the respective hospitals. Samples from Italy (TransHit) and Germany (Wissenschaftliche Leiterin der Stiftung PATH – patients’ Tumor Bank of Hope) were obtained through Biobanks as per their standard approvals.

2.2. Patient selection

Patients were diagnosed between 2007 and 2016. FFPE blocks of women diagnosed with stage I-II hormone receptor positive, HER2/neu negative disease with a minimum of 5-year follow-up post-diagnosis were used. The patient information, treatment and follow-up details-age, year of diagnosis, clinical parameters, hormone receptor status, histology, endocrine/chemotherapy/radiotherapy details, date of distant recurrence/death were obtained from the hospital.

Patients negative for ER & PR and with either positive or negative for HER2/neu were excluded. Patients treated with neoadjuvant chemotherapy, with loco/locoregional recurrence and died due to reasons other than breast cancer were excluded. Metastasis at a distant site other than loco-regional, contralateral, and ipsilateral breast was considered an event. Either distant metastasis free survival (DMFS) for five years or time to first event at a distant site within five years were the study end points.

2.3. Immunohistochemistry

Stainings for five CAB biomarkers and grading were carried out as described [12,15]. ER/PR/HER2 gradings were done as per ASCO/CAP guidelines and Ki67 as described [16].

2.4. IHC4

IHC4 equation entails the IHC gradings of four biomarkers ER, PR, HER2/neu and Ki67 [17].

\[
\text{IHC4 Score} = 94.7 \times \left( -0.100 \times \text{ER} - 0.240 \times \text{PR} + 0.586 \times \text{HER2/neu} + 0.100 \times \text{Ki67} \right)
\]

2.5. Statistical analyses

Kaplan-Meier (KM) curves (GraphPad 8), univariate hazard ratios (HR) and P-values (Log-rank test), C-index (MedCalc) were used to assess the association between CAB risk predictions and clinical outcomes. DMFS for the risk groups was estimated from KM curves.

3. Results

Study cohort: The European cohort had 864 women. As shown in Table 1, 29% were aged ≤50 years with median age of 59 (range: 28–92). Thirty nine percent had T1 tumors (0–2 cm) and rest 31% had T2 tumors (2.1–5 cm). The median tumor size was 1.6 cm (0.2–5.4 cm). Twenty nine percent had N1 disease. All patients received endocrine therapy (ET). Sixty percent were treated with aromatase inhibitors (AI), 37% were treated either with tamoxifen alone or in combination with GnRH analog or switched to AI. Thirty one percent took chemo endocrine therapy (CET) while 69% were treated with ET alone. The median follow-up was 78 months (2.4–171). European cohort (n = 864)

| parameter                          | n (%) |
|------------------------------------|-------|
| Total European cohort              | 864   |
| Age at diagnosis, years            |       |
| ≤40                                | 43 (5) |
| 41–50                              | 206 (24)|
| 51–60                              | 223 (26)|
| 61–70                              | 243 (28)|
| >70                                | 149 (17)|
| Histology                          |       |
| IDC                                | 799 (92)|
| ILC                                | 65 (8) |
| Tumor size, cm                     |       |
| 0.1–1                              | 113 (13)|
| 1.1–2                              | 487 (56)|
| 2.1–3                              | 216 (25)|
| 3.1–4                              | 37 (4) |
| 4.1–5                              | 10 (1) |
| >5                                 | 1      |
| n (%)                              | 699 (71)|
| Number of nodes with tumor cells   |       |
| 0 (NO)                             | 168    |
| 1                                  | (19.4) |
| 2                                  | 55 (6.3)|
| 3                                  | 29 (3.3)|
| Histological grade                 |       |
| Highly differentiated, G1          | 137 (16)|
| Moderately differentiated, G2       | 573 (66)|
| Poorly differentiated, G3          | 154 (18)|
| ER/PR status                       |       |
| ER+/PR-                            | 825 (95.48)|
| ER+/PR+                            | 37 (3.84)|
| ER-/PR-                            | 2 (0.29)|
| Therapy                            |       |
| Endocrine therapy alone            | 595 (69)|
| Chemoendocrine therapy             | 269 (31)|
| Radiation                          | 759 (88)|
| Endocrine therapy drug details      |       |
| Tamoxifen (alone up to 5 years, switch over) | 326 (38)|
| Tamoxifen followed by AI, along with GnRH | |
| Aromatase Inhibitor (AI)           | 524 (61)|
| Other (GnRH analog, unknown)       | 14 (1) |
| Follow-up (in months)              |       |
| Maximum                            | 171    |
| Median                             | 78     |

*For 3 patients nodes could not assessed.

