Journal of Cardiovascular Disease Research

ISSN:0975-3583,0976-2833 VOL13, ISSUE04,2022

Original research article

A prospective study to compare the role of mammography and ultrasound in predicting themolecular subtypes of breast cancer with immunohistochemistry

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Abstract

Background: Breast cancer is the largest cause of cancer-related death in women globally, and its prevalence is rising. Although not all diagnosed breast lesions are dangerous and not all benign masses advance to cancer, radiological imaging (mammography, ultrasonography) and pathological diagnostics can substantially improve the precision of the final diagnosis.The goal of this study was to see if there was a link between mammography as well as ultrasound features and breast cancer molecular status.

Material and Methods: Present study was single-center, prospective, observational study, conducted in females, age above 30 yrs,with clinical suspicion of breast cancer.All cases were subjected to mammographic and ultrasound examination. The findings were correlated with histopathology and Immunohistochemistry reports.

Results: Mean age of the study participants 53.04 ± 13.63 years. Mammographic features were irregular margins (90.7%), angular margins (20%), microlobulated (11%), microlobulated and spiculated (47%). Common USG features were posterior acoustic enhancement (37.3%), mixed pattern (40%) and posterior acoustic shadowing (22.7%), calcification (60%), peritumoral echogenic halo (85.3%), TNBC was positive in 29.3% of the study participants, HER-2Negative ER Positive in 37.3% and HER 2+ve in 33.3% of the study participants.Among posterior acoustic shadowing features, 86% of cases were HER-2 Negative ER,PR Positive and 13.30% of cases were HER 2 +VE. Among posterior enhancement 100% of cases were under TNBC subtype, 10.7% were ER PR +VE, HER 2 –VE and 3% were HER 2 +VE.

Conclusion: In areas where receptor testing is not commonly available, knowledge of imaging features could assist clinicians in stratifying breast cancer patients, potentially allowing for earlier treatment or assisting in therapeutic decisions in those areas.

Keywords: Imaging features, mammography, ultrasound,genetic subtypes of breast cancer, immunohistochemistry,therapeutic decisions

Introduction

Breast cancer is the largest cause of cancer-related death in women globally, and its prevalence is rising. It is a category of diseases with a wide range of natural histories, histological subtypes, biological properties and treatment responses[1]. Since 2008, the incidence of the disease has increased by more than 20%, while the fatality rate has increased by 14%[2]. Breast carcinoma is one of the most common causes of cancer-related death in women, accounting for around 522 000 deaths in 2012[3].

Although not all diagnosed breast lesions are dangerous and not all benign masses advance to cancer, radiological imaging (mammography, ultrasonography) and pathological diagnostics can substantially improve the precision of the final diagnosis[4]. Breast cancer molecular subtyping has become a prerequisite for therapy planning, disease prognosis, and avoiding overtreatment. Based on gene expression patterns, the St. Gallen International Expert Consensus has divided breast cancer into five molecular subtypes: luminal A (LA), luminal B [(LB; HER2)], LB (HER2+)], human epidermal growth factor receptor 2 (HER2)-enriched, as well as basal-like (triple-negative)[5].

The principal imaging modalities utilised for breast cancer screening, diagnosis, staging, therapy response assessment and follow-up of treated breast cancer patients include mammography and ultrasound. Ultrasound features offer significant predictive values in separating triple-negative breast cancer (TNBC) versus non-triple-negative breast cancer (NTNBC), as well as determining tumour grades, according to a few retrospective studies conducted in the past[6, 7]. Cen *et al*.[8] found a strong link

ISSN:0975-3583,0976-2833 VOL13, ISSUE04,2022

between mammographically identified worrisome microcalcifications with HER2 overexpression status in a study.
The goal of this study was to see if there was a link between mammography as well as ultrasound features and breast cancer molecular status.

Material and Methods
Present study was single-center, prospective, observational study, conducted in department of radiodiagnosis, at Government Rajaji Hospital, Madurai, Tamilnadu, India. Study duration was of 1 year (July 2018 to June 2019). Study was approved by institutional ethical committee.

Inclusion criteria
- Females, age above 30 yrs, with clinical suspicion of breast cancer.

Exclusion criteria
- Patients with contralateral breast mass, metastasis, H/O prior cancer treatment and other malignancies.
- Age less than 30yrs.

