International Journal of Women's Health

Dovepress
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ORIGINAL RESEARCH

# IDO-Dependent Tryptophan Metabolites and Endocan as Effective Diagnostic Biomarkers for Pregnancy with Pulmonary Hypertension

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**Objective:** Pregnancy with pulmonary hypertension (PPH) is one of the main causes of maternal heart failure and death. Endocan is a well-established soluble proteoglycan biomarker for endothelial dysfunction and inflammation. The Indoleamine 2, 3 dioxygenase (IDO) enzyme family comprises IDO1, IDO2, and TDO2, with IDO1-dependent tryptophan metabolites being particularly promising as candidate biomarkers for pulmonary hypertension (PH). However, there have been no reports on the relationship between endocan and IDO1-dependent metabolite and the progression of PPH. The study enrolled 62 patients with PPH and 86 age- and sex-matched healthy people. The concentrations of endocan, B-type natriuretic peptide (BNP), and N-terminal prohormone of BNP (NT-pro BNP) in the serum were detected by enzyme-linked immunosorbent assay.

**Methods:** The Pearson correlation coefficient analyzed the correlation between endocan level and BNP and NT-pro BNP. The concentrations of IDO-dependent tryptophan metabolites in the serum were measured by high-performance liquid chromatography-tandem mass spectrometry. The diagnostic value of endocan and IDO-dependent tryptophan metabolites for PPH was evaluated by the receiver operating characteristic curve (ROC).

**Results:** Results suggested that endocan (PPH vs control: 30.56±3.61 vs 24.53±3.00 ng/mL), BNP (243.40,63.03 vs 98.60,93.35 pg/mL), NT pro-BNP (654.65,363.80 vs 141.70,158.55 pg/mL), kynurenine (1.80±0.23 vs 1.53±0.28 AU), kynurenate (5.17±0.24 vs 4.91 ±0.27 AU), anthranilate (5.15,2.13 vs 3.00,1.33 AU), and quinolinate (3.62±0.80 vs 2.77±0.81 AU) (all $p<0.001$) were upregulated in PPH patients. Moreover, Endocan levels showed strong positive correlations with both BNP ($r=0.590$, $p<0.001$) and NT pro-BNP ($r=0.801$, $p<0.001$). ROC curve showed that the combination of endocan and IDO-dependent tryptophan metabolites had diagnostic value to PPH (AUC: 0.935; 95% CI: 0.896–0.974).

**Conclusion:** In short, we demonstrated that IDO-dependent tryptophan metabolites and endocan could be used as diagnostic biomarkers of PPH.

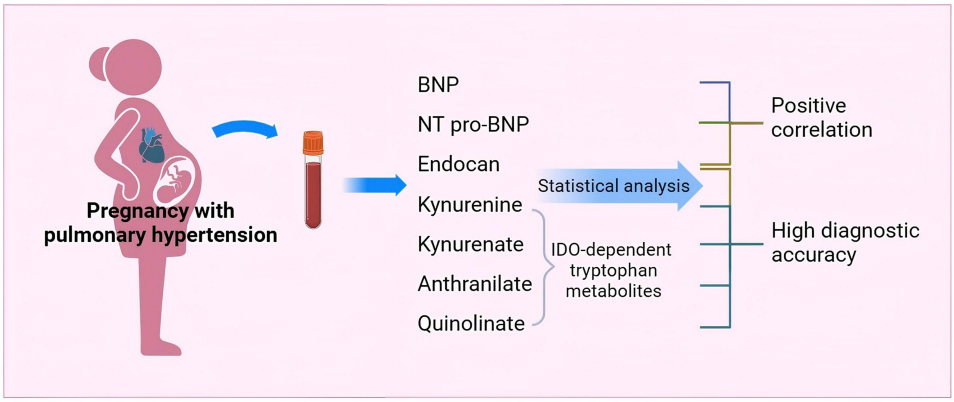
**Keywords:** pregnancy with pulmonary hypertension, endocan, IDO-dependent metabolites, kynurenine, biomarker, diagnosis

