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Risk Stratification of Early Breast Cancer (HR +/HER 2–) by CanAssist Breast and Its Corelation with Other Online Prognostic Tools: Experience from a Single Center

Ajay Bapna[1] Sanjeev Patni[2] Nidhi Patni[3] Anil Kumar Gupta[2] Aseem Kumar Samar[1] Naresh Ledwani[2] Tarachand Gupta[1] Pawan Agarwal[1]

[1] Department of Medical Oncology, Bhagwan Mahaveer Cancer Hospital and Research Centre (BMCHRC), Jaipur, Rajasthan, India
[2] Department of Surgical Oncology, Bhagwan Mahaveer Cancer Hospital and Research Centre (BMCHRC), Jaipur, Rajasthan, India
[3] Department to Radiation Oncology, Bhagwan Mahaveer Cancer Hospital and Research Centre (BMCHRC), Jaipur, Rajasthan, India

Address for correspondence Ajay Bapna, MD, Department of Medical Oncology, Bhagwan Mahaveer Cancer Hospital and Research Center, Jaipur 302017, Rajasthan, India (e-mail: ajaybapna74@gmail.com).

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Abstract

Introduction Risk assessment by various methods for HR +/HER2– early-stage breast cancer (EBC) patients help clinicians stratify risk and tailor individual treatment. Multiple prognostic tests are available, both free and expensive. Free prognostic tools, the Nottingham Prognostic Index (NPI), and modified Adjuvant Online (mAOL) rely on clinical parameters. CanAssist Breast (CAB) considers both clinical parameters and tumor biology for assessing the risk of recurrence.

Objectives The objective is to assess risk by CAB, NPI, and mAOL and discern the differences in the risk stratification in the EBC cohort of Bhagwan Mahaveer Cancer Hospital and Research Centre, Jaipur, Rajasthan, India.

Methods Study cohort comprises 100 patients. Risk concordance was assessed by the kappa correlation coefficient and restratification analysis between risk groups of CAB, NPI, and mAOL was assessed using a two-sided *p*-value.

Results Cohort was predominated by patients aged above 50, with T2/N0/G2 tumors. Low-risk (LR) and high-risk (HR) proportions by CAB, NPI, and mAOL were 67:33, 19:81, and 14:86, respectively. Across both age groups, CAB stratified more patients as LR compared with NPI and mAOL. In subgroups of patients with N0, G2, and T2 tumors, CAB identified significantly ($p < 0.0001$) higher (3–8 times) patients as LR than NPI and mAOL. In patients with T1/G1 tumors, risk proportions were similar by all three tools. Interestingly, CAB LR (57%) was four times that of NPI (14%) in the N1 subgroup. In G3 tumors CAB LR was 13%. mAOL failed to identify LR in the N1 and G3 subgroups and NPI in the G3 subgroup. There was poor agreement between CAB and NPI/mAOL (k 0.14 [95% confidence interval: 0.03–0.24]/0.11 [0.02–0.20]). Up to 11% of mAOL/NPI LR were detected as HR by CAB and up to 63% of mAOL and NPI HR as LR by CAB.

Keywords
- breast cancer
- prognosis
- CanAssist Breast
- Nottingham Prognostic Index
- modified Adjuvant
- hormone receptor-positive
- early-stage breast cancer

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Conclusion Prognostication by tools that use clinical parameters alone might be inadequate. Prognostication using CAB that integrates critical biomarkers indicative of tumor biology along with clinical parameters could be significant. The earlier published data on CAB across various ethnic cohorts and its comparable performance with Oncotype DX makes CAB a relevant prognostic test in HR+/HER2– EBC to make decisions on chemotherapy use.

Introduction

The burden of breast cancer (BC) is ever-increasing in developed and developing countries equally. In 2020, there were 2.3 million women diagnosed with BC and more than half a million died due to BC globally. By the end of 2020, there were 7.8 million women alive who were diagnosed with BC in the past 5 years, making it the world's most prevalent cancer. There are more lost disability-adjusted life years for women diagnosed with BC globally than for any other type of cancer.[1] BC is a heterogeneous disease with six distinct molecular subtypes: luminal A (progesterone receptors (PR)+, estrogen receptor (ER)+, human epidermal growth factor receptor 2 (HER2)–, and low Ki67), luminal B (ER+, HER+/−, low PR/high Ki67, and high PR/low Ki67), HER2+, basal-like subtype (ER–, PR–, and HER2−), normal breast-like, and claudin-low type (where low expression of cellular adhesion genes can be detected),[2] each subtype warranting a different course of treatment.

