

Serum NLRP3 and S1P as Potential Diagnostic and Prognostic Biomarkers in Female Metastatic and Non-metastatic Breast Cancer Patients

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ABSTRACT

Breast cancer (BC) is considered the main cause of death in women worldwide. Overexpression of Sphingosine 1-phosphate (S1P) receptors in estrogen receptor (ER) negative BC patients is linked to poor prognosis. In vitro, the bioactive lipid metabolite S1P induced Nod-like receptor3 (NLRP3) dependent activation of caspase-1 and secretion of interleukin-1beta (IL-1). The object of this study is to evaluate the serum levels of S1P and NLRP3 to examine their potential as diagnostic and prognostic biomarkers for BC. The study involved 26 metastatic BC patients, 30 non-metastatic BC patients, and 20 healthy control volunteers. NLRP3, S1P, and cancer antigen15.3 (CA 15.3) serum levels were analyzed using Enzyme-linked immunosorbent assay ELISA. Statistical analysis involved Mann-Whitney U, Kruskal-Wallis tests, and Receiver operating curve (ROC) analysis. Serum levels of NLRP3 were significantly lower in non-metastatic than in metastatic BC ($P < 0.001$) and serum levels of S1P were significantly lower in metastatic than in non-metastatic BC and control ($P < 0.001$). Single ROC analysis showed that NLRP3 has a very good diagnostic value in early and late-stage BC ($P < 0.0001$), but a poor prognostic value in predicting metastasis ($P = 0.1392$). In contrast, S1P has an excellent diagnostic value in late-stage BC ($P < 0.0001$), and an excellent prognostic value in predicting metastasis ($P < 0.0001$), but a poor diagnostic value in early-stage BC ($P = 0.1781$). The diagnostic and prognostic value of the studied biomarkers improved by combining every two markers ($P < 0.001$). In conclusion, NLRP3 and S1P could be promising novel diagnostic and prognostic biomarkers of BC either used alone or in combination with CA 15.3.

Keywords: *NLRP3; S1P; diagnosis; breast cancer; metastasis.*

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Citation | Soltan AR, Kamal AM, El-Shimy RAA, El-Mesallamy HO, 2023. Serum NLRP3 and S1P as Potential Diagnostic and Prognostic Biomarkers in Female Metastatic and Non-metastatic Breast Cancer Patients. Arch Pharm Sci ASU 7(2): 334-347

DOI: [10.21608/aps.2023.226758.1130](https://doi.org/10.21608/aps.2023.226758.1130)

Print ISSN: 2356-8380. Online ISSN: 2356-8399.

Received 16 August 2023. Accepted 22 October 2023.

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Published by: Ain Shams University, Faculty of Pharmacy

1. Introduction

For the past two decades and till now, BC has been the most commonly diagnosed cancer worldwide [1]. Approximately 60% of BC deaths occur in developing countries. About 2.3 million cases were diagnosed in 2020 presenting about 11.7% of total cancer cases and accounting for almost 1 in every 4 cancer cases among women [2, 3]. In Egypt, BC is the most prevalent cancer among

Egyptian females, representing 16.4% of total cancer cases. The incidence rates of BC far exceed those for other cancers in both transitioned and transitioning countries, followed by colorectal cancer (CRC) in transitioned countries, and cervical cancer in transitioning countries [4]. Diagnosis of BC usually begins with screening by mammography or by using traditional techniques by hand (finding a lump in any place within the breast) or blood-based tests where the most prominent tumor biomarker

used in diagnosis is CA 15.3 which is a glycoprotein derived from Mucin-1 gene (MUC1) [5, 6]. There is a direct relation between levels of CA 15.3 and the number of cancer cells. The level of CA 15.3 is linked with the degree of spread of BC [7, 8]. However, tumor marker sensitivity in primary BC is very low, invalidating their use in early diagnosis [9]. Also, CA 15.3 lacks specificity as its concentration could be elevated in liver, ovarian, pancreatic, and colon cancer [10].

Activation of steroid hormone receptors [ER or PR] or activation of HER-2 receptors causes major mutations in breast cells [11]. As a result of these mutations, tissue damage is activated and promotes inflammation, which releases damage-associated molecular patterns (DAMPs) alone or in aggregation with pathogen-associated molecular patterns (PAMPs). This process is referred to as a cell death promotion mechanism [12]. Inflammasomes are defined as a group of cytosolic protein complexes that are formed to mediate host immune responses to microbial infection and cellular damage by activation of inflammatory responses [13] in which procaspase-1 is activated into caspase-1 and then converts the cytokine precursor's pro-interleukin-1 β (pro-IL-1 β) and pro-interleukin-18 (pro-IL-18) into active IL-1 β and interleukin-18 (IL-18). Mature IL-1 β is a potent pro-inflammatory mediator, which is important for the production of interferon-gamma (IFN- γ) [14].

