The Value of Serum Nestin in Monitoring the Effects of Surgery and Chemotherapy in Female Breast Cancer Patients: A Comparison with Serum CA15.3

Taha I. Hewala♦, PhD, Mohamed S. Kamel**, PhD, Yasmin N. Elwany***, PhD, Noha A. Zekry*, MSc**

Department of Radiation Sciences, Medical Research Institute, Alexandria University, Alexandria, Egypt
**Department of Experimental and Clinical Surgery, Medical Research Institute, Alexandria University, Alexandria, Egypt*
***Department of Cancer Management and Research, Medical Research Institute, Alexandria University, Alexandria, Egypt*

Abstract

Background: Traditional tumor markers such as cancer antigen 15.3 (CA15.3) and carcinoembryonic antigen (CEA) exhibit limited clinical utility in breast cancer due to their lack of sensitivity and specificity, particularly for detecting low-volume tumors. Other serum markers, such as nestin, may offer more promise. This study aimed to assess the clinical significance of serum nestin and CA15.3 in breast cancer patients.

Method: This case-control study enrolled 80 normal control females and 80 females with breast cancer. Serum samples were collected from both control and breast cancer groups. The serum nestin and CA15.3 levels were measured in all samples using enzyme-linked immunosorbent assay (ELISA) kits.

Results: The serum levels of nestin and CA15.3 were found to be significantly elevated in the breast cancer patient group compared with the control group. Preoperative serum nestin levels exceeding 9.9 ng/ml demonstrated a substantial odds ratio of 27 (confidence interval: 4.57-159.67; $P = 0.0003$). In receiver operating characteristic curve analysis, serum nestin exhibited the highest significant area under the curve at 85.2% ($P < 0.001$), followed by serum CA15.3 at 70% ($P = 0.021$). Post-surgery serum nestin levels significantly decreased compared with pre-surgery levels ($P = 0.045$).

Conclusion: Serum nestin outperforms serum CA15.3 in diagnosing breast cancer patients. Elevated serum nestin levels may represent a significant risk factor for the development of breast cancer. Furthermore, serum nestin can monitor the effects of surgery, whereas none of the assessed biomarkers exhibit a significant role in monitoring the effects of chemotherapy on breast cancer patients.

Keywords: Chemotherapy, Adjuvant, Breast neoplasms, Diagnosis, Nestin

♦**Corresponding Author:**
Taha I. Hewala, PhD
Department of Radiation Sciences, Medical Research Institute, Alexandria University, Alexandria, Egypt
Tel: +201273199504
Email: tahahewala@hotmail.com

Introduction

Worldwide, breast cancer is the most common malignancy and the second most frequent reason for death in women.[1] Breast cancer accounts for 24% of all cancers occurring in women.[2]

Cancer antigen 15.3 (CA15.3) is a glycoprotein belonging to a family of proteins called mucins (MUC). Mucins are large glycoproteins classified into 7 families (MUC 1 - MUC7) based on their genetic and biomolecular properties. MUC-1 is found in nearly all epithelial cells, with its overexpression in colon, breast, ovarian, lung, and pancreatic cancers.[3]

The main disadvantage of CA15.3 is its lack of sensitivity and specificity for low-volume tumors. So, it is of no value in either screening or diagnosing early breast cancer. Other markers for breast cancer, like nestin, may look promising, but further studies are required to be carried out before their clinical utility becomes well established.[4,5]

Nestin is one of the intermediate filament proteins in class VI. It was initially detected in neural stem cells during development. Its expression has also been found in different tissues with various pathological conditions. Many cancers express nestin protein, which correlates with the clinical course of breast cancer.[6]

Under pathological conditions, nestin is expressed upon wounding and tissue injury when cells undergo hyper-proliferation.[7] Nestin overexpression has been reported in various tumor cells, including breast cancer.[8] Nestin is also expressed in cancer stem cells, which promote cancer resistance.[9]

The current study aimed to compare the roles of serum levels of nestin and CA15-3 in breast cancer patients concerning the differentiation of breast cancer patients from controls, monitoring the effects of surgery and chemotherapy on breast cancer patients, and correlating the serum levels of each biomarker with the clinicopathological data of breast cancer patients.

For the time being, this is the first study that was performed to measure the serum levels of nestin to figure out the clinical role that may be played by this cytoskeleton protein in female breast cancer patients who were treated by surgery followed by chemotherapy.

