

Elevated Serum Nidogen-2 and Dysregulated Physiological Markers in Iraqi Women with Breast Cancer: A Case-Control Study with In Silico Validation

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Abstract

Background: Breast cancer is one of the major health concerns worldwide, which needs biomarkers for proper diagnosis. Serum *Nidogen-2* (NID2), along with other physiological and inflammatory biomarkers, has been proposed as a non-invasive method for breast cancer diagnosis. The current study was conducted to assess the diagnostic value of serum *Nidogen-2* and physiological parameters in Iraqi breast cancer patients. **Materials and Methods:** This is a case-control study done in Ramadi, Iraq, where 60 women were divided into two groups: 30 women suffering from breast cancer and 30 control women. This study focused on serum *Nidogen-2* (NID2), Estrogen, C-reactive protein (CRP), kidney function markers (urea, uric acid, and potassium), and anthropometric measurements. The Receiver Operating Characteristic (ROC) curve test was carried out to determine their diagnostic efficacy. Furthermore, NID2 mRNA expression data in breast tumors versus breast normal tissues were determined using the TCGA BRCA dataset. **Results:** Significant differences were discovered in serum levels of *Nidogen-2*, as breast cancer patients had increased serum levels (154.17 ± 25.42 pg/ml) compared to the healthy controls (108.85 ± 20.10 pg/ml, $p < 0.0001$). Estrogen levels were also significantly decreased in breast cancer patients (48.34 ± 11.62 pg/ml) compared to healthy controls (99.83 ± 11.59 pg/ml, $p < 0.0001$). Other markers of renal function and inflammation, Urea, Uric acid, and Potassium (K⁺), were also significantly higher in breast cancer patients compared to healthy controls. Excellent diagnostic performance for breast cancer was determined by Receiver Operating Characteristic (ROC) curve analysis for Estrogen (AUC = 1.000), *Nidogen-2* (AUC = 0.938), and CRP (AUC=0.963). In silico validation for NID2 mRNA was completed using the Cancer Genome Atlas (TCGA) Breast Cancer (BRCA) dataset, where NID2 gene expression was $\uparrow$ in breast cancer tissue compared to normal breast tissue ($p=0.00012$), demonstrating breast cancer identified by the use of *Nidogen-2*, Estrogen, and CRP. **Conclusion:** Serum *Nidogen-2*, Estrogen, and CRP demonstrate high diagnostic capacity for breast cancer in Iraqi women, backed by bioinformatic validation of NID2 tissue expression, suggesting their efficacy as valuable diagnostic biomarkers.

Keywords: *Nidogen-2*, Breast cancer diagnosis, Estrogen dysregulation, TCGA-based in silico validation

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Introduction

The high mortality rate of breast cancer in women has resulted in a heightened effort to identify novel diagnostic and therapeutic biomarkers to facilitate the treatment of this disease. The tumor microenvironment (TME), and the extracellular matrix (ECM) in particular, are critical components of the mechanism of cancer progression, metastasis, and haphazard growth of cancer. The ECM is a complex network of proteins and proteoglycans that

undergoes extensive remodelling during the course of tumorigenesis [1-5].

Nidogens (NIDs) are important components of basement membranes (specialised types of ECM) and help to maintain the integrity of basement membranes. *Nidogen-2* (NID2) is an important component of many cancers, and this indicates that it may be useful as both a diagnostic and prognostic biomarker for breast cancer.

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Alterations in the expression of *NID2* have been observed in many tumours; however, the precise roles *NID2* plays in breast cancer have yet to be delineated. The ECM influences systemic physiology in cancer through uncharacterized mechanisms [6-8].

C-Reactive Protein (CRP) measures systemic inflammation, a recognized component of cancer. High CRP levels correlate with worse outcomes in breast and other cancers, but the marker's prognostic value is inconsistent. Altered estrogen levels drive the development of many breast cancers. The estrogen receptor status of a tumor is a critical determinant of treatment response.

Cancer and its treatments alter renal function and electrolyte balance. Impaired kidney function can affect the metabolism and clearance of therapeutic agents and may also be a consequence of paraneoplastic syndromes. Managing breast cancer patients requires evaluating renal and electrolyte markers.

We measured serum *Nidogen-2*, hormonal, inflammatory, and renal markers in Iraqi women with breast cancer. We will test the diagnostic utility of these biomarker profiles by comparing them against healthy controls and correlating them with clinical parameters. We will confirm our findings by analyzing tissue expression in The Cancer Genome Atlas (TCGA) data.

