

Diagnostic performance of a combined TAP, TAM, CA125 and CA153 panel for stage I breast cancer in patients with unilateral nipple discharge

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Received November 4, 2026; Accepted September 25, 2026

DOI: 10.3892/ol.2026.15915

Abstract. Minimally invasive biomarkers that complement imaging may improve risk stratification in patients presenting with unilateral nipple discharge. The present study evaluated the diagnostic performance of tumor-abnormal protein (TAP), tumor-associated material (TAM), carbohydrate antigen 125 (CA125) and cancer antigen 153 (CA153) for breast cancer, individually and in combination, with a particular emphasis on stage I disease. The present retrospective single-center study included 104 untreated patients with breast cancer and unilateral nipple discharge, including 25 patients with stage I disease, and 99 healthy controls. TAP, TAM, CA125 and CA153 were measured using standardized laboratory assays. Group differences were assessed using parametric or non-parametric statistical tests as appropriate. Receiver operating characteristic analyses were performed for individual biomarkers, whereas a multivariable logistic regression model was used for the combined panel. Optimal cut-off values were determined by maximizing Youden's index. TAP and TAM levels were significantly higher across all breast cancer stages compared with healthy controls, whereas CA125 and CA153 showed weaker stage-specific differences. For comparing all

breast cancer cases with controls, the area under the curve (AUC) values for TAP, TAM, CA125, CA153 and the combined model were 0.919, 0.862, 0.648, 0.607 and 0.963, respectively. For distinguishing stage I breast cancer from controls, the corresponding AUC values were 0.923, 0.891, 0.552, 0.531 and 0.959. Furthermore, the combined model achieved 92.0% sensitivity and 93.9% specificity for stage I disease. TAP and TAM biomarkers provided stronger diagnostic discrimination than conventional tumor antigens, and their integration with CA125 and CA153 improved diagnostic identification of early-stage breast cancer compared with single-biomarker assays. As such, the combined panel may serve as an adjunctive risk-stratification tool in symptomatic patients with breast cancer, although prospective validation against benign breast disease is required.

Introduction

Breast cancer remains a major challenge to women's health because of its high incidence, and early detection and timely diagnosis are important for improving clinical outcomes and reducing breast cancer mortality (1,2). In 2022, female breast cancer accounted for ~2.3 million new cases of cancer worldwide, making it one of the most frequently diagnosed malignancies among women globally (1). In China, breast cancer was responsible for 429,105 instances of new cancer diagnoses in 2022, exhibiting a notable increase compared with previous years (3). International cancer-control strategies emphasize early detection, timely diagnosis and accessible clinical management as key components for reducing breast cancer mortality and improving long-term patient outcomes (4). These requirements are particularly relevant for patients presenting with suspicious breast-related symptoms, such as unilateral nipple discharge, for whom efficient and minimally invasive diagnostic support may help guide subsequent imaging, pathological evaluations and clinical decision-making (5).

Currently, breast cancer diagnosis relies on an integrated approach that combines clinical examination, imaging assessment, histopathological confirmation and molecular

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Key words: breast cancer, tumor-abnormal protein, tumor-associated material, carbohydrate antigen 125, cancer antigen 153

characterization (6). Mammography remains a cornerstone of population-based breast cancer screening. Current recommendations support regular mammographic screening beginning at ~40 years of age; for example, the US Preventive Services Task Force recommends biennial screening for women aged 40-74 years, while the American Cancer Society recommends that women aged 40-44 years have the option to begin annual screening and that regular annual screening begin at age 45 years (7,8). However, imaging-based breast-cancer detection can be affected by breast density, lesion size, lesion location, patient age and differences in clinical resources. Dense breast tissue, for example, may reduce the sensitivity of mammography and has also been associated with an increased risk of breast cancer (7). For patients with pathological nipple discharge, diagnostic evaluation often requires a combination of mammography, ultrasonography, magnetic resonance imaging, duct-related assessments and pathological examination when clinically indicated (5,9). Therefore, given that the diagnostic evaluation of unilateral nipple discharge often relies on a combination of imaging modalities and pathological assessment, complementary biomarkers that are convenient, reproducible and minimally invasive may provide additional value in clinical risk stratification without replacing established diagnostic methods.

Serum tumor biomarkers are widely used in oncology because they can reflect tumor-associated biological alterations through relatively simple laboratory testing. Conventional markers, including carbohydrate antigen 125 (CA125) and cancer antigen 153 (CA153), have been applied to breast cancer monitoring, treatment-response assessment and recurrence surveillance (10,11). However, the diagnostic performance of CA153 and related circulating tumor markers in early breast cancer is limited, largely because of their insufficient sensitivity and specificity in patients with low tumor burden. Accordingly, clinical recommendations have advised against using these markers alone for breast cancer screening or diagnosis (10,12). These limitations have encouraged the development of multi-marker strategies that combine conventional tumor antigens with biomarkers reflecting broader tumor-associated metabolic and glycosylation-related changes (13,14).

Tumor-abnormal protein (TAP) and tumor-associated material (TAM) are two potential auxiliary indicators that may capture biochemical alterations associated with malignant transformation. TAP reflects abnormal glycoprotein-related changes and has been investigated as a diagnostic biomarker in patients with breast masses, showing potential value for distinguishing breast cancer from benign conditions (15). TAM has also been evaluated alongside conventional serum tumor markers in patients with malignant tumors, and has been reported to provide additional diagnostic information in comparison with routinely used biomarkers (16). Although much of the existing evidence supporting these indicators remains exploratory, the biological rationale for TAP and TAM as tumor biomarkers is consistent with the increasing recognition that aberrant glycosylation, altered sialylation and abnormal carbohydrate-chain structures are involved in tumor progression, invasion, immune escape and clinically measurable serum signatures (17-20). Thus, combining TAP, TAM, CA125 and CA153 may provide a broader diagnostic signal than any single marker alone.

Despite the clinical appeal of serum tumor markers, their value in early breast cancer diagnosis remains insufficiently defined. Namely, CA125 and CA153 are accessible and routinely measurable, but their limited sensitivity and specificity as biomarkers restrict their usefulness as independent early diagnostic indicators (10). Notably, TAP and TAM may reflect broader tumor-associated biochemical alterations, particularly changes in glycoproteins and glycosylation patterns, rather than relying solely on the detection of specific tumor-associated antigen epitopes (17,21). However, evidence regarding their stage-specific diagnostic performance in breast cancer, especially in patients with stage I disease, remains limited. Additionally, although previous studies have evaluated combinations of circulating biomarkers for improving breast cancer detection, evidence regarding the combined diagnostic performance of TAP, TAM, CA125 and CA153 remains limited (13,22). In the present study, biomarkers were measured before anticancer or hormonal therapy to minimize potential treatment-related alterations in circulating biomarker levels and to assess their diagnostic performance at baseline. The limited evidence regarding the diagnostic performance of the combined TAP, TAM, CA125 and CA153 panel in treatment-naïve patients is clinically relevant because early-stage breast cancer may present with subtle symptoms, and a minimally invasive serum biomarker panel could provide complementary information for initial risk stratification and diagnostic decision-making before treatment, without replacing imaging or histopathological confirmation.

The present study aimed to evaluate the diagnostic value of serum TAP, TAM, CA125 and CA153, both individually and in combination, in patients with breast cancer. Using pre-treatment serum samples from patients with breast cancer and healthy controls, the present retrospective study compared biomarker levels between groups and assessed the diagnostic performance of these biomarkers through receiver operating characteristic (ROC) curve analysis. Particular attention was given to the diagnostic efficacy of these biomarkers in patients with stage I breast cancer to determine whether this biomarker panel exhibited potential value for early diagnostic support.