BC initial detection and regular monitoring play a key role in optimum treatment planning.[3] Prognostication of BC helps clinicians in effective decision-making and thereby tailoring individual treatment plans. Multiple prognostication models are available: online free prognostic tools and expensive Western multigene tests. Commonly used prognostic indicators include the Nottingham Prognostic Index (NPI), and the online tool, modified Adjuvant! Online (mAOL).[4] The NPI essentially functions based on clinical parameters considering the size of the tumor, the number of lymph nodes involved, and tumor grade, stratifying patients into six groups that predict 5- and 10-year survival.[5] mAOL, in contrast, is an online tool used to project the outcomes of patients at 10 years from disease diagnosis with or without adjuvant systemic therapy based on tumor characteristics.[6] Currently, this web site is under revision, so we used the risk category classification method that was used for clinical risk assessment in the MINDACT trial.[7] The major disadvantage of these two tools is that they essentially rely only on clinical parameters and do not consider additional tumor biomarkers beyond hormonal indices and proliferation markers. This might result in misinterpretation of recurrence risk assessment. CanAssist Breast (CAB) in comparison to NPI and mAOL uses clinical parameters and five tumor biomarkers that provide additional useful prognostic information about cancer recurrence risk prediction. Further, it is less expensive compared with other multigene prognostic tests, like Oncotype DX, MammaPrint, Prosigna, and EndoPredict. Additionally, CAB has been extensively validated in Indian patients and patients from the United States and Europe with comparable performance to that of Oncotype DX, Mamma Print, etc.[8–11]

CAB is a five-protein marker immunohistochemistry (IHC)-based prognostic test used to stratify the risk of distant recurrence of BC patients over 5 years from the time of diagnosis of BC. IHC gradings for membrane localization of CD44, ABCC4, and ABCC11, cytosolic localization of N- and pan-cadherins along with three clinical parameters (node status, tumor size, and tumor grade) were used as inputs into an artificial intelligence (AI)-based algorithm. The algorithm generates a risk score between 0 and 100. With a predefined cutoff of 15.5, each patient is assigned either low-risk (LR) (≤ 15.5) or high-risk (HR) (> 15.5).[12]

The objective of the present investigation is to evaluate the risk assessment between CAB and NPI and mAOL in BC patients from a single center of Bhagwan Mahaveer Cancer Hospital and Research Centre (BMCHRC), Jaipur, Rajasthan, India. We discerned the differences in the risk stratification of these three tools from this BMCHRC cohort of BC patients.

Materials and Methods

Study Design and Patient Selection

This study involved the analysis of the tumor specimens of 100 BC patients treated at a single center the BMCHRC over a period of 2.5 years (February 2020–June 2022). These patients underwent CAB testing to plan their therapy in the real world. Samples with early-stage (stage I and II) disease, who are positive either for ER or PR and negative for HER2/neu, tumor specimens of patients who have not received neoadjuvant chemotherapy, and who underwent CAB testing for their treatment planning were considered for the study. Tumor specimens of advanced stage (stage III and IV) disease with triple-negative (ER–/PR–/HER2–) disease and who did not have CAB risk score were excluded from the study. The treating clinician obtained all the information about the patients (such as age, year of diagnosis, clinical parameters, and hormone receptor [HR] status) and treatment follow-up details (treatment received and regimen). Patients had undergone either mastectomy or breast-conserving surgery or lumpectomy. The study endpoint is the generation of risk categories by CAB and other online prognostic tools.

Tumor Sample Processing

Tumor content of every block was assessed by hematoxylin and eosin staining and blocks with 30% tumor were used to perform CAB.

Immunohistochemistry and CanAssist Breast (CAB) Based Risk Categorization

CAB test was performed on formalin-fixed, paraffine-embedded (FFPE) blocks as described earlier.[12–14] Five consecutive sections of 3 µm each were used for IHC staining of CAB's five biomarkers. All the IHCs were performed at OncoStem's CAP and ISO 15189-accredited central laboratory. Briefly, IHC grading for CAB protein biomarkers was performed as described earlier on an automated Ventana IHC machine. This IHC information along with tumor size, grade, and node status was used to arrive at a CAB risk score that ranges between 0 and 100, using the CAB algorithm. A cutoff of 15.5 is used to stratify the patients into LR ($\leq$ 15.5) and HR ($>$ 15.5) categories for distant recurrence.

