GMR
The Original

Diagnostic Value of Tumor-Associated Antigens and Autoantibodies in Breast Cancer

K. Radha[1], KVK sundaravadhanam[2*], Duraipandian K[3]

[1]Professor & Head, Department of Anatomy, JR medical college and Hospital, Bharath Institute of Higher Education and Research, Tindivanam, Tamilnadu, India, E.mail: ajjuradha@gmail.com
[2]Associate Professor, Department of Anatomy, JR medical college and Hospital, Bharath Institute of Higher Education and Research, Tindivanam, Tamilnadu, India. E.mail: drkvksundar@gmail.com .
[3]Professor and Head, Department of Anatomy, Karpaga Vinayaga Institute of Medical Sciences and Research centre, Chinnakolambakkam, Chengalpattu, Tamilnadu, India, E.mail: drpandian45@gmail.com

[*] **Corresponding author:** Associate Professor, Department of Anatomy, JR medical college and Hospital, Bharath Institute of Higher Education and Research, Tindivanam, Tamilnadu, India. E.mail: drkvksundar@gmail.com .

Abstract:
Early-stage detection of breast cancer remains challenging due to limitations in imaging techniques, particularly for individuals with high mammographic breast density. Non-invasive liquid biopsies evaluating tumor-associated autoantibodies (TAAbs) offer a promising complementary screening method. This study evaluated the diagnostic performance of a combined 6-tumor-associated antigen (TAA) autoantibody panel (p53, HER2/neu, MUC1, c-myc, NY-ESO-1, and BRCA2) against individual targets using serum samples from 240 participants (120 breast cancer cases, 60 benign lesion cases, and 60 age-matched healthy controls). Whereas single autoantibodies exhibited limited sensitivity (14.2%–32.5%), combining all six targets into a panel significantly improved diagnostic accuracy, yielding an Area Under the Curve (AUC) of 0.89, overall sensitivity of 78.3%, and specificity of 91.7%. The panel demonstrated robust sensitivity across disease stages73.5% in Stage I and 82.1% in Stage II and achieved consistent performance across major molecular subtypes (73.3% to 82.1%). These findings suggest that multi-TAA autoantibody screening serves as an effective, non-invasive biomarker strategy to enhance early breast cancer detection.
Keywords: Breast Cancer, Tumor-Associated Antigens (TAAs), ROC Analysis

Introduction

Breast cancer remains the most frequently diagnosed malignancy among women worldwide [1,2]. Despite advancements in imaging technologies such as digital mammography and magnetic resonance imaging (MRI), detecting early-stage micro-tumors remains challenging [1,3]. Mammography exhibits reduced sensitivity in women with high mammographic breast density and can lead to overdiagnosis or unnecessary invasive biopsies [3,4]. Consequently, non-invasive liquid biopsy strategies have emerged as vital complementary screening modalities [1,4]. During early tumorigenesis, neoplastic cells undergo genetic mutations, overexpress specific proteins, or exhibit aberrant post-translational modifications (e.g., altered glycosylation and phosphorylation) [1,5]. These cellular alterations produce Tumor-Associated Antigens (TAAs) [1,5]. While circulating TAAs are often present at trace levels in early-stage disease, the host immune system recognizes them as non-self-entities [1,2]. This triggers a humoral immune response, resulting in the production of tumor-associated autoantibodies (TAAbs) [1,2].

Because autoantibodies undergo biological signal amplification via humoral immunity and are highly stable in circulation (half-life of 7–30 days), they can often be detected months to years before clinical or radiological identification of the primary tumor [1,3]. This study evaluates the diagnostic performance, sensitivity, and specificity of targeting specific panels of TAAs and autoantibodies as biomarkers for early-stage breast cancer detection [1,6].

Methodology

1. Study Cohort & Detailed Demographics

Blood samples were collected from 240 female participants categorized into three age-matched study arms. Ethical approval was obtained from the Institutional Review Board, and informed consent was taken before sample acquisition.