Subjects and Methods
Subjects

This case-control study involved 160 female participants segregated into two distinct groups. group I consisted of 80 healthy control females, while group II comprised 80 recently diagnosed breast cancer patients in clinical stages II and III. The individuals in group II were carefully matched with those in group I regarding age and menstrual status. The inclusion criteria for the patients in
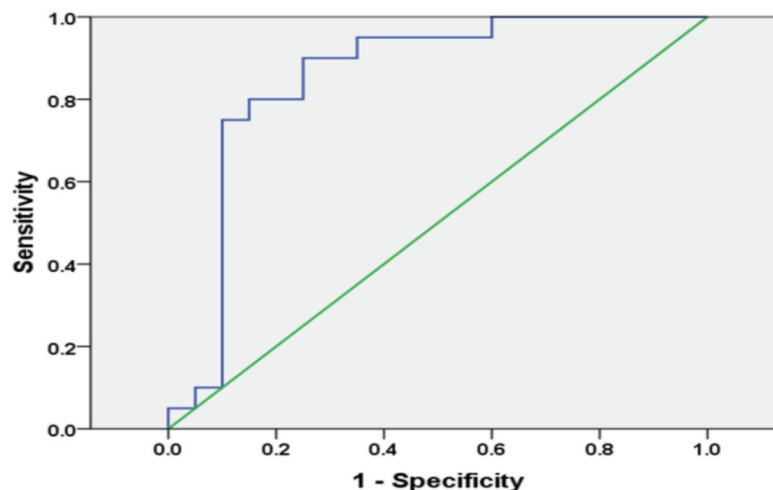
Figure 1. This figure shows the receiver operating characteristic curve for human serum nestin. The area under the curve was 85.2% (*P* < 0.001), with a sensitivity of 80% and specificity of 75% at a 9.90 ng/ml cut-off.

Table 1. The median (range) serum levels of nestin in the control group and the breast cancer patients group

Serum nestin levels (ng/ml)	Control group (n=80)	Breast cancer patients group (n=80)		
		Before surgery	After surgery (before chemotherapy)	After chemotherapy
Minimum	6.10	8.32	2.01	7.56
Maximum	13.68	15.06	12.69	11.06
Median	8.58	10.50	8.87	8.72
P- Values		P1 = 0.000*	P2 = 0.045*	P3 = 0.307

P1: Compared with control group; P2: Compared with the levels before surgery; P3: Compared with the levels after surgery (before chemotherapy); *: Statistically significant compared with the normal control group; Significance was considered at P-value < 0.05

group II were as follows: they had undergone breast conservative therapy, were node-positive, and exhibited a high-risk node-negative status.

Patients were selected among individuals admitted to the Experimental and Clinical Surgery Department and the Cancer Management and Research Department at Alexandria University, Egypt's Medical Research Institute (MRI). This data collection occurred from January 2021 to June 2022.

Methods

After approval from the ethical committee of the Medical Research Institute, Alexandria University, Egypt (ethics code: IORG0008812), signed informed consent was obtained from all subjects who agreed to participate in the current study. A complete history was recorded, and each patient underwent a thorough clinical examination, routine laboratory investigations, mammography of both breasts, radiological investigations including chest X-ray, ultrasonography of the abdomen and the liver, a computed tomography (CT) scan of the chest and abdomen, a bone scan when needed, and a needle biopsy of a breast mass to establish the pathological diagnosis of the patients.

The clinicopathologic data were obtained from patients' pathology reports. The collected data included tumor size, tumor pathological grade, axillary lymph node involvement, vascular invasion, estrogen receptor (ER) and progesterone receptor (PR) status, and human epidermal growth factor receptor 2 (HER-2) expression. For each patient, the clinical stage was determined by the oncologist according to the tumor-node-metastasis (TNM) classification system.[10]

All 80 breast cancer patients underwent modified radical mastectomy and then received adjuvant combination chemotherapy (5-fluorouracil, Adriamycin, and cyclophosphamide) for six cycles.[11] The patients were re-evaluated after three and six cycles of chemotherapy to estimate the clinical response.