ER-estrogen receptor, PR-progesterone receptor, IDC-invasive ductal carcinoma, ILC-invasive lobular carcinoma.
constituted patients from four countries: Spain (n = 286), Italy (n = 50), Austria (n = 317) and Germany (n = 211).

3.1. Prognosis by CanAssist Breast

CAB stratified 77% of this cohort as low-risk (Fig. 1a) with a HR of 3.32 (1.85–5.93) and a low-risk DMFS of 94.76 ± 0.86 with a C-index of 0.695 (0.66–0.73) (Table 2). The risk stratification was comparable in a sub-cohort of patients treated with ET alone (n = 595) (Fig. 1b), where 87% were low-risk with a DMFS of 95.38 ± 0.92 (Table 2) and HR of 3.67 (1.34–10) and C-index of 0.706 (0.68–0.74) (Table 2).

The ET given was either as a single agent or part of CET protocol. 326 patients (37%) were treated with tamoxifen. Eighty three percent were low-risk and 17% were high-risk by CAB. The recurrence rates (RR) of patients with tamoxifen were double in high-risk (12.6%) compared to low-risk (6.5%). In the AI subgroup, 74% were low-risk and 26% were high risk by CAB with 3.5 times higher RR in high-risk (16%) compared to low-risk (4.6%) (Appendix Table 1).

3.2. Risk stratification in young patients

A quarter (n = 249) of the cohort were young, aged ≤50 (Table 1). Young women are perceived to have high risk of recurrence [18]. In line with this, young women had higher RR of 9.65% compared to patients aged above 50 years with RR of 7% (Fig. 2). 80% of young women were low-risk and 20% were high-risk by CAB (P < 0.0001, Fig. 1c). These young CAB low-risk patients had RR of 6% whereas high-risk patients had RR of 24% (HR: 4.42 (1.58–12.3) (Fig. 2 and Table 2). Similarly, in the cohort aged above 50, (P = 0.0002) (Fig. 1d) 76% were low-risk and 24% were high-risk. These CAB low-risk patients had RR of 4.9% and high-risk had 13.8% (HR: 2.93 (1.44–5.96) (Fig. 2 and Table 2).

Information on menopausal status was available for 520 women, of which 66% (n = 341) were postmenopausal. Risk stratification was significant in these postmenopausal women (Fig. 1e), with an HR of 3.33 (1–10) (P = 0.0019) (Table 2). As expected, the premenopausal women had bad prognosis with a lower DMFS of 70.8 ± 9.28 in high-risk group compared to postmenopausal with a DMFS of 81.63 ± 5.53 (Table 2).
Table 2
Univariate HRs with 95% CIs and DMFS in CanAssist Breast low and high-risk patients and C-indices of various clinical sub-groups