## Introduction

Pulmonary hypertension (PH) is defined as a syndrome characterized by abnormally elevated pulmonary artery pressure as a clinical manifestation, and then pulmonary artery overload, which may eventually lead to hypertrophy of the right ventricle and even the development of right heart failure.[1] Pregnancy with pulmonary hypertension (PPH) is the most critical type of pregnancy complicated with heart disease in obstetrics, the most common types being arterial PH and congenital heart disease-related PH, and the main causes of death include PH crisis, pulmonary embolism, and right heart failure.[2] According to previous literature reports, the mortality rate of pregnant women with PH is as high as 16%~30%.[3] In clinical practice, PPH is an important cause of adverse pregnancy outcomes and death, and once pregnant, a cesarean

Received: 10 March 2025
Accepted: 15 July 2025
Published: 19 July 2025

**Graphical Abstract**




section is required to terminate the pregnancy.[4,5] Therefore, identifying simple and reliable biomarkers of PH and cardiac function in PPH will help detect disease early, monitor disease progression, and develop appropriate treatment strategies.

To date, a large number of PH biomarkers have been discovered, including angiopoietin (Ang), B-type natriuretic peptide (BNP), N-terminal prohormone of BNP (NT-pro BNP), growth differentiation factor-15 (GDF-15), endothelin-1 (ET-1), vascular smooth muscle cells (VSMC), and microRNAs (miRNAs).[6,7] Endocan also known as endothelial cell-specific molecule-1 is a soluble proteoglycan mainly secreted by activated vascular endothelial cells and detected in serum/plasma.[8] The protein is encoded by the endothelial cell-specific molecule-1 (ESM-1) gene located on chromosome 5 and is mainly expressed in the liver, lungs, and kidneys. Endocan expression is regulated by pro-angiogenic factors (FGF-2, VEGF) and inflammatory cytokines (TNF-α, HIF-1α, IL-1β).[9] Endocan participates in inflammatory processes as well as endothelial cell remodeling, such as cell adhesion, migration, proliferation, and angiogenesis, properties that illustrate its potential role as a biomarker of endothelial dysfunction and inflammation.[10] Endothelial dysfunction is an important pathophysiological change in PH.[11] A recent study has shown that the signal disturbance between endocan and different molecules may lead to vascular fibrosis and increased pressure and resistance in the pulmonary arteries, so it can be used as a marker of PH.[12] However, there are no reports of the association and disease progression of endocan with PPH.

Indoleamine 2, 3 dioxygenase (IDO) is an essential intracellular monomer heme-dependent oxidase. The IDO enzyme family, comprising IDO1, IDO2, and the structurally related yet genetically distinct TDO2 (tryptophan 2,3-dioxygenase), primarily catalyzes the initial step of the kynurenine pathway (KP) in tryptophan metabolism.[13] IDO1 acts as a rate-limiting enzyme that is responsible for initiating degradation in the first step of the KP.[14] The KP leads to the production of kynurenine as well as other neuroactive compounds such as quinolinic acid, anthranilate, kynurenate, and quinolinate, which provides metabolites for de novo NAD biosynthesis and carbon metabolism.[15] The KP bridges tryptophan metabolism to NAD+ synthesis, neuroregulation, and immunometabolism, making it a pivotal pathway in physiology and disease. Targeting KP enzymes (IDO1/TDO2) or metabolites offers therapeutic potential for cancer, neurodegeneration, and inflammatory disorders.[16–18] IDO is expressed in many tissues, such as lung endothelial cells, female reproductive tract epithelial cells, placenta, and dendritic cells in lymphoid tissues.[19] Notably, IDO1-dependent tryptophan metabolites showed significantly higher levels in PH patients and had significant diagnostic value for PH.[20] It can be seen that it not only has the potential to be a diagnostic marker of PH but also has the potential to become a new diagnostic and therapeutic target. However, the potential of IDO1-dependent tryptophan metabolites in the diagnosis of PPH has not been reported.

In this work, the serum level of endocan and IDO-dependent tryptophan metabolites in patients with PPH was detected to determine its clinical value for PPH and to provide a new biomarker for the diagnosis of PPH.

# Materials and Methods

## Ethics Statement

Our research was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Changzhou Maternal and Child Health Hospital. Before enrollment, all participants were fully informed of the purpose of the study, needed to be cognitively capable of independently adhering to the protocol, and signed an informed consent. All methods of this study were carried out following relevant reporting guidelines and regulations.

## Study Participants and Diagnostic, Inclusion, and Exclusion Criteria

This prospective study enrolled 62 patients diagnosed with PPH from Changzhou Maternal and Child Health Hospital as the research group, along with 86 healthy pregnant women matched for age and gender from the same hospital as the control group.