Laboratory investigations

All laboratory assays were conducted at the Radiation Science Department, Medical Research Institute, Alexandria University, Egypt. Blood samples were collected once from the control group (group I) and three times from cancer patients (group II): prior to surgery, post-surgery (pre-chemotherapy), and post-chemotherapy. The blood samples were utilized to isolate sera for subsequent biochemical analyses. Serum levels of nestin and CA 15.3 were quantified in all study groups utilizing pre-packaged ELISA kits following the manufacturer's guidelines.

Determination of human serum nestin levels using ELISA kit

Assay procedure

The levels of human serum nestin were quantified employing a readily accessible ELISA kit, in strict accordance with the manufacturer's specified protocol (EIAAB-SCIENCE INC., China). Optical density at 450nm was assessed for each well using an ELISA reader. The concentration of human serum nestin within each serum sample was compared with a calibration curve.

Determination of human serum CA15-3 levels using an enzyme immunoassay kit

assay procedure

The levels of human serum CA15-3 were assessed by employing a commercially available EIA kit, specifically designed for immediate use, by the manufacturer's protocol (Bio-Inteco; UK). The optical density of each well was meticulously measured at 450 nm utilizing an ELISA reader.

A calibration curve accurately ascertained the concentration of human serum CA15.3 within each serum sample.

Histopathological examination

In group II, clinicopathological data were extracted from patients' data sheets. This dataset encompassed information about tumor size, tumor grade, ER status, PR status, HER-2 status, and the count of axillary lymph nodes involved. The attending oncologist assessed the clinical staging of each patient. These clinicopathological data were cross-referenced with pre-surgical serum levels of nestin and CA15-3.

Statistical analysis

Statistical analyses were conducted using the SPSS 21 software package. Quantitative data were characterized utilizing the median and range. The Mann-Whitney U-test was employed to examine disparities between the breast cancer patient group and the control group regarding serum levels of nestin and CA15.3. The Kruskal-Wallis test assessed differences in serum parameters before and after surgery and before and after chemotherapy. Spearman's test was employed to explore correlations between the examined serum biomarkers and breast cancer clinicopathological data. The diagnostic efficacy of various serum biomarkers was compared through receiver operating characteristic (ROC) curve analysis. Significance was established at a $P < 0.05$.

Results

### Age distribution among normal control females and females with breast cancer

The median (range) age of breast cancer patients was 47 (30-59) years, while controls had an age distribution of 48 (31-61) years. Statistical analysis of this dataset revealed a non-significant difference between breast cancer patients and controls regarding age ($P = 0.551$).

Menopausal status distribution among normal control females and breast cancer patients

Among breast cancer patients, 32 (40%) were premenopausal females, and 48 (60%) were postmenopausal females. Similarly, among the normal healthy controls, there were 24 (30%) premenopausal females and 56 (70%) postmenopausal females. Statistical analysis of these results revealed a non-significant difference between the control group and the breast cancer patients' group regarding menopausal status ($P = 0.507$).

Serum levels of nestin in the normal control group and breast cancer patient group

Table 1 displays the serum levels of nestin in both the standard control group and the breast cancer patient group. Within the control group, the median (range) serum levels of nestin were 8.58 (6.10-13.68 ng/ml). In the breast cancer patients group, the median (range) serum levels of nestin were 10.50 (8.32-15.06 ng/ml) before surgery, 8.87 (2.01-12.69 ng/ml) after surgery
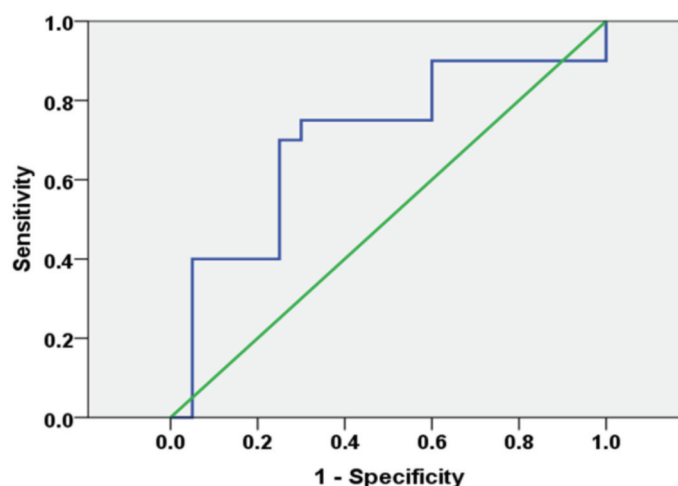
Figure 2. This figure shows the receiver operating characteristic curve for human serum CA15-3. The area under the curve was 70% ($P = 0.021$), with a sensitivity of 45% and specificity of 85% at a cut-off of 22.05 U/ml.