| Number of patients | Variable | HR (95% CI) | DMFS in low-risk ± SE | DMFS in high risk ± SE | P-value | C-index (95% CI) |
|--------------------|----------|-------------|------------------------|------------------------|---------|-----------------|
| Mixed cohort (n = 1,974) | Chemoendocrine + endocrine therapy alone | 1.974 | 3.43 (2.34-4.93) | 94.89 ± 0.58 | 83.48 ± 1.66 | <0.0001 | 0.696 (0.68-0.72) |
| | Endocrine therapy alone | 880 | 3.51 (1.64-7.5) | 95.45 ± 0.76 | 84.68 ± 3.15 | <0.0001 | 0.715 (0.68-0.75) |
| Indian Ethnic origin (n = 925) | Chemoendocrine + endocrine therapy alone | 925 | 3.093 (1.83-5.21) | 95.03 ± 0.843 | 85.31 ± 2.2 | <0.0001 | 0.67 (0.63-0.7) |
| European (n = 864) | Chemoendocrine + endocrine therapy alone | 864 | 3.32 (1.85-5.93) | 94.76 ± 0.86 | 83.56 ± 2.66 | <0.0001 | 0.695 (0.66-0.73) |
| | Endocrine therapy alone | 595 | 3.67 (1.34-10) | 95.38 ± 0.92 | 83.97 ± 4.24 | <0.0001 | 0.706 (0.68-0.74) |
| Age ≤50 years | 249 | 4.42 (1.58-12.3) | 93.96 ± 1.69 | 76 ± 6 | <0.0001 | 0.763 (0.71-0.82) |
| Age >50 years | 615 | 2.93 (1.44-5.96) | 95.1 ± 0.99 | 86.12 ± 2.87 | 0.0002 | 0.659 (0.62-0.7) |
| Pre-menopausal | 179 | 5 (1.19-21.7) | 93.55 ± 1.97 | 70.8 ± 9.28 | 0.0002 | 0.764 (0.7-0.82) |
| Post-menopausal | 341 | 3.33 (1-10) | 94.18 ± 1.37 | 81.63 ± 5.53 | 0.0019 | 0.674 (0.62-0.72) |
| *Node-negative | 612 | 3.35 (1.21-9.28) | 95.18 ± 0.92 | 84.69 ± 4.24 | 0.0004 | 0.672 (0.63-0.71) |
| N1 | 252 | 2.57 (1.26-5.26) | 93 ± 2.24 | 82.89 ± 3.39 | 0.013 | 0.668 (0.61-0.72) |
| One node positive | 168 | 3.19 (1.16-8.79) | 95.45 ± 2.2 | 86.25 ± 3.85 | 0.03 | 0.722 (0.65-0.79) |
| G2 tumors | 573 | 2.07 (0.76-5.63) | 94.24 ± 1.04 | 88.41 ± 3.85 | 0.06 | 0.641 (0.6-0.68) |

Abbreviations: HR-Hazard ratio, DMFS-distant metastasis free survival, CI-Confidence Interval.

*The patients for whom nodes could not be assessed are considered as N0 for this analysis.

3.3. Risk stratification in clinical sub-groups

In 612 women with node-negative (N0) disease CAB low- and high-risk groups (n = 0.0004) (Fig. 1f) were distinct (HR: 3.35 (1.21-9.28) (Table 2) with a low-risk DMFS of 95.18 ± 0.92 (Table 2) with RR of 83.48 ± 1.66 in CAB low-risk patients and 15.3% in CAB high-risk patients (Fig. 2). In ET alone cohort of 488 women with N0 disease, low-risk DMFS was same at 95% (Appendix 1a). In 168 one node positive women (treated with ET + CET), risk stratification was significant (P = 0.03) (Fig. 1g) with a low-risk DMFS of 95.45 ± 2.2 (Table 2) and a HR of 3.35 (1.16-8.79) (Table 2). In women treated with ET alone with one node positive disease (n = 83) low and high-risk groups were well segregated (P = 0.01) with a low-risk DMFS of 100% (Appendix 1b). In women with up to 3 nodes positive (N1) (n = 252) HR was 2.57 (1.26-5.26) (P = 0.013) (Fig. 1h, Table 2), with RR of 7% in CAB low-risk patients and 17.1% in high-risk patients (Fig. 2) along with a low-risk DMFS of 93 ± 2.24 (Table 2). In women treated with ET alone (n = 107), DMFS in low-risk was 96% (Appendix 1c). The prognostic information provided by CAB in N0 and N1 sub-groups was similar with identical C-index of 0.67 (0.63-0.71) and 0.67 (0.61-0.72) (Table 2). In patients with histological grade 2 tumors distant recurrence rates were 5.7 in CAB low-risk and 11.6 in CAB high-risk (Fig. 2) with a DMFS of 94.24 ± 1.04% in CAB low risk (Appendix Fig. 1d, Table 2), with a HR of 2.07 (0.76-5.63) and C-index of 0.641 (0.6-0.68) (Table 2).