Materials and methods

Study participants. The retrospective analysis employed a convenience sampling approach to identify patients with breast cancer and healthy controls at The Fifth Clinical Medical College of Henan University of Chinese Medicine (Zhengzhou People's Hospital) (Zhengzhou, China), between January 2022 and April 2024. Patients with breast cancer were identified from individuals receiving clinical evaluation and treatment at the hospital, whereas healthy controls were recruited from individuals undergoing routine physical examinations during the same period. The inclusion criteria for healthy controls were: i) Individuals undergoing routine physical examination during the study period; ii) no known history of malignant disease; iii) no clinically diagnosed breast disease; and iv) availability of the biomarker measurements required for the present analysis. For the healthy control cohort, the exclusion criteria were: i) A known history of malignant disease; ii) clinically diagnosed breast disease; or iii) incomplete biomarker or clinical data required for the present analysis. The inclusion criteria

for the breast cancer cohort included: i) Histopathologically confirmed breast cancer, with tumor classification based on the World Health Organization classification of breast tumors (23); ii) the presence of unilateral nipple discharge; iii) availability of the clinical, pathological and laboratory data required for the present study, including TAP, TAM, CA125 and CA153 measurements, age at diagnosis, tumor site and size, histological grade, TNM stage, recurrence and metastatic status, and estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 and Ki-67 status; and iv) no prior anticancer or hormonal therapy. Histological grading was performed according to the Nottingham modification of the Scarff-Bloom-Richardson system (24), and TNM staging was assigned according to the eighth edition of the American Joint Committee on Cancer staging system (25). For the breast cancer cohort, exclusion criteria were: i) The presence of additional systemic malignancies; ii) incomplete clinical data; or iii) pituitary prolactinoma or other neoplasms associated with lactation. Ethical approval was granted by the ethics committee of The Fifth Clinical Medical College of Henan University of Chinese Medicine (Zhengzhou, China; approval no. 2024011194), and written informed consent to participate in the study was obtained from all patients. A total of 203 participants were included in the present study, comprising 104 patients with breast cancer and 99 healthy controls. The breast cancer group consisted of 1 male patient and 103 female patients. Age data were available for 102 patients with breast cancer, whose median age was 50.5 years (range, 25-84 years). The healthy control group consisted of 99 female patients and no male patients, with a median age of 44 years (range, 13-83 years).

Biomarker measurements. All blood samples from patients with breast cancer were collected prior to the initiation of any anticancer or hormonal therapy. Samples from healthy controls were collected on the day of routine physical examination.

Measurement of CA125 and CA153. Fasting venous blood was collected once from each participant between 6:00 and 8:00 a.m. at the baseline assessment. This single blood-draw session provided the samples required for all biomarker assays, with aliquots collected or processed according to the specific requirements of the TAP, TAM, CA125 and CA153 assays. No repeated or serial blood sampling was performed. A total of 3 ml venous blood was collected into dry additive-free tubes and centrifuged at 1,200 x g for 15 min at 4°C. The separated serum was used for biomarker measurement. Serum CA125 and CA153 concentrations were measured using an AutoLumo A2000 Plus chemiluminescence analyzer, and the corresponding CA125 magnetic microparticle chemiluminescence assay kit (cat. no. 06970150900151) and CA153 magnetic microparticle chemiluminescence immunoassay kit (cat. no. 06970150904913) supplied by Autobio Diagnostics Co., Ltd. All procedures were conducted in accordance with the operating instructions of the analyzer and assay-kit manufacturers.

Measurement of TAP. The Abnormal Glycoprotein (TAP) Detection Kit (cat. no. 06975865440114) was supplied by Zhejiang Ruisheng Medical Technology Co., Ltd. For the TAP

assay, venous blood was collected into heparin-containing tubes to prevent clot formation and preserve the whole-blood sample required for the slide-based aggregation assay. A 25- μ l aliquot of heparin-anticoagulated whole blood was then spread evenly over more than two-thirds of a glass slide. Lectin-containing reagents, including concanavalin A, *Datura stramonium* agglutinin, *Lens culinaris* agglutinin and E-type *Phaseolus vulgaris* erythroagglutinin, were subsequently added to the slide. The slides were allowed to stand at room temperature for 2 h to permit the formation of circular aggregated particles. Particle images were acquired using a light microscopy-based RS-2011ATAP integrated slide-reading system (Zhejiang Risheng Medical Technology Co., Ltd.), and the areas of the aggregated particles were quantified using the system.

Measurement of TAM. A total of 3 ml fasting venous blood was collected into dry additive-free tubes and centrifuged at 1,200 x g for 10 min. The separated serum was subsequently used for TAM measurement. The Serum Tumor-Associated Substance Detection Kit (cat. no. 06975894410003), standards and quality-control materials were supplied by Qingdao Bosin Biotechnology Co., Ltd. Absorbance was measured at 450 nm using a GF-EII biochemical analyzer manufactured by Shandong Gaomi Caihong Analytical Instruments Co., Ltd. TAM concentrations were calculated as follows:

$$\text{TAM concentration} = (\text{absorbance of the test sample} / \text{mean absorbance of the standard}) \times 100 \text{ U/ml.}$$
 All assays were performed in accordance with the manufacturers' instructions.

Statistical methods. Statistical analyses were performed using SPSS version 19.0 (IBM Corp.) and Python version 3.12.7 (Python Software Foundation). Python-based analyses were conducted using NumPy version 2.1.0 (<https://numpy.org/doc/2.1/>), SciPy version 1.14.1 (<https://docs.scipy.org/doc/scipy-1.14.1/>), statsmodels version 0.14.4 (<https://www.statsmodels.org/v0.14.4/>), scikit-learn version 1.5.2 (<https://scikit-learn.org/1.5/>) and scikit-posthocs version 0.10.0 (<https://pypi.org/project/scikit-posthocs/0.10.0/>). All statistical tests were two-tailed and $P < 0.05$ was considered to indicate a statistically significant difference. For the laboratory assays, each measurement was performed in triplicate. No repeated biological sampling was performed unless otherwise stated.

Continuous variables were analyzed according to their distribution. The normality of age data were formally assessed using the Shapiro-Wilk test. Age was approximately normally distributed and was therefore presented as the mean $\pm$ standard deviation. For comparisons between healthy controls and patients with breast cancer, age was analyzed using an unpaired Welch's t-test. Comparisons of age among healthy controls and patients with stage I, II and III breast cancer were performed using one-way ANOVA, followed by Tukey's post hoc test for pairwise comparisons.

Because TAM, CA125 and CA153 concentrations and TAP-aggregate sizes showed non-normal distributions and/or influential outliers, these variables were presented as the median and interquartile range. Comparisons between healthy controls and patients with breast cancer for these indicators were performed using the Mann-Whitney U test. For these non-normal data, comparisons among healthy controls

and the three breast cancer stage groups were conducted using the Kruskal-Wallis test, followed by Dunn's pairwise test with Holm adjustment for multiple comparisons.

ROC curves were constructed to evaluate the diagnostic performance of TAP, TAM, CA125 and CA153 for distinguishing: i) Patients with breast cancer from healthy controls; and ii) patients with stage I breast cancer from healthy controls. Individual-marker ROC analyses were performed using all available observations for the corresponding biomarker. Among the 104 patients with breast cancer, complete TAP, TAM and CA125 data were available for all patients, whereas CA153 data were unavailable for one patient. Among the 99 healthy controls, TAP, TAM, CA125 and CA153 data were available for 93, 85, 99 and 82 participants, respectively. Missing biomarker measurements were not imputed. The area under the curve (AUC) values were calculated from the ROC curves, and their 95% confidence intervals (CIs) were estimated using the DeLong method. The DeLong method was used for confidence-interval estimation only; no formal pairwise comparisons of AUC values were performed.

