BMJ Open

Multicentric study to evaluate the effectiveness of Thermalytix as compared with standard screening modalities in subjects who show possible symptoms of suspected breast cancer

Akshita Singh,[1] Venkatraman Bhat,[2] S Sudhakar,[3] ArunKumar Namachivayam,[4] Charitha Gangadharan ,[5] Candice Pulchan,[6] Alben Sigamani[7]

To cite: Singh A, Bhat V, Sudhakar S, *et al*. Multicentric study to evaluate the effectiveness of Thermalytix as compared with standard screening modalities in subjects who show possible symptoms of suspected breast cancer. *BMJ Open* 2021;**11**:e052098. doi:10.1136/bmjopen-2021-052098

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2021-052098).

Received 15 April 2021
Accepted 06 September 2021

For numbered affiliations see end of article.

Correspondence to
Dr Alben Sigamani;
albens1227@gmail.com

ABSTRACT

Introduction Machine learning in computer-assisted diagnostics improves sensitivity of image analysis and reduces time and effort for interpretation. Compared to standard mammograms, a thermal scan is easily scalable and is a safer screening tool. We evaluate the performance of Thermalytix (an automated thermographic screening algorithm) compared with other standard breast cancer screening modalities.

Methods A prospective multicentre study was conducted to assess the non-inferiority of sensitivity of Thermalytix (test device) to that of standard modalities in detecting malignancy in subjects who show possible symptoms of suspected breast cancer. Standard screening modalities and Thermalytix were obtained and interpreted independently in a blinded fashion. A receiver operating characteristic (ROC) curve was constructed to identify the best cut-off point, non-inferiority margin of ≥10% to demonstrate the non-inferiority.

Results We recruited 258 symptomatic women who first underwent a thermal scan, followed by mammogram and/or ultrasound. At Youden's Index of ROC curve, the test device had a sensitivity of 82.5% (95% CI 73.2 to 91.9) and specificity of 80.5% (95% CI 75.0 to 86.1) as compared with diagnostic mammogram, which had sensitivity of 92% (95% CI 80.7 to 97.8) and specificity of 45.9% (95% CI 34.3 to 57.9) when BI-RADS 3 (Breast Imaging-Reporting and Data System) was considered as test-positive. The overall area under the curve (AUC) was 0.845. For women aged <45 years, the test device had a sensitivity and specificity of 87.0% (95% CI 66.4 to 97.2) and 80.6% (95% CI 72.9 to 86.9), respectively. For women aged ≥45 years, the sensitivity and specificity were 80.5% (95% CI 65.1 to 91.2) and 86.5% (95% CI 78.0 to 92.6, respectively).

Conclusion We evaluated Thermalytix, a new AI-based modality for detecting breast cancer. The high AUC in both women under 45 years and above 45 years shows the potential of Thermalytix to be a supplemental diagnostic modality for all ages. Further evaluation on larger sample size is needed.

Strengths and limitations of this study

- This was the first-ever systematic blinded study that prospectively compared a computer-assisted thermal technology with standard imaging modalities to detect breast abnormalities .
- This is also the first-ever study evaluating a computer-assisted thermal technology to detect breast abnormalities in a study population that was not previously diagnosed with breast cancer through standard imaging modalities.
- The study also gives an indication of the benefit of combining thermal imaging with breast ultrasound.
- Women who screened positive on mammography/ultrasound were confirmed by biopsy, but lesions identified by Thermalytix but not reported on mammography as positive were excluded from biopsy, which may have impaired the sensitivity and specificity.
- As the study was performed only on symptomatic women, a large-scale screening study needs to be undertaken to evaluate on asymptomatic population as well.

Trial registration number CTRI/2017/10/010115;

INTRODUCTION

Globally, 18 million new cases of cancers were recorded in 2018. Nearly 2.1 million (11.6%) cases were attributed to breast cancer alone,[1] the leading cause of cancer-related death in women.[1] Breast cancer among young women has been on the rise and is increasing cancer-related mortality despite advances in diagnosis and treatment.[2] Breast cancers must be detected at an early stage to increase progression-free survival. Effective screening of women in their reproductive age group

needs regular community surveillance for breast cancer, with tools that exceed the sensitivity or specificity of clinical or self-breast examination.[3]

A screening mammogram reduces breast cancer mortality by 23% but has major limitations in women with high breast density and young women. Women with dense breasts have higher risk for cancer[4] and nearly a 18-fold risk for interval cancer.[3] A screening ultrasound has higher sensitivity and specificity than a mammogram when performed on women with dense breasts. However, ultrasound requires skilled radiologists to perform the scans for arriving at any clinical decision.[5] In certain countries, like India, the use of ultrasound is regulated (Pre-Conception and Pre-Natal Diagnostic Techniques Act, 1994) proving a major impediment to scaling up ultrasound for community screening. Artificial intelligence (AI) can alleviate subjectivity and need for expertise in interpretation of medical images.[6] The introduction of machine learning (ML)-enabled digital mammograms and automated breast ultrasound devices have alleviated the technical burden of performing higher volumes of breast cancer screening, but the cost is prohibitive for adoption in larger programmes.[7]

Though thermography was introduced in the latter part of the 20th century as an adjunct to screening mammograms, it has not been fully adopted.[8] Thermography along with an ML classifier, trained by giving extracted features, specifically corresponding to metabolic, physical, structural, symmetrical and vascular properties of the tumour could reduce the false-positive and false-negative rates.[9]

The first clinical study to evaluate the performance of thermography was done in 1980[10] in over 58 000 symptomatic women. Among these women, 1245 women diagnosed normal or benign by conventional screening exhibited questionable thermal anomaly using thermography.[10] More than a third of these women developed cancer in the 5-year follow-up period.[10] This study generated a spike in thermography-based breast cancer screening studies using primitive technology before being discredited and eventually seeing a lack of enthusiasm from practitioners as interpretations of thermal images suffered from subjectivity. Although approved as an adjunctive modality for breast cancer screening by Food and Drug Administration (FDA) in 1982, thermography was not widely accepted due to the complexity involved in interpreting thermograms with naked eye and resulting high false positivity. However, in recent years, infrared breast thermography is re-emerging as a modality with promising results for detecting breast cancer because of better thermal sensors, improved algorithms and reducing manual error by using AI.[11]

The sensitivity of thermography in detecting breast cancer in previous studies ranged from 53% to 100%. In the study that yielded 100% sensitivity, the study sample had prior mammography, ultrasonography (USG), Fine needle aspiration (FNA) and was scheduled for surgery, thus bringing in a bias.[12] Similarly, the disease characteristics of most study samples were known as they included women with suspicious breast lesions identified by mammography or ultrasound or Magnetic resonance imaging (MRI),[12–14] or for whom a diagnostic biopsy was recommended,[15] or diagnosed with breast cancer.[16–18] Also, in some studies, the comparator test/reference standard tests were performed before thermography,[8] which could result in an interpretation bias. In other studies, the order of testing was appropriate,that is, index test was performed before comparator test, but the study design involved retrospective incorporation of healthy and cancer cases only and excluding Breast Imaging-Reporting and Data System (BI-RADS) 2 3 cases[19] or including only abnormal cases.[20] In one of the studies, only thermography-positive tests were followed up with further investigations, while the journey of patients with normal thermogram was not mapped.[21] In another study where thermography was performed before other tests, thermography had a high sensitivity of 95.24%, although in a small sample of 54 symptomatic women.[22] To the best of our knowledge, this study addresses all these limitations.

In this study, we evaluated a novel AI-based thermography solution and compared it with standard-of-care tests in a multisite, prospective cohort of women with symptomatic breast lesions, where order of testing was followed. We hypothesised that using ML algorithms to analyse and interpret thermograms will result in a test that is non-inferior to mammography in general and superior to mammography in women with dense breasts (American College of Radiology categories C & D). The test device Thermalytix, a computer-assisted diagnostic (CAD) platform, was compared against mammography and combined with ultrasound for dense breasts. While most ML algorithms have been evaluated retrospectively in a non-clinical setting, this study would be the first of its kind study evaluating the novel ML algorithm in a prospective scientific study in a clinical setting.[23]

METHODS

Study design and study population

A prospective two centre cohort study was conducted at cancer care centres, located in Bangalore, South India. The study was registered in Clinical Trial Registry. The Clinical study protocol approved by Ethics Committee is submitted as ‘online supplemental file'.

At a tertiary care hospital, younger women with breast symptoms also seek care. Hence, we included women aged 18 years and above with a report of any one of the following breast symptoms: palpable lump, swelling or mass in breast; persistent breast pain or tenderness or increase in breast size that is unrelated to menstrual cycle; inverted or tender nipple; nipple that is painful, scaly or with discharge (clear or bloody); skin on breast that is dimpled, red, blotchy, prickly, itchy or has changed in the texture; thickening in or around the breast or underarm

area or any other symptoms leading to suspicion of breast cancer.

Pregnant and/or lactating women or those who had undergone either lumpectomy or mastectomy earlier, or had chemotherapy for any cancer in the last 2 weeks or had active illness, psychological and/or pathological conditions that could interfere with study participation were excluded. All participants provided written informed consent for participation before performing the procedures in the study.

All women underwent a clinical breast examination before they presented to the radiology department to undergo a diagnostic mammogram and/or a breast ultrasound. All patients were approached by a clinical research staff member to seek consent for thermal imaging prior to the intended imaging procedure. Before thermal imaging, the upper body of the participant is cooled for around 15 minutes in a room, which was maintained between 22°C and 24°C using an external cooler. This precooling ensures accurate interpretation by the AI-based software, irrespective of the ambient temperature in the screening room.

Thermal images from the participant obtained from five different views were uploaded into the AI-based software system and results were generated. Any suspicious lesion as per clinician's discretion was biopsied. Results of all standard screening tests and biopsy remained blinded till the end of study, to the technician obtaining the thermal image and also to the analytical team recording the Thermalytix score from the software.

Patient and public involvement statement

Patients and/or the public were not involved directly in the design, or conduct, or reporting or dissemination plans of this research. Patient consent was taken for enrolling them into the study, and information on the possibilities of publication of the study results was included in the informed consent form.

A brief overview of study device

Thermalytix is a CAD software built on AI algorithms to automate interpretation of thermal images to generate a report with quantitative scores. An earlier conducted retrospective study using Thermalytix for automated diagnosis based on breast thermal images had shown the feasibility of automated interpretation.[24]

The study device has an infrared camera connected to a laptop used to access the patented Thermalytix software (Patent numbers: US 9898817, US 10307141, US 10055542, US 9622698). The thermal image is a representation of temperature variations on the skin captured using an infrared camera with a resolution of 320×240 pixels and thermal sensitivity of 0.02 °C (degree centigrade).[9] The software runs a pretrained AI-model to analyse breast thermal images and to provide a score that classifies a specific lesion as malignant or benign. The generated score is a real number between 0 and 1, indicating the probability of malignancy, with 0 being normal and 1 having a high probability of malignancy. Additional parameters such as thermal symmetry between the breasts, temperature and structure of blood vessels were also available in the report in addition to an overall score (algo2) and a separate vascularity score, to indicate any asymmetry in vascular structures between the breasts. Figure 1 represents the picture of the Thermalytix device.

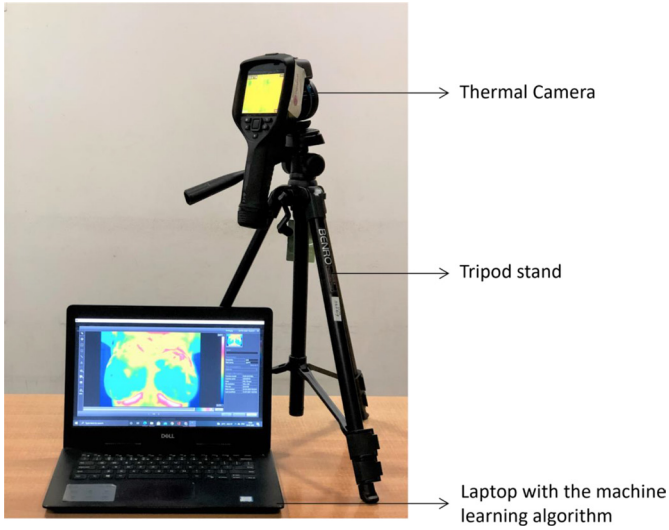


Figure 1 Test device—Thermalytix and its accessories.

Outcomes and statistical analysis

The conclusions drawn from mammogram,USG, and the thermal AI score were evaluated against the histopathology results for sensitivity and specificity. Continuous variables are described using median, categorical variables using frequency and percentages. The sensitivity of Thermalytix and that of standard modalities were compared with assessing non-inferiority. The primary objective of non-inferiority was assessed by constructing a 95% CI around the difference in sensitivities (Thermalytix—standard modalities). If the lower limit of the 95% CI was greater than −10%, then non-inferiority was established. In women aged 45 years and above, we compared the score to mammography alone and in women younger than 45 years of age, we combined ultrasound and/or mammography. During the study, any subject with a score greater than an arbitrary cut-off point of 0.5 was considered as malignant. On study completion, we constructed a receiver operating characteristic (ROC) curve to identify the best cut-off point at Youden Index. ROC curve analysis on Thermalytix scores was performed using this threshold .

Monitoring

Both clinical study sites were monitored by an independent team of clinical monitors. All procedures were monitored for compliance to protocol and Good Clinical Practices.

