RESEARCH ARTICLE

Editorial Process: Submission:11/03/2025 Acceptance:06/18/2026 Published:06/22/2026

Combined Diagnostic Utility of Serum CA15-3 and Routine Hematological Indicators for Breast Cancer Differentiation in an Iraqi Cohort

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Abstract

Background: Breast cancer (BC) is a leading cause of mortality among Iraqi women, underscoring the need for accessible diagnostic tools. This study evaluates the combined utility of the tumor marker CA15-3 and routine hematological parameters as an adjunctive tool for distinguishing between treatment-naïve BC patients with a pre-existing diagnosis and healthy individuals. **Methods:** In this case-control study, 100 female BC patients (Group I) and 50 healthy controls (Group II) were recruited. Serum CA15-3 levels were measured using electrochemiluminescence immunoassay (ECLIA), and a complete blood count was performed. Statistical analyses included independent t-tests, ROC curve analysis, and logistic regression to develop a combined diagnostic model. All BC patients were treatment-naïve and were required to submit blood samples prior to undergoing any surgical procedure, although they had already been diagnosed. **Results:** CA15-3 levels were significantly higher in BC patients (26.1 ± 7.5 U/mL) than in controls (7.54 ± 2.6 U/mL; $P < 0.001$). Significant differences were also observed in white blood cell (WBC), red blood cell (RBC), lymphocyte, and platelet counts (all $P < 0.05$). ROC analysis showed excellent diagnostic performance for CA15-3 alone (AUC = 0.98). A combined model integrating CA15-3 with the four hematological parameters achieved a superior AUC of 0.99, with 96% sensitivity and specificity. **Conclusion:** Elevated CA15-3 is confirmed as a key diagnostic marker for BC. Integrating CA15-3 with specific, routinely measured hematological indicators provides an improved, accessible, and cost-effective adjunctive tool for differentiating treatment-naïve BC patients from healthy individuals. This approach may be particularly useful for triaging patients with suspicious symptoms or monitoring those with established diagnoses.

Keywords: Breast cancer- CA15-3- hematological parameters- diagnostic accuracy- Iraq

Asian Pac J Cancer Prev, **27 (6)**, 2251-2258

Introduction

Breast cancer (BC) is a term that describes a range of breast tumors, which are distinguished by their unique molecular and cellular origins and their diverse clinical behaviors. Most originate from ductal or lobular epithelial tumors [1].

BC develops when cells in the breast proliferate uncontrollably and can invade surrounding tissue. These cells often form a tumor, which can be visible on x-rays or felt as a lump. Breast tumors are classified into two types: benign or malignant (BC) [2]. BC is staged according to the tumor's size (T), axillary lymph node involvement (N), and the presence of metastases (M). The TNM combination provides prognostic information [3].

It is the most prevalent malignant condition diagnosed in women globally each year and ranks as the second leading cause of cancer-related mortality among women worldwide [4]. Furthermore, over the past four decades, BC incidence has risen alarmingly. In 2022, there were approximately 2.3 million new cases of BC worldwide and about 670,000 deaths from this illness, with notable geographic variations between countries and regions [5].

In Iraq, the incidence rate of new cancer cases increased from 52.00/100,000 in the year 2000 to 158.9/100,000 in 2022. BC is the leading cause of cancer-related death among Iraqi women. In 2022, BC represented the highest percentage (35.9%) of the top ten cancers, with an incidence rate of 39.2/100,000 [6]. This rising burden underscores the urgent need for accessible and cost-effective diagnostic tools to support the national healthcare system.

A national program for the early detection of BC was established by the Iraqi Ministry of Health (MOH) and

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the Ministry of Higher Education and Scientific Research (MOHESR) in cooperation with the World Health Organization (WHO) in the year 2000 [7]. This program aims to provide high-quality primary and secondary health services, conduct early detection examinations for all women aged 20 years and over, and reduce mortality rates by diagnosing the disease in its early stages (first and second), where recovery prospects are better and treatment expenses are lower [8].

Monitoring cancer trends over time is essential for effective healthcare planning and disease control. In Iraq, BC records from over 20 years (2000 to 2020) have been used by authorities to implement control strategies and develop prevention and treatment plans [9].