Nottingham Prognostic Index-Based Risk Categorization

The NPI was calculated with the following equation[5]:

$$\text{NPI} = \text{Tumor (T) Size (cm)} \times 0.2 + \text{Node Status (N)} + \text{Tumor Grade (G)}$$

$$\text{NPI} = \text{T (cm)} \times 0.2 + \text{N} + \text{G}$$

Based on the value obtained from the above equation, the NPI risk groups are classified into six classes, namely, excellent prognostic group (EPG, NPI score $\leq$ 2.40), good prognostic group (GPG, NPI score $>$ 2.4 to $\leq$ 3.40), moderate prognostic group (MPG, NPI score $>$ 3.4 to $\leq$ 5.40; MPG is further subdivided into two groups as moderate group 1 [NPI score $>$ 3.4 to $\leq$ 4.40] and moderate group 2 [NPI score $>$ 4.4 to $\leq$ 5.40]), and poor prognostic group (PPG, NPI score $\geq$ 5.4; PPG is further subdivided into two groups as poor group [NPI score $>$ 5.4 to $\leq$ 6.40] and very poor group [NPI score $\geq$ 6.40]).[15] In this article, for all the comparative analysis with CAB which does "LR versus HR" stratification, the NPI-based prognostication was segregated into "LR and HR" as follows: GPG and EPG were considered as LR and MPGs (MPG-1 and MPG-II) along with PPGs (poor and very poor) were considered HR.

Modified Adjuvant! Online-Based Risk Categorization

The mAOL (ver8) criteria as described in the MINDACT trial[7] were used for assigning risk categories to BC patients based on tumor grade, node status, tumor size, and receptor status (►**Supplementary Table S1**, online only).

Primary Outcome

The correlation between all three prognostic tools studied here at the level of risk stratification is the study's primary outcome.

Inclusion and Exclusion Criteria

Inclusion criteria: The patients diagnosed with early-stage (stage I and II as per the American Joint Committee on Cancer staging system), positive for HR status (ER or PR), and negative for HER2/neu are eligible to undergo CAB. Patients should not have undergone neoadjuvant chemotherapy.

Exclusion criteria: Patients diagnosed with advanced stage (stage III and IV) HR-positive and HER2-negative BC, patients diagnosed with triple-negative BC, and with negative for hormone receptor status (ER and PR) and positive for HER2/neu are not eligible to undergo CAB. CAB cannot be performed on the surgical tumor FFPE specimens of patients who have received chemotherapy before surgery (however, CAB can be performed on the biopsy specimens in those patients taken before surgery).

Statistical Analyses

The kappa correlation coefficient between the risk groups of these three tools was computed by MedCalc software. A p-value of < 0.05 is considered statistically significant.

Results

Cohort Description/Patient Characteristics

This study cohort consisted of 100 early-stage BC (EBC) patients in total, with 33 and 67% $\leq$ 50 and $>$ 50 years of age, respectively (median age 55; range: 35–79). Ninety-nine percent of patients were ER-positive and 1 patient who was negative for ER was positive for PR. According to tumor size, 78% of the cohort had T2 tumors (median T size 3 cm; range: 0.8–6.9 cm). Eighty-six percent of the cohort had a node-negative disease and only 14% had N1 tumors. The majority of the patients (66%) had G2 tumors followed by G3 and G1 having 23 and 11%, respectively (►**Table 1**).

Risk Proportions by All Three Prognostic Tools

CAB stratified significantly higher patients as LR, 67% ($n = 67$); 33% as HR ($p < 0.0001$). Interestingly, NPI and mAOL tools had an exact opposite trend with a greater number of patients in the HR group, 81 (81%) and 86 (86%), and a lesser number of patients in the LR group having

Table 1 Patient demographics

Parameters		Number of patients/%[a]
Patients	Total	100
Age (y)	$\leq$ 50	33
	$>$ 50	67
	Median	55 (35–79)
Tumor (T) size (cm)	T1 (0–2 cm)	17
	T2 (2.1–5 cm)	78
	T3 ($>$ 5 cm)	05
	Median	3 (0.8–6.9)
Node (N) status	N0	86
	N1 (1–3 nodes)	14
Tumor grade (G)	G1	11
	G2	66
	G3	23

[a]As the cohort size is 100, number of patients and % will remain the same.

