A Multi- institutional Prospective Analysis of Impact of CanAssist Breast (Morphometric Immunohistochemistry Based Test) on Adjuvant Chemotherapy Decisions in Early Breast Cancer

Somashekhar SP (somusp@yahoo.com)
Manipal Comprehensive Cancer Centre https://orcid.org/0000-0002-7898-1625
Shekhar Patil
HCG, Bangalore
Ravi Thippeswamy
Shri Shankara Cancer Foundation
Rajeevkumar Rajeevkumar
Senior Consultant, Rajeev Gandhi Cancer Institute, New Delhi
Chaturbhuj R Agrawal
Rajeev Gadhi Cancer Insitute, New Delhi
Dinesh Chandra Doval
Rajeev Gandhi Cancer Insitute, New Delhi
Vijay DG
HCG, Ahmedabad, Gujarat
Ajay Bapna
Bhagawan Mahaveer Cancer Hospital, Rajasthan
Kiran Shankar
Clearmedi Radiant Hospital, Mysore
Avinash CB
Clearmedi Radiant Hospital, Mysore
Karthik S Udapa
KMC, Manipal
Krishna Prasad
Mangalore Institute of Oncology, Mangalore

Research Article

Keywords: CanAssist Breast (CAB), Early Breast Cancer, adjuvant chemotherapy, morphometric Immunohistochemistry based test, ER positive/HER2 negative

Posted Date: May 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-520955/v1

Abstract

Purpose: CanAssist Breast (CAB) has been validated retrospectively for assessing risk of recurrence and thereby usefulness of chemotherapy in HR+/HER2- breast cancer. The objective of this study is to assess the agreement between physician's treatment plan and CAB risk stratification and evaluate whether CAB results aid in the physician's treatment decision.

Methods: The data on the physician's treatment plan before and after the CAB test was collected prospectively between 2016 and 2021 in 249 patients. Changes in treatment recommendations and compliance with CAB reports were analyzed.

Results: Based on conventional clinicopathological features physicians planned to treat 46% of patients with endocrine therapy (ET) (low-risk-LR)), 24% with chemoendocrine therapy (CET) (high-risk-HR)) and in 30% physicians were uncertain of prescribing chemotherapy (intermediate-risk-IR)) before CAB testing. The correlation between clinical risk assessment and CAB risk stratification (k=0.2 (0.05-0.35) was nonsignificant. CAB classified 64% as LR, which was 18% (9.3-25, *P*=0.0001) higher compared to clinical LR. In the clinical IR category, CAB risk proportions were 55:45 (LR: HR). We observed a substantial shift in treatment recommendation from CET to ET in 54% (40.75- 66.84, *P*<0.0001) of clinical HR and ET to CET in 26% (18.27- 35.01, *P*<0.0001) of clinical LR patients. Overall CAB lead to change in treatment recommendation in 42% of the cohort.

Conclusions: There was a significant impact of CAB on the physician's treatment decision. CAB provided definite treatment recommendation to IR patients where the physician had dilemma on prescribing chemotherapy and provided precise treatment plan to clinical LR and HR patients.

Introduction:

The inclusion of chemotherapy in the treatment strategy in the ER positive/HER2 negative early-stage breast cancer patients is judged by the risk of cancer relapse. The risk of cancer relapse is assessed by tumor anatomical features like tumor size, node status, histological grade; age of the patient; hormone receptor expression status and Ki-67 expression [1–9]. The emerging evidence from different studies indicate that the tumor pathological features do not provide accurate and reliable prognostic information on the disease aggressiveness [10–11]. These features miss on providing the critical information predictive of cancer recurrence. Studies have shown that some node-negative patients require chemotherapy for a better prognosis while some node-positive patients tumors have good prognosis without chemotherapy [12, 13]. In fact it has been reported that some patients with small tumors < 2cms benefit of chemotherapy [14, 15]. This showcases that prognosis based on clinical factors alone could lead to over or undertreatment in these patients.

Prognostic tests have largely addressed this issue with accurate prognostication with the optimum treatment recommendations [16–19]. The multi-gene tests with validation data on Caucasian women, might not be appropriate for selection of Asian women who would benefit from chemotherapy due to

underlying inherent racial factors beyond clinical parametes of standard of care, contributing to the differences in the prognosis of these patients [20, 21]. CanAssist Breast, a prognostic test using immunohistochemistry platform encompassing the crucial 5 biomarkers (CD44, N-Cadherin, pan-Cadherin, ABCC4 and ABCC11) of cancer progression, recurrence and therapy resistant pathways along with clinical parameters (node status, tumor size and histological grade) predicts risk of cancer relapse with the use of a machine learning algorithm [22, 23]. The test with considerable validation data on women from India, USA [23] and European countries (under review) has helped a greater number of women from South East countries plan their treatment [24]. The test has shown to have greater than 83% concordance in the low-risk category with the other widely used test, Oncotype DX [25].