For the combined-panel analysis, a multivariable binary logistic regression model incorporating TAP, TAM, CA125 and CA153 was constructed. Only participants with complete measurements for all four biomarkers were included in the combined model. For each participant, the logistic regression model generated a predicted probability of breast cancer, which was then used as the continuous variable to construct the ROC curve for the combined biomarker model.

The optimal cut-off value for each individual biomarker and the optimal predicted-probability threshold for the combined model were selected by maximizing Youden's index, calculated as sensitivity + specificity-1. This criterion was used to identify the threshold that provided the best overall balance between sensitivity and specificity for distinguishing patients with breast cancer from controls.

Results were classified as positive when the biomarker value or predicted probability was greater than or equal to the corresponding optimal cut-off. Sensitivity, specificity, positive predictive value, negative predictive value and Youden's index were calculated at each optimal threshold. The 95% CIs for sensitivity, specificity, positive predictive value and negative predictive value were estimated using the Wilson method. Positive and negative predictive values were calculated according to the proportion of breast cancer cases in the present analytical dataset and should therefore be interpreted as sample-specific estimates.

Missing observations were analyzed using an available-case approach for individual variables and a complete-case approach for the combined logistic regression models. No missing values were imputed.

Results

Basic information and characteristics. The dataset contained 99 healthy controls and 104 patients with breast cancer, including 25 patients with stage I disease, 56 patients with stage II disease and 23 patients with stage III disease. As some assay results were unavailable in the retrospective records, the number of available observations differed according to the variable analyzed; missing values were not imputed. Among

healthy controls, complete data regarding age, TAP-aggregate size, and TAM, CA125 and CA153 concentration were available for 99, 93, 85, 99 and 82 participants, respectively. Among patients with breast cancer, age was available for 102 patients, whereas TAP area, and TAM and CA125 concentration were available for 104 patients and CA153 concentration was available for 103 patients. Missing observations were not imputed (Fig. 1; Tables I and II).

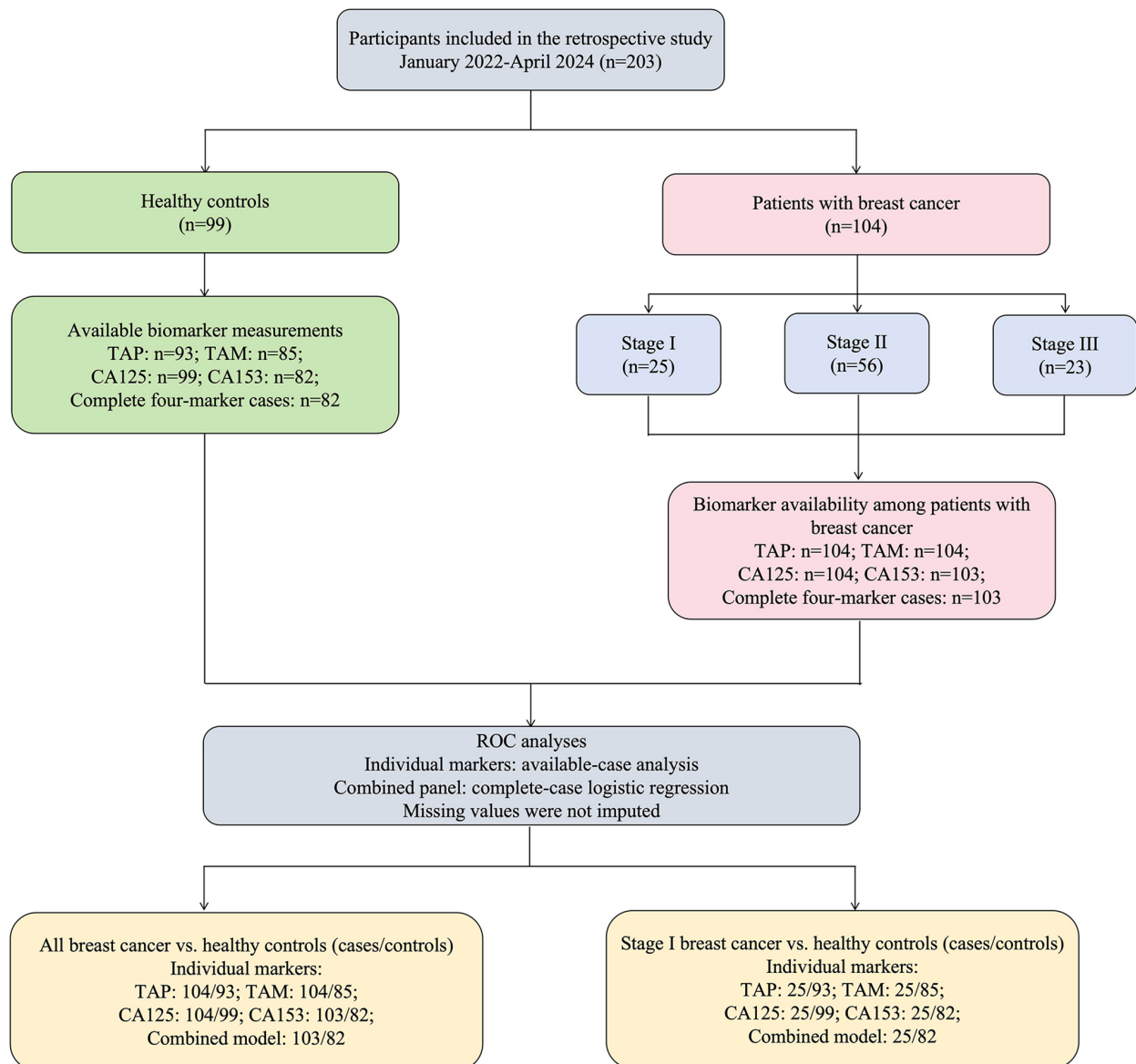
Comparison of TAP, TAM, CA125 and CA153 levels. The results showed that the ages of patients with breast cancer were significantly higher than those of healthy controls (51.20 ± 11.31 vs. 45.62 ± 13.51 years; $t, 3.170$; $P=0.002$) (Table I). TAP-aggregate sizes and TAM, CA125 and CA153 levels were also significantly higher in patients with breast cancer than in healthy controls, with U-values of 787.0 ($P<0.001$), 1,223.0 ($P<0.001$), 3,627.5 ($P<0.001$) and 3,319.0 ($P=0.013$), respectively (Fig. S1; Table I). Additionally, the overall between-group differences were significant for age ($F, 3.337$; $P=0.020$), TAP ($H, 103.116$; $P<0.001$), TAM ($H, 74.126$; $P<0.001$), CA125 ($H, 17.653$; $P<0.001$) and CA153 ($H, 10.086$; $P=0.018$) (Table II). The post hoc analyses showed that TAP and TAM levels were significantly higher in all three breast cancer stage groups compared with healthy controls (Table II). CA125 concentration was significantly higher in patients with stage II and stage III disease compared with healthy controls, whereas the difference between patients with stage I disease and healthy controls was not statistically significant (Table II). CA153 concentration was significantly higher in patients with stage III breast cancer than in healthy controls, whereas CA153 concentration in patients with stage I and stage II disease did not differ significantly from the control group (Table II). For age, a significant difference was only observed between the stage II breast cancer group and healthy controls (Table II). Notably, TAM levels in the stage I breast cancer cohort were significantly higher compared with those in the healthy controls and the stage II and III cohorts (Table II).