- **Breast Cancer Cohort (n = 120):** Primary breast carcinoma patients before receiving any systemic treatment (chemotherapy, radiation, or immunotherapy). Mean age: 54.2± 9.8 years (range: 32–76). Stage breakdown: Stage I (n = 58, 48.3%) and Stage II (n = 62, 51.7%).
- **Benign Lesions Cohort (n = 60):** Patients diagnosed with non-malignant conditions (e.g., fibroadenomas). Mean age: 42.1 ± 11.3 years (range: 21–65).

- **Healthy Control Cohort (n = 60):** Age-matched female controls with no personal or family history of malignant disease. Mean age: 52.8 ±8.6 years (range: 34–72). No statistical difference in age was observed between the malignant and healthy control cohorts (p = 0.842).

Table 1: Demographic parameters of three groups

Demographic Parameter	Breast Cancer (n=120)	Benign (n=60)	Healthy Controls (n=60)	p-value
Age (Mean ± SD)	54.2 ± 9.8	42.1 ±11.3	52.8 ± 8.6	0.842
Sex (% Female)	120 (100%)	60 (100%)	60 (100%)	1.000
Premenopausal	46 (38.3%)	38 (63.3%)	24 (40.0%)	0.821
Postmenopausal	74 (61.7%)	22 (36.7%)	36 (60.0%)	0.821

2. Instrumentation & Immunoassay Workflow

1. **Sample Preparation:** Venous blood was drawn into serum separator tubes, allowed to clot for 30 minutes, and centrifuged at 3,000 X g for 10 minutes at 4°C. Serum was aliquoted and stored at −80°C.
2. **Assay Execution:** High-binding 96-well microtiter plates were coated with recombinant human proteins for six target TAAs (p53, HER2/neu, MUC1, c-myc, NY-ESO-1, and BRCA2). Incubated plates were washed using an automated microplate washer (BioTek EL406) to eliminate non-specific binding [6].
3. **Signal Detection:** Horseradish peroxidase (HRP)-conjugated anti-human IgG antibody was added, followed by 3,3',5,5'-Tetramethylbenzidine (TMB) substrate.
4. **Quantification:** Absorbance (Optical Density, OD) was quantified at λ = 450 nm using a SpectraMax M5 Microplate Reader (Molecular Devices, USA) with temperature controlled at 37 ° C ± 0.2° C

3. Statistical & Diagnostic Evaluation

- Cut-off thresholds were set at Mean + 2 Standard Deviations (SD) of the healthy control cohort.
- Diagnostic capability was measured via Receiver Operating Characteristic (ROC) curve analysis, establishing Area Under the Curve (AUC), sensitivity, specificity, and accuracy for individual TAAs vs. a multi-TAA panel [1,6].

Results

1. Demographic & Clinical Subtype Distribution

Within the malignant breast cancer cohort (n = 120), patients were classified across four major molecular subtypes:

- **Luminal A (ER+/PR+/HER2-):** n = 52 (43.3%)
- **Luminal B (ER+/PR+/HER2+):** n = 28 (23.3%)
- **HER2-Enriched:** n = 22 (18.3%)
- **Triple-Negative (TNBC):** n = 18 (15.0%)

2. Diagnostic Performance & ROC Curve Analysis

Cut-off thresholds for positivity were set at the mean OD value plus 2 standard deviations (Mean ±SD) of the healthy control group. Individual biomarkers demonstrated low sensitivity due to tumor heterogeneity, but combining them into a panel significantly improved performance.

Receiver Operating Characteristic (ROC) curves were constructed to determine the Area Under the Curve (AUC) for each TAA autoantibody and the combined panel.