Although the test has been in use since 2016, influence of CAB on physician's advice to offer chemotherapy has not been reported. In this current prospective observational study, we report the impact of CanAssist Breast on individual physician prescribing adjuvant chemotherapy and change in physician's decision about chemotherapy before and after CAB testing in early-stage breast cancer patients.

Methods:

Study population and study design:

The study was a prospective, observational, multi-centric involving physicians across the country. The data used for this study was on patients for whom CAB test was prescribed between the period, 2016 to 2021. Before CAB was performed on the tumor samples, the information on the treatment plan for each patient before and after CAB testing was collected through a questionnaire with physician's considerations for giving or avoiding chemotherapy. The physician had no obligation to treat the patient as per CAB test results. The data for the study has been collected on 249 patients from 35 physicians practicing in 30 different hospitals across India. Of these 16 were medical oncologists, 18 were surgical oncologist and one was radiation oncologist. For the study as only the physician's opinion on the treatment plan was obtained from the CAB prescribing physicians as part of their routine clinical practice and did not intervene with the patient's treatment choices, ethics approval or patient consent were not required. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Sample processing and CAB testing: After the physician referred the patient for CAB testing, the FFPE tumor blocks were shipped to centralised OncoStem laboratory along with histopathology report. Information on tumor characteristics, ER, PR, HER2/neu and Ki-67 staining were as per the histopathology report shared with OncoStem. Tumor content of the FFPE blocks was assessed by haematoxylin and eosin staining. Blocks with ≥ 30% of tumor content were processed for CAB testing. Immunohistochemistry of the five CAB biomarkers was performed and CAB risk score was obtained using a machine learning algorithm as described earlier [22].

Statistical analysis: The change in percentage of patients who had change in treatment recommendations by CAB and Odd's ratio for estimating the probability of CAB low risk predictions for clinical intermediate and high-risk groups with clinical low-risk group as a reference were analysed by logistic regression using MedCalc.

Results:

Baseline characteristics of patients: The current prospective study consisted of 249 subjects. All were women except two. The median age of diagnosis of the cohort was 59 (26–81 years). Twenty nine percent of the cohort were aged below or equal to 50 years and 71% above 50 years. Thirty one percent of the cohort had patients with T1 tumors, 6% of the patients had tumors greater than 5cm (T3) and 65% had T2 tumors. Median tumor size of the cohort was 2.5cm (range: 0.4-11cm). Seventy nine percent of the cohort had node-negative tumors and 21% with N1 tumors. Sixty seven percent of the patients had tumors with moderately differentiation (G2) and 22% were with poorly differentiated tumors (G3). All patients were with ER positive disease, 94% had PR positive disease. 51% of patients expressed Ki67 greater than 14%. (Table 1).

Table 1

Patient demographics of the study cohort

Parameters		No. of Patients (%)
	Total	**249**
Sex	Females	247 (99.2)
	Males	2 (0.8)
Age	≤ 50	72 (29)
	> 50	177 (71)
*Tumor size	0.0–1.0	14 (6)
	1.1-2.0	62 (25)
	2.1-3.0	120 (48)
	3.1-4.0	34 (14)
	4.1-5.0	8 (3)
	> 5.0	6 (2)
Node status	0 nodes	194 (79)
	1 node	40 (16)
	2 nodes	9 (3)
	3 nodes	6 (2)
Histological Grade	G1	28 (11)
	G2	167 (67)
	G3	54 (22)
**Hormone receptor status	ER+/PR+	233 (94)
	ER+/PR-	16 (6)
^^Ki67	≤ 14	34 (14)
	> 14	126 (51)
	NA	88 (35)

*exact tumor size unavailable for 6 patients; ^^ Ki67 IHC information available only for 161 patients

**PR was inconclusive for one patient; ^ Each bilateral patient is sampled as two

Treatment plan by the physician and CanAssist Breast (CAB) risk stratification: The physician's opinion on the treatment plan before CAB testing was categorised into three groups. The patients to be treated with endocrine therapy as low risk (LR), with chemotherapy as high risk (HR), and for whom the physician is in a dilemma on treating the patient with chemotherapy are categorised as intermediate risk (IR). CanAssist Breast stratifies the patients into two risk categories, low- and high-risk without any ambiguous intermediate risk category. The risk proportions as per the physician were: 46:30:24 (LR:IR:HR). By CAB, the risk proportions were 64:36 (LR:HR) (Fig. 1a). There was a weak agreement between the two methods of risk assessment with a kappa coefficient of 0.2 (95% CI: 0.05–0.35). CAB assigned 18% more patients to low-risk category ($P = 0.0001$) (95% CI:9.3 to 25) compared to physician's risk assessment.