The C-indices of CAB ranged between 0.66 and 0.76 in various sub-groups of this cohort indicating that the amount of prognostic information provided by CAB was significant across various sub-groups tested here (Table 2).

4. Comparison of CAB with other prognostic tools

4.1. IHC4

IHC4 risk stratification was compared to that of CAB in 715 patients. 55% (n = 415) of patients were identified as low risk by IHC4, 35% (n = 266) as intermediate risk and the remaining 10% (n = 70) as high-risk. Both intermediate and high-risk had DMFS of 89% and 87% respectively (Fig. 3a). In this IHC4 cohort, CAB stratified 77% (n = 579) of the cohort as low risk (P = 0.0002) (Fig. 3b).

Further on re-stratification of IHC4 intermediate-risk group by CAB, low: high risk proportions were 82:18 with a high-risk DMFS of 79% (P = 0.03) (Fig. 3c). In ET alone sub-cohort (n = 514), IHC4 risk stratification was like that of the total (CET + ET) cohort, with similar risk proportions of 58% in low-risk, 36% in intermediate-risk and 9% in high-risk (Fig. 3d). Out of this ET alone IHC4 sub-cohort CAB segregated 10% more patients as low risk (87%) compared to total cohort (Fig. 3e).

4.2. CAB vs MammaPrint

The Spain cohort had 43 patients tested with MammaPrint. CAB and MammaPrint classified 65% and 56% of the cohort into low risk respectively (Appendix Table 2). MammaPrint (MP) and CAB had 83.3% concordance in low-risk and 58% concordance in the high-risk category (Appendix Table 2) with a kappa correlation coefficient of 0.42 (0.15-0.69), indicating a moderate agreement. The DMFS in the low-risk was also comparable for both the tests (MP- 100%; CAB- 96.4% (86.9-99.1)). Due to small sample size of this sub-cohort we could not draw Kaplan-Meier survival curves for this analysis and is a limitation of this analysis.

4.3. Prognostic performance of CAB across different cohorts

Risk stratification by CAB in a mixed cohort (n = 1974) of patients from ethnic Indian origin (n = 925), USA (n = 185) and Europe (n = 864) treated with CET + ET (Fig. 4a) and ET alone (n = 880) (Fig. 4b) was similar with a DMFS of 94.89 ± 0.58 and 95.45 ± 0.76 respectively (Table 2). Risk stratification in ethnic Indian (13) and European cohorts independently showed similar accuracy with a low-risk DMFS of 95.03 ± 0.84 and 94.76 ± 0.86 respectively (Figs. 4c and 1a, Table 2). HR and C-indices were also similar (ethnic Indian-HR-3.09 (1.83-5.21); C-0.67
Table 2 proving that the prognostic value of CAB across these cohorts was similar (Table 2).

5. Discussion

Use of genomics based prognostic tests has helped early-stage HR+/HER2-breast cancer patients plan optimum therapy in North America and Europe [1]. The increasing use of these tests is due to the extensive validation data by retrospective and prospective studies [1] and reimbursement of cost of prognostic tests [19]. Among prognostic tests, CAB is a relatively new test that uses proteomics approach [11]. CAB uses five biomarkers along with clinical parameters for distant recurrence risk prediction. CAB biomarkers are unique as they are key players in critical signaling pathways involved in the spread of tumor cells from primary site to a distant metastatic site thus representing tumor biology beyond proliferation [11]. These biomarkers contributed to the enhanced prognostic value over and above the clinical parameters as CAB risk score showed much higher and significant hazard ratio compared to clinical parameters in the previous analysis [11,12].

Different ethnic backgrounds are known to differ in breast cancer incidence and survival as exemplified by the differences between Asian and Caucasian women. Asian women are diagnosed more at premenopausal age, with larger luminal B tumors, with node positive disease, have active tumor micro-environments with frequent TP53 mutations vs Caucasian women [20–24]. In addition to clinicopathological features, tumor biology environment also contributes to disease progression and relapse [25]. With these reported differences between Asian and Caucasian women, the current study reports similar performance of CAB in European and ethnic Indian cohorts. This is unlike the varied performance of some of the genomic prognostic tests in certain Asian populations [8–10].