The study population included patient presenting to the Surgery and Oncology OPD of Government Rajaji Hospital with Breast cancer. Clinical history was taken for every patients followed by careful breast examination. The patients were subjected to mammographic and ultrasound examination. The findings were correlated with histopathology and Immunohistochemistry reports.
After obtaining consent from the patients, they were subjected to Mammographic examination. Study will be performed by our mammographic unit, Planmed Sophie Model. Features such as Breast composition, Mass (Shape-oval/round/irregular, Margin-circumscribed/indistinct, Density-high/low, asymmetry-global/focal), Architectural distortion, Calcifications, Other associated features-skin retraction/ axillarylymphadenopathy were noted.
Routine ultrasound examination was done using Mindray DC-60 Machine, USG was done with the patient lying in supine position. The high frequency linear probe (transducer 7.5-12,MHz) had been used to image the breast tissues clearly. Both the breasts are exposed and the transducer was swept in radial and anti-radial direction to look for any abnormality. Features such as Breast composition, Mass (shape, margin, orientation, echo-pattern, posterior features), calcifications & associated features were noted.
Data was collected and compiled using Microsoft Excel,analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi- square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant.

Results
Majority of the study participants were in the age group of 41-50 years (30.7%) followed by 51-60 years (25.3%), 31-40 years (16%), 61-70 years (!2%), 71-80 years (10.7%), >80 years (4%) and 20-30 years (1.3%). Mean age of the study participants 53.04 ± 13.63 years

Table 1: Distribution of age among the study participants (N=75)

Age (in years)	Frequency	Percentage
20-30	1	1.3
31-40	12	16.0
41-50	23	30.7
51-60	19	25.3
61-70	9	12.0
71-80	8	10.7
>802	3	4.0

Around 90.7% were irregular and 9.3% regular. Around 20% of the lesions were having angular margins, 11% microlobulated, 47% microlobulated and spiculated, 20% spiculated and 3% microlobulation with angulations. Around 37.3% were showing posterior acoustic enhancement, 40% showing mixed pattern and 22.7% showing posterior acoustic shadowing features on ultrasonogram.

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Table 2: Distribution of shape of the breast lesion

Shape of the lesion	Frequency	Percentage
Irregular	68	90.7
Regular	7	9.3
Margin		
Angular	15	20
Micro lobulated	8	10.7
Microlobulated, spiculated	35	46.7
Spiculated	15	20
Microlobulations, angulations	2	2.7
Posterior features of the breast		
Enhancement	17	22.7
Mixed pattern	30	40.0
Shadowing	28	37.3

Around 60% of the patients showed calcification on ultrasonogram. Around 85.3% of cases showed peritumoral echogenic halo on ultrasonogram. Around 30.7% of the breast masses were irregular and 69% were round.

Table 3: Distribution of ultrasonography findings

Calcification	Frequency	Percentage
Present	45	60
Absent	30	40
Peritumoral echogenic halo of the breast	**Frequency**	**Percentage**
Present	64	85.3
Absent	11	14.6
Shape of the mass	**Frequency**	**Percentage**
Irregular	23	30.7
Round	52	69.3

Around 85.3% were having spiculated margins, 12% regular margins and 2.7% are having Spiculated, Microlobulation margins. Around 68% of the breast lesions were having calcification within the mass lesion.

Table 4: Distribution of margins of the breast mass by mammogram findings

Margin	Frequency	Percentage
Spiculated	64	85.3
Regular	9	12.0
Spiculated, Microlobulation	2	2.7
Calcification of the breast		
Present	51	68
Absent	24	32

TNBC was positive in 29.3% of the study participants, HER-2Negative ER Positive in 37.3% and HER 2+ve in 33.3% of the study participants.

Table 5: Distribution of molecular subtypes

Molecular subtypes	Frequency	Percentage
TNBC	22	29.3
HER-2 Negative ER, PRPositive	28	37.3
HER 2+ve	25	33.3

Among posterior acoustic shadowing features, 86% of cases were HER-2 Negative ER,PR Positive and 13.30% of cases were HER 2 +VE. Among posterior enhancement 100% of cases were under TNBC subtype,10.7% were ER PR +VE,HER 2 –VE and 3% were HER 2 +VE. Among mixed features 83.3% were HER 2 +VE and 4% were ER PR +VE,HER 2 –VE.