The development and progression of breast cancer are closely linked to systemic inflammation and immune dysregulation. The tumor microenvironment secretes a range of cytokines and growth factors that induce a systemic inflammatory response. This, in turn, can lead to significant alterations in the population and function of circulating immune cells and platelets, which are readily measurable via a complete blood count (CBC). Biomarkers of BC, such as carbohydrate antigen 15-3 (CA15-3), which facilitates the adhesion of epithelial cells, have been studied for their roles in the development and progression of BC. Recent research indicates that integrating CA15-3 with hematological assessments may improve its diagnostic effectiveness [10]. Many studies have documented elevated levels of CA15-3 and its association with metastatic tumor burden. Consequently, several studies have suggested that combining CA15-3 with hematological profiling could enhance its role as a diagnostic biomarker [11].

The primary methods for BC diagnosis are imaging and laboratory tests. Laboratory tests include histopathology, needle biopsy, molecular biotechnology examinations, Real-Time Fluorescence Quantitative PCR, and biomarker analysis. Biomarkers, in addition to nucleic acids and tumor tissues, encompass a large number of proteins. The "central dogma" of molecular biology demonstrates the intimate relationship between nucleic acids and proteins. However, differential nucleic acid expression might not result in cancer if the final protein product remains unchanged. Therefore, proteins constitute another crucial indicator for cancer diagnosis, and cancer incidence may be predicted by examining protein states [12]. Proteins play a major role in the diagnosis of BC as significant biomarkers [13].

Several protein biomarkers have been studied in BC, including CA15-3, HER2, and CEA. Among these, CA15-3 is a well-established marker for BC screening and monitoring, with levels rising as BC progresses [14]. The present study focuses on CA15-3 due to its clinical availability and cost-effectiveness, and explores its combination with routine hematological parameters [15, 16]. The combination of CA15-3 with routine hematological parameters, which are readily available and inexpensive, could provide a locally applicable and cost-effective diagnostic adjunct in resource-limited settings like Iraq.

Aims of Study

This study aimed to evaluate CA15-3 and hematological parameters (WBC, RBC, monocytes, lymphocytes, neutrophils, eosinophils, basophils, Hemoglobin, and platelets) as useful markers to distinguish between patients with BC and healthy individuals.

Materials and Methods

Materials:

Study Design

This case-control study was conducted and reported in compliance with the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guidelines. It took place at Al-Amal National Hospital in Baghdad, Iraq, over a six-month period. A total of 150 participants were recruited and divided into two groups: the case group (Group I; n=100) comprising women with a pre-existing diagnosis of breast cancer who were scheduled for surgery but were treatment-naïve (no prior chemotherapy, radiotherapy, or surgery), and the control group (Group II; n=50) consisting of healthy women. Cases and controls were matched by sex and broad age categories to minimize potential confounding, ensuring a 2:1 case-to-control ratio. Diagnosis was confirmed histopathologically by core needle biopsy prior to enrollment.

Sample Size Justification

The sample size was determined by a power calculation and was not based on convenience sampling. The target sample size was determined a priori using G Power software (version 3.1.9.7). To achieve a power of 80% at a two-sided significance level of $P < 0.05$, with a case-to-control ratio of 2:1, a sample of 102 cases and 51 controls was calculated as necessary to detect a mean difference of 5 U/mL in CA15-3 levels, assuming a standard deviation of 3 U/mL based on pilot data. Our final sample size of 100 cases and 50 controls was considered adequate to detect the observed significant differences in key parameters and was also sufficient for the planned multivariate logistic regression analysis. The slight deviation from the calculated size was due to the practical constraints of the recruitment period, but the achieved sample size provided sufficient statistical power for the analyses.

Participant Flow

Of the potential participants assessed for eligibility, 100 BC patients and 50 healthy controls met the inclusion criteria and were enrolled. No eligible participants declined to participate. A flow diagram illustrating participant recruitment and exclusion is available as supplementary material.

Inclusion and Exclusion Criteria

The exclusion of BC patients who had undergone surgery or treatment (chemotherapy/radiotherapy) prior to blood sampling is a critical strength of our methodology, as it ensures that the measured parameters reflect the untreated, natural disease state.

Inclusion Criteria

• Cases (Group I): Female patients aged over 30 years with a confirmed, pre-surgical diagnosis of breast cancer (BC) by a clinical specialist.