19 (19%) and 14 (14%), respectively. Among HR of NPI no patient was assigned the poor prognostic risk (PPG) category by NPI. The differences in LR and HR groups within and between NPI/mAOL and CAB risk groups were found to be statistically significant with a p-value of < 0.05 (►**Table 2**).

LR Patients by CAB versus NPI/mAOL across Various Clinical Subgroups

When we looked at LR percentages across various clinical subgroups we found that these three tools behave very differently, and these differences were statistically significant. CAB identified 64% of patients aged $\leq$ 50 years as LR while by NPI they were 27% and by mAOL they were 21%. In the $>$ 50 years age group LR proportions were 69% by CAB, 15% by NPI, and 10% by mAOL. Across various clinical subgroups tested here, CAB had higher LR percentages than NPI and mAOL except in the T1 and G1 subgroups. CAB stratified more than 50% of the subgroups of patients with T2 (71%), N0 (69%), and G2 (85%) tumors as LR when compared with NPI (T2 8%, N0 20%, and G2 15%) and mAOL (T2 4%, N0 16%, and G2 15%) (►**Table 3**). The differences between LR patients of CAB and NPI/mAOL in subgroups of patients aged above 50 years and patients with T2/N0/G2 tumors were statistically significant with p-value < 0.05 (►**Table 3**).

In T1 and G1 subgroups the LR percentages were almost similar for all three tools (T1: CAB: 71%, NPI 76%, mAOL 65%; G1: CAB 73%, NPI 82%, mAOL 36%) with no statistical significance (►**Table 3**). It was interesting to see a decent number of patients as LR by CAB in the N1 and G3 subgroups (N1 57% and G3 13%), whereas mAOL did not identify any of these patients as LR while NPI had few patients (14%) as LR in the N1 subgroup alone (►**Table 3**).

Concordance between the 3 Tests

Between NPI and CAB, there was 89% of concordance in the LR category, whereas only 38% of concordance was observed in the HR category with an overall concordance of 48% (k: 0.14; 95% confidence interval [CI]: 0.036–0.245). Similarly, with respect to mAOL and CAB, there was 93% of concordance in the LR category and 37% concordance in the HR category with an overall concordance of 45% (k: 0.11; 95% CI: 0.029–0.202), representing the poor agreement between NPI/mAOL and CAB. Whereas NPI and mAOL had 74% of concordance in LR, 100% of concordance in HR, and an overall concordance of 95% (k: 0.81; 95% CI: 0.667–0.971) representing a strong agreement between NPI and mAOL (►**Table 4**).

Restratification of Risk Groups of NPI and mAOL by CAB

Restratification analysis revealed that approximately 11% (2 out of 19) of NPI LR and 7% (1 out of 14) of mAOL LR were identified as HR by CAB (►**Fig. 1A**). Similarly, approximately 62% (50 out of 81) of the NPI HR group and 63%

Table 2 Risk proportions by all three tests

Tests	LR/EPG and GPG (*n*/%)	HR/MPG (*n*/%)	*p*-Values for risk groups within the test	*p*-Values for risk groups between CAB vs. NPI/mAOL risk groups
CAB	67	33	0.0014	–
NPI	19	81	0.0001	0.0002
mAOL	14	86	0.0001	0.0003

Abbreviations: CAB, CanAssist Breast; EPG, excellent prognostic group; GPG, good prognostic group; HR, high-risk; LR, low-risk; mAOL, modified Adjuvant Online; MPG, moderate prognostic group; NPI, Nottingham Prognostic Index; PPG, poor prognostic group.
Note: Comparison of risk proportions by CAB vs. NPI and mAOL. In NPI test EPG and GPG is merged as LR and MPG and PPG is merged as HR.

Table 3 Low-risk proportions by all three tests across clinical subgroups

Clinical parameters	LR [*n* (%)]			*p*-Value for low-risk groups	
	CAB	NPI	mAOL	CAB vs. NPI	CAB vs. mAOL
Age $\leq$ 50	21 (64)	9 (27)	7 (21)	0.0674	0.0525
Age $>$ 50	46 (69)	10 (15)	7 (10)	**0.0018**	**0.0031**
T1	12 (71)	13 (76)	11 (65)	0.9131	0.7629
T2	55 (71)	6 (8)	3 (4)	**0.0023**	**0.0167**
N0	59 (69)	17 (20)	14 (16)	**0.0003**	**0.0003**
N1	8 (57)	2 (14)	0	0.3018	NA
G1	8 (73)	9 (82)	4 (36)	0.6657	0.2363
G2	56 (85)	10 (15)	10 (15)	$<$ **0.0001**	$<$ **0.0001**
G3	3 (13)	0	0	NA	NA

Abbreviations: CAB, CanAssist Breast; LR, low risk; mAOL, modified Adjuvant Online; NPI, Nottingham Prognostic Index.
Note: Tumor anatomical characteristics features of LR patients by CAB vs. NPI and mAOL.
Significant *P* values are highlighted in bold.