The diagnostic criteria are as follows: Based on echocardiography, the PH is diagnosed as a systolic pulmonary artery pressure (sPAP) ≥ 30 mmHg in the resting state. The degree of systolic pulmonary artery pressure increase is classified as mild (30–49 mmHg, n = 31), moderate (50–79 mmHg; n = 22), and severe (more than 80 mmHg; n = 9). The PH classification is based on the World Health Symposium on Pulmonary Hypertension.[21] The heart function grading method of the New York Heart Association (NYHA) was adopted, which was divided into grades I to IV. Class I: Patients with heart disease are not restricted in their daily activities. Grade II: General physical activity is slightly limited. Level III: Physical activity is significantly limited. Grade IV: Symptoms of heart failure also appear at rest, aggravated after physical activity.

The inclusion criteria were as follows: (1) Pregnant women who met the diagnostic criteria for PH. (2) Stay in our hospital until termination of pregnancy and have complete clinical data. (3) There were no complications during pregnancy or before and after delivery. (4) Participants had to be ≥18 years old and had undergone regular prenatal check-ups.

The exclusion criteria are as follows: (1) Not meeting the diagnosis of PPH. (2) incomplete medical records. (3) Pregnant women whose reasons for termination of pregnancy are not related to PH. (4) Patients with other pregnancy complications. (5) Those with liver and kidney dysfunction.

## Bioinformatics Analysis

Differential gene microarray datasets of patients with PH and control people (GSE113439) were downloaded from the NCBI GEO DataSets site (https://www.ncbi.nlm.nih.gov/).

## Data Collection and Clinical Indicators Detection

The basic data of the tested pregnant women were recorded: age, height, weight, pregnancy, gestational age, enrollment time, systolic blood pressure, and diastolic blood pressure. Subjects were collected in the morning with fasting venous blood, and centrifuged at 2,000 g for 15 min, the supernatant was aspirated, aliquoted in sterile centrifuge tubes, and stored in a freezer at −80 degrees. The concentrations of endocan (ab278119, Abcam, Cambridge, MA, USA), BNP (ab193694), and NT pro-BNP (ab263877) were detected using the enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols. Briefly, standards and samples were incubated in antibody-coated wells, followed by sequential additions of biotinylated detection antibodies and Horseradish peroxidase (HRP)-streptavidin conjugate. After tetramethylbenzidine (TMB) substrate incubation, the reaction was stopped with sulfuric acid, and absorbance was measured at 450 nm. Concentrations were determined from standard curves, with all samples run in duplicate.

## High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) Analysis

HPLC-MS/MS was used to quantify tryptophan metabolites, and the specific method was reported by Aurore Desmons et al.[22] Serum sample preparation and calibration preparation: An equal volume of tryptophan metabolites (kynurenine, anthranilate,

kynurenate, and quinolinate) was mixed and continuously diluted with methanol to prepare a calibration standard. The 20 μL calibration standard and 50 μL serum samples were mixed with 50 ng L-Tryptophan-d8, 50 ng anthranilic acid, and 450 μL of chilled methanol, respectively, and centrifuged at 18000 g at 4 °C for 20 min. The 2 μL supernatant was taken for HPLC-MS/MS analysis. All chemicals and reagents used in this experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The HPLC instrument setup consisted of a CTC HTC PAL Autosampler (CTC Analytics AG, Zwingen, Switzerland) and an LC-20ADXR chromatographic system (Shimadzu, Kyoto, Japan). The MS inspection system uses the API 4000$^{TM}$ triple quadrupole mass spectrometer (AB Sciex, Darmstadt, Germany). Chromatographic separation was performed with a Waters HSS T3 2.1×100 mm, 2.6 μm column (Waters, Wilmslow, U.K). Data acquisition and processing were carried out using Analyst software (Version 1.6.3) and were quantified using Multiquant (Version 3.0.2) software.

## Statistical Analysis of Data

The data was analyzed and mapped using the SPSS 22.0 statistical software and GraphPad Prism 8.0 software. Shapiro Wilk was used for the normal distribution test. Normally distributed metrics were expressed as mean ± SD and were tested using a Student's *t*-test or Chi-square test. Comparisons among groups were using one-way ANOVA. Measures that do not conform to the normal distribution were expressed as median and interquartile range and were tested using the rank-sum test. The chi-square test was analyzed the relationship between PPH severity and NYHA score. Pearson correlation analysis was evaluated the association between clinical variables and sPAP. Logistic regression was applied to determine independent predictors of PPH severity. The receiver operating characteristic (ROC) curve assesses diagnostic efficacy by calculating the area under the curve (AUC), and an AUC greater than 0.5 indicates a predicted value. A two-tailed $P < 0.05$ was considered statistically significant.