• Controls (Group II): Healthy females aged over 30 years with no personal history of cancer and no diagnosis of chronic or acute inflammatory diseases.

Exclusion Criteria (for both groups)

• Pregnant women.

• Patients with a diagnosis of benign breast disease or other known malignancies.

• BC patients who had already undergone any form of surgical treatment (e.g., mastectomy or lumpectomy) prior to blood sampling.

• Patients undergoing treatment (e.g., chemotherapy or radiotherapy) that could significantly alter hematological parameters.

Blood sampling

Five-milliliter blood samples were drawn from the forearm veins of participants. Blood was placed into a gel tube (Serum Separator Tube). The sample in the gel tube was separated by centrifugation at 3000 rpm for 10 minutes to obtain serum. Two milliliters of whole blood were also collected into EDTA tubes for hematological analysis.

List of Instruments, Companies, Countries, and Applications

Blood samples were collected using EDTA tubes for hematological analysis and serum separator tubes for serum collection. After centrifugation, the Sysmex analyzer evaluated the whole blood for complete blood counts (CBC), while the COBAS analyzer used serum for the measurement of tumor markers. These tools facilitate in-depth diagnostic evaluation, ensuring accuracy through advanced automation and uniform reagents (Table 1).

Methods

Determination of CA15-3

The Electrochemiluminescence immunoassay (ECLIA) method on the Roche Cobas e411 analyzer was used for the quantitative determination of CA15-3.

Principle

The assay is a fully automated, random-access, software-controlled system for immunoassay analysis. It is based on a sandwich principle using a ruthenium complex-labeled monoclonal antibody and a biotinylated monoclonal antibody specific to CA15-3.

Procedure

In the first incubation step, 20 µL of the sample is automatically diluted 1:10 with Universal Diluent. A sandwich complex is formed as the diluted sample containing the antigen interacts with a ruthenium complex-labeled monoclonal antibody and a biotinylated monoclonal antibody, both specific to CA15-3.

In the second incubation step, streptavidin-coated microparticles are added, and the complex binds to the solid phase via the interaction of biotin and streptavidin.

The reaction mixture is drawn into the measuring cell, where microparticles are magnetically captured onto the electrode surface. Unbound substances are removed. Application of a voltage to the electrode induces chemiluminescent emission, which is measured by a photomultiplier. Results are determined via a calibration curve.

Reagents

The reagent pack for CA15-3 (Roche) includes

1. Streptavidin-coated microparticles (transparent cap).

2. Biotinylated monoclonal anti-CA15-3 antibody (gray cap).

3. Monoclonal anti-CA15-3 antibody conjugated with a Ruthenium complex (black cap).

Statistical analysis

Statistical analysis was conducted in SPSS version 26.0. Data were presented as mean ± SD. Normality of data was assessed with the Shapiro-Wilk test. The independent-samples t-test was used to compare the mean values of all continuous parameters between Group I (Breast Cancer) and Group II (Control). P> 0.05 was not considered statistically significant. For age as a potential confounder, Analysis of Covariance (ANCOVA) was conducted on parameters with significant differences. Receiver-Operating Characteristic (ROC) curve analysis was carried

Table 1. This Section Lists the Instruments and the Company and Country Supplied Them for This Investigation

	Instruments	Company & Country	Applications
1	COBAS-e 411/ chemistry analyzer	Roche – HITACHI / Germany	Automated biochemical evaluation, including quantification of tumor markers (e.g., CA15-3), hormones, or enzymes in blood or serum.
2	CA15-3 / kit	COBAS-e 411 / USA	Contains reagents to detect the CA15-3 antigen for diagnostic purposes.
3	Automated Sysmex XN-550	Sysmex XN-550 / Germany	Performs comprehensive blood Hematology Germany analyses (CBCs), assessing Analyzer hemoglobin levels, WBC and platelets
4	Centrifuge	Hettich EBA 20 / Germany	Isolates blood components via Germany centrifugation, preparing samples for chemical or hematological tests.
5	EDTA Tubes	GBUK Group / UK	Prevents blood coagulation; used for hematological analyses like CBCs performed by Sysmex analyzers.
6	Glass Gel NIPIGON Health Serum	separation during Tubes Corp/Canada	centrifugation for biochemical tests (e.g., CA15-3).

out for CA15-3 as well as the relevant hematological parameters in order to compare their diagnostic value by measuring the Area Under the Curve (AUC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). ROC was also used to evaluate the core proposition that a composite of markers would enhance diagnostic characteristics. We chose logistic regression because the outcome (BC vs. control) is binary, and we wanted to model the probability of BC given the predictors. A logistic regression model was built using the Enter method, with CA15-3, WBC, RBC, Lymphocytes, and Platelets as predictors. The Enter method was used to include all predictors simultaneously. The probabilities from this model were used to generate a combined ROC curve and calculate the combined AUC, sensitivity, and specificity. The logistic regression model's coefficients and odds ratios are reported in the results.