Nucleotide-binding oligomerization domain and leucine-rich repeat-containing receptors protein family represent important components of the inflammasomes. It consists of nod-like receptor pyrin 1 (NLRP1), NLRP2, NLRP3, and NLRP4, of which NLRP3 is considered the most expressed in inflammatory and infectious diseases [15]. Meanwhile, S1P, a product of sphingosine kinases (SK), is a bioactive lipid that can be released from cells to act predominantly

on a family of G-protein-coupled receptors that mediate its action on cell growth, migration, transcription, and signal transduction [16]. It is emerging as a key regulator of proliferation, inflammation, vasculogenesis, and resistance to apoptotic cell death. There is increasing evidence of a role for S1P receptors (e.g., S1P4 and SK1) in cancer, where over-expression of these proteins in ER-negative BC patients is linked with poor prognosis [17]. It has been discovered that the bioactive lipid metabolite S1P can act as a DAMP and *in vitro*, it induced NLRP3-dependent activation of caspase-1 and secretion of IL-1 β [18].

2. Subjects and Methods

2.1. Subjects

From October 2018 till December 2019, a total of 56 treatment-naive BC female patients (30 patients (age median= 47.5) diagnosed with non-metastatic BC (stages 0, 1, 2, and 3) and 26 patients (age median= 51) diagnosed with metastatic BC (stage 4), were recruited from the National Cancer Institute, Cairo University. The diagnosis was based on the mammogram and cell biopsy. All patients were classified by tumor, node, and metastases (TNM) classification system. Moreover, the study involved 20 healthy age and sex-matched volunteers as the healthy control group (age median= 46). The clinicopathological data were collected from patient files including ER, PR, and HER-2 expression.

Five milliliters of peripheral blood samples were withdrawn from all subjects. Blood was collected on plain vacutainer tubes for serum separation. Serum samples were divided into 3 aliquots and stored at -80 °C for subsequent assays. This study was approved by the Ethical Committee of Research, Faculty of Pharmacy, Ain Shams University, approval number (244)

09-05-2019, and by the Ethical Committee of The National Cancer Institute, Cairo University. Inclusion criteria included middle age, female BC patients who haven't started their treatment protocol. All patients who received chemotherapy, suffering from coronary heart diseases, any cancer other than breast cancer, liver diseases, neurodegenerative diseases, immune deficient diseases, inflammatory bowel diseases, or acute ischemia-reperfusion injury in the brain, heart, retina, or kidneys were excluded from the study.

2.2. Methods

Serum levels of NLRP3 and S1P were measured by Enzyme-linked immunosorbent assay (ELISA) assay using commercially available kits: Human E3980Hu, and E1860Hu, respectively from Bioassay Technology Laboratory (Shanghai, China), as well as CA15.3 using a commercially available kit from Immunospec (Livonia, USA). All ELISA procedures were done by ELISA chromate micro-plate reader (Awareness Technology, USA) according to the manufacturer's instructions.

2.3. Statistical analysis

GraphPad Prism 8.0.2 (GraphPad Software, CA, USA) was used for data analysis. Data was expressed as median and intraquartile range (IQR). For nonparametric data, the Mann-Whitney U test was used for comparing two independent groups, and the Kruskal-Wallis test was used for comparing more than two groups. Diagnostic and prognostic sensitivity and specificity were calculated using the ROC analysis by SPSS software version 26.0 (SPSS Inc. Chicago, IL, USA). 95% confidence intervals (CIs) were calculated. Multivariable binary logistic regression models were built using various combinations of CA15.3, S1P, and NLRP3 to calculate predicted probabilities for

discrimination between the three study groups using each model. The discriminative value of these combinations was assessed by examination of the area under the receiver-operating characteristic (ROC) curves derived from the predicted probabilities of the corresponding models. The best cut-off criterion was identified as the predicted probability associated with the highest Youden (J) index. The probability of error was considered significant at $p < 0.05$, while $p < 0.01$ and $p < 0.001$ was considered highly significant.

3. Results

3.1. Statistical parameters of CA15.3, NLRP3 and S1P, Demographic data and Clinicopathological characteristics of the studied groups

Statistical parameters of CA15.3, NLRP3, and S1P, Demographic data, and Clinicopathological characteristics of the studied groups are summarized in **Table 1**.

3.2. Serum level of CA 15.3 in the studied groups

Serum level of CA15.3 was significantly elevated in non-metastatic BC and metastatic BC as compared to the control group ($P < 0.0001$). Also, it was significantly elevated in metastatic BC as compared to the non-metastatic BC group ($P = 0.0029$) as shown in **Fig. 1**.

3.3. Serum level of NLRP3 in the studied groups

Serum level of NLRP3 was significantly decreased in both non-metastatic BC ($p < 0.001$) and metastatic BC patients ($p < 0.0001$) as compared to the control group. No significant difference was detected between non-metastatic BC and metastatic BC groups ($P = 0.5275$) as shown in **Fig. 2**.

3.4 Serum level of S1P in the studied groups

There was no significant difference regarding serum level of S1P between the control and non-metastatic BC groups (p=0.9187) but S1P was

significantly decreased in the metastatic BC group than in the control and non-metastatic BC groups (p<0.0001) as shown in **Fig. 3**.