Table 6: Distribution of molecular subtypes with posterior features

Molecular subtypes	Shadowing(N=28)	Enhancement(N=17)	Mixed(N=30)
TNBC (n=22)	3 (10.7)	17 (100)	1 (3.3)
HER-2Negative ER PR Positive (n=28)	24 (85.7)	0	4 (13.3)
HER 2+ve (n=25)	1 (3.6)	0	25 (83.3)

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Mean age among TNBC is 56.48 ± 13.63, among HER-2 Negative ER Positive 54.82 ± 13.83 and HER 2+ve 48.35 ± 11.69.

Table 7: Distribution of molecular subtypes with posterior features

Molecular subtypes	Mean±SD
TNBC	56.48±13.63
HER-2Negative ER Positive	54.82±13.83
HER 2+ve	48.35±11.69

In present study we noted that sensitivity, specificity, positive predictive value, negative predictive value & accuracy values were 94.12%, 79.17%, 90.57%, 86.36%& 89.33% respectively for mammography as compared to immunohistochemistry in breast cancer diagnosis.

Table 8: Correlation between mammography with immunohistochemistry (N=75)

Mammography Calcification	Immunohistochemistry	
	Present (HER 2+VE,ER PR +VE HER2 –VE)	Absent (TNBC)
Present	48	5
Absent	3	19
Sensitivity	94.12% (83.76% to 98.77%)	
Specificity	79.17% (57.85% to 92.87%)	
Positive predictive value	90.57% (81.44% to 95.46%)	
Negative predictive value	86.36% (67.46% to 95.09)	
Accuracy	89.33% (80.06% to 95.28%)	

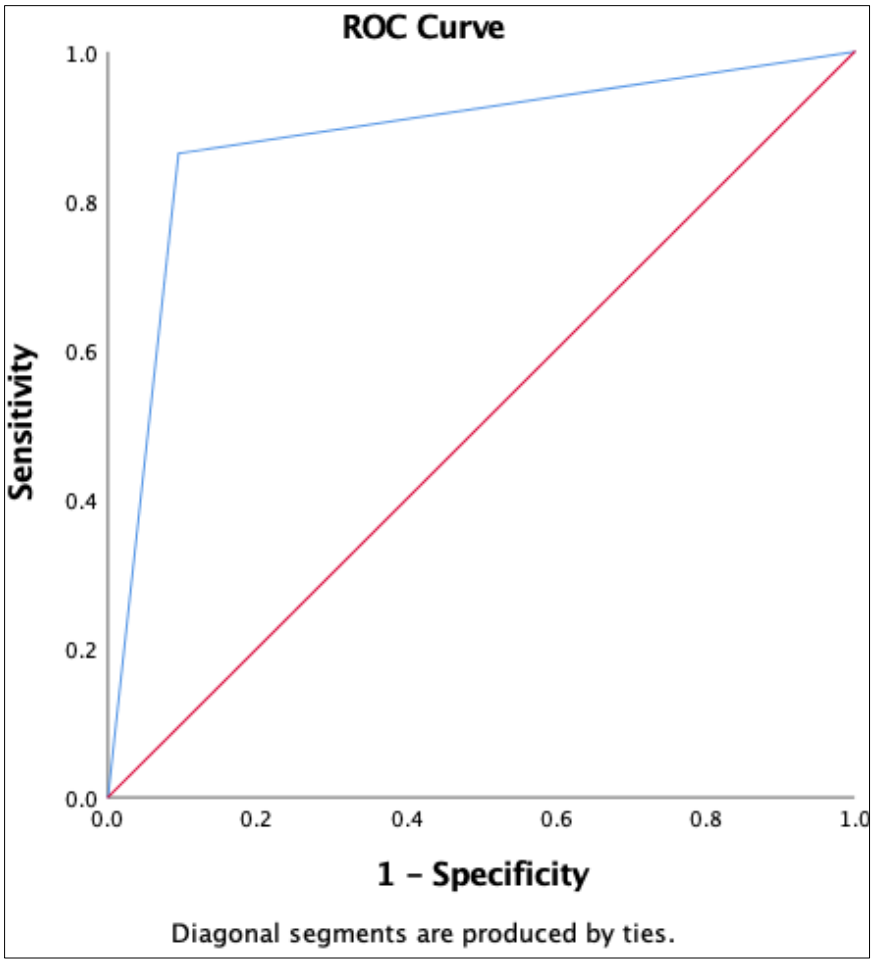

Fig 1: Correlation of between mammography features of breast cancer with immunohistochemistry