Upon restratification of clinical risk groups by CAB, we found 26% (n = 30/114) of 'clinical low-risk' were stratified as high risk by CAB and 54% (n = 33/61) of 'clinical high-risk' as low-risk by CAB. Of the intermediate risk category (n = 74), 55% of the patients were low-risk and 45% high-risk by CAB (Fig. 1b).

In the sub-group analysis, CAB based low-risk proportions were significantly ($P \leq 0.05$) higher compared to high-risk in all except in N1 subgroup where low-risk and high-risk proportions were 44% and 56% respectively. However, in the sub-groups generated as a result of the physician's risk assessment, the difference in low- and high-risk proportions was nonsignificant in subgroups with G3 tumors, in patients aged below 50 years and in patients with T3N0 and T1N1 tumors (Table 2). With the combination of tumor size and node status, in patients with N0 tumors, the low-risk proportions were similar in patients with T1N0 (51%) or T2N0 (49%) tumors while the intermediate risk proportion were significantly ($P = 0.013$) higher in patient sub-group with T2N0 tumors (38%) vs sub-group with T1N0 tumors (20%), thus reducing the high-risk proportions significantly in patients with T2N0 tumors (13%) compared to T1N0 patients (26%) ($P = 0.02$). Contrary to this, CAB classified 85% of T1N0 sub-group and 64% of T2N0 sub-group with higher proportions of 36% as high-risk in patients with T2N0 tumors vs 15% in patients with T1N0 tumors (Table 2). In N1 subgroup in patients with T1N1 tumors clinical low-risk were almost double (42%) compared to patients with T2N1 tumors (22%), while the intermediate risk proportions were slightly higher in patients with T1N1 (26%) tumors vs T2N1 (22%) tumors ($P = 0.7$) (Table 2). Even with CAB the pattern was similar, in T1N1 tumors (66%) the CAB LR were more than double to that of CAB LR in T2N1 patients (31%).

Table 2
Risk groups by the physician and CAB in various sub-groups of patients

	clinical risk assessment (% of patients)			P-value LR vs HR	CAB (% of patients)		P-value LR vs HR
	LR	IR	HR		LR	HR	
Total	46	30	24	< 0.0001	64	36	< 0.0001
N0	50	31	18	< 0.0001	69	31	< 0.0001
N1	29	24	47	0.05	44	56	0.2
T1	50	21	29	0.008	84	16	< 0.0001
T2	43	34	23	0.0001	57	43	0.01
G1	64	21	15	0.0002	93	7	< 0.0001
G2	48	30	22	< 0.0001	72	28	< 0.0001
G3	30	34	36	0.5	23	77	< 0.0001
Low Ki67 (14%)	70	24	6	< 0.0001	74	26	0.0001
High Ki67	40	33	27	0.02	62	38	0.0001
below 50	40	32	28	0.1	72	28	< 0.0001
above 50	48	29	23	< 0.0001	60	40	0.0002
T1N0	51	23	26	0.009	85	15	< 0.0001
T2N0	49	38	13	< 0.0001	64	36	< 0.0001
T1N1	42	26	32	0.5	66	34	0.05
T2N1	22	22	56	0.003	31	69	0.0014

Change in the treatment recommendations: The physician's decision of not giving chemotherapy (clinical LR) was majorly based on node-negative status, grade and age of the patient and low Ki67 (Table 3). Similarly, node-positive tumors, grade 3 tumors, age of the patients ≤ 50 years at the time of diagnosis, high ki67 are perceived to be high risk features for prescribing chemotherapy. Patients with a combination of one or more low-risk feature and high-risk features makes physician's decision of prescribing chemotherapy more complicated thereby putting these patients in the 'intermediate risk' category (Table 3).