Table 2. Association of preoperative serum nestin levels with the risk of breast cancer incidence

Serum nestin (ng/ml)		Control group (n=80)	Breast cancer group (n=80)	Odds ratio	95% CI	*P*-value
Negative	≤ 9.9[®]	72	18	27.00	4.57 – 159.67	0.0003 *
Positive	> 9.9	8	62			

n: Sample size; [®]: Reference group; CI: Confidence interval; *: Significance was considered at *P*-value < 0.05

(before chemotherapy), and 8.72 (7.56-11.06 ng/ml) after chemotherapy. Statistical analysis revealed that the serum levels of nestin were significantly higher in the breast cancer patients' group compared with the standard control group (*P* < 0.001). There was also a significant decrease in nestin levels after surgery compared to before surgery (*P* = 0.045), with no significant differences before and after chemotherapy (*P* > 0.05).

Association of preoperative serum nestin levels with the risk of breast cancer incidence

Table 2 shows that 8/80 (10%) of controls exhibited elevated levels of serum nestin (> 9.9 ng/ml), while 72/80 (90%) of controls had decreased levels of serum nestin (≤ 9.9 ng/ml). In contrast, 62/80 (77%) of cases had elevated levels of preoperative serum nestin (> 9.9 ng/ml), while 18/80 (23%) had decreased levels of preoperative serum nestin (≤ 9.9 ng/ml). Statistical analysis revealed that females with preoperative nestin levels more significant than the cut-off value (9.9 ng/ml) had a significant odds ratio of 27 (95% confidence interval = 4.57-159.67; *P* = 0.0003) compared with those with preoperative nestin levels less than the cut-off value (9.9 ng/ml). This indicates that serum levels of nestin greater than 9.9 ng/ml may act as a risk factor for the development of breast carcinogenesis.

Serum levels of CA15.3 in the normal control group and breast cancer patient group

Table 3 displays the serum levels of CA15.3 in the standard control and breast cancer patient groups. In the control group, the median (range) serum levels of CA15.3 were 10.90 (9.11-20.30 U/ml). In the breast cancer patients group, the median (range) serum levels of CA15.3 were 19.2 (8-39 U/ml) before surgery, 14.67 (10-34.33 U/ml) after surgery (before chemotherapy), and 8.17 (2.67-36.33 U/ml) after chemotherapy. Statistical analysis revealed that the serum levels of CA15.3 were significantly higher in the breast

cancer patients' group compared with the standard control group (*P* = 0.021), with no significant differences before and after surgery or before and after chemotherapy (*P* > 0.05).

Comparing the diagnostic values of preoperative serum nestin and CA15.3 in breast cancer patients using ROC curve analysis

Figures 1 and 2 illustrate the results of the ROC curve analysis, presenting the area under the curve (AUC), cut-off values, sensitivity, and specificity for comparing serum nestin and CA15-3 as diagnostic markers in breast cancer patients. The assessment of diagnostic performance relies on the AUC, where a higher AUC indicates a superior diagnostic test. Serum nestin exhibited a noteworthy AUC of 85.2% (*P* < 0.001), with a sensitivity of 80% and specificity of 75% at a 9.90 ng/ml cut-off value. Conversely, serum CA 15.3 demonstrated a significant AUC of 70% (*P* = 0.021), with a sensitivity of 45% and specificity of 85% at a cut-off value of 22.05 U/ml.

Correlation between the studied serum biomarkers and clinicopathological data in breast cancer patients prior to surgery

Based on the data derived from our current study, it is evident that none of the investigated serum biomarkers display a significant correlation with any of the clinicopathological parameters associated with breast cancer (*P* > 0.05).