Table 1. Statistical parameters of CA15.3, NLRP3, and S1P, Demographic data, and Clinic pathological characteristics of the studied groups

Marker\ parameter	Group	Number	Median	I.Q.R	negative	Positive	Birads4	Birads5	P value
CA15.3	Control	20	28.98	27.83 - 31.93					
	Non-metastatic	30	37.81	35.04 - 41.05					<0.0001**
	Metastatic	26	47.36	39.46 - 49.4					<0.0001**
S1P	Control	20	44.13	39.14-48.83					
	Non-metastatic	30	41.75	35.1-47.97					0.1806
	Metastatic	26	28.08	26.11-29.42					<0.0001**
NLRP3	Control	20	27.11	22.02-30.05					
	Non-metastatic	30	20.24	18.11-21.87					<0.0001**
	Metastatic	26	18.45	15.22-20.2					<0.0001**
Age	Control	20	46	41.25-54.00					
	Non-metastatic	30	47.5	42.25-60.25					0.2894
	Metastatic	26	51.00	45.25-63.50					0.06
ER	Non-metastatic	30			3	27			
	metastatic	26			4	22			>0.9999
PR	Non-metastatic	30			4	26			
	metastatic	26			4	22			>0.9999
HER-2	Non-metastatic	30			22	8			
	Metastatic	26			18	8			>0.9999
BIRADS score	Non-metastatic	30					10	20	
	Metastatic	26					5	21	>0.9999

Statistical parameters of CA15.3, NLRP3, and S1P expressed as median and IQR, Demographic data, and clinicopathological characteristics of the studied groups compared using Mann-Whitney U test. *p< 0.05, **p < 0.0001.

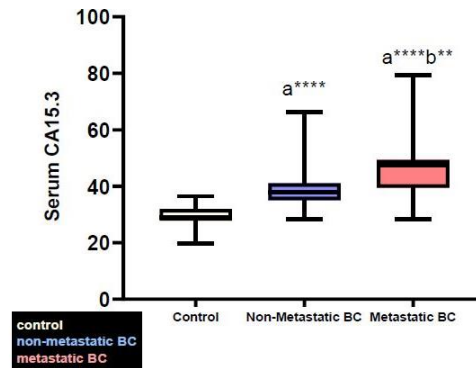


Fig. 1. serum level of CA15.3 in the studied groups

Box plot illustrating serum concentrations of CA15.3 in the studied groups using Kruskal Wallis test. Control group: median=28.98, I.Q.R (27.83-31.93), Non-metastatic BC: median= 37.81, I.Q.R (35.04-41.05) and Metastatic BC median= 47.36, I.Q.R (39.46-49.40). Box represents the interquartile range. Line inside the box represents the median.

Bars represent minimum and maximum values.

a: significantly different as compared with control group. b: significantly different as compared with non-metastatic group.

c: significantly different as compared with metastatic group.

*:P<0.05, **:P<0.01, ***:P<0.001, ****P<0.0001

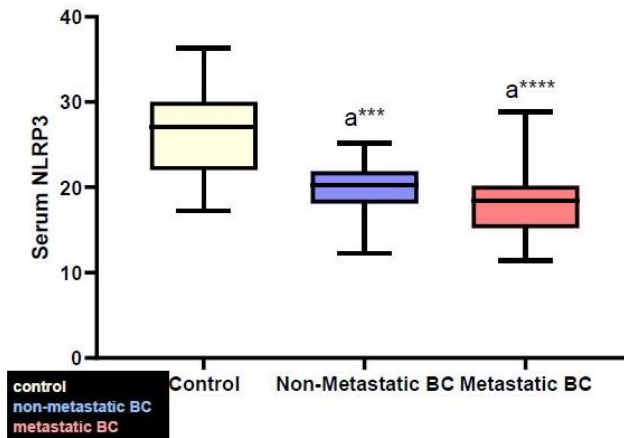


Fig. 2. serum level of NLRP3 in the studied groups

Box plot illustrating serum concentrations of NLRP3 in the studied groups using Kruskal Wallis test. Control group: median=27.11, I.Q.R (22.03-30.05), Non-metastatic BC: median= 20.24, I.Q.R (18.11-21.87) and Metastatic BC median= 18.45, I.Q.R (15.22-20.20). Box represents the interquartile range. Line inside the box represents the median. Bars represent minimum and maximum values.

a: significantly different as compared with control group. b: significantly different as compared with non-metastatic group. c: significantly different as compared with metastatic group.