Table 3
Parameters on which clinical risk was based

	Clinical Low-risk (n = 114) *(% expressed as fraction of total low-risk)*	*Clinical Intermediate risk (n = 74)* *(% expressed as fraction of total intermediate-risk)*	*Clinical High-risk (n = 61)* *(% expressed as fraction of total high-risk)*
node negative	99 (86%)	61 (82%)	35 (57%)
N1	16 (14%)	13 (18%)	26 (43%)
G1	18 (16%)	6 (8%)	4 (7%)
G2	81 (70%)	50 (66%)	38 (62%)
G3	16 (14%)	18 (24%)	19 (31%)
Age under 50	29 (25%)	20 (27%)	20 (33%)
T1N0	31 (27%)	14 (19%)	14 (23%)
T2N0	63 (55%)	46 (62%)	15 (25%)
T3N0	5 (4%)	1 (1%)	3 (5%)
T1N1	8 (7%)	5 (7%)	5 (8%)
T2N1	8 (7%)	7 (9%)	18 (30%)
Low Ki67	24 (21%)	8 (11%)	2 (3%)
High Ki67	51 (44%)	42 (57%)	34 (56%)

In the Clinical low- and high-risk category, in about 63 patients there was change in treatment option either from ET to CET or from CET to ET. In 26% (30/115) (18.27%- 35.01%, $P < 0.0001$) of patients to whom low-risk was assigned by the physician (clinical LR), there was a change from ET to CET. Similarly, in 54% (33/61) (40.75%- 66.84%, $P < 0.0001$) of patients to whom high-risk was assigned by the physician (clinical high-risk), there was a change in the treatment recommendation from CET to ET (Table 4). And in 74 patients (29.7%) in whom the physician was uncertain of prescribing chemotherapy, with CAB test results 41 patients (55%) would be treated with endocrine therapy alone and in 33 (45%) patients' treatment plan would include chemotherapy along with endocrine therapy (Fig. 1b). Assuming that the physicians tend to treat these patients with chemotherapy in the absence of a prognostic test, with 55% (41/74) as low-risk by CAB the overall change in the treatment recommendations was observed in 42% of the cohort (n = 104/249).

Table 4
Change in treatment by CAB in clinical low and high risk groups

	change in treatment recommendation		No change in treatment recommendation-%
Parameter	From CET to ET in % of Clinical HR	From ET to CET in % of Clinical LR	
Total	54	26	45
N0	60	21	47
N1	36	56	38
T1	86	10	49
T2	38	30	44
G2	68	21	45
G3	16	69	40
High Ki67	59	29	39
below 50	65	21	44
above 50	49	28	47
T1N0	88	3	51
T2N0	44	24	46
T1N1	83	38	32
T2N1	35	75	43

In node-negative patients, in about 21% (21/98) (13.46–30.34%, $P < 0.0001$) patients change from ET to CET in clinical LR and in 60% (21/35) (42.1–76.13%, $P < 0.0001$) of patients from CET to ET in clinical HR was suggested by CAB. Likewise, in N1 patients, in 56% (9/16) of clinical LR (29.66–80.06%, $P < 0.0001$) patients change from ET to CET and in clinical HR in 36% (12/26) (18.28–57.03%, $P < 0.0001$) patients change from CET to ET was suggested by CAB (Table 4).

Patients who were assigned intermediate risk by the physician showed higher likelihood of being classified as low risk by CAB with an Odds ratio (OR) of 0. 44 (0.24–0.81) compared to patients who were assigned high-risk by the physician (OR: 0.42 (95% CI:0.22–0.8) (Table 5).

Table 5
Likelihood of Clinical IR/HR being stratified as LR by CAB

Clinical risk groups	Odds ratio	95% CI	P-value
IR	0.44	0.24–0.81	0.0091
HR	0.42	0.22–0.8	0.0085

Physician's treatment decisions concurrent with that of CAB test results:

In 97% of CAB LR patients, physician decided to treat the patient with endocrine therapy and 88% of CAB HR patients, physician opted for chemo endocrine therapy. In about 13 patients the physician did not adhere to the CAB test results. The physician did not prescribe chemotherapy in 142 of 146 CAB low-risk patients. In 4 patients the physician decided to treat the patient with chemotherapy. In 69 out of 78 CAB high risk patients, physician offered chemotherapy. Thus, there was an overall adherence of physician to CAB results was 94% (Table 6).

Table 5
Adherence by Physician to CAB test results

	CAB LR	CAB HR
Total (n = 224)	146	78
Endocrine therapy alone	142 (97%)	9 (12%)
Chemo endocrine therapy	4 (3%)	69 (88%)
Overall concordance	211 (94%)	

Discussion

Precise treatment plan is the key for improved clinical outcomes in early-stage hormone receptor positive, HER2/neu negative breast cancer patients. Oncologists depend on conventional prognostic determinants like age, clinicopathological features, Ki-67, hormone receptor expression levels for making adjuvant chemotherapy decisions. Beyond these factors, data on the prognostic tests have shown that the proliferation markers and other biological features reflective of aggressive tumor biology and cancer relapse are accurate determinants of clinical outcomes [16–19, 22, 23]. CanAssist Breast is one of such a test developed with the incorporation of inputs from tumor biology and clinical features for prediction of risk of recurrence using artificial intelligence-based approach. The study was taken up with the intention to assess agreement on risk assessment made by the physician and CAB. Here we report the impact of CAB on the physician and the change in his/her treatment plan with CAB test results in a prospective cohort.