Diagnostic value of single and combined detection of TAP, TAM, CA125 and CA153 in stage I breast cancer. For comparisons between all breast cancer cases and healthy controls, the AUC values for models based on TAP, TAM, CA125 and CA153, as well as the combined model, were 0.919 (95% CI, 0.881-0.957), 0.862 (95% CI, 0.812-0.912), 0.648 (95% CI, 0.572-0.723), 0.607 (95% CI, 0.525-0.689) and 0.963 (95% CI, 0.939-0.987), respectively (Fig. 2). The corresponding optimal cut-off values, determined by maximizing Youden's index, were $106.00 \mu\text{m}^2$, 87.70, 17.28 and 14.19 U/ml for TAP, TAM, CA125 and CA153, respectively, and the optimal predicted-probability cut-off for the combined logistic regression model was 0.60 (Table SI). For stage I breast cancer vs. healthy controls, the AUC values for TAP, TAM, CA125, CA153 and the combined model were 0.923 (95% CI, 0.857-0.989), 0.891 (95% CI, 0.817-0.965), 0.552 (95% CI, 0.413-0.690), 0.531 (95% CI, 0.393-0.668) and 0.959 (95% CI, 0.913-1.000), respectively (Fig. 3). The corresponding optimal cut-off values were $118.00 \mu\text{m}^2$, 89.70, 15.99, 17.56 U/ml and a predicted-probability threshold of 0.2878 for the combined model (Table SI). At this optimal threshold, the combined model achieved a sensitivity of 92.0% and a specificity of

Table I. Comparison of age and biomarker levels between healthy controls and patients with breast cancer.

Variable	Healthy controls (overall n=99)	Breast cancer (overall n=104)	Test statistic	P-value
Age, years	45.62±13.51 (n=99)	51.20±11.31 (n=102)	t=3.170	0.002
TAP, μm^2	83.00 [72.00,98.00] (n=93)	135.00 [113.67,160.75] (n=104)	U=787.0	<0.001
TAM, U/ml	80.10 [74.50,86.30] (n=85)	91.95 [86.05,97.08] (n=104)	U=1,223.0	<0.001
CA125, U/ml	10.75 [7.17,13.46] (n=99)	13.05 [9.01,18.93] (n=104)	U=3,627.5	<0.001
CA153, U/ml	11.44 [9.17,14.91] (n=82)	14.53 [10.28,19.99] (n=103)	U=3,319.0	0.013

Values are presented as mean $\pm$ standard deviation for age and median [quartile 1, quartile 3] regarding biomarkers. Age was compared using Welch's independent-samples t-test, whereas biomarkers were compared using the Mann-Whitney U test. The number in each cell represents the available sample size. Missing values were not imputed. TAP, tumor-abnormal protein; TAM, tumor-associated material; CA125, carbohydrate antigen 125; CA153, cancer antigen 153.



Numbers differ across analyses because some biomarker measurements were missing.

Figure 1. Flow diagram of participant inclusion and statistical analyses in the present study. A total of 203 participants were included in the present retrospective study, comprising 99 healthy controls and 104 patients with breast cancer. The breast cancer group included 25 patients with stage I disease, 56 patients with stage II disease and 23 patients with stage III disease. As some biomarker measurements were missing, sample sizes differed among individual-marker analyses; individual biomarkers were analyzed using all available observations, whereas the combined logistic regression models included only participants with complete data for TAP, TAM, CA125 and CA153. Missing values were not imputed. ROC, receiver operating characteristic; TAP, tumor-abnormal protein; TAM, tumor-associated material; CA125, carbohydrate antigen 125; CA153, cancer antigen 153.

Table II. Comparison of age and biomarker levels among healthy controls and breast cancer stages.

Variable	Healthy controls	Stage I	Stage II	Stage III	Test statistic	P-value
Age, years	45.62±13.51 (n=99)	51.13±12.39 (n=24)	51.30±11.69 (n=56) ^a	51.00±9.48 (n=22)	F=3.337	0.020
TAP, μm^2	83.00 [72.00,98.00] (n=93)	136.00 [120.00,152.00] (n=25) ^a	131.50 [111.00, 161.00] (n=56) ^a	141.00 [124.50, 172.50] (n=23) ^a	H=103.116	<0.001
TAM, U/ml	80.10 [74.50,86.30] (n=85)	94.60 [89.70,100.20] (n=25) ^a	91.75 [85.57,97.65] (n=56) ^{a,b}	88.70 [85.85,93.50] (n=23) ^{a,b}	H=74.126	<0.001
CA125, U/ml	10.75 [7.17,13.46] (n=99)	11.64 [5.69,16.22] (n=25)	14.08 [9.53,19.11] (n=56) ^a	12.67 [10.89,22.73] (n=23) ^a	H=17.653	<0.001
CA153, U/ml	11.44 [9.17,14.91] (n=82)	11.50 [9.72,19.47] (n=25)	14.79 [9.84,20.52] (n=56)	14.93 [13.64,19.90] (n=22) ^a	H=10.086	0.018

^aP<0.05 compared with healthy controls; ^bP<0.05 compared with the stage I group. Values are presented as mean ± standard deviation for age and median [quartile 1, quartile 3] for biomarkers. Age was analyzed using one-way ANOVA followed by Tukey's post hoc test. Biomarkers were analyzed using the Kruskal-Wallis test followed by Dunn's pairwise comparisons with Holm correction. The number in each cell represents the available sample size. Missing values were not imputed. TAP, tumor-associated material; TAM, tumor-associated material; CA125, carbohydrate antigen 125; CA153, cancer antigen 153.

93.9% for distinguishing patients with stage I breast cancer from healthy controls (Table SI).

Discussion

The present study evaluated the diagnostic performance of serum TAP, TAM, CA125 and CA153 in untreated patients with breast cancer, with particular emphasis on stage I disease. The findings indicated that TAP aggregation and TAM concentration levels reflected diagnostically informative tumor-associated biochemical alterations in early-stage breast cancer, whereas diagnostic models based on CA125 or CA153 provided limited discriminatory power between patients with stage I breast cancer and controls. The combined diagnostic model integrating the four aforementioned markers demonstrated the strongest capacity to differentiate between patients with breast cancer and healthy controls. These results supported the efficacy of a complementary biomarker strategy, in which indicators of aberrant glycosylation were combined with conventional circulating tumor antigens to improve auxiliary diagnostic assessment.

The notable diagnostic performance of TAP and TAM was biologically plausible because malignant transformation is accompanied by notable protein remodeling via glycosylation, glycan branching, fucosylation and terminal sialylation (26). These alterations have been shown to arise from changes in glycosyltransferase activity, substrate availability, Golgi organization, tumor metabolism, and the release or shedding of glycoproteins into the circulation. Notably, aberrant glycans are not merely secondary products of tumor growth, as they have been shown to regulate receptor signaling, cell adhesion, extracellular-matrix interactions, invasion and immune recognition (17,19,20). The biomarkers TAP, which was detected through the aggregation of abnormal sugar-chain glycoproteins, and TAM, which incorporated measurements associated with sialic acid and hydroxyproline (16), therefore interrogated a broader biochemical phenotype than markers discriminating a single antigen. Their marked elevation in stage I disease suggested that systemic glycoprotein and carbohydrate-chain abnormalities can become detectable before the development of a large tumor burden. This interpretation was consistent with the results of glycomic serum studies, which have shown that breast cancer pathogenesis is associated with altered N-glycan branching and sialylation patterns and that these patterns vary across tumor stages and molecular subtypes (27,28).

