A Novel Adipokine, Asprosin, may be a serum biomarker for breast cancer diagnosis

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Abstract

OBJECTIVE: Breast cancer is the most common type of cancer in women. Diagnosis in early stage is very important for cancer treatment. There is no good biomarker to diagnose breast cancer in T1-2 or N0 stage. The aim of this study was to evaluate the diagnostic efficacy of asprosin as a biomarker within all stages of breast cancer.

PATIENTS AND METHODS: An enzyme-linked immunosorbent assay (ELISA) was used to evaluate serum asprosin levels in 40 patients with breast cancer and 40 healthy women. The Cancer group included T1-4, N1-3, and M0-1 patients.

RESULTS: Asprosin showed good discrimination (Area under curve (AUC) = 0.767, 95% confidence interval (CI):0.657–0.878) between breast cancer and the healthy group and acceptable discriminating ability (sensitivity = 0.825; specificity = 0.750) at the optimal cut-off value of 1.82 ng/mL. Asprosin indicated no difference for T, N, and M stages (p= 0.919, p= 0.859, and p= 0.225 respectively).

CONCLUSIONS: Asprosin may be a good diagnostic biomarker for breast cancer at all stages, regardless of T, N, and M stages. Larger prospective clinical studies are required to validate the utility of this method.

Introduction

Breast cancer (BC) is the most common type of cancer in women and the second most common cause of death [1]. Therefore, early diagnosis is important and the rate of early diagnosis has increased via screening mammograms and new imaging modalities [2]. As early diagnosis is not always possible, this situation has led us to search for early diagnostic methods. One of these methods is biomarkers. Ca 15-3 is a marker used for BC diagnosis but it is not sufficient to reach a good discrimination[3].

Biomarkers for cancer screening have been the subject of increasing research in recent years. Asprosin (ASP), an adipokine particularly effective in glucose metabolism, has been extensively studied [4-6]. It is an orexigenic peptide released from the white adipose tissue and is found in most tissues. [4]. Its levels are increased in disorders, such as type 2 diabetes mellitus (T2DM) and insulin resistance. Insulin resistance plays an important role in the molecular pathogenesis of various pathological conditions, including T2DM, cardiovascular diseases, esophageal adenocarcinoma, and cancers of the colon, rectum, pancreas, and gallbladder[7, 8]. Most of cancers have impaired energy metabolism and glucose consumption is increased in most cancers[9]. This metabolic disorder may affect the ASP levels.

This is the first study in which ASP was evaluated as a serum biomarker in breast cancer. The aim of this study is to evaluate the efficacy of ASP as a biomarker in the diagnosis of all stages of breast cancer.

Patients And Methods

Patient selection

Prior to commencing the study, approval was obtained from the Firat University Ethics Committee (Protocol No:2018-19). The target population was women diagnosed with primary invasive BC, excluding those suffering from metabolic diseases, major cardiovascular diseases (such as unstable angina, stroke, or myocardial infarction), liver disease, diabetes mellitus, severe psychiatric conditions, chronic kidney disease, diseases of the central nervous system, and polycystic ovary syndrome, as well as women undergoing immunosuppressive agent therapy or exhausting exercise regimen and those with a history of other malignancies. As 40 female patients recruited from the Surgical Oncology and Oncology Clinic met these criteria, they formed the BC sample, whereas 40 healthy female volunteers served as the controls (Figure 1).

Determination of Human Serum Asprosin and Cancer Marker Levels

Serum ASP levels were measured using sandwich enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's protocol (Abbexa Ltd., Cambridge, UK; Cataolog no.: abx257694). The serum levels of the cancer markers CEA, CA 15–3, and CA 125 were determined using an Advia Centaur Immunology Analyzer (Advia 2400, Automatic Siemens Healthcare Diagnostics Inc., Tarrytown, USA). Peripheral venous blood samples were collected from all patients and healthy volunteers and centrifuged at 4000 rpm for 5 min at 4 ^{o}C. The resulting serum samples were aliquoted and kept at (Bray et al., 2018)–80 ^{o}C until needed for analyses.