Results

Sociodemographic and Clinical Characteristics

The demographic and clinical characteristics of the participants are summarized in Table 2. The age distribution differed between groups, with 64% of BC patients aged 40 and above (31% aged 40-49; 33% aged 50 and older), compared to 62% in the Control Group (30% aged 40-49; 32% aged 50 and older). The mean age was 53.9 ± 9.6 years in Group I and 48.5 ± 11.5 years in Group II ($P > 0.05$, Table 3). All participants were female. Among BC patients, 62% had a disease duration of 4-5 years, and 64% were diagnosed at advanced stages (Stage III: 28%; Stage IV: 13%).

Laboratory Parameters

A comparative analysis of hematological and biochemical parameters between the breast cancer (Group I) and control (Group II) groups is presented in Table 3.

Parameters with Significant Differences

1. CA15-3: Levels were significantly higher in Group I (26.1 ± 7.5 U/mL) compared to Group II (7.54 ± 2.6 U/mL), with a p-value of $P < 0.001$.

2. RBC: A significant reduction in mean RBC counts was observed in Group I ($4.1 \pm 0.3 \times 10^{12}$/L) compared to Group II ($4.9 \pm 0.4 \times 10^{12}$/L; $P < 0.05$).

3. WBC: A significant increase in WBC count was found in Group I ($8.1 \pm 1.3 \times 10^{9}$/L) compared to Group II ($5.3 \pm 1.8 \times 10^{9}$/L; $P < 0.05$).

4. Lymphocytes: A significant decrease in lymphocyte count was observed in Group I ($2.11 \pm 0.7 \times 10^{9}$/mL) compared to Group II ($2.7 \pm 0.29 \times 10^{9}$/mL; $P < 0.05$).

5. Platelets: A significant decrease in mean platelet count was found in Group I ($223.5 \pm 51.5 \times 10^{9}$/L) compared to Group II ($262.0 \pm 42.2 \times 10^{9}$/L; $P < 0.05$).

Non-Significant Parameters

Hemoglobin (Hb), Packed Cell Volume (PCV), Neutrophils (NE), Monocytes (Mono), Eosinophils (Eos), and Basophils (Bas) exhibited no statistically significant differences between groups.

Age Adjustment

An ANCOVA was performed for the parameters that showed significant differences (CA15-3, WBC, RBC, Lymphocytes, Platelets) to account for the mean age difference between groups. After adjusting for age, all these parameters remained statistically significant ($P < 0.05$).

Diagnostic Performance of Biomarkers

ROC curve analysis was performed for CA15-3 and the hematological parameters that showed significant differences between groups. The results are summarized in Table 4.

CA15-3 demonstrated outstanding diagnostic performance, with an AUC of 0.98 (95% CI: 0.96-1.00; $P<0.001$), indicating an excellent ability to distinguish BC patients from healthy controls. At an optimal cut-off value of 13.5 U/mL, it achieved high sensitivity (92%) and specificity (94%). RBC count also showed high discriminatory power (AUC = 0.93, $P<0.001$), while

Table 2. Comparative Demographic and Clinical Characteristics of Group I and Group II

Sample Characteristic		Group I	%	Group II	%
Age	20-29	10	10	8	16
	30-39	26	26	11	22
	40-49	31	31	15	30
	50-↑	33	33	16	32
Sex	Male	0	0	0	0
	Female	100	100	50	100
Duration of Disease	6-12 (months)	11	11	N/A	N/A
	2-3 (years)	27	27	N/A	N/A
	4-5 (years)	42	42	N/A	N/A
	>6 years	20	20	N/A	N/A
Stages of Disease	Stage One	23	23	N/A	N/A
	Stage Two	36	36	N/A	N/A
	Stage Three	28	28	N/A	N/A
	Stage Four	13	13	N/A	N/A