Materials and Methods

Sample Collection

We compared 30 women with breast cancer to 30 healthy controls from Ramadi, Iraq. We measured demographic variables (Age, Body Mass Index) and serum analytes. These included *Nidogen-2* (pg/ml), Estrogen (pg/ml), C-Reactive Protein (mg/L), renal markers (Urea, Creatinine, Uric Acid in mg/dl), and electrolytes (Potassium, Sodium in mEq/L).

Statistical Analysis

We reshaped the raw data into a long format for group comparisons using Python's Pandas library. The Shapiro-Wilk test was used to assess the normality of each

variable. For normally distributed data (Age, K⁺, Na⁺), Welch's t-test was employed for statistical comparison. We used the Mann-Whitney U test for non-normally distributed variables such as *Nidogen-2*, Estrogen, and CRP. The Spearman Rank Correlation was used to assess monotonic relationships between variables. We calculated the Area Under the Curve (AUC) from Receiver Operating Characteristic (ROC) curves to determine the diagnostic accuracy of each biomarker. It should be noted that corrections for multiple comparisons, such as the False Discovery Rate (FDR) or Bonferroni correction, were not applied to the statistical tests. We generated box-and-whisker plots, bivariate regression plots, and heatmaps using Seaborn and Matplotlib.

Expression Validation in TCGA Dataset

We validated our serum findings by analyzing The Cancer Genome Atlas Breast Cancer (TCGA-BRCA) dataset, which included 1,102 primary tumor samples and approximately 100 normal adjacent tissues, using the UCSC Xena platform. We analyzed the differential expression of *NID2* mRNA in primary tumor versus normal mammary tissue. Prognostic value was assessed using Kaplan-Meier analysis for Overall Survival (OS) and Progression-Free Interval (PFI), stratifying patients into high and low *NID2* expression groups based on the median. We used Python 3.11 for analysis and R 4.4.2 for bioinformatics validation.

Results

Demographic and Anthropometric Characteristics

Age and Body Mass Index (BMI) did not differ between study groups. The mean age for the breast cancer patient group was 46.33 ± 9.65 years, while the control group had a mean age of 47.60 ± 9.87 years; this difference was not statistically significant ($p=0.6171$), as determined by a Welch's t-test. Patient and control groups did not differ in BMI (27.86 ± 3.01 vs 26.92 ± 3.45 , $p=0.2700$, Mann-Whitney U test, Figure 1).

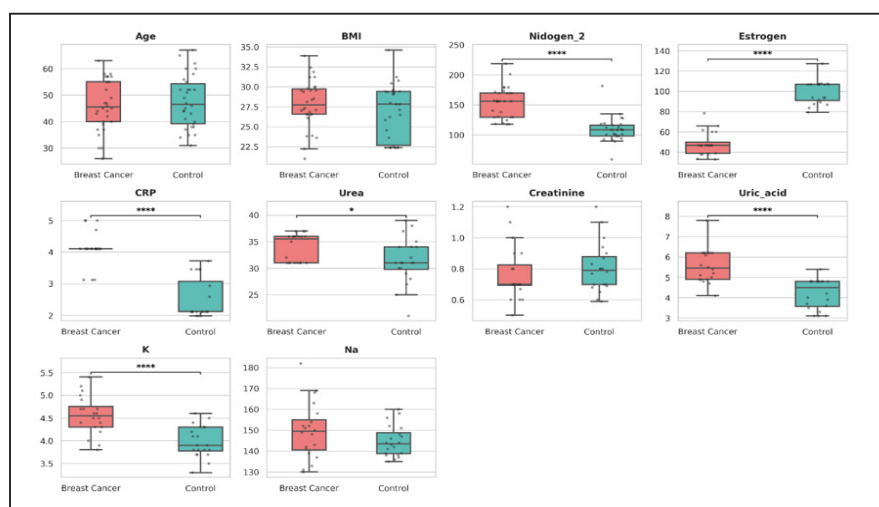


Figure 1. Comparative Distribution of Serum Biomarkers between Breast Cancer and Control Groups. Asterisks denote the level of significance ($p < 0.05$, **** $p < 0.0001$).*