The performance of CAB was not confounded by chemotherapy as evident from the data from ET alone sub-cohort. Even in young (≤50 years) (Fig. 1c) and pre-menopausal women (P = 0.0002) (Appendix 1e) CAB risk stratification was significant which would help plan appropriate additional hormonal therapy alongside chemotherapy. Moreover, CAB risk stratification was independent of node status with significant HR in one node positive as well as other N1 patients.

IHC4 test was developed and validated on post-menopausal cohort,
Although, in Europe incidence is more in post-menopausal women, there were significant number of pre-menopausal women being diagnosed with this disease. In the current study cohort, 34% (n = 520) were in pre- and perimenopausal condition at the time of diagnosis. CAB segregated pre- (Appendix 1e) and post-menopausal (Fig. 1e) women equally well and segregated 87% of pre-menopausal women into low risk with 94% DMFS (Appendix 1e).

TransATAC [17]. Overall, not only CAB prognostication could prevent overtreatment in 20% of more patients compared to IHC4 (IHC4 low-risk, 55% vs CAB low risk, 77%), it also stratified significantly higher percentage of patients, 82% of IHC4 intermediate risk patients as low-risk in whom otherwise treatment strategy is dilemmatic. This data drives home the point that prognostication based on tumor biology ‘beyond’ proliferation makers and/or hormonal indices is more relevant without belittling importance of ER, PR, HER2 and Ki67.

Assessment of risk prediction by integrating the features of tumor biology is further substantiated by the finding that CAB provided similar prognostic information with similar C-index in cohorts that are diverse by clinical parameters. Indian cohort had more young patients with big tumors and node positive tumors compared to European cohort (Appendix Table 3). Moreover C-index of CAB was comparable to the reported C-indices of multi-gene tests in secondary analysis of TransATAC cohort or in ABCSG6 or ABCSG 8 or GEICAM 9906 trials for EPClin or in ABCSG 8 for Prosigna [26–30]. In addition to this it is encouraging to see a concordance of 83.3% between MammaPrint and CAB, 83% between Oncotype DX and CAB in low-risk category [31].

The differences in genetic make-up, disease diagnosis per se are the main contributing factors for the diverse outcomes of patients with different ethnicities. Dissemination of tumor cells from the site of origin involves key players of various signaling pathways whose interactions are independent of ethnicity/racial factors. CAB encompassing the biomarkers of these critical signaling pathways, performs unaltered in cohorts of various ethnicities emphasizing the pivotal role of tumor biology in prognostication.

The strengths of the study are large cohort size, large numbers treated with ET alone, significant representation of N1 patients and clinical outcomes being kept blinded to the team performing CAB. However, the test is limited by lack of chemotherapy benefit predictive ability, data on long term, ten-year predictions and on a prospective cohort.

Fig. 3. Kaplan–Meier survival curves showing low, intermediate, and high-risk groups by IHC4 in patients treated with chemoendocrine and endocrine therapy alone: IHC4 risk groups in 751 patients (a), CanAssist Breast risk groups in same 751 patients (b), CanAssist risk groups in IHC4 intermediate and high-risk patients (c), IHC4 risk groups in endocrine therapy only treated - 514 patients (d), CanAssist Breast risk groups in same 514 patients (e).

Fig. 4. Figure S1 Kaplan–Meier survival curves for CanAssist Breast risk groups across various cohorts. Distant metastasis free survival rates of low and high-risk groups of the entire mixed cohort comprising patients from India, Europe and USA (a) endocrine therapy alone patients represented in earlier ‘a’ panel (b), Indian cohort (c).
This is the first report showing CAB validation in European women. Performance of CAB in European cohort is like that of Indian ethnic origin, showcasing CAB performs independent of racial/ethnic differences. CAB showed its usefulness in taking decisions on chemotherapy use in young and in women with N1 tumors as well. This study along with earlier publications show the applicability of CAB risk predictions for universal patients.