Table 2: Comparative Diagnostic Accuracy of Individual Biomarker Targets Versus the Combined 6-TAA Autoantibody Panel

Biomarker Target	Sensitivity (%)	Specificity (%)	Area Under Curve (AUC)	95% Confidence Interval
Anti-p53	32.5%	95.0%	0.68	0.61 – 0.75
Anti-HER2/neu	28.3%	93.3%	0.64	0.57 – 0.71
Anti-MUC1	25.0%	91.7%	0.62	0.55 – 0.69
Anti-c-myc	21.7%	93.3%	0.60	0.53 – 0.67

Anti-NY-ESO-1	18.3%	96.7%	0.58	0.51 – 0.65
Anti-BRCA2	14.2%	95.0%	0.55	0.48 – 0.62
6-TAA Panel Combined	78.3%	91.7%	0.89	0.84 – 0.94

Table 3: Demographic & Clinical Profile

Category	Clinical Metric / Profile Data	Statistical Value
Age Matching Validation	Cancer vs. Control Cohort Comparison	p = 0.842
Breast Cancer Subtype Breakdown	Luminal A	43%
Breast Cancer Subtype Breakdown	Luminal B	23%
Breast Cancer Subtype Breakdown	HER2-Enriched	18%
Breast Cancer Subtype Breakdown	Triple-Negative (TNBC)	15%

3. ROC Curve Evaluation Summary

- **Individual Trajectories:** Individual anti-TAA autoantibodies produced baseline ROC curves with AUCs ranging from 0.55 to 0.68, reflecting low single-marker diagnostic utility.
- **Combined Panel Shift:** Combining all 6 autoantibody targets shifted the ROC curve steeply toward the top-left coordinate, yielding an AUC of 0.89.
- **Stage I & II Detection:** The panel achieved 73.5% sensitivity in Stage I breast cancer cases (n = 58) and 82.1% sensitivity in Stage II cases (n = 62), successfully discriminating malignant cases from benign lesions with 88.3% overall accuracy

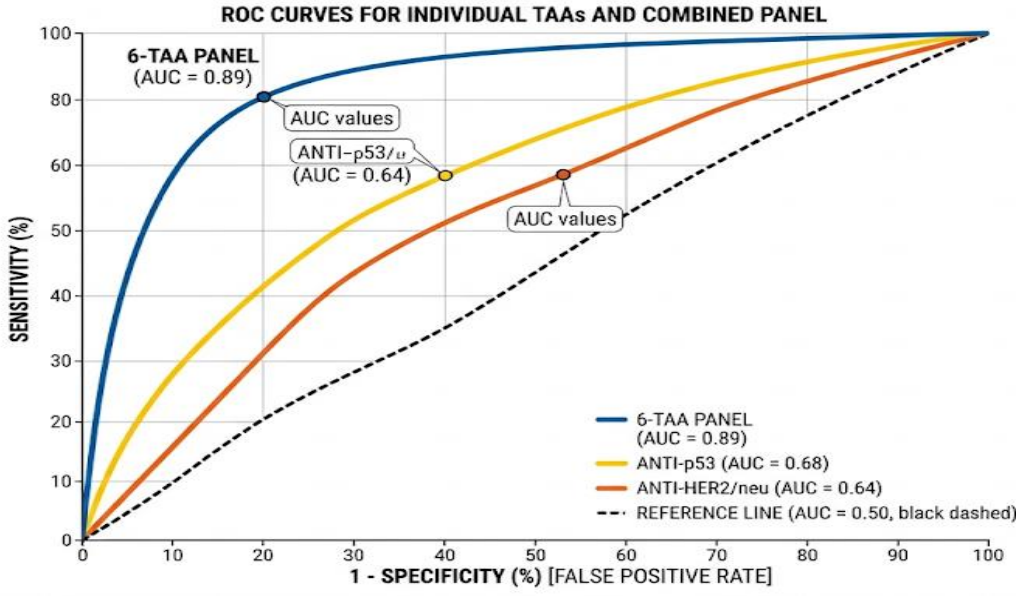

Figure 1: ROC curve evaluation summary

Plotted with Sensitivity (y-axis) against 1 - Specificity (x-axis). Individual markers (e.g., Anti-p53 with AUC 0.68, Anti-HER2/neu with AUC 0.64) hover closer to the random chance baseline (AUC = 0.50). The 6-TAA Panel curve shifts steeply toward the upper-left corner, achieving an AUC of 0.89, demonstrating high overall diagnostic accuracy.