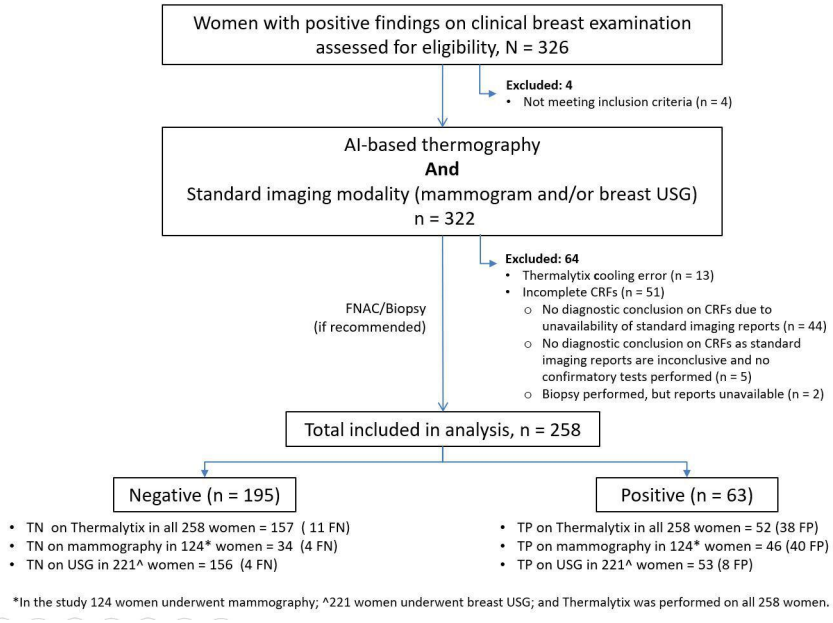


Figure 2 Flowchart illustrating the number of women enrolled and included in the analysis. *In the study, 124 women underwent mammography; ˆ221 women underwent breast USG; and Thermalytix was performed on all 258 women. USG, Ultrasonography: Case Report Form (CRF), Fine Needle Aspiration Cytology (FNAC), False Positives (FP), False Negatives (FN), True Positives (TP), True Negative (TN)

RESULTS

Between 21 September 2017 and 31 July 2018, 326 women who had symptoms on a clinical breast examination were consecutively recruited from two centres in Bangalore, Karnataka. Figure 2 provides an overview of the study flow.

Among all women recruited at the two centres during the study period, 68 women could not be included in the study analysis for the following reasons: 4 women did not meet inclusion criteria, 44 women were not included in the study as they did not have a diagnostic conclusion in the case report form, 5 women did not undergo a confirmatory test after an inconclusive imaging test, biopsy reports were unavailable for two women, and in 13 women, the thermal images obtained could not be used for interpretation due to insufficient cooling. Among the cohort of 258 women, 63 women (24.4%) were diagnosed with malignant breast cancer. Table 1 describes basic demographics and symptoms of all women by diagnosis (malignant or benign).

The median age of the cohort was 41 years with a range between 18 and 80 years. The median age among women diagnosed with cancer was 55 years (range: 26–78 years) compared with women without cancer, 40 years (range: 18–80 years), p<0.001. Detailed information on age categorisation on this cohort is provided in table 2. Among the cohort of 258 women, 149 women (57.8%) were aged <45 years, 92 women (35.7%) had attained menopause and 38 women (14.7%) had a family history of cancer. A palpable lump was the most common symptom on presentation in 71.7% of the study population followed by 57.0% women

Table 1 Study population characteristics based on final diagnosis

Parameters	Total (n=258)	Final diagnosis		P value
		Negative (n=195)	Positive (n=63)	
Age: median (range)	41 (18–80)	40 (18–80)	55 (26–78)	<0.001
Menopause	92 (35.7%)	54 (27.7%)	38 (60.3%)	<0.001
Relevant medical history	17 (6.6%)	10 (5.1%)	7 (11.1%)	0.139
Family history of cancer	38 (14.7%)	31 (15.9%)	7 (11.1%)	0.418
Lump, swelling or mass in breast	185 (71.7%)	129 (66.2%)	56 (88.9%)	<0.001
Breast pain/tenderness unrelated to menstrual cycle	147 (57.0%)	126 (64.6%)	21 (33.3%)	<0.001
Texture change on breast	12 (4.7%)	10 (5.1%)	2 (3.2%)	0.736
Increase in breast size unrelated to menstrual cycle	4 (1.6%)	4 (2.1%)	0 (0.0%)	0.575
Thickening in and around breast or underarm area	4 (1.6%)	3 (1.5%)	1 (1.6%)	0.999
Other symptoms of breast cancer	3 (1.2%)	2 (1.0%)	1 (1.6%)	0.575

Table 2 Age categorisation in recruited patients

	Final diagnosis					
	Negative		Positive		Total	
	Number	%	Number	%	Number	%
Age (years)						
<30	27	13.8	2	3.2	29	11.2
30–45	107	54.9	21	33.3	128	49.6
>45	61	31.3	40	63.5	101	39.1
Total	195	100.0	63	100.0	258	100.0

reporting of breast pain/tenderness unrelated to the menstrual cycle.

In the entire cohort of 258 women, 124 (48.1%) women underwent diagnostic mammograms and 221 (85.7%) had a breast USG. Among them, only 67 women had a biopsy for the suspicious lesion detected either on mammogram, or USG or both. Four women were disease-negative on biopsy even though they had a positive scan report in USG/mammogram. Thirty-eight women with breast cancer (60.3%) had attained natural menopause in the disease-positive group compared with 54 women (27.7%) in disease-negative group (p<0.001). Other significant variables that differed between the disease-positive and disease-negative groups were lump, swelling or mass in breast, breast pain or tenderness unrelated to menstrual cycle (p<0.001). Among the 63 women who were diagnosed with malignant breast lesions, lesion size information was available for 53 women. Of these, 15 women had T1 lesions that measured 2 cm or less on a breast USG.

The diagnostic mammogram had a sensitivity of 92% (95% CI 80.7 to 97.8) and specificity of 45.9% (95% CI 34.3 to 57.9) assuming BI-RADS (Breast Imaging-Reporting and Data System) 3 as test positive (table 3). The initial sensitivity of the test device, with cut-off score of 0.5, for detecting malignancy, was 74.6% (95% CI 63.9 to 85.4) and specificity was 82.1% (95% CI 76.7 to 87.4). With the post hoc cut-off score of 0.41, the sensitivity of the test device was 82.5% (95% CI 73.2 to 91.9) and specificity was 80.5% (95% CI 75.0 to 86.1) (figure 3 and table 3).

In women younger than 45 years of age, the sensitivity of test device was 87.0% (95% CI 66.4 to 97.2) and specificity was 80.6% (95% CI 72.9 to 86.9) at a cut-off score of 0.43 and the area under the ROC (AUROC) of 0.846. For women with age greater than or equal to 45 years, we observed a sensitivity of 80.5% (95% CI 65.1 to 91.2) and a specificity of 86.5% (95% CI 78.0 to 92.6) at a cut-off value of 0.41 and AUC was found to be 0.875 (figure 4). The test device was within the non-inferiority margin of 10% in sensitivity as compared with mammography.

Among 124 cases who underwent mammograms, 30 women were tagged as BI-RADS 0 (inconclusive). When they underwent an USG examination, three women were reported as positive and were confirmed as breast malignancies on biopsy. All these three breast malignant cases were also detected by Thermalytix as positive for malignancy.

In order to evaluate possible clinical workflows that can complement the gaps in current modalities, the sensitivity and specificity of a workflow where a person found positive on Thermalytix undergoes a breast ultrasound examination were computed. This workflow is analogous to standard

Table 3 Comprehensive table showing the results of mammogram and Thermalytix against biopsy findings

	Malignancy (Final Diagnosis)			Estimate (%) 95% CI				
	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV	Accuracy
Mammography positive (BI-RADS 3 considered as positive)	46	40	86	92.0 (80.7 to 97.8)	45.9 (34.3 to 57.9)	53.5 (47.9 to 59.0)	89.5 (76.3 to 95.7)	64.5 (55.4 to 72.9)
Mammography negative	4	34	38					
Total	**50**	**74**	**124**					
Thermalytix positive (cut-off 0.41)	52	38	90	82.5 (73.2 to 91.9)	80.5 (75.0 to 86.1)	57.8 (47.6 to 68.0)	93.5 (89.7 to 97.2)	81.0 (76.2 to 85.8)
Thermalytix negative	11	157	168					
Total	**63**	**195**	**258**					

Total women underwent mammography124 (50 Positive + 74 Negative for malignancy) Total Women underwent Thermalytix 258 (63 Positive + 195 Negative for malignancy)
BI-RADS, Breast Imaging-Reporting and Data System; NPV, Negative Predictive Value; PPV, Positive Predictive Value.

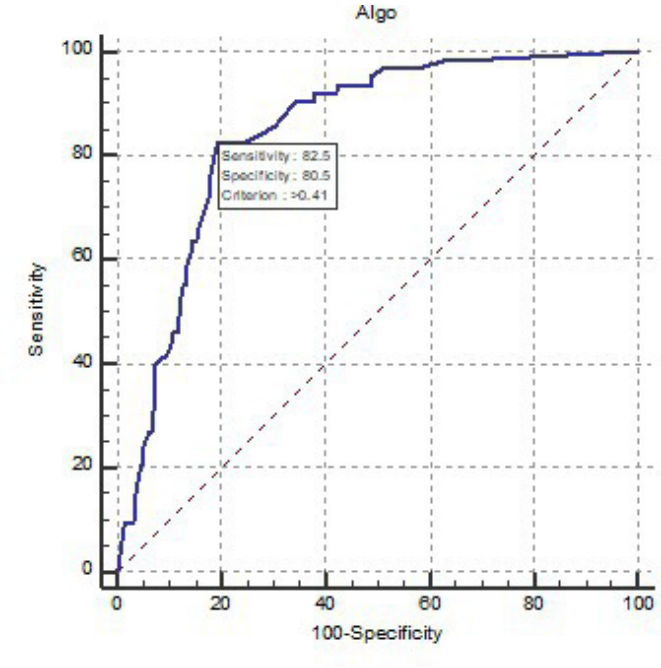


Figure 3 ROC curve with sensitivity and specificity at a cut-off of >0.41 for Thermalytix score and the AUC was found to be 0.845. AUC, area under the curve; ROC, receiver operating characteristic.

practice followed in India, where a positive mammography is sent for a diagnostic correlation with an ultrasound. If the test device is used instead of mammogram to screen women before referring them for an ultrasound, the sensitivity of the combined modality would have been 81.0% and specificity would be 96.4%.

DISCUSSION

Nearly, a quarter of the women who had symptomatic breast lesions had malignancy confirmed. The study was conducted in two centres both located in Bangalore, acity that has reported a recent spike in breast cancers among women.[25] Thermalytix performed non-inferior to mammography . Its sensitivity or specificity was also similar to ultrasound in women aged 45 years or younger, the typical age group with dense breasts. There are various key strengths in this study. We prospectively screened women coming to a tertiary care hospital with a symptom and demonstrated a feasible workflow for the CAD to perform as an adjuvant to existing breast screening modalities. The study demonstrated the simplicity and scalability of the solution, as Thermalytix tests in both centres were conducted by low-skilled technicians with just 2 days of training. The technicians who captured the thermal images and uploaded them to the Thermalytix test device were blinded from results derived from the mammogram and/or the ultrasound which were conducted after Thermalytix test. The tool automatically computed the scores on the thermograms. We derived a new cut-off for differentiating malignant lesions from benign lesions through construction of ROC curve, which was based on the prospective study results. The new cut-off improved the sensitivity and negative-predictive value of CAD in breast cancer screening. Being a simple to use and easy to integrate device with automated reporting, the study gives confidence to adopt the same for any setting. It further restores faith in use of thermography for screening at greater frequency among women across all age groups.

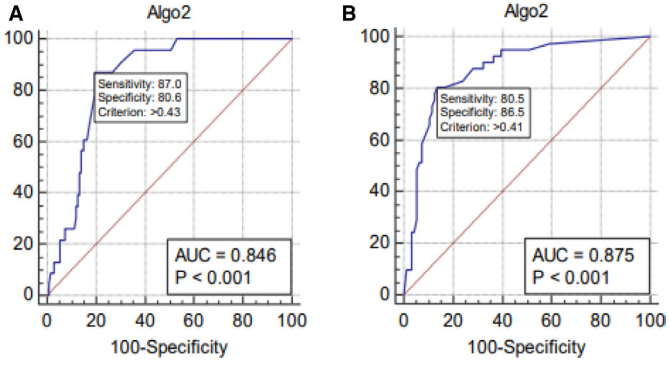


Figure 4 ROC curves find optimal cut-off points of Thermalytix score for breast cancer diagnosis in women aged (A) 45 years or younger and (B) above 45 years. AUC, area under the curve; ROC, receiver operating characteristic.

Thermalytix also has the potential to be used as a supplement to mammography in cases of inconclusive diagnosis (eg, women with dense breasts). Thermalytix could provide a definitive diagnosis in women reported with BI-RADS 0 on mammography. Thus, based on this clinical study, Thermalytix could be recommended as a first-line modality for diagnostic screening. Those who are found positive by Thermalytix can be sent to an ultrasound test for confirmatory diagnosis before recommending for histopathology examination. This workflow complements the gap in current breast cancer screening procedures.

Our study did have certain limitations. Inability to include asymptomatic women seen during the study period may have affected our sensitivity or specificity. A predetermined stratum for recruiting symptomatic and asymptomatic women with dense breasts (American College of Radiology Category C and D- ACR category Cand D) could have increased their sample numbers and provided a better insight into utility in this group. We were limited to recommending a biopsy only in women with a suspicious lesion on mammography and/or ultrasound. This may have led to missing women with early malignant lesions that were not picked up on mammography. Further studies may have to be done to prove the true sensitivity and specificity for picking up early stage breast cancer.