*:P<0.05, **:P<0.01, ***:P<0.001, ****P<0.0001

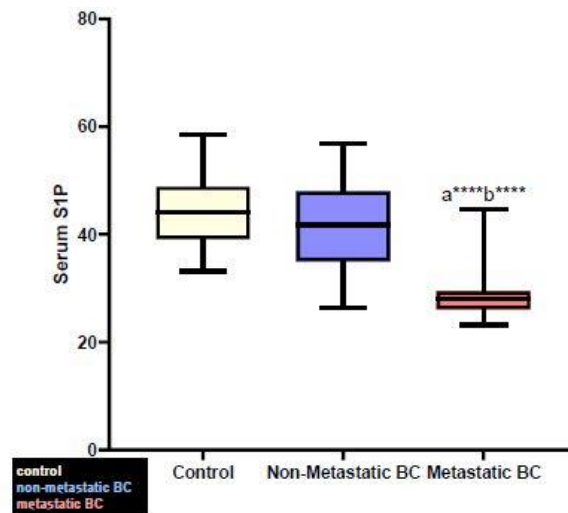


Fig. 3. serum level of S1P in the studied groups

Box plot illustrating serum concentrations of S1P in the studied groups using Kruskal Wallis test. Control group: median=44.13, I.Q.R (39.14-48.83), Non-metastatic BC: median= 41.75, I.Q.R (35.10-47.97) and Metastatic BC median= 28.08, I.Q.R (26.11-29.42).

Box represents the interquartile range. Line inside the box represents the median.

Bars represent minimum and maximum values.

a: significantly different as compared with control group. b: significantly different as compared with non-metastatic group.

c: significantly different as compared with metastatic group.

*:P<0.05, **:P<0.01, ***:P<0.001, ****P<0.0001

3.5. The diagnostic value of CA15.3, NLRP3 and S1P serum levels in non-metastatic BC patients

The significance of CA15.3, NLRP3, and S1P serum levels as potential diagnostic biomarkers for de novo non-metastatic BC was assessed using ROC analysis. CA15.3 showed excellent diagnostic value (AUC= 0.9583, $P < 0.0001$), NLRP3 showed very good diagnostic value (AUC= 0.8483, $P < 0.0001$) while S1P showed poor diagnostic value (AUC= 0.6133, $P = 0.1781$) as shown in Fig. 4.

3.6. The diagnostic value of CA15.3, NLRP3 and S1P serum levels in metastatic BC patients

The significance of CA15.3, NLRP3, and S1P serum levels as potential diagnostic biomarkers

for metastatic BC was assessed using ROC analysis. Both CA15.3 and S1P showed excellent diagnostic value (AUC= 0.9692 and 0.9596 respectively, P value < 0.0001), while NLRP3 showed very good diagnostic value (AUC= 0.8769, $P < 0.0001$), as shown in Fig. 5.

3.7. The prognostic value of CA15.3, NLRP3 and S1P serum levels in BC patients

The significance of CA15.3, NLRP3, and S1P serum levels as potential prognostic biomarkers for discriminating metastatic BC from non-metastatic BC was assessed using ROC analysis. CA15.3 showed good prognostic value (AUC= 0.7776, $P = 0.0004$), while NLRP3 showed poor prognostic value (AUC= 0.6154, $P = 0.1392$), and S1P showed excellent prognostic value (AUC= 0.9269, $P < 0.0001$), as shown in Fig. 6.

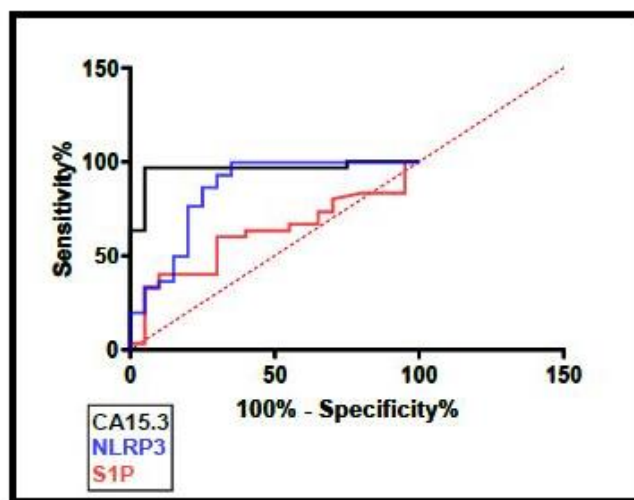


Fig. 4. Receiver operating curves (ROC) for discriminating non-metastatic BC from control using CA15.3, NLRP3 or S1P Roc curve showing CA15.3 with excellent diagnostic value (AUC=0.9583, 95%CI [0.8992-1.017], $P < 0.0001$, cut-off= 32.89, sensitivity= 96.67% and specificity= 95.00%), NLRP3 with a very good diagnostic value (AUC=0.8483, 95% CI [0.7267-0.97], $P < 0.0001$, cut-off= 23.10, sensitivity=86.67% and specificity= 75.00%) and S1P with poor diagnostic value (AUC=0.6133, 95%CI [0.4559- 0.7708], $P = 0.1781$, cut-off=42.34, sensitivity= 60.00% and specificity= 70.00%) AUC>0.9 is excellent, 0.8-0.9 is very good, 0.7-0.8 is good and <0.7 is poor.