The area under the curve shows 0.88 with 95% CI (0.78 to 0.99); p=<0.001
In present study we noted that sensitivity, specificity, positive predictive value, negative predictive value & accuracy values were 77.27%, 100%, 100%, 91.38%& 91.33% respectively for radiological feature of

ISSN:0975-3583,0976-2833 VOL13, ISSUE04,2022

posterior acoustic enhancement as compared to immunohistochemistry in breast cancer diagnosis.

Table 9: Correlation between posterior acoustic enhancement with immunohistochemistry (N=75)

Posterior acoustic enhancement	IHC (TNBC)	
	Present	Absent
Present	17	0
Absent	5	53
Sensitivity	77.27% (54.63% to 92.18%)	
Specificity	100% (93.28% to 100%)	
Positive predictive value	100% (91.44% to 100%)	
Negative predictive value	91.38% (83.07% to 95.82%)	
Accuracy	93.33% (85.12% to 97.80%)	

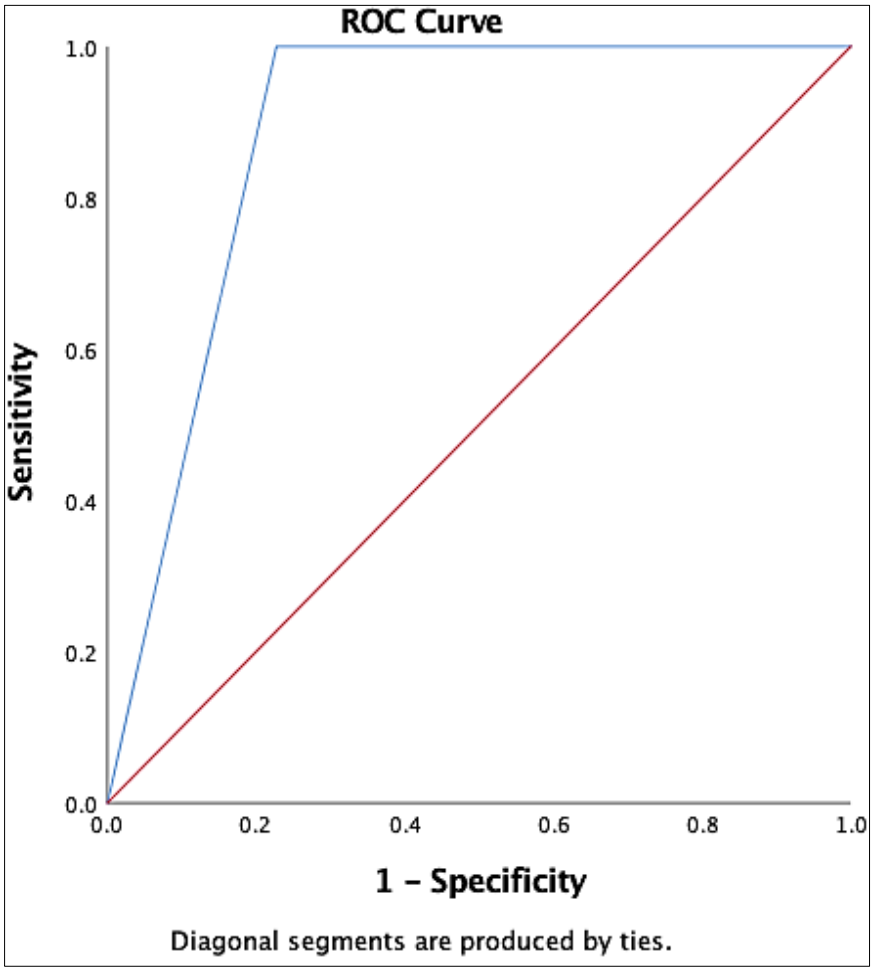

Fig 2: Correlation between posterior acoustic enhancement of breast cancer with immunohistochemistry (N=75)

The area under the curve shows 0.88 with 95% CI (0.79 to 0.97); p=<0.001

In present study we noted that sensitivity, specificity, positive predictive value, negative predictive value & accuracy values were 85.71%, 91.49%, 85.71%, 91.49%& 89.33% respectively for radiological feature of posterior acoustic shadowing as compared to immunohistochemistry in breast cancer diagnosis.