By contrast, the modest diagnostic performance of CA125 and CA153 in stage I breast cancer was consistent with their established clinical limitations. CA153 is a circulating tumor marker that primarily detects epitopes associated with mucin (MUC)1, whereas CA125 detects epitopes associated with MUC16. The circulating concentrations of these markers may be influenced by tumor-associated antigen expression and shedding, as well as by tumor burden and access of shed antigens to the circulation (29,30). Early-stage localized tumors may release insufficient levels of these antigens to clearly differentiate the early pathological state from the physiological range. Consequently, CA153 is used principally for disease monitoring and the evaluation of advanced or recurrent breast cancer rather than population screening or initial diagnosis (10). Furthermore, meta-analysis results have indicated

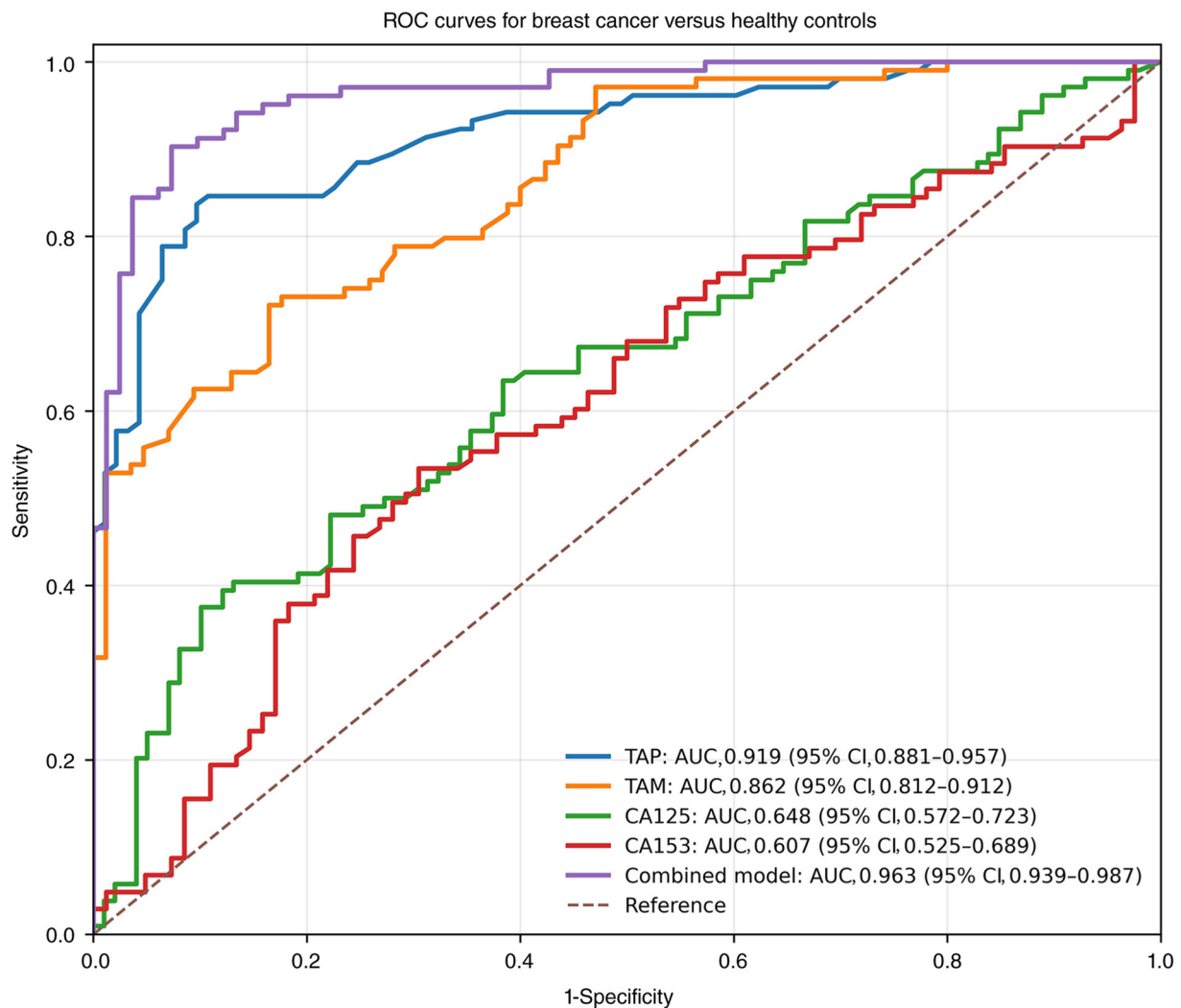


Figure 2. ROC curves of TAP, TAM, CA125, CA153 and the combined biomarker model for distinguishing patients with breast cancer from healthy controls. AUC and 95% CI values are shown for each individual biomarker and the combined model. The diagonal dashed line represents the reference line. ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; TAP, tumor-abnormal protein; TAM, tumor-associated material; CA125, carbohydrate antigen 125; CA153, cancer antigen 153.

that increases in CA153 levels are more closely associated with increasing disease stage and tumor burden than with the presence of small, localized tumors (31). An important feature of the data generated in the present study was that, compared with controls, statistically significant increases in median CA125 and CA153 concentrations in patients with stage I breast cancer did not translate into useful individual-level discriminatory power, as reflected by AUC values close to 0.5. This apparent discrepancy exemplified the fact that a significant difference in group averages can coexist with notable overlap between patient and control distributions. The statistical significance of differences between values alone should therefore not be interpreted as evidence of clinical diagnostic utility.

The stage-specific behavior of TAM requires a different interpretation from that of a conventional tumor-burden marker. Unlike TAM, which was highest in the stage I breast cancer subgroup and did not show a monotonic stage-dependent pattern, CA153 tended to be higher in the more advanced-stage groups, whereas TAP and CA125 did not exhibit a strictly monotonic increase across disease stages. This finding suggests that TAM may not be a reliable surrogate marker

for tumor volume or disease progression. Instead, this early peak in TAM levels may reflect early host-tumor interactions, extracellular-matrix remodeling, inflammatory glycoprotein turnover or stage-dependent changes in sialylated molecules. A previous study has demonstrated that TAM levels can be used to distinguish patients with malignant tumors from non-cancer populations (16); however, its tissue specificity and the specific molecular components contributing to the measured TAM signal remain incompletely characterized. The non-monotonic pattern observed in the present cohort may also have been influenced by the limited numbers of patients with each individual stage of breast cancer that were included in the present study, as well as by variation in histological or molecular subtype. Accordingly, the results of the present study supported the diagnostic relevance of TAM in early disease but did not indicate that TAM would be applicable as a marker of breast cancer progression. Future studies should determine which specific proteins and glycan structures within the molecular components contributing to the TAM signal are responsible for the measured TAM levels and whether these components differ among breast cancer subtypes.