Globally, the incidence of breast cancer is on the rise with breast cancer in women aged between 15 and 49 years in low and middle-income countries (LMICs) like India being two times than seen in developed countries.[26] Predominantly, younger women have dense breasts in which a mammogram has low sensitivity (62% to 68%).[27] In this study, the AUROC curve for subgroup of women aged 45 years or younger is 0.846 and is sensitive in picking up malignancy in symptomatic women.

For successful implementation of any population-based breast cancer screening programme, it is important for

a healthcare system to provide an accurate diagnosis (benign vs malignant), for women with clinically detectable disease.[28] Mammography-based screening programmes are expensive for developing and under-developed countries.[29] According to the 2003 World Health Survey, only 2% of women aged 40–69 years had received any breast cancer screening in LMIC.[30] This low yield of screening could be attributed to many factors including lack of accessible, affordable solutions to detect breast cancers at an early stage.[31]

Task sharing and task shifting initiatives adopted in many in LMICs employ low-skilled personnel to perform traditionally complex procedures.[31 32] The focus on population screening in LMICs, currently, is to downstage the disease at presentation rather than establishing a mortality benefit.[28] Compared with the use of mammograms and/or ultrasound, Thermalytix is a feasible and efficient system. A high-resolution thermal camera can be procured at US$10 000 as compared with a 2D mammogram that costs US$350 000.[33] Not only does the Thermalytix device cost less than a 2D mammography machine, it also requires minimal infrastructure. It does not need radiation protection, is easily portable, weighs less than 3 Kilogram (kg) and can be accommodated in a cabin-size bag. It takes less than 15 minutes to complete the setting up of the screening area using a foldable tripod, a laptop housing the software to analyse thermal images and a printer to generate the quantitative report. Furthermore, the test can be performed easily by even low-skilled personnel. As the test is performed in a closed room/booth, and the technician does not see or touch the participant, the test ensures privacy and, thus, reduces the inhibition a woman may have in taking a test that assesses her private parts. This is a very important factor for adoption in a country like India.

Thermalytix is a solution that can be deployed even during an emergency such as the pandemic (COVID-19). This solution could provide screening in the population as the device is a no-see, no-touch, privacy aware solution. Even maintaining safe social distance between the operator and the woman seeking breast cancer screening is feasible, as the operator need not come in close contact to instruct the subject wanting to be screened.

CONCLUSION

While screening mammograms help to reduce mortality due to breast cancer, there are some limitations of the technique. A routine mammography is not feasible in a community setting due to its high cost, challenge of accessibility and lack of experienced professionals required to interpret the images. In this study, we evaluated the clinical performance of a new device called Thermalytix, a modality based on AI over thermal imaging, which showed higher sensitivity for detecting breast cancer in women who presented with symptomatic breast lesions. Thermalytix with high AUC of 0.846 and 0.875 for women <45 years and ≥45 years, respectively, is a promising screening tool among women in all age groups. Further studies with larger sample size are required to evaluate the efficacy and performance of Thermalytix for its definitive role in routine screening. New screening modalities like Thermalytix, a CAD platform that demonstrated non-inferior sensitivity and specificity to mammogram and ultrasound in women with symptomatic breast lesions are likely to be used more extensively in the near future.

Author affiliations
[1]Department of Surgical Breast Oncology, Mazumdar Shaw Medical Centre, Narayana Hrudayalaya Limited, Narayana Hrudayalaya Health City, Bangalore, Karnataka, India
[2]Department of Radiology, Mazumdar Shaw Medical Centre, Narayana Hrudayalaya Limited, Narayana Hrudayalaya Health City, Bangalore, Karnataka, India
[3]Department of Radiology, HCG Cancer Hospital, HealthCare Global Enterprises Ltd, Bangalore, Karnataka, India
[4]Department of Data Science, Alke Research Private Limited, Bangalore, India
[5]Department of Clinical Research, Narayana Hrudayalaya Limited, Narayana Hrudayalaya Health City, Bangalore, Karnataka, India
[6]Department of Radiology (Ultrasonographer III (Ag)), South-West Regional Health Authority, San Fernando, Trinidad and Tobago
[7]Department of Clinical Research, Narayana Hrudayalaya Health City, Bangalore, Karnataka, India

Acknowledgements The authors like to thank the sponsor for financial support. Dr Geetha Manjunath and Dr Lakshmi Krishnan for reviewing and providing valuable suggestions and comments in shaping this article. We would also thank Srirang Ramamoorthy for the constant support in the trial and the patients who participated in the trial. We would like to thank Ms Mala Srivastava and her team from Nextvel Consulting for independent data management and coordination of the multi-site clinical study.

Contributors Conceptualisation,study design and overall content guarantor : Alben Sigamani. Clinical inputs and patient resources: Akshita Singh. Investigations (radiological imaging): Venkatraman Bhat and Sudhakar S. Statistical analysis: ArunKumar Namachivayam. Drafting of the manuscript: Charitha Gangadharan, Candice Pulchan. Accountable for all aspects of the work: all the authors have read and approved the final version for publication.

Funding The study was sponsored by Niramai Health Analytix which manufactures the device being tested in this study, Thermalytix. While the sponsors provided inputs to the study design that was submitted and approved later by an independent ethics committee, the study was conducted with no interference from the sponsors. Furthermore, representatives from the sponsor had only view-access to the manuscript before the submission. The writing of the manuscript was not influenced by the sponsors and only the authors were involved in the process of writing and publication. Award/grant number: MST2017

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval Ethical approval for the study was gained from the Narayana Health Medical Ethics Committee. Ethics committee approvals were obtained from both the institutions prior to the start of the study (EC approval number: NHH/MEC-CL-2017-466).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. Data are available upon request

ORCID iD

Charitha Gangadharan http://orcid.org/0000-0002-4623-1694

REFERENCES

1 Ferlay J, Colombet M, Soerjomataram I, *et al*. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019;144:1941–53.
2 Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Abate D, *et al*. Global, regional, and National cancer incidence, mortality, years of life lost, years lived with disability, and Disability-Adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the global burden of disease study. *JAMA Oncol* 2019;5:1749–68.
3 Linver MN. 4–19 mammographic density and the risk and detection of breast cancer. *Breast Dis* 2008;18:364–5.
4 Yaghjyan L, Colditz GA, Collins LC, *et al*. Mammographic breast density and subsequent risk of breast cancer in postmenopausal women according to tumor characteristics. *J Natl Cancer Inst* 2011;103:1179–89.
5 Weigert J, Steenbergen S. The Connecticut experiment: the role of ultrasound in the screening of women with dense breasts. *Breast J* 2012;18:517–22.
6 Gampala S, Vankeshwaram V, Gadula SSP. Is artificial intelligence the new Friend for radiologists? A review article. *Cureus* 2020;12:e11137.
7 Al-Antari MA, Al-Masni MA, Kim T-S. Deep learning computer-aided diagnosis for breast lesion in digital mammogram. *Adv Exp Med Biol* 2020;1213:59–72.
8 Omranipour. Comparison of the Accuracy of Thermography and Mammography in the Detection of Breast Cancer,", 2016. Available: http://files/751/Omranipour_2016.pdf
9 Zuluaga-Gomez J, Zerhouni N, Al Masry Z, *et al*. A survey of breast cancer screening techniques: thermography and electrical impedance tomography. *J Med Eng Technol* 2019;43:305–22.
10 Gautherie M, Gros CM. Breast thermography and cancer risk prediction. *Cancer* 1980;45:51–6.
11 Ghobadi H, Thainimit S, Sugino N. Comparative accuracy of digital infra-red thermal imaging (DITI) in breast cancer diagnosing. *J Chem Pharm Res* 2016;8:577–83.
12 Brkljacić B, Miletić D, Sardanelli F. Thermography is not a feasible method for breast cancer screening. *Coll Antropol* 201;37:589–93.
13 Arora N, Martins D, Ruggerio D, *et al*. Effectiveness of a noninvasive digital infrared thermal imaging system in the detection of breast cancer. *Am J Surg* 2008;196:523–6.
14 Wishart GC, Campisi M, Boswell M, *et al*. The accuracy of digital infrared imaging for breast cancer detection in women undergoing breast biopsy. *Eur J Surg Oncol* 2010;36:535–40.
15 Moskowitz M. Efficacy of computerized infrared imaging. *AJR Am J Roentgenol* 2003;181:596.
16 Head JF, Wang F, Lipari CA, *et al*. The important role of infrared imaging in breast cancer. *IEEE Eng Med Biol Mag* 2000;19:52–7.
17 Arena, Francis, Clement Barone, and Thomas DiCicco, "Use of digital infrared imaging in enhanced breast cancer detection and monitoring of the clinical response to treatment," Proceedings of the 25th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (IEEE Cat. No. 03CH37439).Vol. 2.IEEE, 2003. Available: https://www.infraredbreasthealth.com/PDF%20Files/digital%20infrared%20imaging%20in%20enhanced%20breast%20cancer%20detection.pdf
18 Prasad SS, Ramachandra L, Kumar V, *et al*. Evaluation of efficacy of thermographic breast imaging in breast cancer: a pilot study. *Breast Dis* 2016;36:143–7 https://content.iospress.com/articles/breast-disease/bd236
19 Sella T, Sklair-Levy M, Cohen M, *et al*. A novel functional infrared imaging system coupled with multiparametric computerised analysis for risk assessment of breast cancer. *Eur Radiol* 2013;23:1191–8.
20 Xiaoli Y. A comparison of mammography, ultrasonography, and far-infrared thermography with pathological results in screening and early diagnosis of breast cancer. *Asian Biomed* 2017;8:11–19 https://www.sciendo.com/article/10.5372/1905-7415.0801.257
21 Rassiwala M, Mathur P, Mathur R, *et al*. Evaluation of digital infra-red thermal imaging as an adjunctive screening method for breast carcinoma: a pilot study. *Int J Surg* 2014;12:1439–43.
22 Sarigoz T, Ertan T, Topuz O. Role of digital infrared thermal imaging in the diagnosis of breast mass: a pilot study: diagnosis of breast mass by thermography. *Infrared Phys Technol* 2018;91:214–9.
23 Kakileti ST. Machine learning for breast cancer diagnosis in developing Countries.ProefschriftMaken, 2020. Available: https://doi.org/10.26481/dis.20201109st
24 Kakileti ST, Madhu HJ, Krishnan L, *et al*. Observational study to evaluate the clinical efficacy of Thermalytix for detecting breast cancer in symptomatic and asymptomatic women. *JCO Glob Oncol* 2020;6:1472–80.
25 Malvia S, Bagadi SA, Dubey US, *et al*. Epidemiology of breast cancer in Indian women. *Asia Pac J Clin Oncol* 2017;13:289–95.
26 Forouzanfar MH, Foreman KJ, Delossantos AM, *et al*. Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. *Lancet* 2011;378:1461–84.
27 Carney PA, Miglioretti DL, Yankaskas BC, *et al*. Individual and combined effects of age, breast density, and hormone replacement therapy use on the accuracy of screening mammography. *Ann Intern Med* 2003;138:168–75.
28 Ginsburg O, Yip Cheng-Har, Brooks A, *et al*. Breast *cancer* early detection: a phased approach to implementation. *Cancer* 2020;126:2379–93.
29 Shyyan R, Masood S, Badwe RA, Shyyanet al R, *et al*. Breast cancer in limited-resource countries: diagnosis and pathology. *Breast J* 2006;12 Suppl 1:S27–37.
30 Dey S. Preventing breast cancer in LMICs via screening and/or early detection: the real and the surreal. *World J Clin Oncol* 2014;5:509–19.
31 Romero Y, Trapani D, Johnson S, *et al*. National cancer control plans: a global analysis. *Lancet Oncol* 2018;19:e546–55.
32 Sankaranarayanan R, Ramadas K, Thara S, *et al*. Clinical breast examination: preliminary results from a cluster randomized controlled trial in India. *J Natl Cancer Inst* 2011;103:1476–80.
33 Patel BK, Gray RJ, Pockaj BA. Potential cost savings of contrast-enhanced digital mammography. *AJR Am J Roentgenol* 2017;208:W231–7.