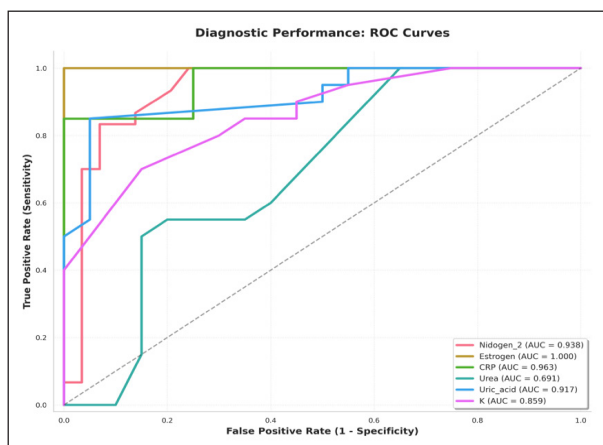


Figure 2. Statistically Significant Biomarkers Demonstrated Predictive Value in Receiver Operating Characteristic (ROC) analysis. *Nidogen-2*, Estrogen, CRP, Urea, Uric acid, and Potassium levels distinguished breast cancer patients from healthy controls.

Comparative Analysis of Serum Biomarkers

Concentrations of key serum biomarkers differed between the breast cancer and control groups (Figure 1). Serum levels of *Nidogen-2* were significantly elevated in breast cancer patients, with a mean concentration of 154.17 ± 25.42 pg/ml, compared to 108.85 ± 20.10 pg/ml in the healthy control group ($p < 0.0001$). Patients had lower serum Estrogen (48.34 ± 11.62 pg/ml) than controls (99.83 ± 11.59 pg/ml, $p < 0.0001$). The inflammatory marker, C-Reactive Protein (CRP), was also significantly higher in the breast cancer cohort, with a mean of 4.12 ± 0.55 mg/L, versus 2.51 ± 0.63 mg/L in the control group ($p < 0.0001$).

Markers associated with renal function were significantly dysregulated in the patient group. Serum Urea was elevated in patients (33.90 ± 2.63 mg/dl) compared to controls (31.40 ± 4.33 mg/dl, $p=0.0358$). A more pronounced and highly significant elevation was seen in Uric acid levels, with 5.56 ± 0.83 mg/dl in patients versus 4.21 ± 0.74 mg/dl in controls ($p < 0.0001$). However, Creatinine levels did not differ significantly between the groups (0.76 ± 0.18 mg/dl vs. 0.80 ± 0.16 mg/dl, $p=0.4036$). Among the measured electrolytes, serum Potassium (K⁺) was significantly higher in the patient group (4.56 ± 0.42 mEq/L) compared to the control group (3.98 ± 0.35 mEq/L, $p < 0.0001$). Sodium (Na⁺) levels

showed no significant difference (149.60 ± 13.58 mEq/L vs. 144.70 ± 7.46 mEq/L, $p=0.1677$).

Diagnostic Performance of Biomarkers

To assess the potential of the significantly altered biomarkers for diagnosing breast cancer, a ROC curve analysis was performed (Figure 2). Estrogen demonstrated perfect diagnostic accuracy with an AUC of 1.000. *Nidogen-2* and CRP also showed excellent diagnostic potential with high AUC values of 0.938 and 0.963, respectively. Uric acid and Potassium also proved to be strong diagnostic markers, with AUCs of 0.917 and 0.859, respectively. Urea yielded a diagnostic AUC of 0.691.

Correlation Analysis

We correlated variables within each group using Spearman's method. In the breast cancer patient group, several significant correlations were identified. Sodium (Na⁺) correlated positively with Potassium (K⁺) ($R=0.56$, $p=0.0095$). Significant negative correlations were found between Estrogen and Creatinine ($R=-0.54$, $p=0.0140$), and between *Nidogen-2* and Potassium (K⁺) ($R=-0.48$, $p=0.0306$). Figure 3 shows these correlations.

The correlation matrices in Figure 4-5 indicate systemic physiological disruption in the breast cancer cohort, a pattern absent from the homeostatic control group. Furthermore, no significant correlations were observed between the key biomarkers (*Nidogen-2*, Estrogen, CRP, Urea, Uric acid, K⁺) and the demographic variables of Age or BMI in either the patient or the control group, as shown in Figure 6 and Figure 7. Patient characteristics do not alter the diagnostic utility of these biomarkers.

In Silico Validation of NID2 Expression and Prognostic Value

NID2 mRNA expression from The Cancer Genome Atlas Breast Cancer (TCGA-BRCA) dataset validated our clinical findings. Primary breast tumor tissue ($n=1102$) expressed significantly more *NID2* mRNA (mean=9.66) than normal adjacent tissue ($n \sim 100$, mean=9.26, $p=0.00012$, Figure 8).