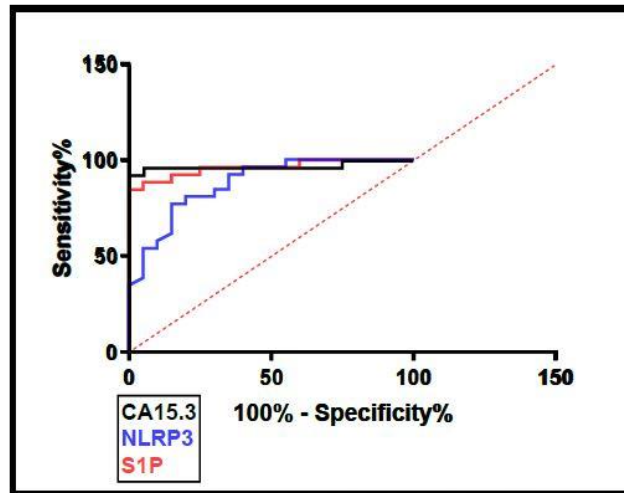


Fig. 5. Receiver operating curves (ROC) for discriminating metastatic BC from control using CA15.3, NLRP3 or S1P

Roc curve showing CA15.3 with excellent diagnostic value (AUC=0.9692, 95%CI [0.9126-1.026], $P<0.0001$, cut-off= 33.21, sensitivity= 96.15% and specificity= 95.00%), NLRP3 with a very good diagnostic value (AUC=0.8769, 95% CI [0.7781-0.9757], $P<0.0001$, cut-off= 20.16, sensitivity=76.92% and specificity= 85.00%) and S1P with excellent diagnostic value (AUC=0.9596, 95%CI [0.9063- 1.013], $P<0.0001$, cut-off=34.51, sensitivity= 88.46% and specificity= 95.00%). AUC>0.9 is excellent, 0.8-0.9 is very good, 0.7-0.8 is good and <0.7 is poor.

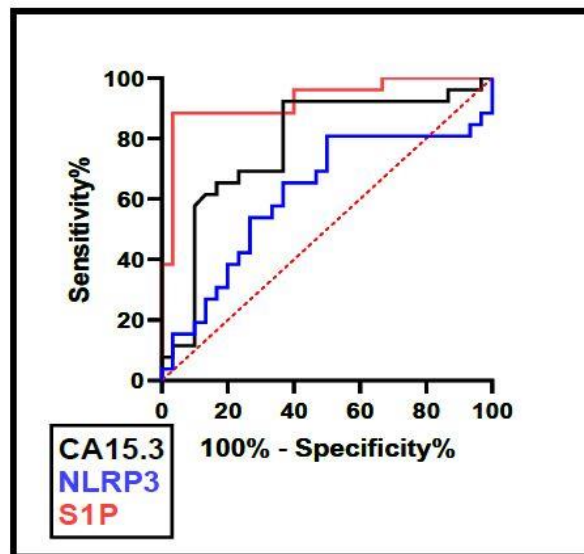


Fig. 6. Receiver operating curves (ROC) for discriminating non-metastatic BC from metastatic using CA15.3, NLRP3 or S1P

Roc curve showing CA15.3 with good diagnostic value (AUC=0.7776, 95%CI [0.6484-0.9068], $P=0.0004$, cut-off= 39.23, sensitivity= 92.31% and specificity= 63.33%), NLRP3 with a poor prognostic value (AUC=0.6154, 95% CI [0.4609-0.7698], $P=0.1392$, cut-off= 19.65, sensitivity=65.38% and specificity= 63.33%) and S1P with excellent prognostic value AUC=0.9269, 95%CI [0.8535- 1.000], $P<0.0001$, cut-off=34.08, sensitivity= 88.46% and specificity= 96.67%). AUC>0.9 is excellent, 0.8-0.9 is very good, 0.7-0.8 is good and <0.7 is poor.

3.8. The diagnostic and prognostic value of various combinations of CA15.3, S1P, and NLRP3 serum levels in the studied groups

Multivariable binary logistic regression models were built using various combinations of CA15.3, S1P, and NLRP3 to calculate predicted probabilities for discrimination between the studied groups using each model, and the discriminative value of these combinations was assessed by ROC analysis derived from the predicted probabilities of the corresponding models.

3.8.1. The diagnostic and prognostic value of combined CA15.3 and S1P serum levels in the studied groups

Combined ROC analysis of serum CA15.3 and S1P showed excellent diagnostic value in discriminating non-metastatic BC from control (AUC= 0.962, $P < 0.001$), excellent diagnostic value in discriminating metastatic BC from control (AUC= 0.916, $P < 0.001$), and excellent prognostic value in discriminating non-metastatic BC from metastatic BC (AUC= 0.927, $P < 0.001$) as shown in **Fig. 7**.