Immunohistochemical (IHC) staining was performed on the samples to determine hormone receptor status. Immunohistochemistry was performed using 4 µm thick histological tissue slides obtained from the paraffin blocks. The Olympus Microscope Digital Camera model DP71 (Olympus Co.; Shinjuku, Tokyo, Japan) software imaging system was used for histological analysis. Estrogen (ER) and progesterone (PR) status was defined as positive when 10% or more nuclei showed positive staining. For human epidermal growth factor 2 (HER2) status, tumors with IHC staining of 3+ (uniform, intense membrane staining of 30% of invasive tumor cells) were considered HER2 positive. Cases with IHC staining of 2+ and positive amplification of HER- 2/neu gene using fluorescence in situ hybridization were regarded as positive. The 1+ HER2 status was considered negative. The Modified Bloom Richardson score was used for the histological grading.

Statistical Analyses

The sample size for the present study was determined by power analysis, indicating that 15 subjects were sufficient to achieve a type 1 error (α) of 0.05, a type 2 error (β) of 0.10, and power of 0.90. Conformity with the normal distribution was evaluated using the Shapiro-Wilk test, and all normally distributed data were presented as mean ± standard deviation (M ± SD), whereas categorical variables were summarized as absolute (n) and relative frequencies (%), and nonparametric data were presented as median (25–75th percentile). Associations between categorical variables were evaluated using contingency tables and chi-squared tests without continuity correction or Fisher's exact test, if applicable. For normally distributed data, two independent groups were evaluated using Student's t-test, while applying the Mann–Whitney U

test in other cases. Three or more categories were compared using one-way analysis of variance (ANOVA) followed by a posthoc Bonferroni correction, while applying the Kruskal-Wallis test followed by a posthoc Bonferroni correction in other cases. Correlations between asprosin levels and continuous variables were evaluated using the Pearson's correlation coefficient (normally distributed data) or Spearman's correlation coefficient (skewed data).

The strength of the association between ASP levels and BC probability was evaluated using logistic regression analysis, while considering other data with significant differences ($p<0.05$) as potential confounders. We also calculated adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for BC risk estimates.

Receiver operating characteristic (ROC) curve analysis was used to identify the optimal ASP cut-off level and determine its sensitivity and specificity for BC. In addition, the area under the ROC curve (AUC) was used to assess the diagnostic performance of ASP for BC. Finally, the Hosmer–Lemeshow fit test was conducted to assess the agreement between the observed and model-predicted proportions of BC. All statistical calculations were performed using SPSS version 21.0 commercial software (SPSS Corp., Armonk, NY, USA).

Results

Demographic comparison of groups

As shown in Table 1, the BC and control groups were similar in terms of age, height, weight, body mass index (BMI), dyslipidemia, and menopausal status ($p> 0.05$). The BC group was more likely to have a family history of breast or gynecological cancer than healthy women ($p= 0.018$, OR:3.769, 95% CI:1.205–11.789).

Comparison of Laboratory data for groups

No statistically significant differences were noted in total cholesterol, HDL, LDL, or TG (dyslipidemia) levels between the BC and healthy groups ($p> 0.05$). Serum levels of CA 125 and CA 15–3 were significantly higher in patients with BC than in healthy controls ($p< 0.05$) (Table 2). There were significant increases in ASP levels in BC group (2.14 (1.88-2.46) ng/mL) compared with the healthy group (1.62 (1.37-1.85) ng/mL) ($p < 0.001$).

Comparison of asprosin levels in pathological data

All pathological variables were analyzed for ASP and tumor marker levels (CEA, CA 15-3, and CA 125). The ASP showed a significant difference in terms of grade ($p= 0.025$). The Posthoc Bonferroni test revealed a significant difference between grades I and III ($p= 0.043$). The difference between grades II and III was close to the significance level ($p= 0.058$). No differences were found between Grades I and II. No differences were found for ASP in terms of ER, PR, HER2, luminal type, T stage, N stage, M stage, and Ki-67 ($p> 0.05$) (Table 3). Moreover, no differences were found in tumor markers (CEA, CA 15-3, and CA 125)

in terms of ER, PR, HER2, luminal type, grade, T stage, N stage, or Ki-67 (p> 0.05). CA 15-3 showed only a significant difference in terms of M stage (p< 0.001).

Univariate and multivariate analyses

Variables that showed differences between the groups were entered into the univariate regression analysis. ASP (OR: 4.714; 95% CI: 1.726–12.876; p= 0.002), CA 15-3 (OR: 1.049; 95% CI: 1.005–1.095; p= 0.029), CA 125 (OR: 1.127; 95% CI: 1.017–1.250; p= 0.023), and family history of breast or gynecological cancer (OR: 3.796; 95% CI: 1.205–11.789; p= 0.023) were significantly associated with a higher risk of BC. Variables with p < 0.05 on univariate analysis were entered into multivariable logistic regression models, and the results indicated that ASP (OR:4.403; 95% CI:1.492–12.995; p= 0.007) was an independent risk factor for BC (Table 4).