Niramai

CLINICAL STUDY PROTOCOL

Protocol Number: NI-THERMA-01
Version 1.1 Dated July 19th 2017
Supersedes Version 1.0 Dated June 2nd 2017

STUDY TITLE Multicentric Study to evaluate the effectiveness of Thermalytix© (automated thermographic screening algorithms developed by NIRAMAI) as compared to standard screening modalities in subjects who show possible symptoms of suspected breast cancer

Name of the Modality Under Investigation
Thermalytix©: Machine Learning based Automated Thermographic Screening Algorithms

CONFIDENTIAL

Principal Contacts		
1. Principal Investigator (PI)	Dr S Sudhakar, Department of Radiology HCG Towers, No 8, Kalinga Rao Road, Sampangi Ram Nagar, Bengaluru, Karnataka 560027 E-mail:dr_sudhakar79@yahoo.com	Dr Raghavendra Rao M, GM Clinical Excellence HCG Towers, No 8, Kalinga Rao Road, Sampangi Ram Nagar, Bengaluru, Karnataka 560027 E-mail:raghav.hcgrf@gmail.com
2. Principal Investigator (PI)	Dr Venkataraman Bhat, Director of Imaging, Head of Radiology Department & Imaging Services Mazumdar Shaw Medical Center Narayana Hrudayalaya Limited No. 258/A, Bommasandra Industrial Area, Anekal Taluk, Bengaluru 560 099, Karnataka, India	Dr. Alben Sigamani, Group Head – Clinical Research, Mazumdar Shaw Medical Center Narayana Hrudayalaya Limited No. 258/A, Bommasandra Industrial Area, Anekal Taluk, Bengaluru 560 099, Karnataka, India Phone: 080 71 222 222 / Fax: +080 2783 2648, 2783 5222 (Dir) E-mail: Alben.sigamani.dr@narayanahealth.org
Statistician	Dr. Jayanti Gupta Consultant Biostatistician, A-1601 Safal Twins, Sion- Trombay Road, Deonar Mumbai 400088, Maharashtra, India Phone: +91 9820228720 E-mail jayantigupta@live.com	
Blinded /Independent Thermologist	Dr Hadonahalli V. Ramprakash, Professor & Head Dept. of Radiology & Imaging Vydehi Institute of Medical Sciences Founder, Anjana Diagnostics, No. 12, 3rd Main, 4th Cross O.M.B.R Layout, Bengaluru – 560043, Karnataka, India E-mail drhvrp@gmail.com	
Clinical Quality Oversight	Ms. Mala Srivastava *MRQA* Co-founder & Managing Partner Nextvel Consulting LLP Novel Business Center #10, 100 Feet Ring Road, BTM Layout 1st Stage Bengaluru -560068, Karnataka, India E-mail: malas@nextvel.com	
Sponsor	Dr. Geetha Manjunath, CEO & Co-founder NIRAMAI HEALTH ANALYTIX PVT. LTD. A7-506, Elita Promenade, J P Nagar 7th Phase, Bengaluru – 560078, Karnataka, India E-mail: geetha@niramai.com	

Table of Contents

1. REVISION HISTORY

Protocol Version	Effected Sections	Amendment/Change	Effected pages numbers:	Reason for Changes	Date
1.1	Study Synopsis-Study Design Section, Number of Study Visits, Subjects (Exclusion Criteria) Section 8.2 Section 10	• The number of Visits is only for convenience; subjects can finish procedures on as early as one day but not go beyond 2 weeks • Exclusion Criteria -removed subject participation in prior Thermalytix© studies -Added Exclusion of patients who have undergone chemotherapy within 2 weeks of study enrolment • Header footer and relevant section where version number and date of protocol has been updated	Pages 7-10, 13, 15, 19-21	Feedback from Scientific Review Committee and Ethics Committees	19th July, 2017

2. STUDY SYNOPSIS:

TITLE	Multicentric Study to evaluate the effectiveness of Thermalytix© (automated thermographic screening algorithms developed by NIRAMAI) as compared to standard screening modalities in subjects who show possible symptoms of suspected breast cancer	
Investigational Site	HCG Cancer Hospital HCG Towers, No 8, Kalinga Rao Road, Sampangi Ram Nagar, Bengaluru, Karnataka 560027 Tel: 080-40206000, 40206521, 40206522	Mazumdar Shaw Medical Center Narayana Hrudayalaya Limited No. 258/A, Bommasandra Industrial Area, Anekal Taluk, Bengaluru 560 099, Karnataka, India Phone: 080 71 222 222
Investigators	Dr S Sudhakar Department of Radiology Dr Raghavendra Rao M, GM Clinical Excellence Dr. Ajai Kumar B S Chairman & CEO	Dr Venkataraman Bhat, Director of Imaging, Dr. Alben Sigamani, Group Head – Clinical Research Dr. Akshita Singh Consultant Oncoplastic Breast Surgeon
Sponsor	NIRAMAI HEALTH ANALYTIX PVT. LTD. A7-506, Elita Promenade, J P Nagar 7th Phase, Bengaluru – 560078, Karnataka, India	
Protocol Number	NI-THERMA-01	
Final Protocol	Version Number 1.1	Date July 19th 2017
Clinical Phase	Pilot	
Objectives	**Primary Objective(s):** 1. To assess non-inferiority of sensitivity of Thermalytix© solution to not more than 10% of the sensitivity of standard screening modalities 2. Compare the diagnostic performance of Thermalytix© with standard screening modalities in breast cancer patients as measured by: 1. Sensitivity of Thermalytix© as compared to sensitivity of standard modalities 2. Specificity of Thermalytix© as compared to specificity of standard modalities 3. Positive Predictive Value (PPV) of Thermalytix© 4. Negative Predictive Value (NPV) of Thermalytix© **Secondary Objective(s)** 1. Influence of patient characteristics on diagnostic accuracy of Thermalytix©. Patient characteristics will include: o Age o Lesion type o Pathologic diagnosis o Menopausal and hormonal status	

	○ Breast density ○ Family history ○ Risk factors 2. Recommend how Thermalytix© can be used to complement standard breast cancer screening procedure.
Study Rationale:	Breast cancer is one of the crucially prevailing cancers among women. Early detection and diagnosis of breast cancer is very important for patient survival. Currently, mammography is the most common method used for detection of breast cancer. However, mammography has certain limitations. It is not recommended to be used on women under the age of 45 years, cannot be used on lactating women and is inconclusive on women with high density of breast. Mammography in correlation with sono-mammography is widely used currently for screening in hospitals and for early diagnosis of women over the age of 45 years. Further, for younger women, mammography based screening is not recommended due to its low sensitivity and likely radiation effects. In emerging markets, many women do not consider routine annual mammography, due to cost, accessibility and other reasons. Infrared imaging of the breast or thermography is an imaging technique which detects suspected malignancy based on thermal changes in the body. Due to increased blood circulation and metabolic activity of the tumour, the temperature of the tissue is higher around the regions where a tumor is present. A high resolution thermal camera can be used to capture this thermal information. This thermal image representing the temperature variations observed on the breast is called a breast thermogram. It is shown as a colored image where specific temperature is represented by a specific color assigned to it. A thermal image provides a visual representation of the temperature distribution and can be used for clinical interpretation of abnormal breast conditions. Particularly, radiologists who are also certified thermologists are able to interpret these breast thermograms to determine its malignancy or benign condition. Early diagnosis through thermal screening offers a way to minimize the mortality risks of breast cancer. Our proposed research is to improve breast cancer screening through low cost, mobile, non-invasive and non-contact thermal screening without causing any discomfort to patients and develop accurate interpretation algorithms using machine learning, image processing and computer vision techniques to give automated screening capabilities. In this study, sensitivity of Thermalytix© (an automated thermographic screening algorithms developed by NIRAMAI) will be compared to the sensitivity of standard screening modalities. The entry criteria for the study will be subjects who show possible symptoms of suspected breast cancer, such as pain, lump and so on. The primary objectives of the study are to assess a non-inferiority of sensitivity of Thermalytix© methodology over the sensitivity of standard screening procedures by not more than 10% and study comparative diagnostic performance of Thermalytix© with standard screening modalities. The endpoint measurements would be sensitivity, specificity, PPV (Positive Predictive Values) and NPV (Negative Predictive Values) in the detection of breast cancer in women.

	Secondary Objectives would be to study the influence of effects of patient characteristics on diagnostic accuracy. Patient characteristics would include, Age, Lesion type, Pathologic diagnosis, Menopausal and hormonal status, Breast density and Family history. Also to study how Thermalytix© can complement the standard modalities in breast cancer screening in future This protocol for human research study will be conducted according to standards of effective Good Clinical Practice (International Conference on Harmonization (ICH) Guidelines), if applicable the Indian regulations and applicable Ethical Guidelines for Biomedical Research involving Human Participants issued by the Indian Council of Medical Research. The trial protocol and subject documentation will be reviewed and approved by the Institutional Ethical Review Boards of the participating trial sites. In order to ensure the conclusions of the study get reviewed by an independent expert group. There is a provision in the study design to have an Independent Data Monitoring Committee (IDMC) which will conduct an independent review of the conclusions and correlations made by the study team. This Committee will consist of minimum of 5 members with the following roles: • Chairman (Independent of the Study), • Secretary (Sponsor /Site Representative), • Member Thermologist (Independent of Study Team), • Member Radiologist (Independent of the Study Team) • Member Statistician (Independent of the Study team) The role and procedural policies of the committee will be outlined in a "Charter" and submitted for review by the Institutional Ethical Committees of the participating sites before any such a committee is constituted.
Study Design	This is a prospective, comparative and multi-centric study to evaluate the effectiveness of the Thermalytix© when compared to the standard screening modalities in subjects having possible symptoms and presentation leading to the suspicion of breast cancer. Subjects eligible for the clinical trial would be women of 18 years and above and who complain of any one of the following symptoms: • Palpable lump, swelling or mass in breast • Persistent breast pain or tenderness that is unrelated to menstrual cycle • Nipple is inverted or tender, painful, scaly or with discharge (clear or bloody) • Skin on breast is dimpled, red, blotchy, prickly, itchy or has change in the texture • Increase in breast size that is not related to menstrual cycle • Thickening in or around the breast or underarm area • Or any other symptoms leading to suspicion of breast cancer Pregnant, lactating subjects, subjects who have undergone either lumpectomy or mastectomy, gone through chemotherapy in the last two weeks from the date of enrollment and those suffering from any active illnesses, psychological and/or

	pathological conditions that would interfere with study participation in the opinion of the Investigator, will be excluded from participation in the trial All subjects who visit the trial site and are found to satisfy the entry criteria will be counselled for participation in the trial. The Principal Investigator or member of his team will explain the trial to the subject as part of the informed consent process. The screening procedure will begin once the informed consent form has been signed and dated personally by the subjects or their legally accepted representative and the person who administers the consent. Screening Procedure will include but not limited to the following as per the discretion of the treating doctor: 1. Past Medical History and Current Complaints 2. Treatment (Past/Current) 3. Family History 4. Physical Examination 5. Risk factors (smoking/alcohol consumption etc.) 6. Thermography (NIRAMAI Thermalytix© screening) 7. Clinical Breast Examination 8. Standard Laboratory Work up (if applicable) 9. Standard Screening Imaging as per standard practice, which may be as follows: o Sono-mammography for subjects below the age of 45 years o Sono-mammography & Mammography for subjects 45 years and above o MRI /PET Scan for confirming inconclusive reports of mammography and sono-mammography as per standard practice o MRI/PET Scan in cases were Thermography shows positive for malignancy when standard modalities reported negative for malignancy 10. Biopsy/FNAC if positive as per standard screening, MRI guided Biopsy will be done in case where the results are positive by MRI 11. Hormonal Status (if possible) The Thermography will be done at the site by a qualified technician. The technician will be trained by the sponsor. The Technician will need to follow the protocol for the same as described in Appendix I of this protocol for taking and uploading the thermal images. The thermal images will be interpreted by the algorithm “Thermalytix© and also read by a blinded and independent Thermologist. The reports of the scans will be reported to the Principal Investigator via a signed report from the thermologist over e-mail. The thermography report and the standard screening reports will be collated by the coordinator and entered in the Case Report Forms. The Correlation results will be analyzed by PI.

<table>
<tr><td></td><td>In case there is inconclusive reports from standard modality subjects will go through confirmatory MRI or PET Scan as determined by the PI.

In case the thermography reports malignancy and the standard screening methodology does not, subject will go for confirmatory MRI or PET Scan as determined by the PI.

When all the results are in, subjects will be contacted telephonically to complete the end of trial procedure and their treatment course will be decided by the treating doctor.

Study Coordinator will transcribe all the data in the CRF.

Data from individual CRFs will be entered in the excel spread sheet by the Study Coordinator and sent to Data Management for analysis</td></tr>
<tr><td>Number of Study Visits</td><td>Efforts will be made to conclude the screening procedure as early as possible for the subjects.

The screening process can also be completed on the same day when subjects are enrolled to the study, but is not to go beyond the allocated time of 14 days.

Mammography and Sono-mammography reports of subjects who have already undergone the investigation prior to study participation will be considered valid if done within one week from the date of subject’s enrollment on to the study or as determined by the Investigator.

Depending on the investigations done the respective CRF pages can be completed.