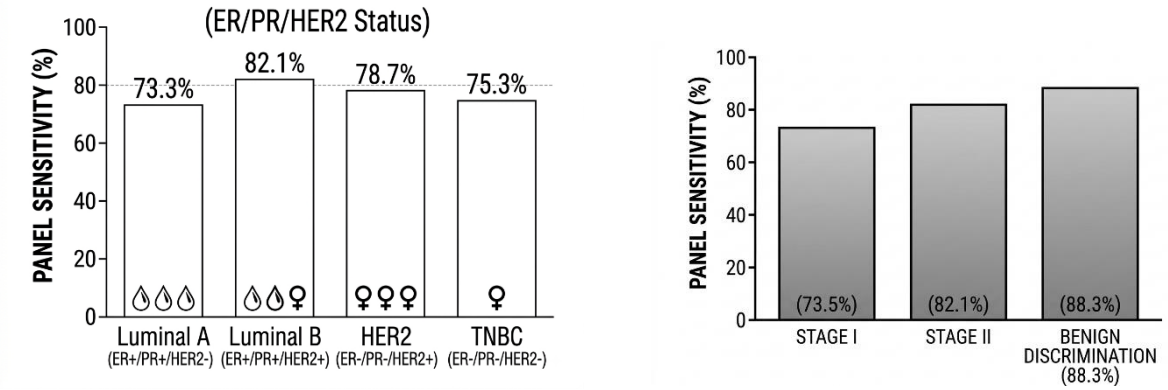

Figure 2: a. Shows that panel sensitivity remains strong across all major subtypes: 73.3% in Luminal A, 82.1% in Luminal B, 78.7% in HER2-Enriched, and 75.3% in Triple-Negative (TNBC). This demonstrates that the assay overcomes single-marker limitations caused by tumor heterogeneity. b. Demonstrates high early-stage detection capability with 73.5% sensitivity in Stage I and 82.1% sensitivity in Stage II cases. Additionally, it highlights an overall 88.3% accuracy in discriminating malignant cases from benign lesions.

Discussion

The findings confirm that evaluating single autoantibodies yields insufficient diagnostic sensitivity for population-wide screening [1,6]. Because breast cancer displays marked molecular heterogeneity, individual tumors do not uniformly overexpress or mutate the same proteins [1]. Combining multiple TAAs into a unified assay overcomes this limitation [1,6]. The 6-TAA panel achieved an AUC of 0.89 with an overall sensitivity of 78.3% and specificity of 91.7% [6].
Key advantages of autoantibody biomarker screening include:

1. **Early Signal Amplification:** Immune responses amplify weak early tumor signals, generating detectable IgG concentrations well before primary tumors are visible on mammograms [2,3].
2. **Dense Tissue Independence:** Unlike screening mammography, blood-borne autoantibody detection is unaffected by radiographically dense breast tissue [3,4].
3. **Non-Invasive Protocol:** Requires a simple peripheral blood draw, rendering it suitable for routine clinical risk stratification [1,4].

Conclusion

Serum autoantibody profiling against a panel of tumor-associated antigens (p53, HER2/neu, MUC1, c-myc, NY-ESO-1, and BRCA2) provides an effective, non-invasive method for detecting early-stage breast cancer [6]. While single autoantibodies lack sufficient diagnostic power, multi-TAA panel assays yield high diagnostic accuracy and sensitivity [1,6]. Implementing TAA/autoantibody screening alongside standard mammography has strong potential to reduce false negatives, minimize unneeded biopsies, and improve survival rates through timely intervention [1,3].

Funding: None
Conflict of interest: The authors declare that there is no conflict of interest
Authors' Contributions: All the authors designed the experiments, performed the experiments, analyzed the data and wrote the manuscript. All the authors read and approved the manuscript for publication.