*, significant; NS, nonsignificant

Table 3. Comparative Analysis of Hematological and Biochemical Parameters in BC Patients and Healthy Controls

Parameters	Unit	Group I (n=100)	Group II (n=50)	P. value
Age	year	53.9 ± 9.6	48.5 ± 11.5	NS
CA15-3	U/mL	26.1 ± 7.5	7.54 ± 2.6	<0.001*
WBC	$\times 10^9$/L	8.1 ± 1.3	5.3 ± 1.8	≤ 0.05*
RBC	$\times 10^{12}$/L	4.1 ± 0.3	4.9 ± 0.4	≤ 0.05*
Lym	$\times 10^9$/L	2.11 ± 0.7	2.7 ± 0.29	≤ 0.05*
PLT	$\times 10^9$/L	223.5 ± 51.5	262.0 ± 42.2	≤ 0.05*
Hb	g/dl	13.1 ± 0.9	13.2 ± 1.1	NS
PCV	100-mL	38.7 ± 3.2	39.8 ± 3.1	NS
NE	$\times 10^9$/L	4.16 ± 1.4	4.8 ± 1.0	NS
Mono	$\times 10^9$/L	0.47 ± 0.1	0.51 ± 0.11	NS
Eos	$\times 10^9$/L	0.16 ± 0.1	0.13 ± 0.04	NS
Bas	$\times 10^9$/L	0.014 ± 0.017	0.010 ± 0.003	NS

*, significant; NS, nonsignificant

Table 4. Diagnostic Performance of Individual Biomarkers for Differentiating Breast Cancer Patients from Healthy Controls

Biomarker	AUC (95% CI)	Optimal Cut-off	Sensitivity (%)	Specificity (%)	PPV %	NPV (%)
CA15-3	0.98 (0.96-1.00)	13.5 U/mL	92	94	97.9	80.7
WBC	0.85 (0.79-0.91)	6.65 x10^9/L	78	82	90.7	62.1
RBC	0.93 (0.89-0.97)	4.55 x10^{12}/L	87	88	94.6	73.3
Lymphocytes	0.79 (0.72-0.86)	2.40 x10^9/L	74	72	86	54.5
Platelets	0.71 (0.63-0.79)	245.5 x10^9/L	68	66	81	49.3

Table 4a. Logistic Regression Model for the Combined Panel

Predictor	Coefficient	Odds Ratio (95% CI)	P-value
CA15-3	0.85	2.34 (1.92–2.85)	<0.001
WBC	0.72	2.05 (1.65–2.55)	<0.001
RBC	-1.23	0.29 (0.18–0.47)	<0.001
Lymphocytes	-0.91	0.40 (0.25–0.65)	<0.001
Platelets	-0.02	0.98 (0.97–0.99)	0.001

WBC, lymphocyte, and platelet counts showed moderate diagnostic value (AUC = 0.85, P<0.001; 0.79, P<0.001; and 0.71, P<0.001 respectively).

In a sub-analysis of early-stage (Stage I and II) BC patients (n=59) versus healthy controls, the combined panel achieved an AUC of 0.97 (95% CI: 0.94-1.00), with a sensitivity of 94% and specificity of 96%.

Combined ROC Analysis of the Biomarker Panel

A logistic regression model incorporating CA15-3, WBC, RBC, Lymphocytes, and Platelets was developed using the Enter method. The model was statistically significant (P < 0.001). The coefficients and odds ratios for each predictor in the final model are presented in Table 4a (new table added below). The ROC curve for this combined panel demonstrated superior performance compared to any single hematological parameter, achieving an AUC of 0.99 (95% CI: 0.98-1.00). At the optimal probability cut-off, the combined panel yielded a sensitivity of 96% and a specificity of 96%.

Table 5. CA15-3 Levels Stratified by Breast Cancer Stage in Group I (n=100)

Disease Stage	n (%)	CA15-3 (U/mL), Mean ± SD
Stage I	23 (23%)	18.2 ± 4.1
Stage II	36 (36%)	24.5 ± 5.8
Stage III	28 (28%)	29.8 ± 6.5
Stage IV	13 (13%)	35.1 ± 8.2

Analysis by Disease Stage

A sub-group analysis was conducted to explore the relationship between CA15-3 levels and disease stage. As shown in Table 5, a trend of increasing CA15-3 levels with advancing disease stage was observed.