# Results

## Clinical Baseline Characteristics of the Participants

Before the study officially began, we first compared the clinical characteristics of subjects between the control group and the PPH group. As shown in Table 1, the data suggested that only age was not statistically significant between the two groups. In the PPH group, sPAP, endocan, BNP, NT pro-BNP, kynurenine, kynurenate, anthranilate, and quinolinate were increased. However, gestational week, caesarean section, vaginal delivery, and birth weight of the newborn were all decreased (all $P < 0.05$). These data determined that PPH patients with higher levels of IDO-dependent tryptophan metabolites and biomarkers of pulmonary hypertension, along with endocan-induced endothelial dysfunction and inflammatory responses.

**Table 1** Baseline Characteristics of the Participants

| Project | Control (n=86) | PPH (N=62) | t/Z/x² | P value |
|---|---|---|---|---|
| Age (years) | 29.80±4.35 | 30.66±4.01 | 1.23 | 0.223 |
| sPAP (mmHg) | 23.07±2.65 | 49.50,19.25 | −10.38 | <0.001 |
| Gestational week | 36.00,2.00 | 33.92±2.67 | −4.33 | <0.001 |
| Mode of delivery (n,%) | | | 17.74 | <0.001 |
| Caesarean section | 62,72.09% | 61,98.39% | | |
| Vaginal delivery | 24,27.91% | 1,1.61% | | |
| Birth weight of the newborn(kg) | 2.57±0.50 | 2.25±0.53 | 3.76 | <0.001 |
| Endocan (ng/mL) | 24.53±3.00 | 30.56±3.61 | 15.84 | <0.001 |
| BNP (pg/mL) | 98.60,93.35 | 243.40,63.03 | −8.17 | <0.001 |
| NT pro-BNP (pg/mL) | 141.70,158.55 | 654.65,363.80 | −8.46 | <0.001 |
| Kynurenine (AU) | 1.53±0.28 | 1.80±0.23 | 6.09 | <0.001 |
| Kynurenate (AU) | 4.91±0.27 | 5.17±0.24 | 6.16 | <0.001 |
| Anthranilate (AU) | 3.00,1.33 | 5.15,2.13 | −6.76 | <0.001 |
| Quinolinate (AU) | 2.77±0.81 | 3.62±0.80 | 6.26 | <0.001 |

**Table 2** Relationship Between PPH Severity and NYHA Score

| Group | Mild (n=31) | Moderate (n=22) | Severe (n=9) | $x^2$ | P value |
|---|---|---|---|---|---|
| NYHA | | | | 92.68 | <0.001 |
| I | 18 | 0 | 0 | | |
| II | 13 | 13 | 0 | | |
| III | 0 | 9 | 0 | | |
| IV | 0 | 0 | 9 | | |

## Relationship Between PPH Severity and NYHA Score

Pulmonary hypertension is classified as mild, moderate, and severe with the increase of sPAP. The NYHA classification is a classification of heart failure that classifies impaired heart function into grades I to IV. From Table 2, we concluded that the severity of PPH disease was significantly associated with heart failure and worsened as an aggravation of heart failure.

## Correlation Analysis Between sPAP and Clinical Indicators of Patients with PPH

Next, we analyzed the correlation between sPAP and clinical indicators of PPH patients. As shown in Table 3, there was no statistical difference between gestational week and birth weight of the newborn and PPH diseases, indicating no correlation between them. Nevertheless, BNP, NT pro-BNP, endocan, kynurenine, kynurenate, anthranilate, and quinolinate were significantly correlated with PPH disease, indicating that these indicators affected the disease to some extent. Meanwhile, linear regression analysis (Table 4) revealed that BNP, NT-pro-BNP, endocan, kynurenine, anthranilate, and

**Table 3** Correlation Analysis Between Clinical Data and sPAP

| Project | PPH | |
|---|---|---|
| | r | P |
| Gestational week | 0.19 | 0.138 |
| Birth weight of the newborn | 0.17 | 0.197 |
| BNP | 0.65 | <0.001 |
| NT pro-BNP | 0.78 | <0.001 |
| Endocan | 0.87 | <0.001 |
| Kynurenine | 0.76 | <0.001 |
| Kynurenate | 0.84 | <0.001 |
| Anthranilate | 0.71 | <0.001 |
| Quinolinate | 0.71 | <0.001 |