For convenience of recording data, the entire study work-up has been divided into 5 visits, so that in the eventuality that all the procedures are not completed within a day it can be covered across the allocated 5 visits (this would be expected in cases where the confirmatory FNAC/biopsy, MRI guided biopsy, MRI or PET scan would be required). The Visit 5 being a Telephonic Visit for all cases:

The Visits have been divided as follows:

<table>
<tr><td>Visit Number</td><td>Visit Type</td><td>Time point</td><td>Subjects Applicability</td></tr>
<tr><td>Visit 1:</td><td>Screening Visit (involving procedures 1-8)</td><td>Day 0-3</td><td>All Subjects</td></tr>
<tr><td>Visit 2</td><td>Sono-mammography & Mammography Imaging as per standard practice)</td><td>Day 3-5</td><td>All Subjects</td></tr>
<tr><td>Visit 3</td><td>Biopsy/FNAC Histopathology and related Laboratory Work up)</td><td>Day 5-7</td><td>Only Cases showing Positive results as per standard screening</td></tr>
</table>
</td></tr>
</table>

	<table><tr><td>Visit 4</td><td>MRI/PET Scan* followed by MRI Guided biopsy will be performed in cases where results are positive for malignancy by MRI.</td><td>Day 7-10</td><td>• Cases showing inconclusive report by standard screening modalities • Cases showing positive as per thermal scan images, which would have negative report by standard screening modalities</td></tr><tr><td>Visit 5</td><td>Telephone Contact-End of Trial</td><td>Day 10-14</td><td>All Subjects</td></tr></table> **Note: The cost for this imaging will be borne by sponsor* Cases negative for cancer would complete: Visits 1, 2 & 5 Cases positive and suspected cancer Cases, would complete all visits, or as applicable. *The patients would be expected to go through the entire screening process in a duration of within* ***2 weeks (14days) of enrollment on the study.***
Subjects	**Inclusion Criteria** 1. Female subjects equal to and above 18 years presenting with the following breast abnormalities: o Palpable lump, swelling or mass in breast o Persistent breast pain or tenderness that is unrelated to menstrual cycle o Nipple is inverted or tender, painful, scaly or with discharge (clear or bloody) o Skin on breast is dimpled, red, blotchy, pricking, itchy or has change in the texture o Increase in breast size that is not related to menstrual cycle o Thickening in or around the breast or underarm area o Or any other symptoms leading to suspicion of breast cancer 2. Subjects who are willing to give written informed consent for study participation 3. Subjects who are ready to comply with the study related visits and procedures **Exclusion criteria** 1. Subjects who are pregnant 2. Subjects who are lactating 3. Subjects who have undergone either lumpectomy or mastectomy 4. Subjects who have undergone Chemotherapy in the last 2 weeks at the time of study enrollment 5. Any active illness, psychological and/or pathological condition that would interfere with study participation in the opinion of the Investigator
Product to be evaluated	Thermalytix©: Machine Learning based Automated Thermographic Screening Algorithms developed by Niramai

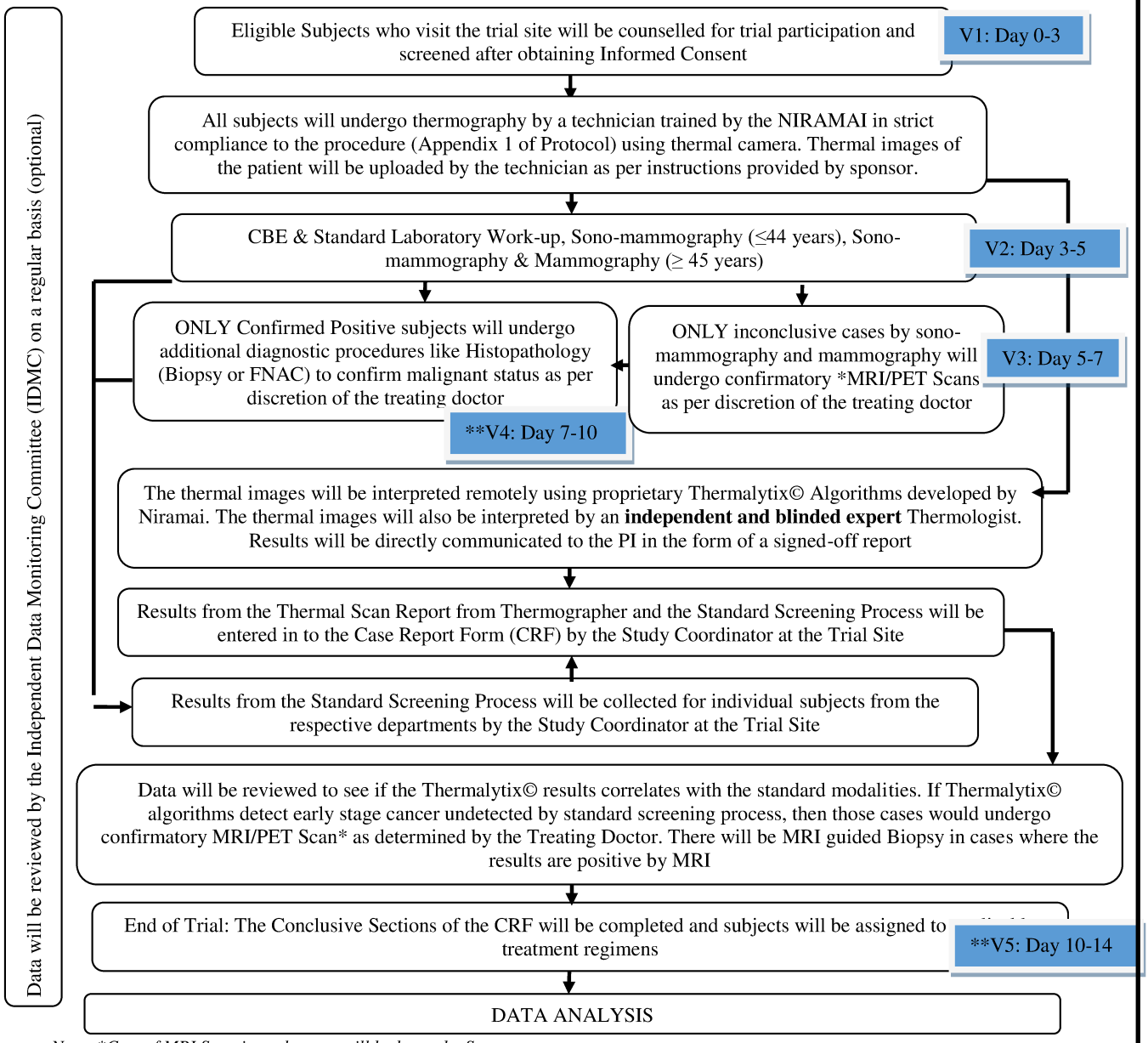
Duration of study	Recruitment Period: 12 weeks Study Period (Breast Cancer Screening): 2 weeks per subject Data Collection/cleaning and Report Compilation 8-10 weeks Total Duration would approximately 22-24 weeks (approximately 6 months)
Endpoints	**Primary Endpoints:** 1. Sensitivity of Thermalytix© as compared to Sensitivity of Standard Modalities 2. Specificity of Thermalytix© as compared to Specificity of Standard Modalities 3. Positive Predictive Value (PPV) 4. Negative Predictive Value (NPV) **Secondary Endpoints:** 1. Influence of patient characteristics on diagnostic accuracy of Thermalytix©. Patient characteristics will include: o Age o Lesion type o Pathologic diagnosis o Menopausal and hormonal status o Breast density o Family history o Risk Factors 2. Recommend how Thermalytix© can be used to complement standard breast cancer screening procedure Analyse Correlation with the following: o Thermalytix© Versus Standard Modality (observation combining all the modalities including clinical breast examination, sono-mammography, mammography, MRI, PET Scan and all modalities applicable to the subject), o Thermalytix© Versus Mammography, o Thermalytix© Versus Sono-mammography o Thermalytix© Versus standard Clinical Breast Examination. o Thermalytix© Versus standard MRI/PET Scan
Sample Size	**Total Subjects:** A total of **275** subjects will be enrolled on the study in order to get **256** evaluable subjects and to compensate for lost to follow-up and drop-outs
Statistical Analysis	**Hypothesis**: Sensitivity of Thermalytix© is non-inferior to sensitivity of standard modalities in detecting malignancy in subjects who show possible symptoms of suspected breast cancer symptoms of suspected breast cancer **Sample Size Justification**: The sample size for the study is computed to assess the non-inferiority of sensitivity of Thermalytix© to that of standard modalities in detecting malignancy in subjects who show possible symptoms of suspected breast cancer. The sensitivity of the standard modalities is assumed to be 95% (sensitivity of mammography or sono-mammography alone is between 75-85%. However, we assume that when multiple

	modalities are correlated the collective sensitivity of the standard of care will be much closer to absolute truth). Using a non-inferiority margin of 10% and significance level of 5%, there is 80% power to demonstrate non-inferiority with a sample size of 59 malignant subjects. From earlier studies, the rate of malignancy in the study population is estimated to be 23%. Using this rate, 256 subjects need to be evaluated in order to get the required number of malignant subjects. Assuming that about 10% of the enrolled subjects may be lost to follow-up, 275 subjects will be recruited in the study. **Statistical Analysis**: The following measures of validity will be computed: **Sensitivity** - probability of correctly identifying a true case of malignancy **Specificity** - probability of correctly identifying a true case of non-malignancy (normal or benign) **Positive Predictive Value** - probability that a labelled positive is a true case of malignancy **Negative Predictive Value** - probability that a labelled negative is a true case of non-malignancy. The sensitivity of Thermalytix© and that of standard modalities will be compared to assess non-inferiority. The primary objective of non-inferiority will be assessed by constructing a 90% confidence interval (CI) around the difference in sensitivities (Thermalytix© – standard modalities). If the lower limit of the 90% CI is greater than -10%, then non-inferiority will be established. All the methods of validity will be computed for Thermalytix© along with their 90% CI.

2.1. Study Schema:

Protocol Number: NI-THERMA-01 /Version 1.1 Dated July 19th 2017

Study Title: Multicentric Study to evaluate the effectiveness of Thermalytix© (automated thermographic screening algorithms developed by NIRAMAI) as compared to standard screening modalities in subjects who show possible symptoms of suspected breast cancer

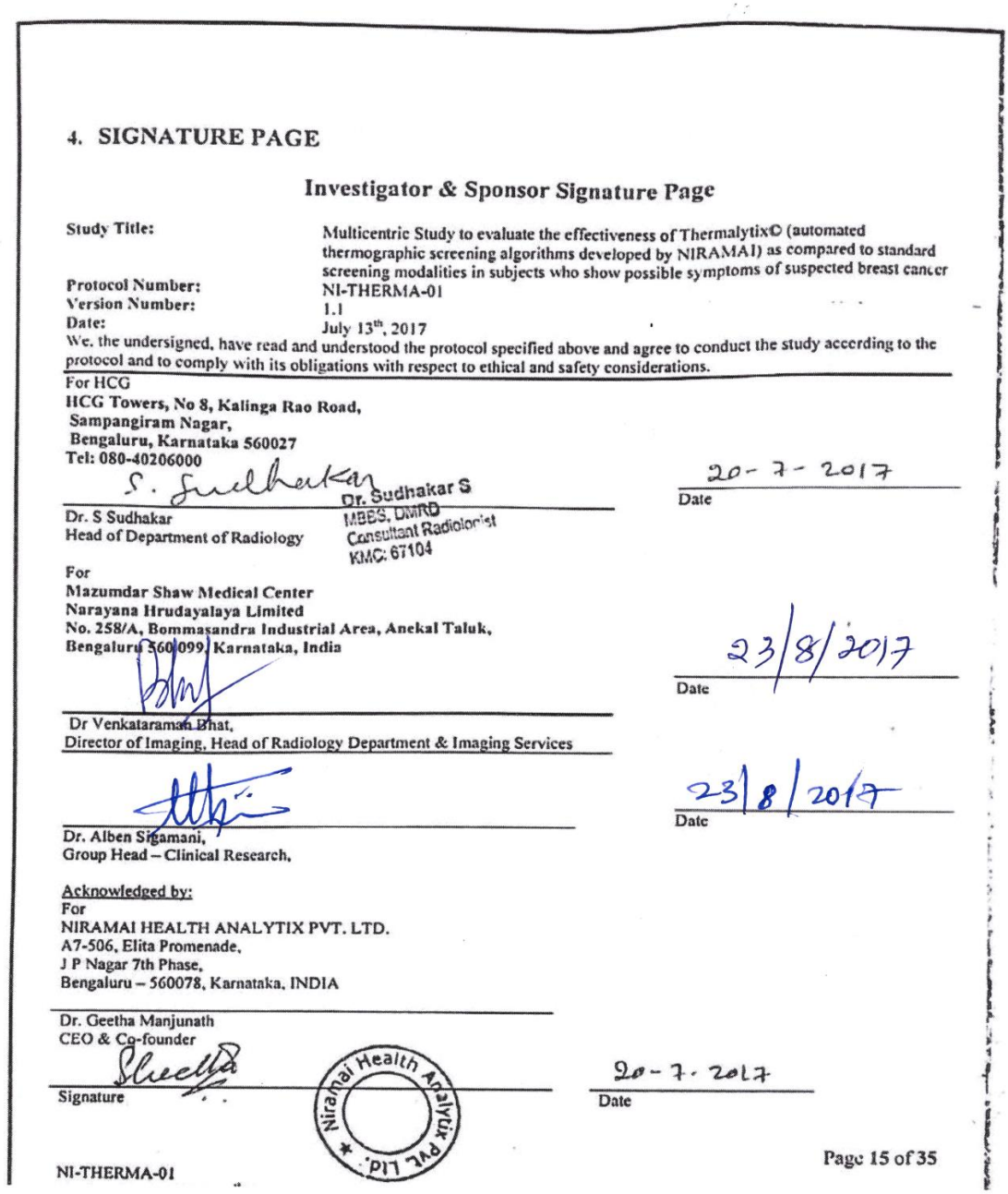


*Note: *Cost of MRI Scan in such cases will be borne by Sponsor*
***This Visit can be conducted telephonically for subjects who are unable to visit the trial site*

3. ABBREVIATIONS

AEs	Adverse Events
CBE	Clinical Breast Examination
CD	Compact Disc
CDSCO	Central Drugs Standards Control Organization
CI	Confidence Interval
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTRI	Clinical Trials Registry India
DICOM	Digital Imaging and Communications in Medicine
EC	Ethics Committee
FNAC	Fine Needle Aspiration Cytology
ICH	International Council for Harmonization
ICH GCP	International Council for Harmonization Good Clinical Practices
ICMR	Indian Council of Medical Research
ID	Identification
IDMC	Independent Data Monitoring Committee
MRI	Magnetic Resonance Imaging
N/D	Note done
NPV	Negative Predictive Value
PET	Positron Emission Tomography
PI	Principal Investigator
PPV	Positive Predictive Value
SAE	Serious Adverse Event

4. SIGNATURE PAGE



4. SIGNATURE PAGE

Investigator & Sponsor Signature Page

Study Title: Multicentric Study to evaluate the effectiveness of Thermalytixⓒⓒ (automated thermographic screening algorithms developed by NIRAMAI) as compared to standard screening modalities in subjects who show possible symptoms of suspected breast cancer

Protocol Number: NI-THERMA-01
Version Number: 1.1
Date: July 13th, 2017

We, the undersigned, have read and understood the protocol specified above and agree to conduct the study according to the protocol and to comply with its obligations with respect to ethical and safety considerations.