As per the physician only 46% of the patients were at low risk for recurrence and could be treated with endocrine therapy alone, whereas with CAB it was 68%. Most importantly CAB could segregate the group of patients for whom the physician was uncertain of treating with chemotherapy, into low and high-risk groups. The physician's choice of treating the patients to give or to withhold chemotherapy largely depended on node status, ki67, grade and age of the patient. In the clinical intermediate risk category, the physicians seem to be perplexed as the patient was associated both with clinical low and high-risk features. It is also noteworthy that in patients with node-negative tumors of size up to 5cm, the physicians seem to be more challenged. With increase in tumor size from T1 to T2 size, the percentage of patients in intermediate risk category increased from 23–38%. However, the physicians do not seem to be so puzzled in patients with N1 tumors, where intermediate risk proportions remained the same at 22% with T1 and T2 tumors suggesting that node positivity dominates over tumor size for treatment decisions and physicians seem to be more perplexed with respect to tumor size. With increase in the size of the tumors (T2) with node positivity, physician's choice of treatment for chemotherapy increased from 32% (T1N1) to 56% (T2N1) indicating that patients with large tumors with involved nodes could be considered for chemotherapy (Table 2).

Moreover, of the clinical HR T1N1 patients, 83% (n = 5/6) were identified as LR by CAB implying chemotherapy could be withheld in these patients, although this data is from small numbers. A large database study on 24,740 patients showed a breast cancer survival of 87.4% at five years in T1N1 patients with hormonal therapy alone, recommending chemotherapy benefit only in few patients [26]. A longterm data with 18 years follow-up suggested that systemic therapy would prove beneficial in patients with T1 tumors (both N0 and N1) who have unfavourable prognostic factors like higher histologic grade, blood vessel invasion, lymphovascular infiltrate, lymphatic tumor emboli [27]. Similary in N0 sub-group, 13% higher patients were classified as HR by CAB compared to clinical HR indicating that CAB would prevent undertreatment in these patients [13].

Other than node status, the size of the tumor equally poses a challenge to physician to decide on chemotherapy. In patients with T2 tumors, CAB LR were significantly higher (57%) ($P = 0.0012$) compared to clinical LR (43%) suggestive of prevention of overtreatment in these patients without CAB test recommendation. SEER database showed five year breast cancer survival rates of 89.4% in women with T2N0 tumors again indicating benefit of chemotherapy in small percentage of patients [26].

Likewise in patients expressing high Ki-67, CAB stratified 22% higher as LR compared to clinical LR, again preventing overtreatment in scenarios where decisions were based on Ki67. It was interesting to note that in patients with G3 tumors CAB classified substantially higher proportions (77%) as HR compared to clinical LR (36%) whereas physicians were in a dilemma in good number of patients, 34% (Table 2).

Considering that clinical IR patients could receive chemotherapy in the absence of use of a prognostic test; with CAB based risk stratification 55% of these clinical IR were LR by CAB.

Of the clinical HR group there was change from chemo endocrine therapy t o endocrine therapy alone with CAB in 54% (n = 33) of patients, thus preventing overtreatment for these patients. Likewise, CAB

advised chemotherapy in 26% (n = 30) of clinical low-risk group wherein otherwise physician would treat these patients with endocrine therapy alone, thereby preventing undertreatment in these patients. Thus overall across all the clinical sub-groups (LR, IR and HR), the change in treatment recommendation (ET to CET; CET to ET) was in 42% (n = 104) of the cohort. CAB which focuses on tumor biological features to determine the aggressiveness of disease provides definite precise treatment options for the patients.

Some physicians expressed that before treating the patient they would prefer a recommendation from a prognostic test (n = 70) like CanAssist Breast or any other prognostic test. Of these it is noteworthy that 42 patients were categorised as clinical intermediate risk suggesting that physician wants to be guided by a prognostic test. Few other reasons expressed by physician for performing CanAssist Breast were validation of their decision, avoid chemotherapy (Fig. 2).

Physician's adherence to prognostic tests reports is an important aspect. We found that adherance to CAB test results in 94% of patients.