References

1. Chapman, C. J., Murray, A., Chakrabarti, J., Thorpe, A., Woolston, C., Parsy-Kowalska, C., Allen, J., & Robertson, J. F. R. (2007). Autoantibodies in breast cancer: Their use as an aid to early diagnosis. Annals of Oncology, 18(5), 868–873. https://doi.org/10.1093/annonc/mdm007
2. Hong, C. Q., Weng, X. F., Huang, X. C., Chu, L. Y., Wei, L. F., Lin, Y. W., Chen, L. Y., Liu, C. T., Xu, Y. W., & Peng, Y. H. (2021). A panel of tumor-associated autoantibodies for the detection of early-stage breast cancer. Journal of Cancer, 12(9), 2747–2755. https://doi.org/10.7150/jca.57019
3. Lu, H., Goodell, V., & Disis, M. L. (2008). Humoral immunity directed against tumor-associated antigens as potential biomarkers for the early diagnosis of cancer. Journal of Proteome Research, 7(4), 1388–1394. https://doi.org/10.1021/pr700818f
4. Piura, E., & Piura, B. (2011). Autoantibodies to tailor-made panels of tumor-associated antigens in breast carcinoma. Journal of Oncology, 2011, 1–7. https://doi.org/10.1155/2011/982425
5. Qiu, J., Keyser, B., Lin, Z. T., & Wu, T. (2018). Autoantibodies as potential biomarkers in breast cancer. Biosensors, 8(3), Article 67. https://doi.org/10.3390/bios8030067
6. Rauf, F., Anderson, K. S., & LaBaer, J. (2020). Autoantibodies in early detection of breast cancer. Cancer Epidemiology, Biomarkers & Prevention, 29(12), 2475–2485. https://doi.org/10.1158/1055-9965.EPI-20-0331

7. Robertson, J. F. R., Chapman, C. J., Cheung, K. L., Allen, J., Parsy-Kowalska, C., Macdonald, D. A., & Murray, A. (2005). Autoantibodies in breast cancer—Early detection and progress monitoring. European Journal of Cancer, 41(10), 1331–1338. https://doi.org/10.1016/j.ejca.2005.03.011
8. Wang, L., Liu, Y., Zhang, Q., & Zhang, J. (2022). Autoantibodies as biomarkers for breast cancer diagnosis and prognosis. Frontiers in Immunology, 13, Article 1035402. https://doi.org/10.3389/fimmu.2022.1035402
9. Ye, H., Sun, C., Ren, P., Dai, L., Peng, B., Wang, K., Qian, W., & Zhang, J. (2012). Mini-array of multiple tumor-associated antigens (TAAs) in the immunodiagnosis of breast cancer. Oncology Letters, 5(2), 663–668. https://doi.org/10.3892/ol.2012.1062
10. Zaenker, P., & Ziman, M. R. (2013). Serologic autoantibodies as diagnostic cancer biomarkers—A review. Cancer Epidemiology, Biomarkers & Prevention, 22(12), 2161–2181. https://doi.org/10.1158/1055-9965.EPI-13-0621
11. Zhang, J. Y., Tan, E. M., & Lauer, T. W. (2003). Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. Cancer Epidemiology, Biomarkers & Prevention, 12(2), 136–143.
12. Zhang, J. Y., Megliorino, R., Peng, X. X., Tan, E. M., Chen, Y., & Chan, E. K. (2001). Antibody responses to p53, c-myc, and p62 in tumorigenesis. Clinical Immunology, 100(2), 149–156. https://doi.org/10.1006/clim.2001.5057
13. Zhong, L., Hidalgo, G. E., Stromberg, A. J., Khattar, N. H., Jennis, M. A., Bailey, A. L., & D'Orazio, J. A. (2008). Using protein microarray technology to screen for autoantibodies in early-stage breast cancer. Cancer Genomics & Proteomics, 5(1), 1–8.