For HCG
HCG Towers, No 8, Kalinga Rao Road,
Sampangiram Nagar,
Bengaluru, Karnataka 560027
Tel: 080-40206000

S. Sudhakar — Dr. Sudhakar S MBBS, DMRD Consultant Radiologist KMC: 67104
Dr. S Sudhakar
Head of Department of Radiology
20-7-2017
Date

For
Mazumdar Shaw Medical Center
Narayana Hrudayalaya Limited
No. 258/A, Bommasandra Industrial Area, Anekal Taluk,
Bengaluru 560099, Karnataka, India

Dr Venkataraman Bhat,
Director of Imaging, Head of Radiology Department & Imaging Services
23/8/2017
Date

Dr. Alben Sigamani,
Group Head – Clinical Research,
23/8/2017
Date

Acknowledged by:
For
NIRAMAI HEALTH ANALYTIX PVT. LTD.
A7-506, Elita Promenade,
J P Nagar 7th Phase,
Bengaluru – 560078, Karnataka, INDIA

Dr. Geetha Manjunath
CEO & Co-founder
Signature
Niramai Health Analytix PVT. Ltd.
20-7-2017
Date

NI-THERMA-01
Page 15 of 35

5. BACKGROUND AND SIGNIFICANCE:

Breast cancer has the highest incidence among cancers in women worldwide [1]. 1.7 million women worldwide had been diagnosed with breast cancer in 2012, and in a five-year prevalence period, 6.2 million women have breast cancer [1]. In India, the incidence is 145 thousand, with a five-year prevalence is 397 thousand in 2012 [1]. Breast cancer is curable if diagnosed in the early stage. Hence, screening is important for early detection. Mammography is the current gold standard for breast cancer screening. However, mammograms are less sensitive on younger women due to denser tissue and the radiation effects due to the X-rays are harmful enough to cause cancer in younger women [2]. The sensitivity of mammography falls from 83% in less dense tissue to 55% in the highest density tissue [2]. Sono-mammography is typically used in correlation as its standalone approach has too many false positives and false negatives for screening [3].

In this clinical trial, feasibility of using Thermography as an alternative breast imaging modality to current modalities is being evaluated with a goal towards screening of the patients having early breast cancer symptoms. Nutrients are delivered to cells via vascular circulation (blood vessels). This circulation generates heat. As a result, the portions of the body that demand the most nutrients give off the most infrared light (heat). Cancer cells divide quickly, demanding an ever-increase supply of nutrients, and further increase in temperature around malignant tissues. Thermography measures the infra-red rays emitted by the body [2]. Malignant cells cause the formation of new blood vessels around them to supply nutrients for their faster growth. The increased blood supply also causes a rise in temperature compared to the surrounding tissue, which is visible in the thermographic image. Thermography captures the functional changes in malignant tissue due to neovascularity and angio-genesis. There was research in the 1970's and 1980's on thermography for breast cancer screening [4,5] and thermography got approval by FDA since 1982 as a risk predictor for cancer [2]. However, the lower sensitivity and specificity compared to mammography and sono-mammography reported in a controversial study in 1977 [6] resulted in a decline in its usage. Over the last 1.5 decades, with the advent of high resolution thermal cameras, there is a relook at thermography [7]. In a study of 100 subjects with carcinoma [7], the sensitivity of thermography was 83%, and its value is in signaling abnormality in younger subjects with carcinoma where mammograms or clinical examination did not detect malignancy. Thermography may be able to detect malignant tumors well before mammography [5]. The thermal cameras are also of lower cost, small and mobile, enabling non-contact and non-invasive screening for large populations in non-hospital settings. These advantages could be useful especially in less developed countries like India where cost and availability of hospitals play a vital role in screening.

NIRAMAI has pioneered an advanced method of screening for breast cancer enhancing thermography with machine intelligence. This combination of thermography and computer aided diagnostics is called Thermlaytix©.

Automatic screening algorithms can help in outreach to large populations, as doctors can focus on a fewer number of suspicious cases for further analysis. There have been several semi-automated algorithms for breast cancer screening with thermography [8,9] whose specificity and sensitivity is comparable with that of mammography, but have been tested on only a small number of subjects. Ng et al [10] report a sensitivity of 86% and specificity of 91% on 25 normal subjects and 25 subjects with malignant tumors in stage 2 or stage 3 cancer. Most of these approaches use textural features and temperature moments for classification with standard classifiers.

As opposed to the prior methods of automated screening algorithms, NIRAMAI Thermalytix© solution uses machine learning (an advanced form of Data Analytics and an upcoming research theme in Computer Science) which use probabilistic modelling of a decision process using statistical analysis of historic data. NIRMAI algorithms have been tried on over 400 patients in a retrospective setting and has obtained a sensitivity and specificity comparable to Mammography and Sono-Mammography [9]. This study will perform a prospective

study of the effectiveness of Thermalytix© algorithm over popular methods of screening used as defacto standard of care.

In this study, sensitivity of Thermalytix© (an automated thermographic screening algorithms developed by NIRAMAI) will be compared to the sensitivity of standard screening modalities. The entry criteria for the study will be subjects who show some symptoms of suspected breast cancer.

The primary objectives of the study are to assess a non-inferiority of sensitivity of Thermalytix© methodology over the sensitivity of standard screening procedures by not more than 10% and study comparative diagnostic performance of Thermalytix© with standard screening modalities. The endpoint measurements would be sensitivity, specificity, PPV (Positive Predictive Values) and NPV (Negative Predictive Values) in the detection of breast cancer in women.

Secondary Objectives would be to study the influence of effects of patient characteristics on diagnostic accuracy. Patient characteristics would include, Age, Lesion type, Pathologic diagnosis, Menopausal and hormonal status, Breast density, Family history and Risk Factors. Also to study how Thermalytix© can complement the standard modalities in breast cancer screening in future

6. SPECIFIC AIMS/OBJECTIVES

Primary Objective(s):

1. To assess non-inferiority of sensitivity of Thermalytix© solution to not more than 10% of the sensitivity of standard screening modalities
2. Compare the diagnostic performance of Thermalytix© with standard screening modalities in breast cancer patients as measured by:
    1. Sensitivity of Thermalytix© as compared to sensitivity of standard modalities
    2. Specificity of Thermalytix© as compared to specificity of standard modalities
    3. Positive Predictive Value (PPV) of Thermalytix©
    4. Negative Predictive Value (NPV) of Thermalytix©

Secondary Objective(s)

1. Influence of patient characteristics on diagnostic accuracy of Thermalytix©.
   Patient characteristics will include:
    - Age
    - Lesion type
    - Pathologic diagnosis
    - Menopausal and hormonal status
    - Breast density
    - Family history
    - Risk Factors
2. Recommend how Thermalytix© can be used to complement standard breast cancer screening procedure.

7. STUDY OVERVIEW

This is a prospective, comparative and multi-centric study to evaluate the effectiveness of the Thermalytix© when compared to the standard screening modalities in subjects having possible symptoms and presentation leading to the suspicion of breast cancer.

Subjects eligible for the clinical trial would be women of 18 years and above and who complain of any one of the following symptoms:

- Palpable lump, swelling or mass in breast
- Persistent breast pain or tenderness that is unrelated to menstrual cycle
- Nipple is inverted or tender, painful, scaly or with discharge (clear or bloody)
- Skin on breast is dimpled, red, blotchy, prickly, itchy or has change in the texture
- Increase in breast size that is not related to menstrual cycle
- Thickening in or around the breast or underarm area
- Or any other symptoms leading to suspicion of breast cancer

Pregnant, lactating subjects, subjects who have undergone either lumpectomy or mastectomy and those suffering from any active illnesses, psychological and/or pathological conditions that would interfere with study participation in the opinion of the Investigator, will be excluded from participation in the trial

All subjects who visit the trial site and are found to satisfy the entry criteria will be counselled for participation in the trial. The Principal Investigator or member of his team will explain the trial to the subject as part of the informed consent process. The screening procedure will begin once the informed consent form has been signed and dated personally by the subjects or their legally accepted representative and the person who administers the consent.

Screening Procedure will include but not limited to the following as per the discretion of the treating doctor:

1. Past Medical History and Current Complaints
2. Treatment (Past/Current)
3. Family History
4. Physical Examination
5. Risk factors (smoking/alcohol consumption etc.)
6. NIRAMAI Thermalytix© screening
7. Clinical Breast Examination
8. Standard Laboratory Work up if applicable
9. Standard Screening Imaging as per standard practice, which may be as follows:
    - Sono-mammography for subjects below the age of 45 years
    - Sono-mammography & Mammography for subjects 45 years and above
    - MRI /PET Scan for confirming inconclusive reports of mammography and sono-mammography as per standard practice
10. MRI/PET Scan in cases were Thermography shows positive for malignancy when standard modalities reported negative for malignancy. MRI Guided biopsy will be performed in cases where results are positive for malignancy by MRI.
11. Biopsy/FNAC if positive as per standard screening
12. Hormonal Status (if possible)

The Thermography will be done at the site by a qualified technician. The technician will be trained by the sponsor. The Technician will need to follow the protocol for the same as described in Appendix I of this protocol for taking and uploading the thermal images.

The thermal images will be interpreted by the algorithm "Thermalytix© and also read by a blinded and independent Thermologist.

The reports of the scans will be reported to the Principal Investigator via a signed report from the thermologist over e-mail.

The thermography report and the standard screening reports will be collated by the coordinator and entered in the Case Report Forms.

The Correlation results will be analyzed by PI.
In case there is inconclusive reports from standard modality subjects will go through confirmatory MRI or PET Scan as determined by the PI.

In case the thermography reports malignancy and the standard screening methodology does not, subject will go for confirmatory MRI or PET Scan as determined by the PI.

MRI Guided biopsy will be performed in cases where results are positive for malignancy by MRI.

When all the results are in and final diagnosis has been confirmed, subjects will be contacted telephonically to complete the end of trial procedure and their treatment course will be decided by the treating doctor.

Study Coordinator will transcribe all the data in the CRF.

Data from individual CRFs will be entered by the Study Coordinator in the excel spread sheet and sent to Data Management for analysis

8. PARTICIPANT SELECTION

8.1. Inclusion Criteria

1. Female subjects equal to and above 18 years presenting with the following breast abnormalities:
    - Palpable lump, swelling or mass in breast
    - Persistent breast pain or tenderness that is unrelated to menstrual cycle
    - Nipple is inverted or tender, painful, scaly or with discharge (clear or bloody)
    - Skin on breast is dimpled, red, blotchy, pricking, itchy or has change in the texture
    - Increase in breast size that is not related to menstrual cycle
    - Thickening in or around the breast or underarm area
    - Or any other symptoms leading to suspicion of breast cancer
2. Subjects who are willing to give written informed consent for study participation
3. Subjects who are ready to comply with the study related visits and procedures

8.2. Exclusion criteria

1. Subjects who are pregnant
2. Subjects who are lactating
3. Subjects who have undergone either lumpectomy or mastectomy
4. Subjects who have undergone Chemotherapy in the last 2 weeks at the time of study enrollment
5. Any active illness, psychological and/or pathological condition that would interfere with study participation in the opinion of the Investigator

8.3. Recruitment and Screening

The recruitment of subjects would be based on the subjects who visit the trial site with the eligibility criteria as specified under Sections 5.1 and 5.2. The subjects will be sourced from the following:

1. Subjects referred by the primary specialist to the department of radiology of the trial sites
2. Referrals from Oncologists
3. Subjects with suspicious findings from screening camps during routine health check-up will be referred to trial sites
4. Other sources as identified from time to time and used after approval from Ethics Committees

9. SITE SELECTION

9.1. Institution Requirements

Sites willing to participate, having the potential to recruit the required subjects, having qualified Principal Investigators and study team, will be selected for the study. Sponsor and its representatives will make all possible efforts to ensure that the sites have the commitment to adhere to the study protocol. The site should have the manpower, resources and infrastructure to conduct the study. The selected sites should have an Institutional Ethics Committee that is registered with the CDSCO (Central Drugs Standardization and Control Organization). All the staff members managing study should be trained in ICH Good Clinical Practices, Ethical Guidelines for Biomedical Research on Human Participants Guidelines issued by Indian Council of Medical Research (ICMR) and the applicable regulations.

9.2. IRB Approval and Informed Consent

All institutions must have study-specific Institutional Review Board (IRB) approval for the protocol and informed consent form. (The informed consent form is included in this protocol as Appendix II)
The investigator and the investigator-designated research staff must follow Ethics Committee (EC) Approved informed consent form and as per ICH GCP defined procedures, as well as those set by the site EC at the institution. A copy of the EC approval letter and a copy of the EC approved study specific consent form must be on file at trial site prior to registering the first participant.

10. STUDY PROCEDURES

Efforts will be made to conclude the screening procedure as early as possible for the subjects.

The screening process can also be completed on the same day when subjects are enrolled to the study, but is not to go beyond the allocated time of 14 days.

Mammography and Sono-mammography reports of subjects who have already undergone the investigation prior to study participation will be considered valid if done within ***one week*** from the date of subject's enrollment on to the study or as determined by the Investigator.

Depending on the investigations done the respective CRF pages can be completed.

For convenience of recording data, the entire study work-up has been divided into 5 visits, so that in the eventuality that all the procedures are not completed within a day it can be covered across the allocated 5 visits (this would be expected in cases where the confirmatory FNAC/biopsy, MRI guided biopsy, MRI or PET scan would be required). The Visit 5 being a Telephonic Visit for all cases:

The Visits have been divided as follows:

.