In conclusion these results emphasize the use of a prognostic test for precise treatment decisions in breast cancer patients. The data evidenced the change in chemotherapy treatment plan and how it resolves the conflict of physician in planning the chemotherapy thereby supporting the clinical utility of this comparatively newer test. With the extensive analytical validation and clinical validation data in patients across geographies we believe that CanAssist Breast will be useful in tailoring the chemotherapy decisions in clinical practice.

Declarations:

Funding:

The principal investigators of the study received educational grant from OncoStem Diagnostics which is privately funded.

Conflicts of interest/competing interests:

Somashekhar S P, MD report of receiving fees for the advisory role from OncoStem Diagnostics. All other authors have no other competing interests to declare.

Code availability:

Not applicable

Availability of data and material:

Available

Acknowledgements:

We thank Aparna Gunda, Ph.D. with data analysis, manuscript writing and review, Mallikarjuna SE, Ph.D. and Krian, Ph.D. with data compilation. We thank Mr. Manoj Kumar Padhiari, Mr. Rudrajit Mohanty, Mr. Ashok Prajapat for coordination with physicians on obtaining data, Ms Vijaya K for following with patients. All are employees of OncoStem Diagnostics Pvt Limited, Bengaluru.

References:

1. Chung M, Chang HR, Bland KI, Wanebo HJ (1996) Younger women with breast carcinoma have a poorer prognosis than older women. Cancer 77: 97-103.
2. Aebi S, Castiglione M (2006) The enigma of young age. Ann Oncol 17: 1475-1477.
3. Nguyen D, Yu J, Reinhold WC, Yang SX (2020) Association of Independent Prognostic Factors and Treatment Modality With Survival and Recurrence Outcomes in Breast Cancer. *JAMA Netw Open* 3(7):e207213. doi:10.1001/jamanetworkopen.2020.7213
4. Lee J, Kim SH, Kang BJ (2020) Prognostic Factors of Disease Recurrence in Breast Cancer Using Quantitative and Qualitative Magnetic Resonance Imaging (MRI) Parameters (2020) Sci Rep 10, 7598. https://doi.org/10.1038/s41598-020-64564-6
5. Nitz U, Gluz O, Christgen M, et al (2017) Reducing chemotherapy use in clinically high-risk, genomically low-risk pN0 and pN1 early breast cancer patients: five-year data from the prospective, randomised phase 3 West German Study Group (WSG) PlanB trial. Breast Cancer Res Treat 165(3):573-583. doi: 10.1007/s10549-017-4358-6.
6. Berry DA, Cirrincione C, Henderson IC, et al (2006) Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. JAMA 12;295(14):1658-67. doi: 10.1001/jama.295.14.1658.
7. Clarke M, Coates AS, Darby SC et al (2008) Early Breast Cancer Trialists' Collaborative Group (EBCTCG) Adjuvant chemotherapy in oestrogen-receptor-poor breast cancer: patient-level meta-analysis of randomised trials. Lancet 5;371(9606):29-40. doi: 10.1016/S0140-6736(08)60069-0.
8. Viale G, Regan MM, Mastropasqua MG, et al (2008) Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. J Natl Cancer Inst 100: 207-12.
9. Nishimura R, Osako T, Okumura Y et al (2010) Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. Exp Ther Med 1:747-54.
10. Esserman LJ, Yau C, Thompson CK et al (2017) Use of molecular tools to identify patients with indolent breast cancers with ultralow risk over 2 decades JAMA Oncol. doi:1001/jamaoncol.2017.1261
11. Sung Ui Jung, Guiyun Sohn, Jisun Kim et al (2019) Survival outcome of adjuvant endocrine therapy alone for patients with lymph node-positive, hormone-responsive, HER2-negative breast cancer. Asian Journal of Surgery 42(10): 914-921, https://doi.org/10.1016/j.asjsur.2019.01.003.