We tested the prognostic value of *NID2* expression in the TCGA-BRCA cohort with a Kaplan-Meier analysis. Patients were stratified into high and low *NID2* expression

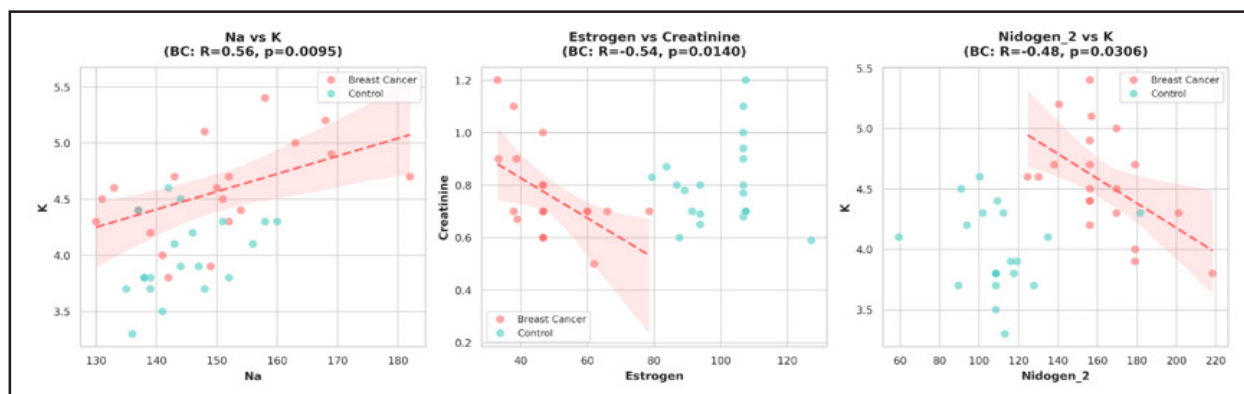


Figure 3. Bivariate Regression Plots Show Significant Correlations within the Breast Cancer Patient Cohort

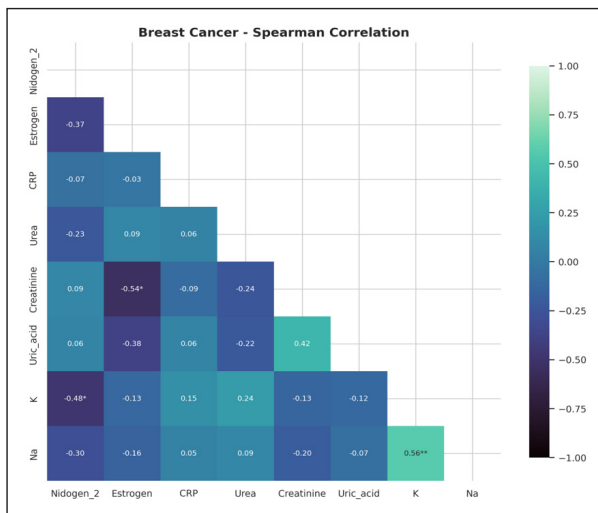


Figure 4. Correlations between Measured Variables in Breast Cancer Patients Indicate Systemic Physiological Disruption

groups based on the median expression value. Although not statistically significant, high *NID2* expression was associated with a shorter Progression-Free Interval (PFI) ($p = 0.10$). There was no significant association between *NID2* expression and Overall Survival (OS) ($p = 0.36$). Figure 9 shows the survival curves.

Discussion

Serum *Nidogen-2*, combined with other physiological markers, indicates breast cancer in Iraqi women. Serum *Nidogen-2* levels are higher in breast cancer patients. Altered expression of the basement membrane protein nidogen contributes to cancer progression. Our results confirm elevated *Nidogen-2* in other malignancies and support its use as a biomarker [9-14]. The in silico validation using the TCGA-BRCA dataset further strengthens this finding by demonstrating a significant upregulation of *NID2* mRNA in breast tumor tissue, suggesting that the tumor itself is a likely source of the elevated serum levels, although previously *NID2* has been reported to be involved to be a key factor in melanoma