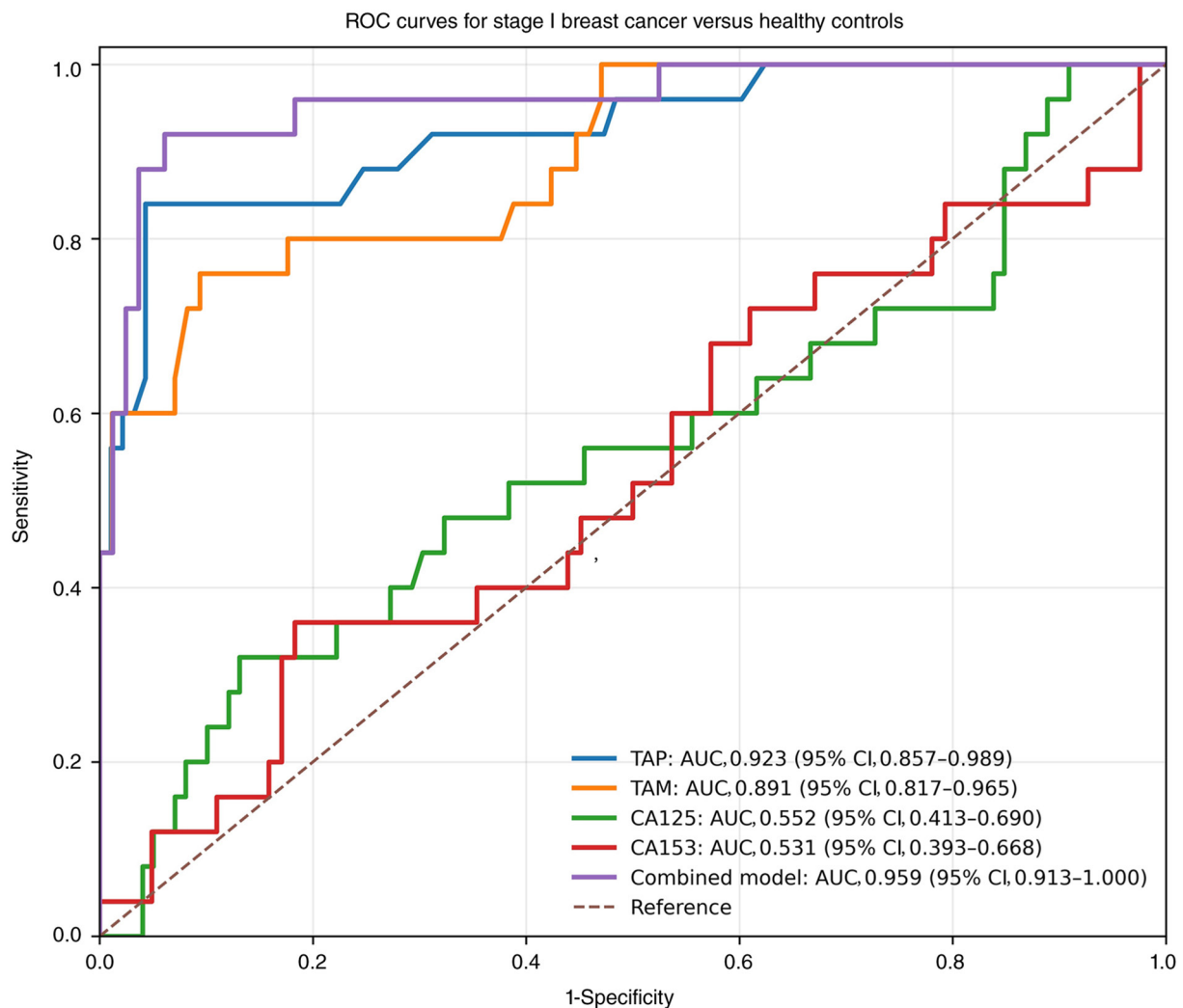


Figure 3. ROC curves of TAP, TAM, CA125, CA153 and the combined biomarker model for distinguishing patients with stage I breast cancer from healthy controls. AUC and 95% CI values are shown for each individual biomarker and the combined model. The diagonal dashed line represents the reference line. ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; TAP, tumor-abnormal protein; TAM, tumor-associated material; CA125, carbohydrate antigen 125; CA153, cancer antigen 153.

The superior performance of the combined panel compared with individual-biomarker models most likely reflected the biological complementarity of these biomarkers rather than equivalent contributions from all four markers. TAP and TAM can be used to detect broad abnormalities in circulating glycoproteins and carbohydrate-related materials, whereas CA125 and CA153 quantify defined tumor-associated antigenic epitopes (32,33). A patient with limited release of conventional antigens could still exhibit detectable systemic glycosylation abnormalities. By contrast, another patient with less pronounced changes to TAP or TAM could be identified through the elevation of tumor-associated antigen levels. This principle is consistent with previous evidence that the addition of TAP to diagnostic panels involving CA125, CA153 and other conventional markers increases diagnostic sensitivity compared with single-marker testing (15). More broadly, glycan-based biomarkers have been shown to improve diagnostic specificity by distinguishing cancer-associated glycoforms from the corresponding proteins present under physiological conditions (21,34).

Previous glycovariant studies have further supported the glycoform-specific diagnostic capacity of CA153. A study reported by Choi *et al* (35) showed that detecting the glycosylation status of CA153 with an antibody-lectin assay improved the discrimination of patients with breast cancer from non-cancer controls compared with conventional measurement of the protein epitope alone. A subsequent study using an N-glycolylneuraminic acid-specific lectin to detect an aberrantly sialylated CA153 glycoform reported notable improvements to cancer-classification performance compared with a routine CA153 assay and identified notable differences in glycoprotein sialylation even in early-stage disease (36). These observations demonstrated that the limited sensitivity of conventional CA153-based assays does not necessarily indicate that samples for which negligible signals are detected exhibit an absence of tumor-specific circulating MUC1-related signals, only that conventional assays may fail to capture the most disease-specific glycoforms. Proteomic evidence identifying abnormally glycosylated proteins in early breast cancer has also provided a rationale for refining these broad assays into more precisely defined biomarker signatures (14).

The present study extended this concept through a clinically accessible panel that integrated broad glycoprotein abnormalities with routinely tested antigens and did not require mass spectrometry or specialized glycoproteomic platforms. This analytical simplicity represents a potential advantage of the panel, although the specific molecular components measured by TAP and TAM and the reproducibility of these assays across different laboratories require further investigation.

The high AUC values of the combined-biomarker panel for distinguishing all patients with breast cancer from healthy controls (AUC, 0.963) and for distinguishing patients with stage I breast cancer from healthy controls (AUC, 0.959) suggest the potential value of this panel as an adjunctive test in patients undergoing evaluation for suspicious breast symptoms, particularly unilateral nipple discharge. However, the panel should not be recommended as an alternative to mammography, ultrasonography, magnetic resonance imaging or histopathological confirmation. Notably, the most plausible clinical role for this biomarker panel is risk stratification, helping to identify symptomatic patients who require accelerated imaging or tissue evaluation or providing complementary evidence when initial imaging findings are indeterminate. The clinical value of this combined panel will depend not only on its discriminatory power but also on: i) The sensitivity of the panel at a clinically acceptable specificity; ii) the predictive values of the panel at the intended disease prevalence; iii) analytical cost; iv) reproducibility; and v) whether using the panel leads to changes in patient management. The present case-control findings therefore established proof of diagnostic potential rather than evidence for population screening.

Advances in breast-cancer diagnosis have increasingly emphasized multimodal and precision-oriented approaches. The integration of photoacoustic imaging with conventional diagnostic modalities, such as mammography, ultrasonography and magnetic resonance imaging, has demonstrated the potential to improve diagnostic sensitivity, specificity and breast-lesion classification (37). In addition, CD47-targeted positron emission tomography/second near-infrared window fluorescence imaging has shown notable promise for precise tumor localization and image-guided surgery in breast cancer, highlighting the value of molecular imaging in tumor detection and surgical management (38). Beyond imaging, 3D bioprinted breast-cancer models have been developed to more accurately mimic tumor morphology and therapeutic responses, providing a potential platform for personalized oncology and drug-sensitivity testing (39). Furthermore, recent studies in other cancer types have highlighted the potential of serum-based metabolic fingerprints and nanoparticle-enhanced mass spectrometry for rapid and cost-effective cancer diagnosis (40,41). These advances suggest that, in the future, early breast cancer diagnosis may benefit from integrating serum biomarkers, imaging findings, metabolic profiling and individualized tumor models. In this context, the combined detection of TAP, TAM, CA125 and CA153 in patient serum may represent a useful auxiliary component of a broader multimodal diagnostic strategy rather than a standalone diagnostic method.