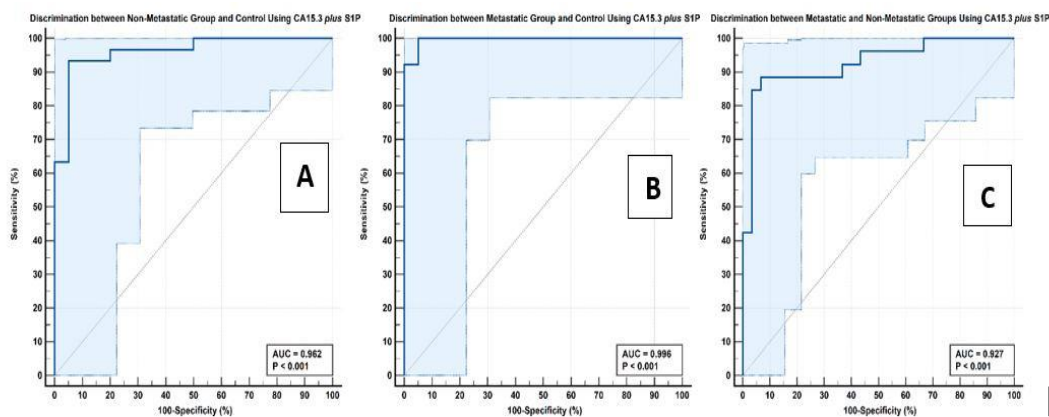


Fig. 7. combined ROC of CA15.3 and S1P for discrimination between A) Non-Metastatic and Control, B) Metastatic BC and Control, C) Non-Metastatic BC and Metastatic BC

A) Combined ROC showing excellent diagnostic value (AUC=0.962, 95%CI [0.865-0.996], $P < 0.0001$, cutoff > 0.619, sensitivity= 93.3% and specificity= 95.0%), (B) Combined ROC showing excellent diagnostic value (AUC=0.966, 95%CI [0.916-1.000], $P < 0.001$, cutoff > 0.123, sensitivity= 100% and specificity= 95.0%), (C) Combined ROC showing excellent prognostic value (AUC=0.927, 95%CI [0.825-0.979], $P < 0.001$, cutoff > 0.429, sensitivity= 88.5% and specificity= 93.3%).

3.8.2. The diagnostic and prognostic value of combined CA15.3 and NLRP3 serum levels in the studied groups

Combined ROC analysis of serum CA15.3 and NLRP3 showed excellent diagnostic value in discriminating non-metastatic BC from control (AUC= 0.988, $P < 0.001$), excellent diagnostic value in discriminating metastatic BC from control (AUC= 0.973, $P < 0.001$) and good prognostic value in discriminating non-metastatic BC from metastatic BC (AUC= 0.774, $P < 0.001$) as shown in **Fig. 8**.

3.8.3. The diagnostic and prognostic value of combined NLRP3 and S1P serum levels in the studied groups

Combined ROC analysis of serum NLRP3 and S1P showed very good diagnostic value in discriminating non-metastatic BC from control (AUC= 0.847, $P < 0.001$), excellent diagnostic value in discriminating metastatic BC from control (AUC= 0.985, $P < 0.001$) and excellent prognostic value in discriminating non-metastatic BC from metastatic BC (AUC= 0.929, $P < 0.001$) as shown in **Fig. 9**.

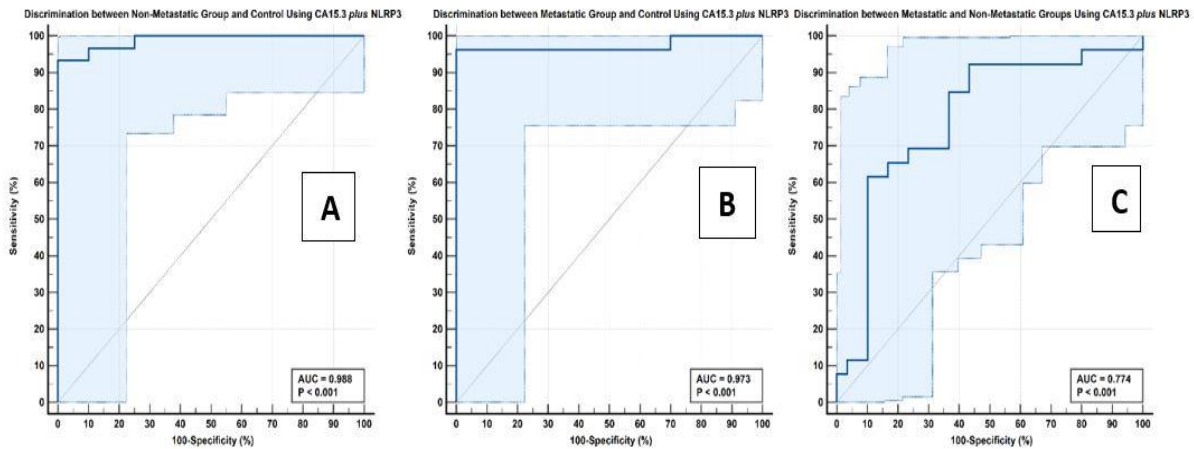


Fig. 8. combined ROC of CA15.3 and NLRP3 for discrimination between A) Non-Metastatic and Control, B) Metastatic BC and Control, C) Non-Metastatic BC and Metastatic BC.