12. Goldhirsch A, Glick JH, Gelber RD. Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005. *Annals of Oncology* 2005; 16: 1569–1583.
13. Fisher B, Dignam J, Wolmark N et al (1997) Tamoxifen and chemotherapy for lymph node-negative, estrogen receptor-positive breast cancer. J Natl Cancer Inst 89: 1673-1682.
14. Wo JY, Chen K, Neville BA et al (2011) Effect of very small tumor size on cancer-specific mortality in node-positive breast cancer. J Clin Oncol 29(19):2619-27. doi: 10.1200/JCO.2010.29.5907.
15. Surabhi Amar, Ann E Mccullogh, Winston Tan et al (2010) Prognosis and Outcome of Small (1 cm), Node-Negative Breast Cancer on the Basis of Hormonal and HER-2 Status The Oncologist 15:1043–1049.
16. Paik S, Shak S, Tang G et al. (2004) A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 351 (27): 2817-26.
17. Chia S, Bramwell VH, Tu D et al (2012) A 50-gene intrinsic subtype test for prognosis and prediction of benefit from adjuvant tamoxifen. Clin Cancer Res 18: 4465–72.
18. Veer LJ, Dai H, Vijver MJ et al (2002) Gene expression profiling predicts clinical outcomes of breast cancer. Nature 415, 530-536
19. Filipits M, Rudas M, Jakesz R et al (2011) 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: 6012–20.
20. Iqbal J, Ginsburg O, Rochon PA et al (2015) Differences in breast cancer stage at diagnosis and cancer-specific survival by race and ethnicity in the United States. JAMA 13;313(2):165-73. doi: 10.1001/jama.2014.17322.
21. Leong SP, Shen ZZ, Liu TJ et al (2010) Is breast cancer the same disease in Asian and Western countries? World J Surg 34(10):2308-24. doi: 10.1007/s00268-010-0683-1.
22. Ramkumar C, Buturovic L, Malpani S et al (2018) Development of a Novel Proteomic Risk-Classifier for Prognostication of Patients With Early-Stage Hormone Receptor-Positive Breast Cancer. Biomark Insights 1930;13:1177271918789100. doi: 10.1177/1177271918789100..
23. Bakre MM, Ramkumar C, Attuluri AK et al (2019) Clinical validation of an immunohistochemistry-based CanAssist-Breast test for distant recurrence prediction in hormone receptor-positive breast cancer patients. Cancer Med 8(4):1755-1764. doi: 10.1002/cam4.2049.
24. Sankaran, S., Dikshit, J.B., Prakash SV et al (2019) CanAssist Breast Impacting Clinical Treatment Decisions in Early-Stage HR+ Breast Cancer Patients: Indian Scenario. Indian J Surg Oncol https://doi.org/10.1007/s13193-019-01014-4
25. Sengupta AK, Gunda A, Malpani S et al (2020) Comparison of breast cancer prognostic tests CanAssist Breast and Oncotype DX. Cancer Med 00:1–9. https://doi.org/10.1002/ cam4.3495
26. Carter CL, Allen C, Henson DE. (1989(Relation of tumor size, lymph node status and survival in 24,740 breast cancer cases. Cancer 63: 181–187.

27. Rosen PR, Gorsehn S, Saigo, PE et al (1989) Patholgoical Prognostic Factors in stage I (T1N0M0) and stage II (T1N1M0) Breast Carcinoma: A study of 644 patients with median follow-up of 18 years. J Clin Oncol 7 (9): 1239-1251

Figures

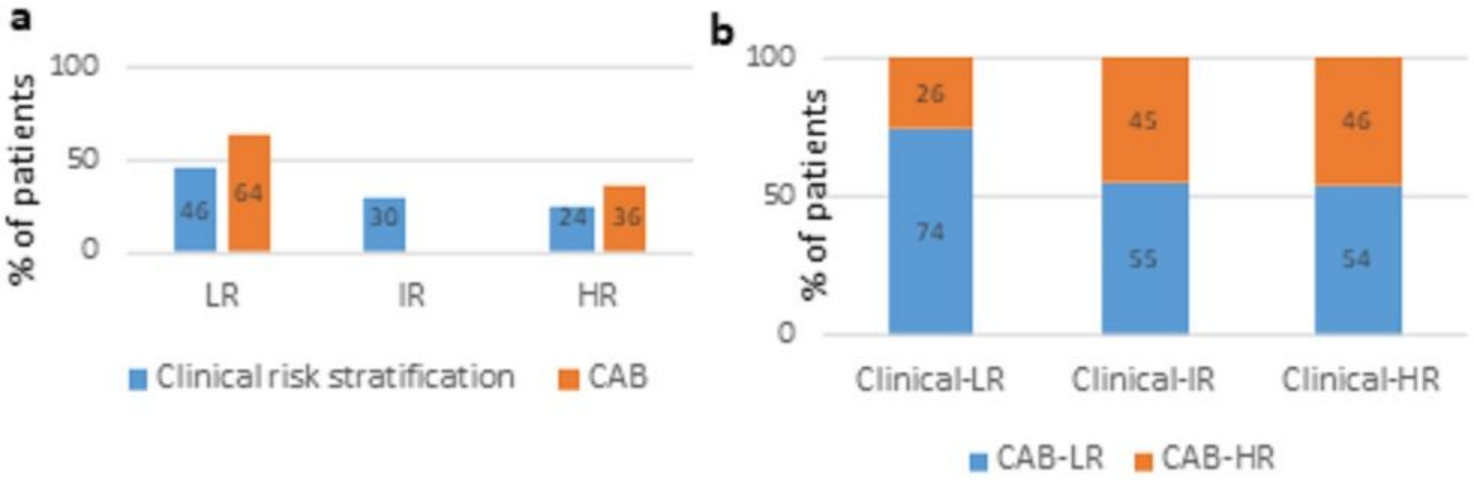

Figure 1

Risk proportions by clinical assessment and CanAssist Breast (a), restratification of clinical risk categories by CAB (b) Abbreviations: LR=low-risk; IR=Intermediate-risk, HR=High-risk