Several limitations should be acknowledged regarding the present study. Primarily, the present study is a retrospective, single-center study based on convenience sampling, which

introduces potential selection bias and limits generalizability across institutions, ethnicities and assay platforms. All patients included in the study presented with unilateral nipple discharge; therefore, the findings of the present study primarily apply to this symptomatic population and cannot be extrapolated to asymptomatic screening populations. Furthermore, the control group consisted of healthy individuals undergoing routine physical examinations rather than patients with benign breast diseases or inflammatory conditions. Comparisons with healthy controls may have overestimated the diagnostic performance of this assay because the intended clinical task of such diagnostic assays is usually to distinguish malignant disease from benign breast abnormalities rather than from individuals with no breast abnormalities. The inclusion of cohorts with fibroadenoma, intraductal papilloma, mastitis, duct ectasia and other causes of nipple discharge will be important in future studies.

Additionally, the stage I patient subgroup was comparatively small, increasing ambiguity concerning stage-specific estimates and potentially contributing to the non-monotonic TAM pattern observed in the present study. Although 95% CIs were reported for the AUC estimates, formal statistical comparisons of AUCs between the individual biomarkers and the combined panel were not performed, preventing conclusions regarding the statistical superiority of the combined panel. The relatively large variability in CA125 and CA153 concentrations also highlighted the importance of accounting for non-normal distributions; accordingly, these biomarkers were analyzed using the Mann-Whitney U test for two-group comparisons and the Kruskal-Wallis test followed by Dunn's pairwise test with Holm adjustment for multiple-group comparisons. Furthermore, clinically relevant factors, such as menopausal status, menstrual phase, liver and renal function, inflammation, molecular subtype, tumor histology, histological grade according to the Nottingham grading system and breast density, were not incorporated into the diagnostic models of the present study. Several of these factors, including menopausal status, menstrual phase, liver function, renal function and systemic inflammation, have been reported to influence circulating tumor marker concentrations, particularly CA125 and CA153, and may therefore have confounded the observed associations (42-45).

Finally, the present study did not include an independent validation cohort, longitudinal sampling or direct comparison with imaging-based diagnostic pathways. Future prospective, multicenter studies should: i) Use pre-specified assay thresholds; ii) enroll healthy participants, benign breast-disease controls and patients across molecular subtypes of breast cancer; and iii) evaluate whether the panel adds diagnostic value beyond age, symptoms and imaging findings. Molecular characterization of the glycoproteins contributing to TAP and TAM signals would further improve biological interpretability and facilitate assay standardization.

Finally, the present study predominantly focused on the diagnostic value of the combined serum biomarker panel and did not evaluate its prognostic efficacy. Although TAP, TAM, CA125 and CA153 may have potential value in monitoring tumor progression, recurrence, metastasis and treatment response, the present retrospective study lacked complete long-term follow-up data, including disease-free

survival, recurrence-free survival, overall survival and progression-related outcomes. Therefore, the prognostic value of this combined biomarker panel could not be fully assessed. Future prospective studies with larger sample sizes and standardized follow-up are warranted to determine whether the combined detection of TAP, TAM, CA125 and CA153 can serve not only as an auxiliary diagnostic tool but also as a prognostic indicator for breast cancer. These limitations constrain immediate clinical translation but do not diminish the central observation that glycosylation-related serum signals provide notable early-stage diagnostic information.

In conclusion, TAP and TAM demonstrated a greater early-stage diagnostic potential than conventional CA125 and CA153, and the integration of these biomarkers yielded a highly discriminative auxiliary biomarker panel in the symptomatic cohort of the present study. Prospective validation against benign breast diseases and the development of expanded multi-variable models incorporating the four biomarkers together with imaging and clinicopathological variables are required to establish whether the strategy evaluated in the present study can improve clinical diagnostic decision-making.

Acknowledgements

Not applicable.

Funding

The present study was supported by grants from the Medical Science and Technology Joint Project of Henan Province (grant nos. LHGJ20210707 and LHGJ20220784) and the Henan Province Science and Technology Research Project (grant no. 252102311186).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YM contributed to study conception and design, data acquisition, data analysis and manuscript drafting. RF contributed to data acquisition, data analysis, statistical analysis and manuscript drafting. BY contributed to data collection, laboratory experiments and data interpretation. YH contributed to data collection and laboratory experiments. PW contributed to data acquisition and interpretation. JZ contributed to data acquisition and analysis. YZ contributed to statistical analysis and data interpretation. HW contributed to data analysis and manuscript revision. YS contributed to study conception and design, supervision, project administration and manuscript revision. CD contributed to study conception and design, supervision, data interpretation and manuscript revision. YS and CD confirm the authenticity of all the raw data. All authors also contributed to drafting, revising or critically reviewing the present study, and agree to be accountable for all aspects of the work. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Fifth Clinical Medical College of Henan University of Chinese Medicine (Zhengzhou, China; approval no. 2024011194). Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71: 209-249, 2021.
2. Henderson JT, Webber EM, Weyrich MS, Miller M and Melnikow J: Screening for breast cancer: Evidence report and systematic review for the US preventive services task force. *JAMA* 331: 1931-1946, 2024.
3. Sun K, Zhang B, Lei S, Zheng R, Liang X, Li L, Feng X, Zhang S, Zeng H, Yao Y, *et al*: Incidence, mortality, and disability-adjusted life years of female breast cancer in China, 2022. *Chin Med J (Engl)* 137: 2429-2436, 2024.
4. World Health Organization: Global breast cancer initiative implementation framework: Assessing, strengthening and scaling up of services for the early detection and management of breast cancer. World Health Organization, Geneva, 2023.
5. Expert Panel on Breast Imaging; Sanford MF, Slanetz PJ, Lewin AA, Baskies AM, Bozzuto L, Branton SA, Hayward JH, Le-Petross HT, Newell MS, *et al*: ACR appropriateness criteria® evaluation of nipple discharge: 2022 update. *J Am Coll Radiol* 19: S304-S318, 2022.
6. Loibl S, André F, Bachelot T, Barrios CH, Bergh J, Burstein HJ, Cardoso MJ, Carey LA, Dawood S, Del Mastro L, *et al*: Early breast cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol* 35: 159-182, 2024.
7. US Preventive Services Task Force; Nicholson WK, Silverstein M, Wong JB, Barry MJ, Chelmow D, Coker TR, Davis EM, Jaén CR, Krousel-Wood M, *et al*: Screening for breast cancer: US preventive services task force recommendation statement. *JAMA* 331: 1918-1930, 2024.
8. Oeffinger KC, Fontham ETH, Etzioni R, Herzig A, Michaelson JS, Shih YC, Walter LC, Church TR, Flowers CR, LaMonte SJ, *et al*: Breast cancer screening for women at average risk: 2015 guideline update from the American cancer society. *JAMA* 314: 1599-1614, 2015.
9. Gupta D, Mendelson EB and Karst I: Nipple discharge: Current clinical and imaging evaluation. *AJR Am J Roentgenol* 216: 330-339, 2021.
10. Harris L, Fritzsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF and Bast RC Jr; American Society of Clinical Oncology: American society of clinical oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol* 25: 5287-5312, 2007.
11. Van Poznak C, Somerfield MR, Bast RC, Cristofanilli M, Goetz MP, Gonzalez-Angulo AM, Hicks DG, Hill EG, Liu MC, Lucas W, *et al*: Use of biomarkers to guide decisions on systemic therapy for women with metastatic breast cancer: American society of clinical oncology clinical practice guideline. *J Clin Oncol* 33: 2695-2704, 2015.
12. Duffy MJ, Evoy D and McDermott EW: CA 15-3: Uses and limitation as a biomarker for breast cancer. *Clin Chim Acta* 411: 1869-1874, 2010.
13. Chen R, Jiang C, Zhu Q, You S, Li Y, Li S, Ding L, Meng H, Yang Y, Zha X and Wang J: Combining the tumor abnormal protein test with tests for carcinoembryonic antigens, cancer antigen 15-3, and/or cancer antigen 125 significantly increased their diagnostic sensitivity for breast cancer. *Medicine (Baltimore)* 99: e21231, 2020.