A) Combined ROC showing excellent diagnostic value (AUC=0.988, 95%CI [0.905-1.000], $P < 0.001$, cutoff > 0.710 , sensitivity= 93.3% and specificity= 100%), (B) Combined ROC showing excellent diagnostic value (AUC=0.973, 95%CI [0.877-0.999], $P < 0.001$, cutoff > 0.564 , sensitivity= 96.2% and specificity= 100%), (C) Combined ROC showing good prognostic value (AUC=0.774, 95%CI [0.643-0.875], $P < 0.001$, cutoff > 0.557 , sensitivity= 61.5% and specificity= 90.0%).

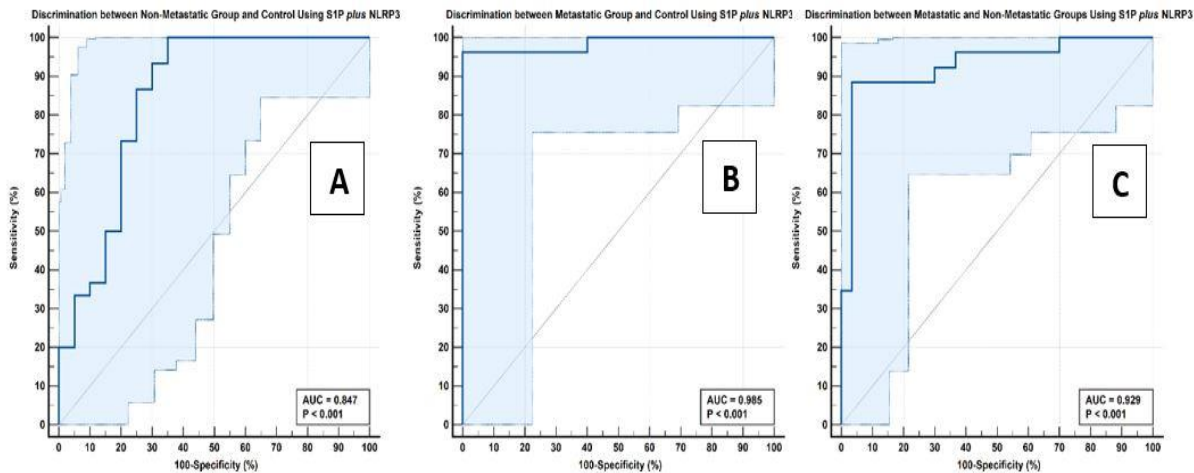


Fig. 9. combined ROC of S1P and NLRP3 for discrimination between A) Non-Metastatic and Control, B) Metastatic BC and Control, C) Non-Metastatic BC and Metastatic BC

A) Combined ROC showing very good diagnostic value (AUC=0.847, 95%CI [0.717-0.933], $P < 0.001$, cutoff > 0.264 , sensitivity= 100% and specificity= 65.0%), (B) Combined ROC showing excellent diagnostic value (AUC=0.985, 95%CI [0.895-1.000], $P < 0.001$, cutoff > 0.613 , sensitivity= 96.2% and specificity= 100%), (C) Combined ROC showing excellent prognostic value (AUC=0.929, 95%CI [0.828-0.981], $P < 0.001$, cutoff > 0.547 , sensitivity= 88.5% and specificity= 96.7%).

4. Discussion

Female BC has now become the most commonly diagnosed cancer worldwide, with

2.26 million new cases estimated in 2020 [19]. That has made the urge to find efficient and rapid ways to diagnose and prognose BC higher than

any time before; to help improve women's health and life [6]. The bioactive pleiotropic sphingolipid metabolite S1P, which is enriched in both blood and lymphatic fluid is involved in both inflammation and carcinogenesis through induction of NLRP3-dependent activation of caspase-1 and secretion of IL-1 β [20]. The NLR family is associated with several human diseases, including cancer, infectious, inflammatory, and autoimmune disorders. Which NLRP3 inflammasome is critical for host immune defenses against bacterial, fungal, and viral infections and also has been linked to the pathogenesis of several inflammatory disorders when dysregulated. The expression of NLRP3 inflammasomes in BC is relatively unknown, while some inflammatory reactions could exert a dual effect on tumor growth and progression [23]. In this context, this study was designed to evaluate the benefits of NLRP3 and S1P serum levels as biomarkers to diagnose and improve detection sensitivity in BC by their combination with the established tumor marker CA15.3, as well as to predict BC metastasis risk in Egyptian female BC patients.

Interestingly, our study showed a significant decrease in serum NLRP3 levels in both the metastatic and non-metastatic groups compared to the control group. This follows the findings of Lasithiotaki *et al.* in 2018, who reported that local lung cancer alveolar macrophages (LCAM) were unable to activate the NLRP3 inflammasome [24]. This could be attributed to low levels of tumor necrosis factor (TNF) produced, which is critical for NLRP3 activation, as TNF- activates its receptor which activates Natural factor- (NF- κ b) then activate proIL-1 β and proIL-18 into IL-1 β and IL-18 which cause inflammation and pyroptosis [14]. Also, in metastatic states cancer cells spread to other organs and sites in the body which could lead to

decreased levels of NLRP3 as in hepatocellular carcinoma (HCC) and CRC [14].