Discussion

In the current study, nestin and CA15.3 serum levels were significantly higher in the breast cancer patient group than in the control group. Serum nestin is superior to serum CA15.3 in diagnosing breast cancer patients. Increased serum levels of nestin may be a significant risk factor for breast cancer development. Serum nestin was able to monitor the effects of surgery. None of the assayed biomarkers has a significant role in monitoring the effect of chemotherapy on breast

Table 3. The median (range) serum levels of CA15-3 in the control group and the breast cancer patients group

Serum CA15.3 levels (U/ml)	Control group (n=80)	Breast cancer patients group (n=80)		
		Before surgery	After surgery (before chemotherapy)	After chemotherapy
Minimum	9.11	8	10	2.67
Maximum	20.30	39	34.33	36.33
Median	10.90	19.2	14.67	8.17
P- Values		*P*1 = 0.021*	*P*2 = 0.073	*P*3 = 0.055

*P*1: Compared with the control group; *P*2: Compared with the levels before surgery; *P*3: Compared with the levels after surgery (before chemotherapy). Significance was considered at a *P*-value < 0.05. *: Statistically significant compared with the standard control group

cancer patients.

Regarding the diagnostic role of serum levels of nestin, the statistical analysis of the current study showed that serum levels of nestin were significantly higher in the breast cancer patients group compared with the standard control group ($P < 0.001$). These results indicate that nestin may have a role in the development of breast cancer. To our knowledge, most studies on nestin in breast cancer analyzed its tissue expression immunohistochemically. Several studies reported significantly increased nestin expression in breast cancer tissues compared with normal breast tissues.[12]

The results of the current study confirmed those found by Sal et al.,[13] who reported that the preoperative serum nestin levels were significantly higher in patients with malignant ovarian tumors compared with patients with benign ovarian tumors. However, the results of the current study disagreed with the results obtained by Aglan et al.,[14] who found that patients with breast cancer had significantly lower levels of serum nestin compared with normal control subjects. It was reported that nestin has the potential to be used as a biomarker in daily clinical practice. Nestin may be a promising prognostic biomarker and a possible therapeutic target for tumor suppression in breast cancer.[8]

In the current study, the preoperative serum levels of nestin were found to be significantly higher in breast cancer patients compared with controls. At the same time, the results of the current study showed that the increased peroperative serum levels of nestin (> 9.9 ng/ml) might increase the risk of females getting breast carcinoma by a factor of 27 compared with females having lower concentrations of serum nestin (≤ 9.9 ng/ml). Because increased serum levels of nestin may increase the risk of breast cancer incidence, this result is strongly compatible with the increased serum levels of nestin in breast cancer patients compared with normal control subjects, which were the results that were found in the current study. To the best of our knowledge, the current study is the first to try to assess the role of serum nestin as a risk factor for the development of breast cancer. A correlation was found between nestin and aggressive growth, angiogenesis, metastasis, and poor prognosis in some tumors, but the roles of nestin in cancer cells have not been characterized.[15]

Regarding the correlation of preoperative serum levels of nestin with breast cancer clinicopathological data, the results of the current study showed a non-significant correlation between serum nestin and any of these clinicopathological data ($P > 0.05$). To the best of our knowledge, the current study is the first to try to assess the correlation of serum nestin with breast cancer clinicopathological data. On the other hand, the current results confirmed those reported by Sal et al.,[13] who found that preoperative serum levels of nestin were non-significantly correlated with the clinicopathological features of ovarian carcinoma.

Regarding the role of serum nestin levels in monitoring the effect of surgery on breast cancer, the current study showed that the serum levels of nestin after surgery were significantly decreased compared with their levels before surgery ($P = 0.045$). To the best of our knowledge, the current study is the first one to try to investigate the role of serum nestin in monitoring the effect of surgery on breast cancer patients.

Regarding the role of serum nestin levels in monitoring the effects of chemotherapy on breast

cancer, the current study showed that serum levels of nestin after chemotherapy were non-significantly decreased compared with their levels before chemotherapy ($P = 0.307$). To the best of our knowledge, the current study is the first to try to investigate the role of serum nestin in monitoring the effects of chemotherapy on breast cancer patients.

Regarding the diagnostic value of serum levels of CA15.3 in the differentiation between controls and breast cancer patients, the statistical analysis of the results of the current study showed that serum levels of CA15.3 were significantly higher in the breast cancer patient group compared with the standard control group ($P = 0.021$). This means that CA15.3 may have a role in the development of breast cancer. These results were in line with those reported by Mohammed et al.,[16] Moazzezy et al.,[17] Hewala et al.,[18] and Fejzič et al.,[19] who found that the serological levels of CA15.3 in breast cancer patients were significantly higher than the serum levels of normal controls.