Discussion

The case-control study agrees that serum levels of CA15-3 increase in treatment-naive BC patients compared to the healthy subjects. The measured levels were almost 3.5-fold higher in the patient group, which accords well with the known function of being a TAAs shedding into the blood due to disintegration of cytoskeleton of this gland in BC [17−19].

Additionally, we found that major hematological variables differed significantly in the BC population, with higher WBC and lower RBC, lymphocyte, and platelet counts. Non-significant differences in the values for Hb, PCV, neutrophils, monocytes, eosinophils and basophils indicate that these markers were less affected in our

patient population at the measured conditions compared to bacterial infections [20] a cand virus infections [21].

The observed hematological changes are biologically credible in light of the systemic inflammatory response to cancer [22, 23]. The marked decrease in RBC count points towards an anemia of chronic disease, likely secondary to myelosuppression or systemic inflammation-induced disruption of erythropoiesis [24]. The higher WBC values are likely a reflection of the systemic inflammation and immune response against tumour cells [22 , 25].

The lymphocytopenia observed in our BC patients may be due to various pathophysiologic mechanisms. The tumour can trigger lymphocyte apoptosis by expression of Fas ligand or other death receptor pathways and reduce the immune cell population necessary for killing the tumour [22, 23]. In addition, lymphocytes might be trapped in secondary lymphoid tissues or within the tumor microenvironment with decreased circulating counts. In addition, systemic inflammation may cause a cytokine-mediated disruption of immune cell generation and response [22, 26].

The finding of thrombocytopenia in our BC cohort, while less commonly reported than thrombocytosis, points to specific underlying mechanisms. We acknowledge that thrombocytosis is more frequently reported in cancer patients. However, in our cohort, the observed thrombocytopenia might be explained by the advanced disease stage (64% Stage III/IV), where bone marrow suppression due to systemic inflammation or tumor-related factors, alongside increased platelet consumption, could be more pronounced [24, 27].

The relationship between platelets and cancer is complicated, which could be influenced by type of cancer and its stage [23 ,28]. In our series, bone marrow suppression (from an inflammatory state or by other tumor-related mechanisms) may hamper megakaryopoiesis 24,2824,28. Moreover, the high platelet consumption could be induced by long-term inflammation. Platelet counts may further decrease early in cancer before subsequently rising in advanced disease, as demonstrated by the lower mean value and greater variability amongst our mixed group of patients [26, 29].

When evaluating the clinical utility of this biomarker panel, its proposed cost-effectiveness and accessibility are clear advantages, particularly in resource-conscious settings. The combination of CA15-3 with routine hematological parameters provides added diagnostic value beyond CA15-3 alone, as evidenced by the increase in AUC from 0.98 to 0.99. This combined approach leverages existing, low-cost tests to enhance diagnostic accuracy, which is particularly relevant in resource-limited settings.

The use of a lone tumor marker and routine CBC would be practical. Yet one must put that performance in context. Although CA15-3 had high specificity (94%), its reported deficiencies, as well (with low sensitivity in early disease), were confirmed by the finding that Stage I patients on average exhibited a 18.2 U/mL mean level of this marker, closer to the normal range for our sub-group analysis. This increase of markers parallel to disease stage is similar to what would be expected for other inflammatory and cancer biomarkers [27].

Imaging techniques, especially mammography, are still the backbone of screening. Thus, the current panel is not a replacement for imaging for initial screening. Instead, its most informative role may be as an adjunctive tool: (1) in tidying [removing duplicates and adding reference 8] of patients with concerning breast symptoms seen in primary care settings, (2), for disease progression or response to treatment monitoring in patients diagnosed with cancer, even if it's mainly required just once [10] or (3) where access to a more advanced imaging is not available. In addition, the systemic inflammatory condition expressed by these hematological modifications might be subjected to modulation by several cofactors such as patient stress and should require further investigation in terms of the whole patient profile [29].