Our findings also confirm the findings of Dupaul-Chicoine *et al.* in 2015, who found that NLRP3 inflammasome-mediated IL-18 production suppresses CRC metastatic growth in the liver [25]. However, our study failed to find any significant difference in NLRP3 levels between metastatic and non-metastatic BC groups.

Another interesting finding of our study is that ROC analysis showed that NLRP3 has a very good diagnostic value regarding discrimination between control and non-metastatic BC patients (AUC 0.8483), as well as control and metastatic BC patients (AUC= 0.8769), so it could be regarded as a promising diagnostic tumor biomarker. However, NLRP3 showed poor prognostic value regarding discrimination between non-metastatic BC and metastatic BC (AUC= 0.6154).

Interestingly, our results showed no significant difference in the serum level of S1P between the non-metastatic BC and control groups. This result is consistent with previous studies that showed the S1P serum level might show slight elevation or no change in BC patients in the first stages [26].

However, our results showed a significant decrease in serum S1P levels in the metastatic BC group than in both the control and the non-metastatic BC groups. This follows the findings of Uranbileg *et al.* in 2016, who found that the tumor promotor, S1P was decreased in the serum of HCC patients. This phenomenon can be easily understood since it was reported that S1P levels in HCC tissues are reduced due to the increased SK and S1P lyase (SPL) activity in HCC tissues resulting in a down-regulation in S1P from tissue and serum since this sphingolipid could be

secreted from cancer cells [27] In another finding, reduced S1P levels has been reported in metastatic pancreatic cancer compared with normal tissues [28].

Another important finding of our study, ROC analysis showed that S1P has an excellent prognostic value in the differentiation between metastatic and non-metastatic BC groups value (AUC= 0.9269) indicating that S1P could be a promising prognostic marker for late-stage metastatic BC as well as an excellent diagnostic value regarding discrimination between control and metastatic BC patients (AUC= 0.9596) suggesting that S1P could be a promising diagnostic marker for late-stage metastatic BC, However, S1P showed poor diagnostic value regarding discrimination between control and non-metastatic BC patients (AUC= 0.6133).

Taking our promising results to the next level, we performed combined ROC analysis based on multivariable binary logistic regression models using various combinations of CA15.3, S1P, and NLRP. Interestingly, the diagnostic and prognostic value of the studied biomarkers improved by combining every two markers where combined CA15.3 with S1P showed excellent diagnostic value in discriminating non-metastatic BC and metastatic BC from control (AUC= 0.962 and AUC= 0.966, respectively) and excellent prognostic value in discriminating non-metastatic BC from metastatic BC (AUC= 0.927) and combined CA15.3 with NLRP3 showed excellent diagnostic value in discriminating non-metastatic BC and metastatic BC from control (AUC= 0.988 and AUC= 0.973, respectively) and good prognostic value in discriminating non-metastatic BC from metastatic BC (AUC= 0.774). Moreover, combined NLRP3 with S1P showed very good diagnostic value in discriminating non-metastatic BC from control (AUC= 0.847), excellent diagnostic value in discriminating metastatic BC from control (AUC= 0.985), and

excellent prognostic value in discriminating non-metastatic BC from metastatic BC (AUC= 0.929). These results enlighten the road to earlier detection of BC and better prognosis of patients which are two crucial goals towards better treatment outcomes and longer life expectancy in BC which are the paramount goals of cancer research worldwide.

Summary and Conclusion

In conclusion, our study highlights the role of NLRP3 and S1P in the development and progression of BC through the down-regulation of these crucial proteins, as well as their potential as novel promising, sensitive, and specific tumor diagnostic and prognostic biomarkers either alone or in combination with the well-established tumor biomarker CA15.3.

Recommendations

Further investigation of serum NLRP3 and S1P on a larger cohort of patients to further ascertain their potential use as novel promising diagnostic and prognostic tumor biomarkers for BC.

Declarations

Ethical approval

This study was approved by the Ethical Committee of Research, Faculty of Pharmacy, Ain Shams University, approval number (244), and by the Ethical Committee of National Cancer Institute, Cairo University. Additionally, the study was carried out following the regulations and recommendations of the Declaration of Helsinki. Written Informed consent was obtained from every patient.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

No potential competing interests are to be reported.

Authors' contributions

AS, setting the hypothesis, sample analysis, statistical analysis, and writing the manuscript. AK, setting the hypothesis, sample analysis supervision, statistics, and manuscript revision. RE, sample collection, manuscript revision. HE, setting the hypothesis, statistics, and manuscript revision

Consent for publication

All authors have read, approved, and agreed on the publication of the final manuscript

Availability of data and material

All data and materials are available on request

Acknowledgments

The authors would like to acknowledge all the patients who participated in the study as well as all colleagues in the Biochemistry Department, Faculty of Pharmacy, Ain Shams University for their support.

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