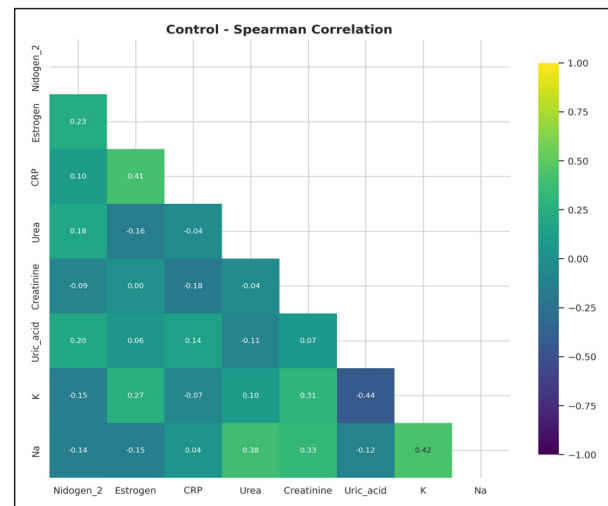


Figure 5. The Patient Cohort Lacked the Stable Homeostatic Correlations among Measured Variables Found in Healthy Controls

and urinary bladder tumor tissues.

The perfect separation (AUC = 1.000) observed for serum Estrogen is biologically implausible and must be interpreted with extreme caution. This result is likely an overestimation due to several major limitations of the current study. The small sample size ($n=30$ per group) increases the risk of overfitting the model. Furthermore, menopausal status, a critical determinant of circulating estrogen levels, was not recorded for the participants. This unmeasured confounding variable could account for the stark difference between groups. Similarly, tumor hormone receptor status (ER, PR, HER2) was not available. Without this information, it is difficult to interpret the systemic estrogen levels in the context of tumor biology, as the relationship between serum estrogen and tissue-level hormone receptor activity is complex and subtype-dependent [15-21]. The lack of these key clinical data points is a major limitation of this study.

Higher CRP in our patients indicates inflammation promotes cancer progression. However, CRP is a non-specific acute-phase reactant, and its elevation is not exclusive to cancer-related inflammation. A limitation

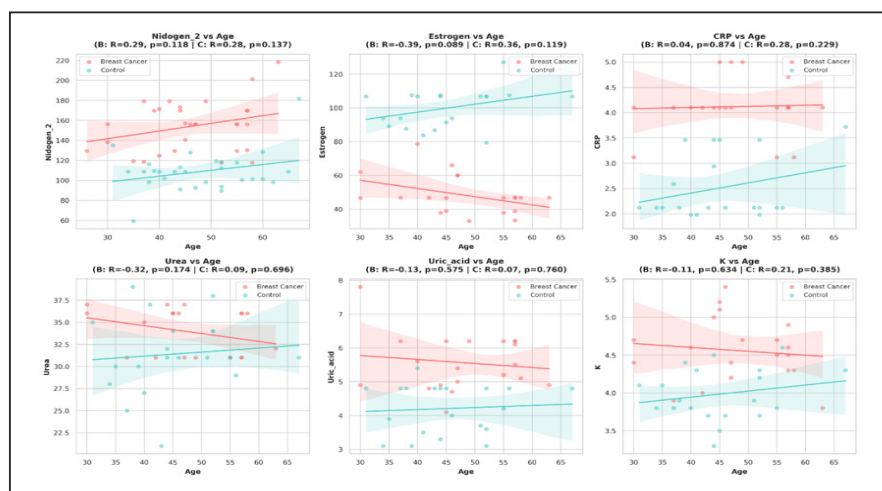


Figure 6. Biomarker Levels Correlate with Patient Age. Neither the breast cancer nor the control group showed significant correlations.

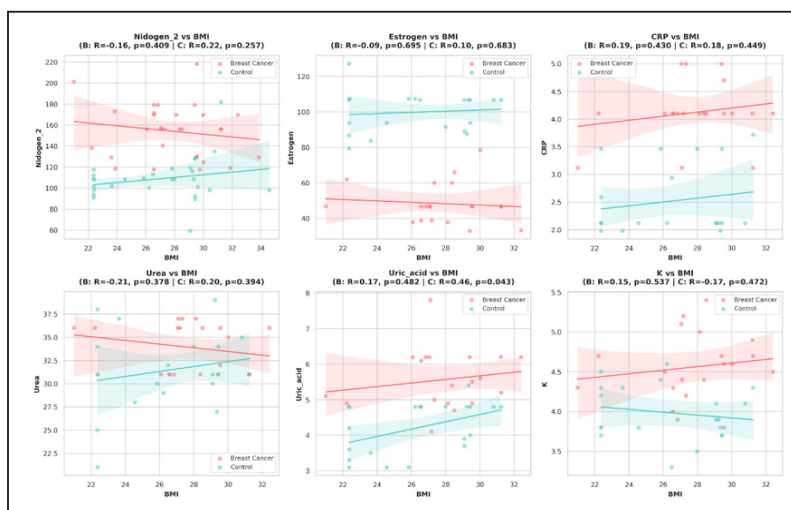


Figure 7. Bivariate Regressions Plot Key Biomarkers against Body Mass Index (BMI). Neither the breast cancer nor the control group showed significant correlations.

of this study is that underlying infection or other inflammatory conditions were not ruled out in the patient or control groups, which tempers the specificity of this finding. Nonetheless, CRP's performance supports its inclusion in a biomarker panel.