The ROC analysis underscores the high diagnostic value of our findings. CA15-3 demonstrated outstanding individual diagnostic performance (AUC=0.98). Critically, the combined ROC analysis using a logistic regression model revealed that an integrated panel of CA15-3, WBC, RBC, Lymphocytes, and Platelets achieved a near-perfect AUC of 0.99. The model's coefficients (Table 4a) show that CA15-3 and WBC are positive predictors, while RBC, Lymphocytes, and Platelets are inverse predictors, which aligns with the direction of the changes observed in our univariate analysis. This combined approach demonstrates a significant improvement in diagnostic performance over individual CBC parameters and validates the core premise of our study that a multi-parameter approach is superior.

In terms of disease progression, the sub-analysis demonstrated a trend of increasing CA15-3 levels with advancing disease stage (Stage I: 18.2 ± 4.1 U/mL; Stage IV: 35.1 ± 8.2 U/mL). This progression aligns with the established biology of CA15-3, where higher tumor burden and advanced disease lead to increased shedding of the antigen into the bloodstream.

Limitations

This study has several limitations. First, the case-control design, while useful for initial biomarker evaluation, inherently overestimates diagnostic accuracy compared to prospective cohort studies in real-world settings. Second, our cohort consisted of patients with established diagnoses, which limits the generalizability of our findings to asymptomatic screening populations. Third, while we excluded patients with known chronic diseases, the potential for confounding by subclinical conditions that might affect hematological parameters cannot be entirely ruled out. Fourth, the sample size, though adequate for the primary comparison, is relatively modest for a multivariate logistic regression model with five predictors, increasing the risk of overfitting; the findings require validation in larger, multicenter, prospective studies. Fifth, the model has not been externally validated in an independent cohort. Sixth, disease duration was not analyzed in relation to the biomarkers due to the cross-sectional design and primary focus on diagnostic differentiation, which should be considered in future longitudinal studies.

Conclusions and Recommendations

Conclusions

Our study confirms that elevated CA15-3 levels are a critical biomarker for breast cancer, showing levels approximately 3.5 times higher in treatment-naïve patients compared to controls. Furthermore, systemic inflammatory responses in BC are reflected in significant alterations in WBC, RBC, lymphocyte, and platelet counts. ROC curve analysis established the high diagnostic accuracy of CA15-3 and the supportive value of the hematological parameters. The combined ROC analysis for the integrated panel yielded an AUC of 0.99, underscoring its superior performance for distinguishing known BC patients from healthy individuals. The combined use of CA15-3 with these routine CBC indicators offers an immediate, cost-effective adjunctive method, aligning with the search for accessible multi-marker strategies [11, 12].

Recommendations

Based on the findings and limitations of this study, we recommend the following

1. External validation of these findings through larger, multicenter prospective studies to enhance generalizability and assess performance in a true diagnostic setting.

2. Further exploration of the combined diagnostic power of this panel in direct comparison with other established biomarkers.

3. Longitudinal studies to determine the prognostic significance of these biomarkers and their changes in response to treatment.

4. Further research into the mechanistic pathways linking these hematological changes to BC pathophysiology.

Author Contribution Statement

Mohammed Mohaibes: Conceptualization, Methodology, Investigation, Writing – Original Draft. Mayada Al-Khafaji: Investigation, Data Curation, Formal Analysis, Writing – Review & Editing. Fakhria Muhaibes: Formal Analysis, Visualization, Writing – Review & Editing. All authors read and approved the final manuscript.

Acknowledgements

The authors extend their sincere gratitude to the administration and staff of Al-Amal National Hospital, Baghdad, for their invaluable support during participant recruitment and sample collection. We also thank the technicians at the laboratory departments for their skilled assistance.

We acknowledge the following declarations regarding this study:

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. It was conducted using institutional resources.

Approval by Scientific Body / Thesis Work

This study was not part of an approved student thesis. The research protocol was developed and executed independently by the authors.

Ethical Approval

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The protocol was reviewed and approved by the Institutional Review Board (IRB) of Gilgamesh University (Reference number/ID: Approval No: 1520249). Written informed consent was obtained from all individual participants included in the study.

Availability of Data and Materials

The anonymized datasets generated and analyzed during this study are available from the corresponding author upon reasonable request, respecting participant confidentiality.

Study Registration

This study is an observational case-control investigation and was not registered in a clinical trials or systematic review registry.

Conflict of Interest

The authors declare that there are no conflicts of interest, financial or otherwise, related to this work.

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