Table 4 Kappa correlation coefficient between CAB vs. NPI and mAOL

Tests	LR concordance %	HR concordance %	Overall concordance %	Kappa correlation (95% CI)	Agreement
NPI vs. CAB	89	38	48	0.14 (0.036–0.245)	Poor/weak
mAOL vs. CAB	93	37	45	0.11 (0.029–0.202)	Poor/weak
NPI vs. AOL	74	100	95	0.81 (0.667–0.971)	Strong

Abbreviations: CAB, CanAssist Breast; CI, confidence interval; HR, high risk; LR, low risk; mAOL, modified Adjuvant Online; NPI, Nottingham Prognostic Index.
Note: LR, HR, and overall concordance by kappa correlation coefficient between all tests.

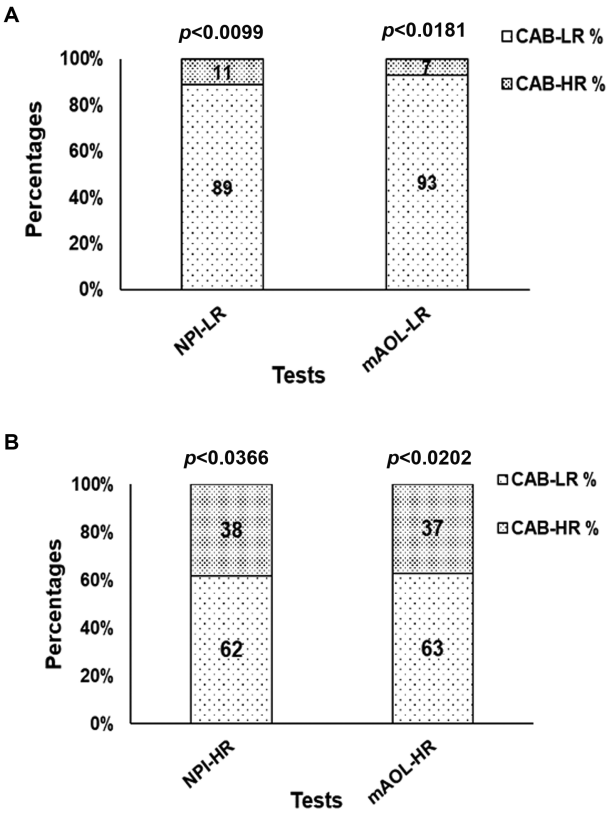

Fig. 1 Restratification of the Nottingham Prognostic Index (NPI) and modified Adjuvant Online (mAOL) risk groups by CanAssist Breast (CAB). Restratification of NPI/mAOL low-risk (LR) by CAB into CAB-LR and CAB high-risk (HR) groups (**A**). Restratification of NPI/mAOL-HR by CAB into CAB-LR and CAB-HR groups (**B**).

(54 out of 86) of mAOL HR patients were identified as LR by CAB (►**Fig. 1B**).

Discussion

Only up to 20% of early HR+/HER2/neu BC patients derive chemotherapy benefit.[16,17] Prognostic tests help to identify these patients. Both expensive multigene tests and free online prognostic tools are used by clinicians across the globe based on the resources. NPI and mAOL are a couple of free prognostic tests often used in resource-limited settings. It is, however, important to ascertain the limitations of these tools before using them to decide on a patient treatment trajectory. Both NPI and mAOL provide overall survival information and may not be precise in exactly predicting the benefit of chemotherapy. Among the multiple prognostic tests currently available for patients, an IHC-based biomarker test, CAB is the new trendsetter that has been developed incorporating the inputs from tumor biology and clinical features for the prediction of risk of recurrence using an AI-based approach.[12]

Significant advances in genetic fingerprints and molecular signaling processes have found a variety of biomarkers in tissues and blood (liquid biopsies) that may be used to predict the likelihood of cancer spread, resurgence, therapy recommendations, recurrence prediction, and medication tolerance.[18,19] As a result, novel and more effective biomarkers have been identified. So, as physicians we believe CAB is an appropriate prognostic test in the current scenario which considers the five protein biomarkers that play a vital role in cancer progression and recurrence pathways, as well as clinical parameters in deciding the prognosis of BC patients for their customized treatment.