Visit Number	Visit Type	Time point	Subjects Applicability
Visit 1:	Screening Visit (involving procedures 1-8)	Day 0-3	All Subjects
Visit 2	Sono-mammography & Mammography Imaging as per standard practice)	Day 3-5	All Subjects
Visit 3	Biopsy/FNAC Histopathology and related Laboratory Work up)	Day 5-7	Only Cases showing Positive results as per standard screening
Visit 4	MRI/PET Scan*	Day 7-10	• Cases showing inconclusive report by standard screening modalities • Cases showing positive as per thermal scan images, which would have negative report by standard screening modalities • MRI guided Biopsy will be done in case where the results are positive by MRI
Visit 5	Telephone Contact- End of Trial	Day 10-14	All Subjects

The patients would be expected complete the entire screening process within 2 Weeks (14days) of enrollment on the study

10.1. Visit 1 (Day 0-3)

All the following procedures will be completed for all the subjects found eligible for participation in the study:

1. Informed Consent Administration and Sign-off
2. Allocate Unique Subject ID to the subject
3. Past Medical History and Current Complaints
4. Treatment (Past/Current)
5. Family History
6. Demographic details
7. Physical Examination
8. Risk factors (smoking/alcohol consumption etc.)
9. Thermography for NIRAMAI Thermalytix© screening (As per Appendix I)
10. Clinical Breast Examination
11. Standard Laboratory Work up required as part of routine screening

10.2. Visit 2 (Day 3-5)

All the following procedures will be completed for all the subjects enrolled on the study:

1. Review of Reports from the previous visit
2. Conduct any tests which were not completed during Visit 1
3. Review any change in how the patient feels from last visit
4. Review any change in complaints
5. Sono-mammography & Mammography Imaging as per standard practice and as per the age of the subject
6. Review the reports of Imaging (Sono-mammography/Mammography and Thermography)

10.3. Visit 3 (Day 5-7)

Only the patients who report positive by the standard screening modalities, will complete the following procedures:

1. Review of Reports from the previous visit
2. Review any change in how the patient feels from last visit
3. Review any change in complaints
4. Biopsy/FNAC Histopathology and related Laboratory Work up
5. Review the reports
6. Correlate the results
7. Confirm Diagnosis

10.4. Visit 4 (Day 7-10)

Only for subjects whose results are:

a) Inconclusive with standard modalities
b) Thermography reports positive and standard modalities report negative

will complete the following procedures:

1. Review any change in how the patient feels from last visit
2. Review any change in complaints
3. Confirmatory *MRI or PET Scan** as per Principal Investigator discretion
4. MRI guided Biopsy will be done in case where the results are positive by MRI
5. Review the reports
6. Correlate the results
7. Confirm Diagnosis

10.5. Visit 5 (Day 10-14)

All the following procedures will be completed for all the subjects enrolled on the study:

1. Review any change in how the patient feels from last visit
2. Review any change in complaints
3. Collect Subject's Feedback of the new screening modality
4. This will be a telephonic contact conducted by the Study Coordinator to convey to the subject on the diagnostic outcome and the course of medical treatment

**Note: The cost for this imaging will be borne by sponsor*

Cases negative for cancer would complete: Visits 1, 2 & 5
Cases positive and suspected cancer Cases, would complete all visits as applicable

The patients would be expected to go through the entire screening process in a duration of within ***2 weeks of enrollment on the study.***

10.6. Study Parameters

Study Procedure	**VISIT 1:** (Day 0-3)	**VISIT 2:** (Day 3-5)	**VISIT 3:** (Day 5-7)	**VISIT 4:** (Day 7-10)	**VISIT 5** (Day 7-14) ***End of Trial
Informed Consent and Sign-off	X				
Allocate Unique Subject ID to the subject	X				
Collect Past Medical History and Current Complaints	X				
Treatment History	X				
Family History	X				
Demographics	X				
Physical Examination	X				
Record Risk Factors	X				
Conduct Thermography (as per Appendix I)	X				
Clinical Breast Examination	X				
Standard Laboratory Work up required as part of routine screening	X				
Review change in complaints		X	X	X	
Review any change in how the patient feels from last visit		X	X	X	
#Sono-mammography/Mammography		X			
Review of Reports		X	X	X	
*Biopsy/FNAC/Histopathology / Hormone Status/related Laboratory Work-up			X		
MRI guided biopsy for cases reported positive by MRI			X	X	
**MRI/PET Scan			X	X	
Correlate Reports		X	X	X	
Confirm Diagnosis			X	X	
Collect Patient Feedback					X
Plan Patient Treatment			X	X	X

Note:

- **#**Sonomammography will done for subjects ≤44 years & Mammography will be done for subjects ≥45 years
- *Biopsy/FNAC/ Histopathology/ Hormone Status/related Laboratory Work up will be done for cases reported positive for malignancy by sonomammogrphy and mammography
- **MRI/PET Confirmatory Scan will be done in two scenarios; 1) in case of inconclusive result by sonomammography and mammography. 2) Also when the thermography reports positive for malignancy and standard modality does not
- ***End of Trial will be a telephone contact
- The screening process will conclude within 2 weeks of date of subject enrollment.
- Subjects confirmed negative for malignancy will complete visits 1,2,5
- Subjects confirmed positive for malignancy will complete all visits 1 through 5

11. DATA COLLECTION AND MANAGEMENT

Data collection and management will be performed by Sponsor or sponsor representative

Participant enrollment and data collection will be captured by the Site Study Coordinators in the individual patient files during the entire screening process. All the imaging and radiological reports hard copies and soft copies will be filed in the individual patient files. After all the reports have been received and collected by the study coordinator. The Coordinator would transcribe the information in the hardcopy CRFs (Case Report Forms) provided by Sponsor.

None of the personalized patient data will be captured in the hardcopy CRF. The subjects will be identified by unique Subject ID provided to them at the time of study enrolment. All the fields in the CRF will be completed within 10 days of patient completed all relevant study visits.

11.1. Clinical Data Submission

There will be data quality oversight visits conducted for the first 10% cases to identify any discrepancies or misunderstanding by Sponsor or sponsor representative. Study Coordinator will enter the fields on the excel spread sheet or software provided by Sponsor or sponsor representative after the CRFs have been verified. The scanned copies of the reviewed and completed CRFs after verification along with the verified excel spread sheet will be sent to Data Management on an on-going basis to the E-mail id provided by Sponsor. Data will be reviewed and queries will be raised by Sponsor or sponsor representative for resolutions to the respective sites, until data is cleaned and data base is locked. The clean data will go for Statistical Analysis and generation of report.

11.2. Data Security

Data collection system will have built-in security features preventing unauthorized access to confidential participant information. Access to the system will be controlled by passwords.

11.3. Data Quality Control

The Data Management at Sponsor end will maintain a study database at its site for monitoring data quality and for performing analyses. Any discrepancies and other data quality issues will be referred to Sites for resolution.

A goal of the monitoring of data is to assess compliance with the protocol and to look for unforeseen trends that may be indicative of procedural differences among clinical sites. If patterns are discovered in the data that appear to arise from causes specific to an institution, Data Management will apprise Sponsor and the site of the problem, and work with the site, along with Sponsor, until the problem has been resolved. If the Data Management and sponsor and site cannot find a resolution to the problem, it will be brought to the IDMC for further discussion and resolution.

12. IMAGE SUBMISSION

All the Images and scans produced as part of the screening procedure will be placed in the individual patient folders and also collected as part of the Case Report Forms. All personal information of the patients will be masked when collected by the sites by the sponsor or sponsor representatives.

All images are requested to be in digital format, unless indicated otherwise.

Film images or images should be e-mailed to geetha@niramai.com, posted on a secure shared drive as advised by the sponsor.

Images on CD should be addressed and sent to:

Kind Attention: Dr. Geetha Manjunath CEO & Co-founder
Address: NIRAMAI HEALTH ANALYTIX PVT. LTD.
A7-506, Elita Promenade,
J P Nagar 7th Phase,
Bengaluru – 560078, Karnataka, INDIA

If required and part of the protocol, images maintained at Site's Archive may be distributed to other parties for review, using CDs or E-mail where appropriate, for purposes of secondary review.

The header recorded on DICOM formatted image data often contains information identifying the participant by name. These identifiers must be masked before the images are transferred.

This involves replacing the following:

- Participant Name tag with the Subject ID
- Participant ID tag with the Hospital ID case number,
- Participant ID tag with Protocol Number.

In the event that the site does not have DICOM capability or is unable to transfer images with masked headers, the images may be sent on a CD or other electronic medium to Sponsor for digitization/transfer to the Image Archive.

13. ADVERSE EVENTS REPORTING

Thermography is virtually free of any adverse side effects.

- Thermography is a pain-free exam.
- It is non-invasive.
- Thermography does not require the use sedatives, anaesthesia, or any other medications.
- There is no need to observe preparatory measures, such as fasting or liquid-dieting, prior to the exam.
- It is passive and just measures the temperature on the skin, like a thermometer.

There is no adverse reaction expected from this imaging procedure, however, in case there is any untoward occurrence it should be reported immediately to sponsor at the following address:

Dr. Geetha Manjunath CEO & Co-founder
Address: NIRAMAI HEALTH ANALYTIX PVT. LTD.
A7-506, Elita Promenade,
J P Nagar 7th Phase,
Bengaluru – 560078, Karnataka, INDIA

E-mail: geetha@niramai.com
Phone Number: +91 98 801 18379

13.1. Definition of Adverse Event

An **Adverse Event (AE)** is any untoward medical occurrence in a participant that does not necessarily have a causal relationship with the study intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory or physiological finding), symptom, or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). Abnormal results of diagnostic procedures are considered to be adverse events (AEs) if the abnormality:

- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- is considered by the investigator to be of clinical significance

13.2. Definition of Serious Adverse Event

A Serious Adverse Event (SAE) is defined as any untoward medical occurrence that:

- results in death, or
- is life-threatening (at the time of the event), or
- requires inpatient hospitalization or prolongation of an existing hospitalization, or
- results in persistent or significant disability or incapacity, or
- is a congenital anomaly/birth defect

Such SAEs if they occur have to be reported to Sponsor Contact given above within ***24 hours*** of its knowledge.

All adverse events that do not meet the criteria of serious should be regarded as **non-serious adverse events.**

13.3. Adverse Event Grading

Grade is used to denote the severity of the adverse event. An AE is graded using the current version of the Common Terminology Criteria for Adverse Events (CTCAE), or the following categories (if the term does NOT appear in the current version of the CTCAE):

1 – Mild
2 – Moderate
3 – Severe
4 – Life-threatening or disabling

5 – Fatal

(For terms listed in the CTCAE, the grade is still recorded as 1, 2, 3, 4, or 5; however, the definition of the various grades will be specific to the term being used.)

13.4. Adverse Event Attribution

Attribution is used to determine whether an adverse event is related to a study treatment or procedure. An adverse event may be considered **associated** with the study treatment/procedure if there is reasonable possibility that the adverse event may be caused by the *"thermography"* An adverse event may be considered **NOT associated** with the study treatment/procedure if there is not a reasonable possibility that the adverse event may have been caused by the *"thermography"* Attribution categories are:

Definite – AE ***is clearly related*** to the study treatment or procedure.
Probable – AE ***is likely related*** to the study treatment or procedure.
Possible – AE ***may be related*** to the study treatment or procedure.
Unlikely – AE ***is doubtfully related*** to the study treatment or procedure.
Unrelated–AE ***is clearly NOT related*** to the study treatment or procedure.

13.5. Reporting of Adverse Events

Prompt reporting of adverse events is the responsibility of each investigator and/or Study Coordinator engaged in clinical research. An adverse event report should be submitted if there is a reasonable suspicion of the imaging procedure.

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse events are otherwise explained. Any death or adverse event (e.g. development of cancer, congenital anomaly in conceived offspring) occurring at any time after a subject has discontinued or terminated study participation that may be reasonably be related to the medical treatment or imaging effect should be reported.

Any adverse events that result in hospitalization or prolonged hospitalization should be documented and reported as serious AEs unless specifically stated otherwise in the protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an adverse event.

The condition, hospitalization, prolonged hospitalization, or surgery should NOT be reported as an AE in the following situations:

- For diagnostic or elective surgical procedures for a pre-existing condition. Surgery should not be reported as an outcome of an adverse event if the surgery was elective or diagnostic and it was uneventful.
- If it is required to allow efficacy measurement for the study.
- For therapy for the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

A pre-existing medical condition is defined as an adverse event if this medical condition worsens after the subject has been entered into the study.

14. ETHICAL CONSIDERATIONS

This protocol for human research study will be conducted according to standards of effective Good Clinical Practice (International Conference on Harmonization (ICH) Guidelines), if applicable the Indian regulations and applicable Ethical Guidelines for Biomedical Research involving Human Participants issued by the Indian Council of Medical Research. The trial protocol and subject documentation will be reviewed and approved by the Institutional Ethical Review Boards of the participating trial sites.

The study will be registered on the Clinical Trial Registry India (CTRI) as per government regulations before the first patient is enrolled.

In order to ensure the conclusions of the study get reviewed by an independent expert group. There is a provision in the study design to have an Independent Data Monitoring Committee (IDMC) which will conduct an independent review of the conclusions and correlations made by the study team. This Committee will consist of minimum of 5 members with the following roles:

- Chairman (Independent of the Study),
- Secretary (Sponsor /Site Representative),
- Member Thermologist (Independent of Study Team),
- Member Radiologist (Independent of the Study Team)
- Member Statistician (Independent of the Study team)

The role and procedural policies of the committee will be outlined in a "Charter" and submitted for review by the Institutional Ethical Committees of the participating sites in case such a committee is constituted.