ROC Analysis for Asprosin of BC

ROC analysis was conducted to determine the sensitivity and specificity of ASP for BC detection. The obtained results indicated that serum ASP levels successfully discriminated BC patients from healthy controls (AUC= 0.767, 95% CI:0.657–0.878) and exhibited acceptable discriminative ability (sensitivity= 0.825; specificity= 0.750) at the optimal cut-off value of 1.82 ng/mL (p< 0.001), as shown in Figure 2.

Discussion

This study indicated that ASP levels in the BC group were significantly higher than in the healthy group. Therefore, elevated ASP levels may be an indicator of an increased risk of BC. Specifically, the ROC analysis results demonstrated that when the cut-off point for ASP was set at 1.82 ng/mL, its sensitivity for BC detection was 82.5% and its specificity was 75%. Thus, ASP may be a useful marker for the detection of BC.

At present, BC screening is performed using mammography and/or USG (mammography may be incapable of detecting interval tumors and suspicious lesions in women with dense breast tissue) [10, 11]. The American Society of Breast Surgeons recommends that average-risk women undergo BC screening every year, starting at the age of 40 years [12, 13]. To improve the early detection of BC, it is essential to identify new biomarkers with high sensitivity and specificity. This new biomarker may be ASP as a novel adipokine .

Recent studies have shown that changes in adipokine levels may be a significant risk factor for BC [14, 15]. Asprosin, a novel glucogenic adipokine, may play a role in tumor development and progression. ASP immunoreactivity is considerably increased in malignant mesothelioma and BC, and can thus serve as a possible diagnostic marker [16, 17]. In a study by Akkuş, the immunoreactivity of ASP was high in BC versus healthy group. There was no difference between histological grades and no data were presented regarding the cancer stages [16]. In the present study, there was a statistically significant difference between the grades. The median ASP level was lower in grade I patients and higher in grade III

patients. Moreover, the ASP level showed no statistical difference between the T, N, and M stages. Therefore, this result indicates that the ASP level increased at all stages of BC. These results suggest that ASP may be a potential early diagnostic marker of BC.

Thus, asprosin may play a role in the development of cancer. It uses cyclic adenosine monophosphate (cAMP) as a secondary messenger and induces hepatic glucose production by activating the G-protein/cAMP/protein kinase A pathway [4]. ASP levels are increased in disorders such as type 2 diabetes mellitus and insulin resistance. Insulin resistance plays an important role in the pathogenesis of metabolic diseases and cancers [18]. The release of ASP may increase the level of insulin-like growth factor (IGF) by increasing insulin resistance, which may lead to cancer development [8]. In addition, it has been reported to participate in the development and metastasis of various cancers [7].

Nuclear factor-κB (Nf- κB) is another mechanism that may explain the relationship between cancer and ASP. The cAMP/protein kinase A pathway may function as a cellular response to hypoxia. This can increase ROS levels of reactive oxygen, which causes oxidative stress. Systemic inflammation resulting from impaired antioxidant balance activates NF-κB [19, 20]. NF-κB regulates the expression of various target genes that promote cell proliferation and contribute to the pathogenesis of various diseases, including cancer [21]. In the present study, we could not determine whether the increase in ASP levels was the cause or result of cancer. Therefore, further studies on molecules such as NF-κB and IGF are required.

Hormone receptors and luminal subtypes are important for BC treatment. In the present study, there were no differences in the ER, PR, HER2, Kİ-67 receptors and luminal types for ASP. There is insufficient data on the association between ASP and these receptors or luminal types. Therefore, we could not compare our results of the receptor and luminal types.

Among the limitations of the present study is the fact that we only measured serum asprosin levels and did not examine ASP expression in detail for prognosis. However, we believe that ASP levels may have a significant effect on our study variables because the target population consisted of women newly diagnosed with primary invasive BC. Traditional prognostic factors continue to have an impact throughout time, and more recent factors require extensive follow-up. The prognosis improves over time for breast cancer patients who have survived for at least a decade. Earlier detection, more effective modern treatment, can raise long-term survival. The study did not include the ASP immunoreactivity, benign lesions or disorders of breast patients to compare them with cancer and healthy groups. Another limitation is that we used ELISA for measuring ASP levels. Western blotting may have increased the reliability of our study.