Regarding the correlation of serum CA15.3 with breast cancer clinicopathological data, the current study's results showed no significant correlation between serum CA15.3 and breast cancer clinicopathological data.

Regarding the role of serum CA15.3 levels in monitoring the effect of surgery on breast cancer patients, the current study showed that serum levels of CA15.3 after surgery were non-significantly decreased compared with their serum levels before surgery ($P = 0.073$). This non-significant decrease in serum CA15.3 levels after surgery may be due to the longer half-life of CA15.3, as previously found by Ali et al.[20] In their study, they reported that since the half-life of CA15.3 is unknown, it remains controversial how to define the optimal interval for tumor marker follow-up.[20]

Regarding the role of serum CA15.3 levels in monitoring the effect of chemotherapy on breast cancer, the current study showed that serum levels of CA15.3 after chemotherapy were non-significantly decreased compared with their levels before chemotherapy ($P = 0.055$). This means that serum CA15.3 has no role in monitoring the

response of breast cancer patients to chemotherapy. Although Yang et al.[21] reported that serum CA15.3 can play a role in monitoring therapy, the contradictory results obtained in the current study may be due to the small sample size included in the current study.

Regarding the comparison of the diagnostic values of serum nestin and CA15.3 in breast cancer using the ROC curve analysis, serum nestin showed a significant AUC (85.2%, $P < 0.001$) with sensitivity (80%) and specificity (75%) at a cut-off 9.90 ng/ml. Serum CA15.3 showed a significant AUC (70%, $P = 0.021$) with sensitivity (45%) and specificity (85%) at a cut-off of 22.05 U/ml. This means that serum nestin is more potent than serum CA15.3 in determining the difference between controls and breast cancer patients.

Aglan et al.[14] applied the ROC curve analysis to estimate the diagnostic value of serum nestin to differentiate breast cancer patients from controls. At a specific cut-off value of 39.9 pg/ml for serum nestin, they found that its diagnostic sensitivity was 84.8% and specificity was 65.1%, with a significant AUC of 81.2% ($P < 0.001$). These results are more or less compatible with the results of the current study.

No published study compared the diagnostic powers of serum nestin and CA15.3 in differentiating breast cancer patients from controls using ROC curve analysis. So, the current study may be the first designed to carry out this aim. The current study reported a significant role for nestin as a risk factor for the development of breast carcinoma. Nestin was also found to have a role in monitoring the effect of surgery on breast cancer. However, due to its relatively small sample size, the results of the current study regarding the roles of serum nestin and CA15.3 in monitoring the effect of chemotherapy need to be verified by performing further investigations, including a larger sample size of cases and controls.

Conclusion

The findings from the present study suggest that serum nestin may play a pivotal role in the pathogenesis of breast cancer and could serve as

a valuable diagnostic tool for identifying breast cancer patients. Serum nestin exhibits superior diagnostic efficacy in discriminating breast cancer patients from control subjects compared to serum CA15.3. Elevated serum nestin levels (> 9.9 ng/ml) are associated with a 27-fold increased risk of developing breast carcinoma in females, in contrast to individuals with lower serum nestin concentrations (9.9 ng/ml or below). Furthermore, serum nestin demonstrates utility in monitoring surgical outcomes, effectively distinguishing between breast cancer patients before and after surgery ($P = 0.045$). However, it is noteworthy that serum nestin does not exhibit a significant role in assessing the effects of chemotherapy in breast cancer patients. No statistically significant correlations were observed between preoperative serum nestin levels and various clinicopathological parameters associated with breast cancer.

On the other hand, CA15.3 also appears to be implicated in the onset of breast cancer and can be employed as a diagnostic marker for identifying breast cancer patients. Nonetheless, serum CA15.3 demonstrates comparatively lower diagnostic accuracy in distinguishing breast cancer patients from healthy controls when contrasted with serum nestin. Similarly, serum CA15.3 does not play a significant role in evaluating the impact of surgery or chemotherapy on breast cancer patients. No statistically significant associations were identified between preoperative serum CA15.3 levels and breast cancer clinicopathological parameters.

It is imperative to note that the current study's findings necessitate validation through subsequent investigations, which should ideally encompass a larger cohort of breast cancer patients and an appropriate control group for comprehensive assessment and confirmation of these observations.

Conflict of Interest

None declared.

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