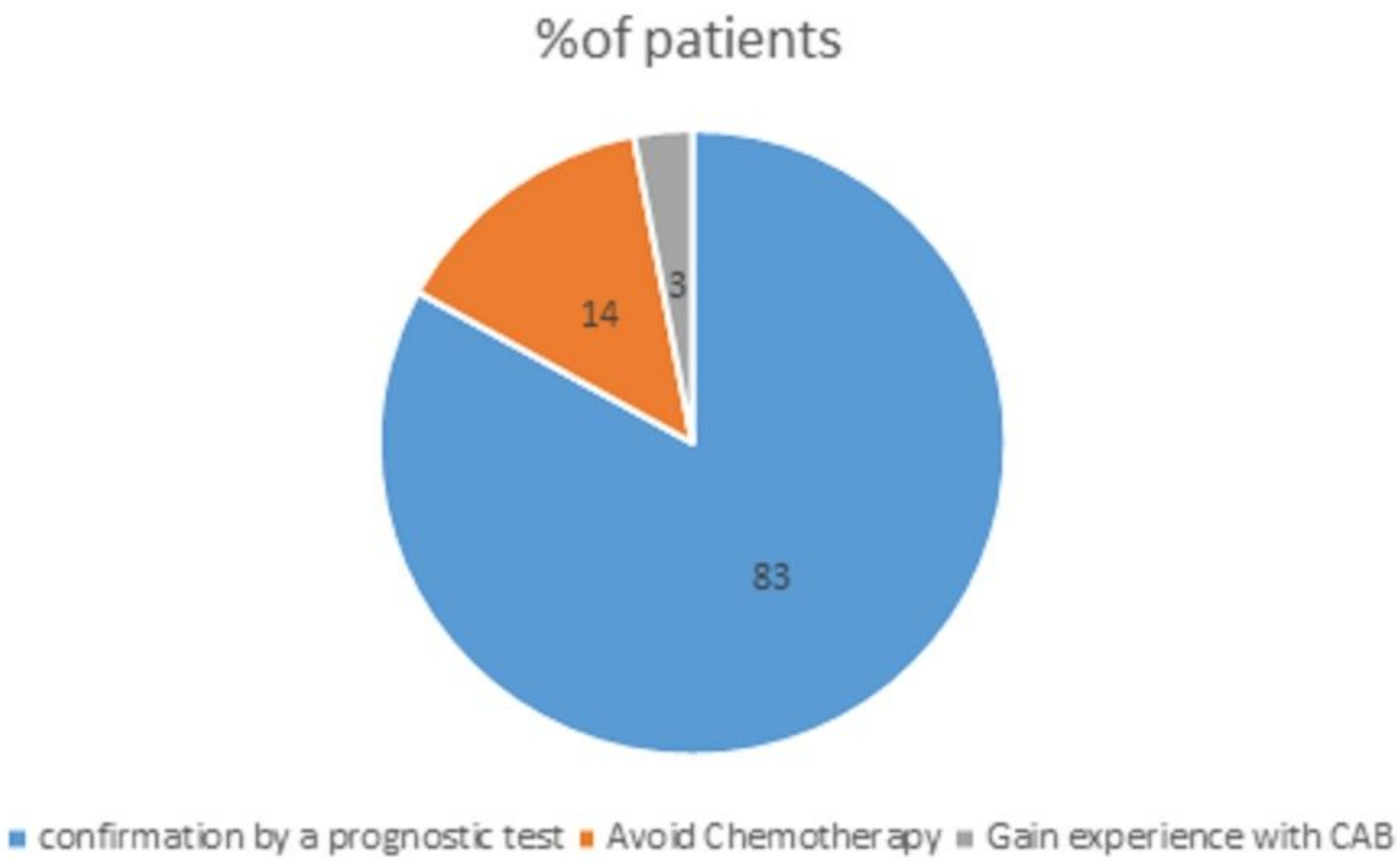

Figure 2

Pie chart showing various scenarios in which CAB test was prescribed.