Conclusion

Serum ASP levels were higher in the BC group than in the control group. Its use may be a biomarker for diagnosis of BC regardless of stage and may indicate the grade. Further studies, including ASP

immunoreactivity, benign breast disorders or lesions, are needed to validate these hypotheses.

Declarations

Ethics declarations

This study was approved by the Firat University Local Ethics Committee (Protocol No:2018-19)

Consent to participate

A written and signed informed consent form was obtained from all the participants of this study.

Fundings

There is no funding

Conflict of Interest

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Availability of Data and Materials

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

MY and MRÖ collected the data and write the study. MÖ, MY and SÖ conducted the study and desingned it. NY, MY and SÖ drafted the article, made critical revisions related to relevant intellectual content of the manuscript. MY conducted the stastical analyses and interpretation of data. All authors read and approved the final manuscript

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Tables

Table 1: Demographic comparison of groups (BC: breast cancer, BMI: body mass index).

Variables	Control group n: 40	BC group n: 40	*p* value
Age (Years)	49.13 ± 14.81	53.33 ± 12.45	0.174
Weight (kg)	75.58 ± 11.40	72.93 ± 10.86	0.290
Height (meter)	1.63 ± 0.8	1.60 ± 0.6	0.075
BMI (kg/m^2)	27.87 ± 5.44	28.64 ± 4.53	0.839
Dyslipidemia	18 (45%)	25 (62.5%)	0.116
Menopausal status;			0.625
Pre-menopausal	18 (45%)	16 (40%)	
Post-menapausal	22 (55%)	24 (60%)	
Cancer History;			
Having benign breast disease	0 (0%)	2 (5%)	0.505
Having a prior neoplasm	0 (0%)	4 (10%)	0.141
Having breast and/or gynecological cancer of family	5 (12.5%)	14 (35%)	**0.018**

Table 2: Comparison of laboratory data for groups (BC: breast cancer, HDL: high-density lipoprotein, LDL: low-density lipoprotein)

Laboratory data	Control group n: 40 median (25–75th percentile)	BC group n: 40 median (25–75th percentile)	*p* value
Asprosin (ng/mL)	1.62 (1.37-1.85)	2.14 (1.88-2.46)	**< 0.001**
HDL (mg/dL)	45.9 (37.1-58.2)	47.9 (41.5-54.1)	0.661
LDL (mg/dL)	123 (100.5-149.5)	129 (113.3-147.4)	0.206
Triglyceride (mg/dL)	103 (83.5-135)	106.5 (91-165.5)	0.233
Total cholesterol (mg/dL)	196.1 (175-216)	205.9 (188.2-239.2)	0.138
CEA (ng/mL)	1.29 (0.85-2.09)	1.08 (0.45-1.95)	0.400
CA 15-3 (IU/mL)	14.65 (8.25-19.20)	18,55 (12.80-33.70)	**0.012**
CA 125 (IU/mL)	7.00 (4.70-10,05)	9.30 (5.90-16.20)	**0.014**

Table 3: Comparison of asprosin in pathological data for cancer patients (HER2: human epidermal growth factor receptor 2, *: Spearman’s correlation analysis performed)

Pathological variables		Asprosin level (ng/dl) median (25–75th percentile)	*p* value
Estrogen	Negative	2.09 (1.94-2.58)	0.976
	Positive	2.25 (1.87-2.43)	
Progesteron	Negative	2.09 (1.94-2.36)	0.804
	Positive	2.25 (1.85-2.49)	
HER2	Negative	2.15 (1.88-2.58)	0.475
	Positive	2.13 (1.85-2.36)	
Luminal type	Type A	2.25 (2.25-2.26)	0.714
	Type B HER2(-)	2.26 (1.87-2.59)	
	Type B HER2(+)	2.05 (1.68-2.35)	
	HER2(+)	2.36 (1.94-2.59)	
	Triple Negative	2.08 (1.91-2.37)	
T stage	T1-2	2.20 (1.90-2.46)	0.919
	T3-4	2.14 (1.88-2.49)	
N stage	N 0	2.28 (1.87-2.46)	0.859
	N (+)	2.11 (1.90-2.50)	
M stage	M 0	2.14 (1.88-2.4)	0.225
	M 1	2.36 (1.94-2.73)	
Grade	I	1.92 (1.76-2.29)	**0.025**
	II	2.11 (1.88-2.38)	
	III	2.60 (2.25-2.63)	
Ki-67			0.209*