14. Chang X, Obianwuna UE, Wang J, Zhang H, Qi G, Qiu K and Wu S: Glycosylated proteins with abnormal glycosylation changes are potential biomarkers for early diagnosis of breast cancer. *Int J Biol Macromol* 236: 123855, 2023.
15. Chen WX, Yang LG, Cheng L and Zhu YL: Tumor abnormal protein in the diagnosis of breast cancer in patients with a palpable mass. *Ann Clin Lab Sci* 49: 297-301, 2019.
16. Shu J, Li CG, Liu YC, Yan XC, Xu X, Huang XE, Cao J, Li Y, Lu YY, Wu XY, *et al*: Comparison of serum tumor associated material (TAM) with conventional biomarkers in cancer patients. *Asian Pac J Cancer Prev* 13: 2399-2403, 2012.
17. Pinho SS and Reis CA: Glycosylation in cancer: Mechanisms and clinical implications. *Nat Rev Cancer* 15: 540-555, 2015.
18. Zhang Z, Wuhler M and Holst S: Serum sialylation changes in cancer. *Glycoconj J* 35: 139-160, 2018.
19. Dobie C and Skropeta D: Insights into the role of sialylation in cancer progression and metastasis. *Br J Cancer* 124: 76-90, 2021.
20. He M, Zhou X and Wang X: Glycosylation: Mechanisms, biological functions and clinical implications. *Signal Transduct Target Ther* 9: 194, 2024.
21. Kailemia MJ, Park D and Lebrilla CB: Glycans and glycoproteins as specific biomarkers for cancer. *Anal Bioanal Chem* 409: 395-410, 2017.
22. Devine PL, Duroux MA, Quin RJ, McGuckin MA, Joy GJ, Ward BG and Pollard CW: CA15-3, CASA, MSA, and TPS as diagnostic serum markers in breast cancer. *Breast Cancer Res Treat* 34: 245-251, 1995.
23. WHO Classification of Tumours Editorial Board: Breast tumours. WHO classification of tumours, 5th edition, volume 2. International Agency for Research on Cancer, Lyon, 2019.
24. Elston CW and Ellis IO: Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. *Histopathology* 19: 403-410, 1991.
25. Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, Compton CC, Hess KR, Sullivan DC, *et al* (eds): *AJCC Cancer Staging Manual*. 8th edition. Springer International Publishing, New York, NY, 2017.
26. Lin Y and Lubman DM: The role of N-glycosylation in cancer. *Acta Pharm Sin B* 14: 1098-1110, 2024.
27. Kirmiz C, Li B, An HJ, Clowers BH, Chew HK, Lam KS, Ferrige A, Alecio R, Borowsky AD, Sulaimon S, *et al*: A serum glycomics approach to breast cancer biomarkers. *Mol Cell Proteomics* 6: 43-55, 2007.
28. Vreeker GCM, Vangangelt KMH, Bladergroen MR, Nicolardi S, Mesker WE, Wuhler M, van der Burgt YEM and Tollenaar RAEM: Serum N-glycan profiles differ for various breast cancer subtypes. *Glycoconj J* 38: 387-395, 2021.
29. Kufe DW: Mucins in cancer: Function, prognosis and therapy. *Nat Rev Cancer* 9: 874-885, 2009.
30. Yin BW and Lloyd KO: Molecular cloning of the CA125 ovarian cancer antigen: Identification as a new mucin, MUC16. *J Biol Chem* 276: 27371-27375, 2001.
31. Fu Y and Li H: Assessing clinical significance of serum CA15-3 and carcinoembryonic antigen (CEA) levels in breast cancer patients: A meta-analysis. *Med Sci Monit* 22: 3154-3162, 2016.
32. Zhang Z, Tian C, Liu Y, Zhang L, Sun H, He S, Liu Y, Fan H, Zhang Y, Gao M and Wu S: Tumor abnormal protein as a promising biomarker for screening solid malignancies and monitoring recurrence and metastasis. *Front Oncol* 13: 1290791, 2023.
33. Winston-McPherson GN, Merrill AE, Lo SY, Hoofnagle AN and Greene DN: Interassay comparison of the tumor markers CA125, CA15.3, and CA27.29. *J Appl Lab Med* 2: 17-24, 2017.
34. Lastovickova M, Strouhalova D and Bobalova J: Use of lectin-based affinity techniques in breast cancer glycoproteomics: A review. *J Proteome Res* 19: 1885-1899, 2020.
35. Choi JW, Moon BI, Lee JW, Kim HJ, Jin Y and Kim HJ: Use of CA15-3 for screening breast cancer: An antibody-lectin sandwich assay for detecting glycosylation of CA15-3 in sera. *Oncol Rep* 40: 145-154, 2018.
36. Nikseresht S, Shewell LK, Day CJ, Jennings MP, Chittoory H, McCart Reed AE, Simpson PT, Lakhani SR, Nabiee R, Moore M, *et al*: Improved breast cancer diagnosis using a CA15-3 capture antibody-lectin sandwich assay. *Breast Cancer Res Treat* 211: 605-615, 2025.
37. Biswas S, Chohan DP, Wankhede M, Rodrigues J, Bhat G, Mathew S and Mahato KK: Photoacoustic-integrated multimodal approach for colorectal cancer diagnosis. *ACS Biomater Sci Eng* 11: 4033-4049, 2025.
38. Shi X, Xu P, Cao C, Cheng Z, Tian J and Hu Z: PET/NIR-II fluorescence imaging and image-guided surgery of glioblastoma using a folate receptor α -targeted dual-modal nanoprobe. *Eur J Nucl Med Mol Imaging* 49: 4325-4337, 2022.
39. Petővári G, Moldvai D, Raffay R, Dankó T, Sztankovics D, Reszegi A, Miyaura R, Gelencsér R, Rókus A, Tolnai-Kriston C, *et al*: Mimicking breast cancer tissue-3D bioprinted models in accurate drug sensitivity tests. *VIEW* 6: 20250092, 2025.
40. Liu W, Hu X, Bao Z, Li Y, Zhang J, Yang S, Huang Y, Wang R, Wu J, Xu X, *et al*: Serum metabolic fingerprints encode functional biomarkers for ovarian cancer diagnosis: A large-scale cohort study. *EBioMedicine* 115: 105706, 2025.
41. Wang R, Yang S, Wang M, Zhou Y, Li X, Chen W, Liu W, Huang Y, Wu J, Cao J *et al*: A sustainable approach to universal metabolic cancer diagnosis. *Nat Sustain* 7: 602-615, 2024.
42. Grover S, Quinn MA, Weideman P and Koh H: Factors influencing serum CA 125 levels in normal women. *Obstet Gynecol* 79: 511-514, 1992.
43. Pauler DK, Menon U, McIntosh M, Symecko HL, Skates SJ and Jacobs IJ: Factors influencing serum CA125II levels in healthy postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 10: 489-493, 2001.
44. Collazos J, Genollá J and Ruibal A: CA 15.3 in nonmalignant liver diseases. *Int J Biol Markers* 6: 188-192, 1991.
45. Estakhri R, Ghahramanzade A, Vahedi A and Nourazarian A: Serum levels of CA15-3, AFP, CA19-9 and CEA tumor markers in cancer care and treatment of patients with impaired renal function on hemodialysis. *Asian Pac J Cancer Prev* 14: 1597-1599, 2013.



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