Table 10: Correlation between posterior acoustic shadowing of breast cancer with immunohistochemistry (N=75)

Posterior acoustic shadowing	IHC (HER-2Negative ER Positive)	
	Present	Absent
Present	24	4
Absent	4	43
Sensitivity	85.71% (67.33% to 95.97%)	
Specificity	91.49% (79.62% to 97.63%)	
Positive predictivevalue	85.71% (69.89% to 93.94%)	
Negative predictive value	91.49% (81.21% to 96.40%)	
Accuracy	89.33% (80.06% to 95.28%)	

ISSN:0975-3583,0976-2833 VOL13, ISSUE04,2022

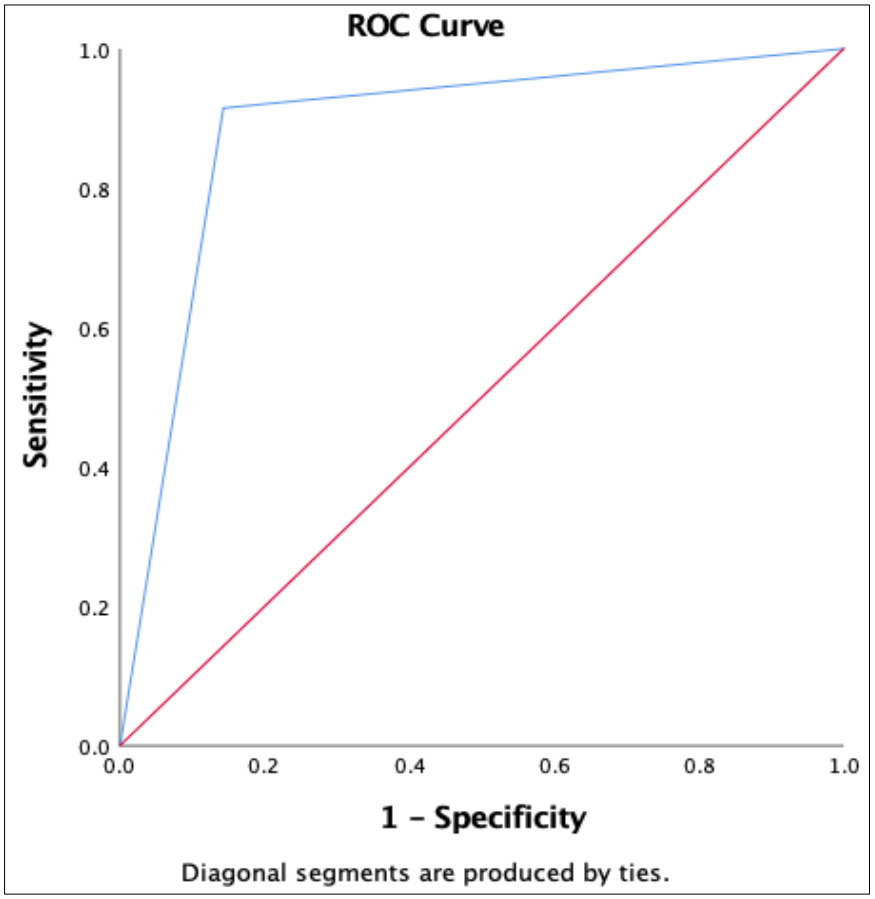

Fig 3: Correlation between posterior acoustic shadowing of breast cancer with immunohistochemistry (N=75). The area under the curve shows 0.88 with 95% CI (0.79 to 0.97); p=<0.001

Discussion

In our study majority of the study participants were in the age group of 41-50 years (30.7%) followed by 51-60 years (25.3%), 31-40 years (16%), 61-70 years (12%), 71-80 years (10.7%), >80 years (4%) and 20-30 years (1.3%). Mean age of the study participant's 53.04±13.63 years. In Ian *et al*.,[9] the mean age of the study participants were 61.1 ± 11.75 years.