Our findings suggest that breast cancer alters renal function and disrupts electrolyte balance. The observed pattern of elevated Urea and Uric acid with normal Creatinine levels is noteworthy. This profile may suggest a state of prerenal azotemia, potentially related to systemic effects of the malignancy, rather than primary renal failure. Additionally, the significant elevation in Uric acid could be related to increased cell turnover or tumor lysis. The increase in serum Potassium (hyperkalemia) in the absence of significant renal failure is uncommon and may be attributable to factors such as pseudohyperkalemia or tumor lysis syndrome, warranting further investigation.

A biomarker panel including Estrogen, *Nidogen-2*, CRP, and Uric acid proved diagnostically accurate by ROC curve analysis. This suggests that a multi-marker approach could be highly effective for the early detection of breast cancer. A key strength is that the biomarkers do not correlate with age or BMI, suggesting a diagnostic value independent of these demographics. However, a further limitation is that statistical corrections for multiple testing were not performed, which increases the possibility of Type I errors. Therefore, the p-values should be interpreted as exploratory.

Higher *NID2* expression correlated with a shorter Progression-Free Interval in the TCGA dataset. *NID2* showed no significant association with Overall Survival in the full cohort, suggesting its prognostic value is restricted to specific breast cancer subtypes.

In conclusion, Iraqi women with breast cancer had higher serum *Nidogen-2* and lower Estrogen levels. These biomarkers, in combination with CRP and markers of renal function, exhibit high diagnostic accuracy. The upregulation of *NID2* in breast tumor tissue provides a biological basis for the observed increase in serum levels. These results support a multi-biomarker panel for early breast cancer detection and monitoring. Confirming

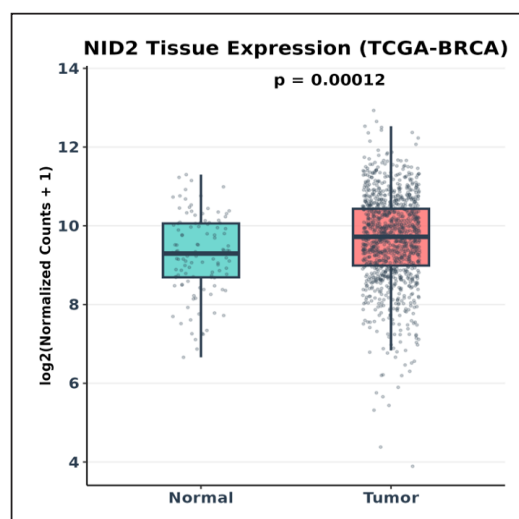


Figure 8. *NID2* mRNA Expression Differs in the TCGA-BRCA Dataset. Tumor tissue showed significantly higher expression than normal tissue.

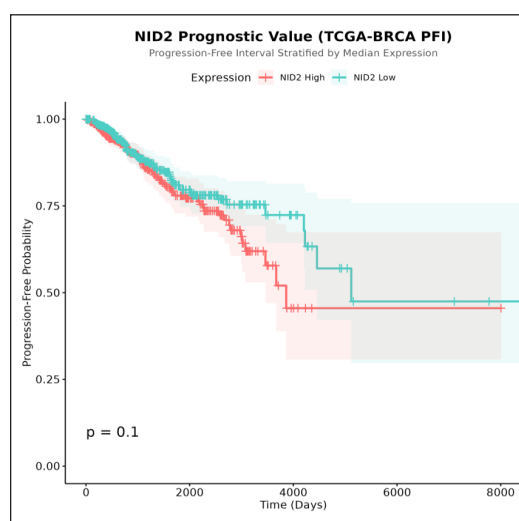


Figure 9. *NID2* Expression Predicts Progression-Free Interval in the TCGA-BRCA Cohort. The median expression value defined high and low expression groups.

these results requires large-scale studies that control for menopausal and tumor receptor status and assess the prognostic significance of *Nidogen-2* across different breast cancer subtypes.

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None.

Conflict of Interest

Author declares no conflict of interest.

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