Table 4: Univariate and multivariate analyses for predictive factors of BC

	Univariate analyses			Multivariate analyses		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Asprosin	4.714	1.726-12.876	**0.002**	4.403	1.492-12.995	**0.007**
CA 15-3	1.049	1.005-1.095	**0.029**	1.052	0.993-1.114	0.083
CA 125	1.127	1.017-1.250	**0.023**	1.118	0.996-1.255	0.058
Family history of breast or gynecological cancer	3.769	1.205-11.789	**0.023**	3.105	0.834-11.563	0.091

Figures

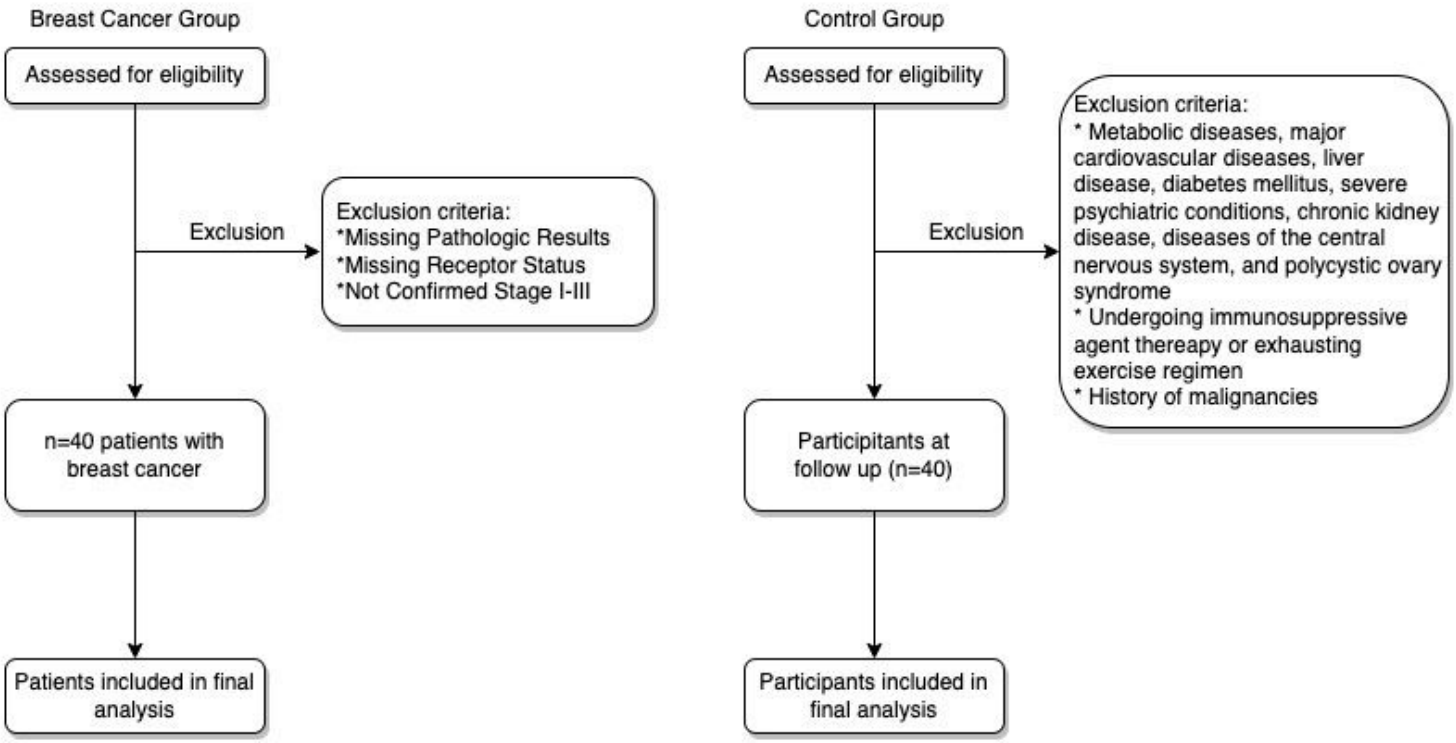

Figure 1: Flowchart of the study

Figure 1

Flowchart of the study