In our study upon ultrasonogram around 20% were having angular margins, 11% microlobulated, 47% microlobulated and spiculated, 20% spiculated and 3% microlobulation with angulations. In Ian *et al*.,[9] 30% had spiculated margins, 60% microlobulated and 5% circumscribed.

In our study posterior acoustic features shows that around 29.3% were showing enhancement, 34.7% mixed pattern and 36% shadowing features on ultrasonogram. In Ian *et al*. [7]23% showed shadow, 26% enhancement and 11% mixed.

In contemporary breast cancer management practise, treatment is tailored to the receptor status of a specific tumour as established by IHC, which is the gold standard test and necessitates the collection of a tissue sample for testing. Many research are being carried out to evaluate whether various imaging modalities like as mammography, ultrasound imaging and magnetic resonance imaging may be used to forecast the molecular subtype of a breast tumour, which would further improve the possibility for preoperative treatment methods[10, 11]. Breast cancer is diagnosed primarily by the use of mammography and ultrasound, which are the principal imaging modalities.

Using in situ hybridization (IHC), we investigated the relationships between mammography and ultrasound features and molecular subtypes of breast cancer classified according to receptor status (ER, PR, HER2+, and Ki67) and other characteristics of the tumour. The presence of non-circumscribed margins and posterior acoustic shadowing on ultrasonography was seen in LA and LB tumours (ER- and/or PR-positive and HER2-negative).Cancers with posterior acoustic shadowing were found to be more than nine times more likely to be associated with hormone receptor positive, according to Irshad *et al*.,[12] Similar findings were made by Celebi *et al*.,[10] who discovered that tumours with combined findings of non-circumscribed margins and posterior shadowing had a 10.58 times higher relationship with the LA and LB subtypes than other cancers[2].The existence of receptor status is associated with a favourable prognosis because they demonstrate hormone sensitivity as well as stromal response,

ISSN:0975-3583,0976-2833 VOL13, ISSUE04,2022

perilesional spiculations, and fibrosis, which result in non-circumscribed margins and posterior shadowing features on ultrasound imaging[13].

Tumors detected to have microcalcifications on mammogram were strongly associated with HER2 overexpression. Sensitivity and specificity in our study in detecting the calcification by mammogram is around 94.12% and 79.17% respectively. HER2-enriched tumours were shown to have more calcifications on ultrasound than other subtypes, however some tumours with microcalcifications may have been missed because ultrasonography is not as sensitive as mammography for detecting microcalcifications[14].

In addition, Cen *et al*.,[8] discovered that HER2-enriched tumours were more likely to exhibit heterogeneous and pleomorphic microcalcifications on mammography than non-HER2-enriched tumours. Amorphous and heterogenous coarse calcifications were shown to be associated with a higher occurrence of the LA subtype, as demonstrated by Cen and colleagues[8].

It was determined that tumours with well-circumscribed margins and posterior enhancement were highly indicative for TNBC type of breast cancer, which was found to be the most aggressive type of breast cancer with rapid growth and necrosis. Triple-negative malignancies are hyper-vascular when compared to non-triple-negative cancers[15]. TNBCs were more common in the younger age group and were found to be related with microcalcifications in a negative way[15].

The IHC test, which is an invasive treatment, is used to determine the receptor status of a patient. This process can cause discomfort and difficulties in the patient. This test's dependability on tissue handling and processing is also dependent on how the tissue is handled and processed, which can result in false-negative results on rare occasions. These tests are quite expensive, and they are not readily available in many developing and underdeveloped nations, particularly in Africa[16].

Mammography and ultrasound, on the other hand, are noninvasive technologies that are widely available and are utilised as the principal imaging modalities for the evaluation of breast cancer[9, 17].Knowing the predictive value of particular imaging modalities radiologists may assist clinicians in stratifying their patients, allowing them to manage their patients in accordance with the resources available to them in their practice. The presence of imaging markers that are strongly suggestive of a positive receptor status can further assist the clinician in deciding on the appropriate therapy in the event of a discrepancy.

Conclusion

Several imaging features were found to be independent predictors of genetic subtypes of breast cancer, and these findings were confirmed. In areas where receptor testing is not commonly available, knowledge of such connections could assist clinicians in stratifying breast cancer patients, potentially allowing for earlier treatment or assisting in therapeutic decisions in those areas.

Conflict of Interest: None to declare.

Source of funding: Nil.

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