Data availability statement

The data will be provided on request.

Declarations

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Author contributions

MB conceived the idea. AG analyzed the data, performed statistical analyses, and drafted the manuscript. MB, AG, and GS reviewed the manuscript. CP and RK was involved in data acquisition. CB and MA performed all the histopathological analysis. MSE coordinated the lab activities. CS, FR, PG, VR, JJ, SS, HF, CB, DE were involved in patient sample and clinical data acquisition. All authors read and approved the final manuscript.

Declaration of competing interest

Authors do not have any conflicts. AG, CB, CP, MA, RK, MSE, MB are employees of OncoStem Diagnostics. Late GS has reported to have received honorarium from OncoStem Diagnostics. All other authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2022.02.010.

Appendix Table 1
Recurrence rates in CAB low- and high-risk patients treated with tamoxifen and aromatase inhibitor (AI)

| Endocrine therapy drug | Total patients | CAB Low-risk (%) | CAB High-risk (%) | Patients who had distant relapse within 5 years from date of diagnosis |
|------------------------|----------------|------------------|------------------|---------------------------------------------------------------|
|                        | No. of patients | CAB Low-risk     | CAB High-risk    | No. of patients (recurrence rates) | CAB Low risk (recurrence rates) | CAB High risk (recurrence rates) |
| Tamoxifen              | 326            | 270 (83)         | 56 (17)          | 24 (7.4)          | 17 (6.3)          | 7 (12.5)          |
| Aromatase inhibitors   | 524            | 388 (74)         | 136 (26)         | 40 (7.6)          | 18 (4.6)          | 22 (16)           |
| (AI)                   |                |                  |                  |                  |                  |                  |
| GnRH + others          | 14             | 11               | 3                | 3 (21)            | 0                | 3 (100)           |

CAB-CanAssist Breast, GnRH-Gonadotropin releasing hormone.

Appendix Table 2
Distribution of CanAssist Breast low and high-risk patients across MammaPrint risk categories

| MammaPrint - Low-Risk (%) | MammaPrint – High-Risk (%) | Total number (%) |
|---------------------------|---------------------------|------------------|
| CAB-Low-Risk              | [8]                       | 28 (65)          |
| CAB-High-Risk             | [1]                       | 15 (35)          |
|                           | 11 (58)                   | 43               |

Appendix Table 3
Comparison of cohorts -European and Indian Ethnic origin

| Parameter                        | Variable | European, n (%) | Indian Ethnic Origin, n (%) | P-value |
|----------------------------------|----------|-----------------|-----------------------------|---------|
| (continued on next page)
Appendix Table 3 (continued)

| Parameter | Variable | European, n (%) | Indian Ethnic Origin, n (%) | P-value |
|-----------|----------|-----------------|----------------------------|---------|
| Age at diagnosis | ≤50 years | 249 (29) | 355 (38) | 0.0001 |
| | >50 years | 615 (71) | 562 (61) | <0.0001 |
| Tumor size | T1 | 600 (69.4) | 223 (23) | <0.0001 |
| | T2 | 263 (30.5) | 642 (70) | 0.0001 |
| | T3 | 1 (0.1) | 60 (7) | 0.0001 |
| Number of nodes with tumor cells | 0 (N0) | 612 (71) | 513 (55) | <0.0001 |
| | 1-3 (N1) | 252 (29) | 312 (34) | <0.0001 |
| Histological grade | Moderately differentiated, G2 | 137 (16) | 83 (9) | <0.0001 |
| | Poorly differentiated, G3 | 154 (18) | 370 (40) | <0.0001 |
| ER/PR status | ER-/PR- | 825 (95.48) | 766 (83) | <0.0001 |
| | ER+/PR- | 37 (3.84) | 136 (15) | <0.0001 |
| Treatment | Endocrine therapy alone | 2 (0.23) | 23 (2) | <0.0001 |
| | Chemoendocrine therapy | 595 (69) | 196 (21) | <0.0001 |

*Age of 8 patients of Indian ethnic origin was unknown.

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