15. CONFLICT OF INTEREST

Any investigator and/or research staff member who has a conflict of interest with this study (such as patent ownership, royalties, or financial gain greater than the minimum allowable by their institution) must fully disclose the nature of the conflict of interest in accordance with Sponsor policies, ethical considerations and local regulations.

16. PUBLICATION POLICY

Neither complete nor any part of the results of the study obtained under this protocol, nor any information provided to the investigator for the purposes of performing the study, will be published or passed on to any third party without the consent of Sponsor. Any investigator involved in this study is obligated to provide Sponsor with complete test results and all clinical data obtained from the participating in this protocol. Investigators will follow Sponsor Publication Policy as per the memorandum of Understanding signed between sponsor and participating trial site.

17. INSTITUTIONAL AUDITS

The investigator will permit study-related auditing and inspections (in case it happens) of all study-related documents by the EC/IRB, government regulatory agencies, and SPONSOR. The investigator will ensure the capability for inspection of all participating site's study-related facilities (e.g. imaging center, satellite sites). The investigator will allocate adequate time for these activities, allow access to all study-related documents and facilities, and provide adequate space to conduct these visits.

To help sites prepare for audits and assure that the investigator and the research staff maintain records appropriately, the Sponsor and its representatives will offer training to sites. This training will cover all aspects of data collection, including special instructions to obtain and file the various source documents needed to verify the accuracy of submitted data for this trial.

17.1. Source Documents

Source data are found in all information, original records of findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source documents represent the first recording of any observations made or data generated about a study participant while he or she is enrolled in a clinical trial. Source documents for each study participant substantiate the data that are submitted to Sponsor.

Source documents must verify the eligibility criteria and data submitted on all case report forms (CRFs). If an item is not mentioned (e.g., history and physical with no mention of a psychological condition), it will be assumed it is not present.

Research records for each case should contain copies of the source documents for the data reported to Sponsor. If data is abstracted from medical charts that are not filed at the investigative sites (e.g. hospital charts), copies of these records should be filed in the research chart. However, every attempt must be made to obtain all records/charts that were used to abstract any study data for this protocol at the time of the audit visit. This will prevent any discrepancies and the inability to verify the document and the data reported.

17.2. Case Report Forms

Case report forms (CRFs) are the primary data collection instruments for the study. All data requested on the CRFs must be recorded, and any missing data must be explained. If a space is left blank because the procedure was not done or the question was not asked, "ND" must be noted. If the item is not applicable to the individual case "NA" must be noted and if Data is not known, it should be entered as "NK". All entries must be printed legibly in black ink on the paper case report forms. In the event of any entry errors, corrections must be made by drawing a **single straight line** through the incorrect entry, writing **the initials of the person making the correction, recording the date** when the correction is being made, and entering the correct data above the strike through and date and initial the correction. Do not use white out or an eraser.

Data elements that are extracted from the medical record (such as participant history or official clinical interpretations of images, pathology, or surgery results) and recorded on the case report forms (CRFs) will be audited against the appropriate component of the medical record. Data elements gathered from signed participant questionnaires may be documented on the CRF. The image interpretation data required by the study that is a more detailed extraction of information from the image and is not typically documented in the standard radiology report may be recorded on the CRF and is accepted as source documentation **if signed by the Investigator**. At the time of audit if it happens, the auditor will verify the occurrence of the imaging examination, the reader, and the date on which the exam took place from the medical record. **Any use of an approved CRF as source documentation requires that the CRF be signed and dated and refer to the source of the information.**

Any use of CRFs as source documentation when the protocol has designated the source data will be medical record documentation will be considered a deficiency.

18. STATISTICAL CONSIDERATIONS

18.1. Hypothesis:

Sensitivity of Thermalytix© is non-inferior to sensitivity of standard modalities in detecting malignancy in subjects who show possible symptoms of suspected breast cancer.

18.2. Sample Size Justification:

The sample size for the study is computed to assess the non-inferiority of sensitivity of Thermalytix© to that of standard modalities in detecting malignancy in subjects who show possible symptoms of suspected breast cancer. The sensitivity of the standard modalities is assumed to be 95% (sensitivity of mammography or sono-mammography alone is between 75-85%. However, we assume that when multiple modalities are correlated the collective sensitivity of the standard of care will be much closer to absolute truth). Using a non-inferiority margin of 10% and significance level of 5%, there is 80% power to demonstrate non-inferiority with a sample size of 59 malignant subjects. From earlier studies, the rate of malignancy in the study population is estimated to be 23%. Using this rate, 256 subjects need to be evaluated in order to get the required number of malignant subjects. Assuming that about 10% of the enrolled subjects may be lost to follow-up, 275 subjects will be recruited in the study.

18.3. Statistical Analysis:

The following measures of validity will be computed:

- **Sensitivity** - probability of correctly identifying a true case of malignancy
- **Specificity** - probability of correctly identifying a true case of non-malignancy (normal or benign)
- **Positive Predictive Value** - probability that a labelled positive is a true case of malignancy
- **Negative Predictive Value** - probability that a labelled negative is a true case of non-malignancy.

The sensitivity of Thermalytix© and that of standard modalities will be compared to assess non-inferiority. The primary objective of non-inferiority will be assessed by constructing a 90% confidence interval (CI) around the difference in sensitivities (Thermalytix© – standard modalities). If the lower limit of the 90% CI is greater than -10%, then non-inferiority will be established. All the methods of validity will be computed for Thermalytix© along with their 90% CI.

19. REFERENCES

1. International agency for research on cancer, WHO, 2012 cancer statistics, http://globocan.iarc.fr
2. D. Kennedy et al, A comparative review of thermography as a breast screening technique. Integrative Cancer Therapies, 8(1), 2009, 09-16.
3. S.V. Sree, E.Y. Ng, R.U. Acharya, O. Faust, Breast imaging: a survey World Journal of Clinical Oncology, 2(4), April 10, 2011: 171-178.
4. Lapayowker MS, Barash I, Byrne R, et al. Criteria for obtaining and interpreting breast thermagrams. Cancer. 1976;38:1931-1935.
5. M. Gautherie, C.M. Gros. Breast thermography and cancer risk prediction. Cancer. 1980;45:51-56.
6. Feig SA, Shaber GS, Schwartz GF, et al. Thermography, mammography, and clinical examination in breast cancer screening. Review of 16,000 studies. Radiology. 1977;122:123-127.
7. Keyserlingk JR, Ahlgren PD, Yu E, Belliveau N, Yassa M. Functional infrared imaging of the breast. IEEE Eng Med Biol Mag, 19. 2000:30-41.
8. Nair et al, A Prospective Study of Computerized Digital Infrared ImageAnalysis (No Touch BreastScanTM) in Biopsy Proven Breast Cancers. Cancer Research, 69 (24), Supl. 3, Dec 15, 2009
9. Himanshu Madhu, Member, IEEE, Siva Teja Kakileti, Member, IEEE, Krithika Venkataramani, Member, IEEE and Susmija Jabbireddy; Extraction of Medically Interpretable Features for Classification of Malignancy in Breast Thermography 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Orlando, USA, on August 17 - 20, 2016.
10. K. Venkataramani, H. Madhu, S. Sharma, H. V. Ramprakash, A. Rajendra, A. K. Parthasarathy, G. Manjunath and L.K. Mestha, "Initial evaluation of semi-automated breast cancer screening using thermography," Quantitative InfraRed Thermography Asia Conference, July 2015.
11. Siva Teja Kakileti, Krithika Venkataramani, Xerox Research Center India, Bengaluru;
12. Automated Blood Vessel Extraction in Two-Dimensional Breast Thermography
13. Krithika Venkataramani, Member, IEEE, Lalit K. Mestha, Fellow, IEEE, L. Ramachandra, S.S. Prasad, Vijay Kumar, Priyanka J. Raja, Semi-automated Breast Cancer Tumor Detection with Thermographic Video Imaging
14. K. Venkataramani*, H. Madhu*, S. Sharma*, H. V. Ramprakash**, A. Rajendra**, A.K. Parthasarathy*, and G. Manjunath*, Initial Evaluation of Human Supervised Automated Breast Cancer Screening Using Thermography
15. Deborah Kennedy, BSc (Hons), MBA, ND, Tanya Lee, BSc, and Dugald Seely, ND, MSc; A Comparative Review of Thermography as a Breast Screening Technique; Integrative Cancer Therapies, Volume 08 Number 1, 2009 09-16, © 2008 Sage Publications, 10.1177/1534735408326171, http://ict.sagepub.com

20. APPENDICES

20.1. Appendix I PROTOCOL FOR TAKING THERMAL PICTURES

Protocol for Thermography

- Position the thermal camera with a height adjustment so that the image is approximately centered at the breast region to be scanned (as shown in figure 1) with its face toward the subject and the camera is positioned about 3 feet from the subject.
- The face of the camera head should be parallel to the surface of the subject.
- There should not be any reflective material (such as glass, metal, tiles, etc) near the subject.
- The room temperature should be set to 22° C and the A/C should be switched on at least 30 minutes before the subject comes into the room.

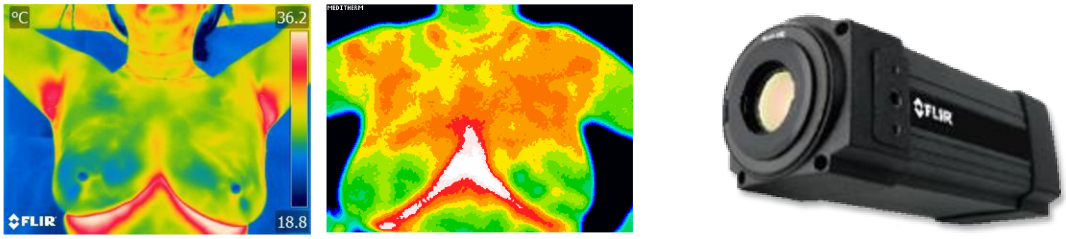
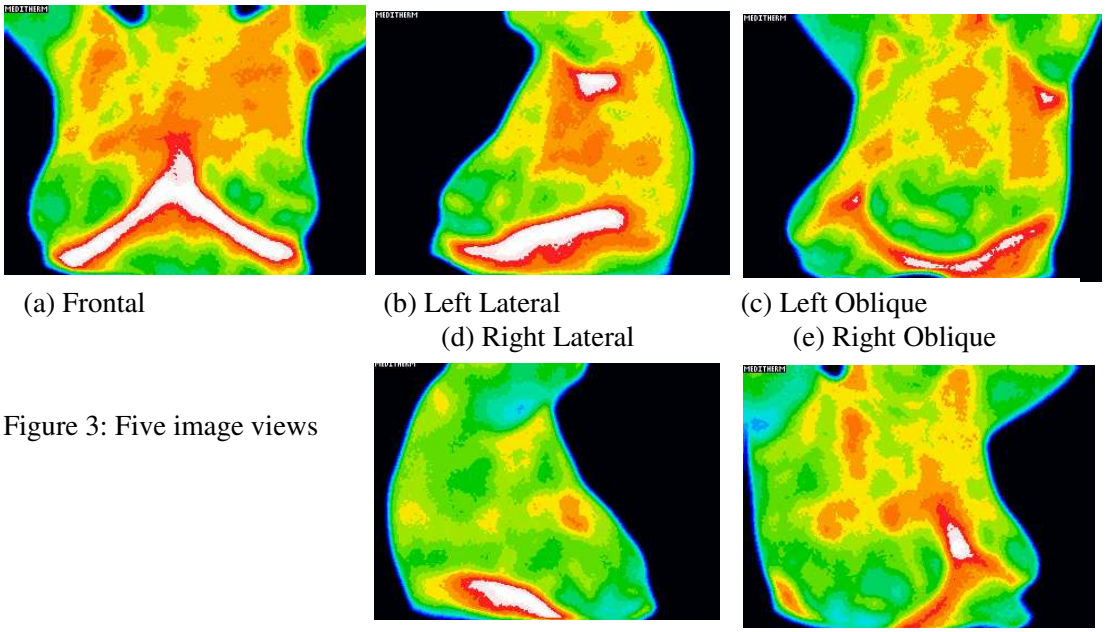


Figure 1: Thermal Camera

- The following are the guidelines on patient preparation before taking the thermography images
  - No yoga massage, or strenuous exercise (physical therapy) for at least three hours before the examination.
  - Avoid lotions, creams, deodorants, powders, or makeup on the breasts or underarms on the day of the exam.
  - No physical stimulation, mammography, ultrasound or any treatment of the breasts, chest, neck, or back for 24 hours prior to the examination
  - No bathing closer than 1 hour before the examination.
  - No coffee or any other hot drink or food to be taken 1 hour before the imaging.
  - The patient can continue to take all prior prescribed medications but should provide a list of such medications and supplements to the technician at the time of the exam.

- The subject should be cooled for a minimum of 10 minutes with all clothes removed from upper body with hands sideways on her waist and with her hair tied above (as in figure 1(b)
- The patient should sit straight, not slouch, take back support or touch the upper part of the body during cooling or imaging.
- Patient should face the source of air flow during cooling such that there is direct and uniform blow of cold air on both breasts.
- The thermal images should be captured with the subject's hands kept behind her head and with her hair tied above and any jewelry on the neck to be removed (as shown in figure 1(a)).

- Five images of the subject are captured for (a) frontal (b) left-lateral (c) left-oblique (d) Right-lateral and (e) Right-oblique views shown in the figure 3. To take the oblique and lateral views, the subject is positioned 45 deg and 90 deg, respectively, from the camera view point.
- Image capture and upload should be as per operating manual provided by Sponsor.



(a) Frontal (b) Left Lateral (c) Left Oblique (d) Right Lateral (e) Right Oblique

Figure 3: Five image views

20.2. Appendix II Informed Consent Document