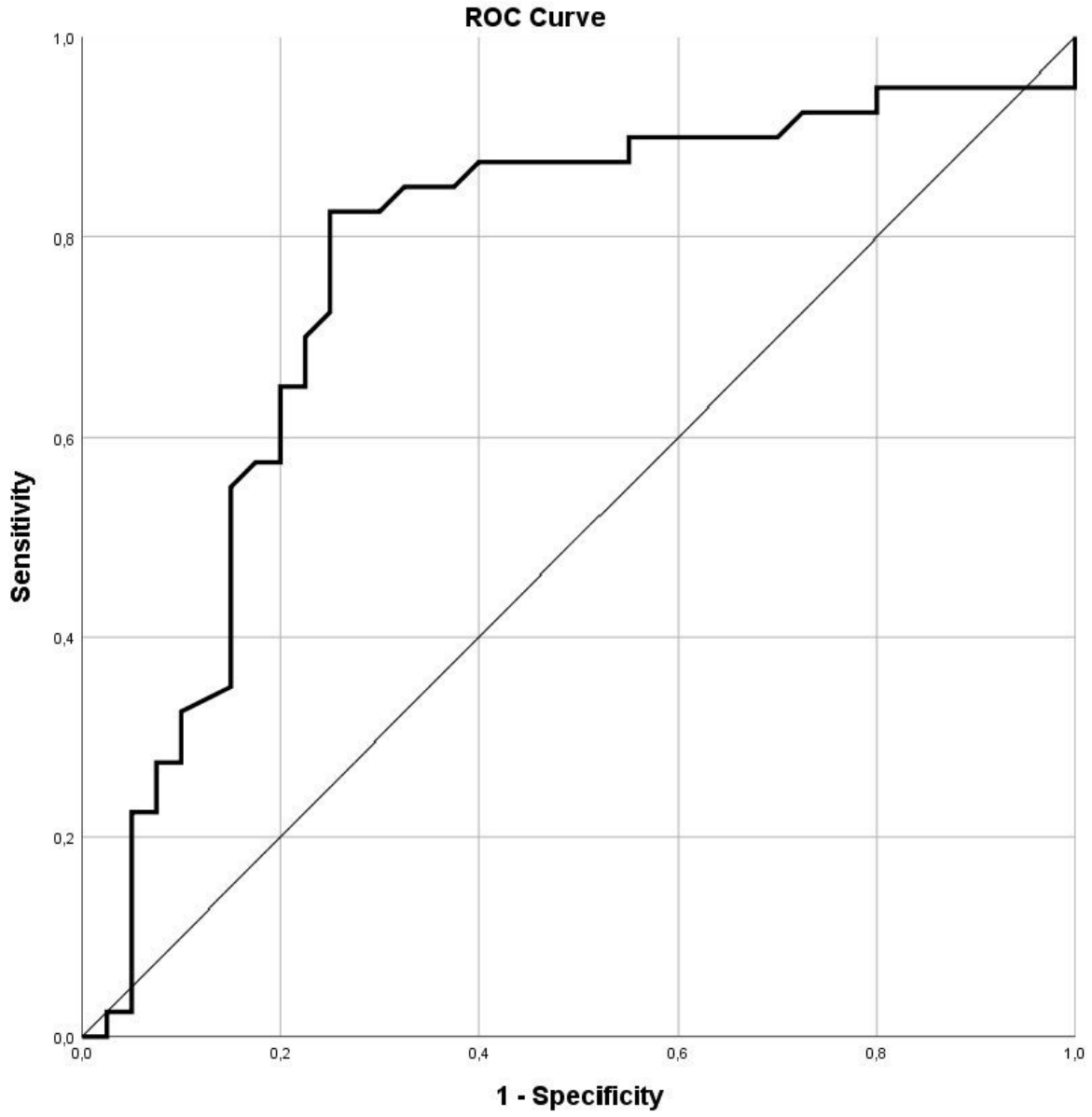

Figure 2: Receiver operating characteristic (ROC) analyses for diagnosis of BC. Serum asprosin can discriminate between breast cancer patients and healthy individuals at a cut-off point of 1.82 ng/mL, with 0.767 area under the curve (AUC), 82.5% sensitivity and 75.0% specificity.

Figure 2

Receiver operating characteristic (ROC) analyses for diagnosis of BC. Serum asprosin can discriminate between breast cancer patients and healthy individuals at a cut-off point of 1.82 ng/mL, with 0.767 area under the curve (AUC), 82.5% sensitivity, and 75.0% specificity ($p < 0.001$).