CAB categorized more than 50% of the cohort into LR as against $<20\%$ of patients by NPI and mAOL. Irrespective of age group more patients were recognized as LR by CAB when compared with NPI and mAOL, demonstrating CAB's usefulness in treating young (≤ 50 years) patients. It is noteworthy that in the subgroups commonly found in India, patients with T2/G2 tumors CAB identified more than 70% as LR while the other two tools identified as low as 4% as LR. Other than these two clinical features, node-positivity is widely observed among Indian women with BC compared with Western women. With the demonstrated importance of tumor biology in patient prognosis, data has suggested not all patients with clinically HR features like node-positivity or higher histological grade would warrant chemotherapy.[20,21] In tune with this, our data showed patients with a lower risk of recurrence exist among the clinically HR group (N1 and G3 tumors), and such patients are identified by CAB who otherwise would be overtreated with NPI and mAOL. This highlights the importance of biomarkers in providing precise treatment plans, thus making the CAB more sensitive than the other prognostic tools. It has been shown previously that CAB risk stratification was accurate with respect to clinical outcomes in young patients and in patients with clinical HR and high proliferative index like in node-positive tumors, patients with G2/G3 tumors, T2N1 tumors, high K_i-67, and luminal-B tumors across multiple cohorts (Indian, European).[8,9]

As expected, there was a weak agreement between NPI/mAOL and CAB and a strong agreement between NPI and mAOL as both functions are based on clinical parameters. The data clearly suggests that a great number of BC patients (62–63%) would be overtreated if treated based on these online prognostic tools, and at the same time, a fraction of patients would be undertreated as up to 11% of NPI/mAOL LR patients were identified as HR by CAB.

All CAB LR received endocrine therapy alone. Premenopausal CAB LR women received tamoxifen alone while postmenopausal women received aromatase inhibitor for a period of 5 years. CAB pre- and postmenopausal HR node-negative patients typically received chemotherapy of four cycles of Taxotere (docetaxel)/cyclophosphamide while node-positive (N1) patients received four cycles of Adriamycin (doxorubicin)/cyclophosphamide followed by four cycles of paclitaxel (Taxol) followed by extended endocrine therapy. We are following up with all these patients until 5 years and then compare the risk categorization of these three tools with respect to clinical outcomes. Early clinical outcomes with a median follow-up of 17 months (8–36 months) showed that all the CAB LR patients are doing well with no events at a distant site.

Regarding CAB validation, the test has recently completed validation in a Dutch subcohort of patients who participated in a prospective trial, TEAM, that showed CAB's risk predictions are valid up to 10 years from disease diagnosis.[22] This data has further enhanced the confidence of physicians in prescribing CAB and encouraged us to use it more on our patients. Regarding the validation data of CAB, its validation in a clinical trial randomized for chemotherapy is the limitation and we hope to see this kind of data from the team (that developed CAB) in the near future.

Conclusion

As a physician, from this single-center cohort data of our BMCHRC, it is evident that clinical parameters alone might not be enough in predicting the recurrence risk, but in addition to it, the contribution of CAB's five protein biomarkers adds great value in prognosticating EBCs. The robust data from various cohorts including ours makes CAB an ideal, effective, and relevant prognostic test that can be used for HR+/HER2– EBC patients in making informed decisions on the use of chemotherapy.

Ethical Statement

The current report involves an analysis of the existing patient data that was captured during regular procedures as part of routine patient care. No additional tissue-based assessments were performed. Institutional Ethics Committee (IEC), Bhagwan Mahaveer Cancer Hospital and Research Centre unanimously granted the waiver of IEC approval for the manuscript submission and analyzed the data for journal publication including consent waiver of study participants.

Authors' Contributions

A.B. has collated the data, analyzed, and written the manuscript. All other authors have reviewed and approved the manuscript. Each author believes that the work represents honest work.

The study was conceptualized and designed by A.B., data acquisition and data analysis was done by S.P., N.P., and A.G., statistical analysis was done by A.B., S.P., and N.P., the manuscript was written by A.B., and all authors edited and reviewed the manuscript.

Source of Grants

None.

Conflict of Interest

No conflict of interest exists among the authors. All are employees of BMCHRC, Jaipur, Rajasthan, India.

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