**Table 4** Linear Regression Analysis of Clinical Data and sPAP

| Project | β | P value | VIF |
|---|---|---|---|
| Gestational week | −0.23 | 0.007 | 6.37 |
| Birth weight of the newborn | 0.17 | 0.036 | 6.21 |
| BNP | 0.17 | 0.001 | 2.07 |
| NT pro-BNP | 0.25 | <0.001 | 3.40 |
| Endocan | 0.32 | <0.001 | 2.41 |
| Kynurenine | 0.12 | 0.003 | 1.52 |
| Kynurenate | 0.06 | 0.163 | 1.65 |
| Anthranilate | 0.10 | 0.030 | 2.12 |
| Quinolinate | 0.11 | 0.009 | 1.66 |

**Table 5** Logistic Regression Analysis

| Project | P value | OR | 95% CI |
|---|---|---|---|
| Endocan | <0.001 | 1.94 | 1.42–2.66 |
| Kynurenine | 0.005 | 47.67 | 3.19–712.72 |
| Kynurenate | 0.153 | 5.02 | 0.55–45.99 |
| Anthranilate | 0.004 | 2.03 | 1.25–3.31 |
| Quinolinate | 0.062 | 1.91 | 0.97–3.77 |

quinolate were all significantly positively correlated with sPAP ($P < 0.05$), with endocan exhibiting the strongest association ($\beta = 0.32$). These findings suggested that these biomarkers may serve as predictive factors for elevated sPAP. In contrast, gestational age showed a significant negative correlation with sPAP, while no significant associations were observed for kynurenate ($P > 0.05$). Notably, the variance inflation factor (VIF) for gestational age and neonatal birth weight was elevated (> 6), indicating potential multicollinearity issues in the model. Overall, these results provide important insights into the factors influencing sPAP.

## Logistic Regression Analysis of Each Factor and Occurrence of PPH

Logistic regression analysis was performed for variables with statistically significant differences (endocan and IDO-dependent tryptophan metabolites) to identify potential risk factors associated with PPH. The results showed that kynurenate and quinolinate in IDO-dependent tryptophan metabolites were not associated with the development of PPH. However, endocan, kynurenine, and anthranilate were risk factors for patients with PPH development (OR>1, P<0.05) (Table 5).

## EMS1, Endocan, BNP, and NT Pro-BNP are Upregulated in PPH Patients

Based on previous results, we measured the levels of endocan and classical biomarkers of PH (BNP and NT pro-BNP) in the patient with PPH. We first downloaded the GSE113439 datasets from the GEO database and analyzed the differential expression of the ESM1 gene between the control group (G1) and the PH group (G2). The results showed that the ESM1 gene was increased in the PH group (Figure 1A). Then, the concentrations of endocan, BNP, and NT pro-BNP in serum were detected by ELISA. We found that endocan, BNP, and NT pro-BNP were remarkably upregulated in the serum of PPH patients (Figure 1B–D).

## Endocan Expression Is Positively Correlated with BNP and NT Pro-BNP

We further analyzed the correlation between endocan expression and BNP and NT pro-BNP. There was a positive correlation between endocan and BNP (r=0.590, $p<0.001$) (Figure 2A) and between endocan and NT pro-BNP (r=0.801, $p<0.001$) (Figure 2B). This funding suggested that endocan expression was positively correlated with classical biomarkers of PH, supporting the potential of endocan as a biomarker for PPH diagnosis.

## The Levels of IDO-Dependent Tryptophan Metabolites are Elevated in the Serum of PPH Patients

Next, we determined the concentrations of kynurenine, kynurenate, anthranilate, and quinolinate in the serum by HPLC-MS/MS. The results showed that compared with the control group, the concentrations of all four metabolites were significantly elevated in the serum of patients with PPH (Figure 3A–D).

## Endocan and IDO-Dependent Tryptophan Metabolites May Be Effective Diagnostic Biomarkers of PPH

To investigate the value of endocan and IDO-dependent tryptophan metabolites as a biomarker of MM, ROC curve analysis was performed to identify their diagnostic value (Table 6). ROC analysis revealed significant diagnostic performance for all

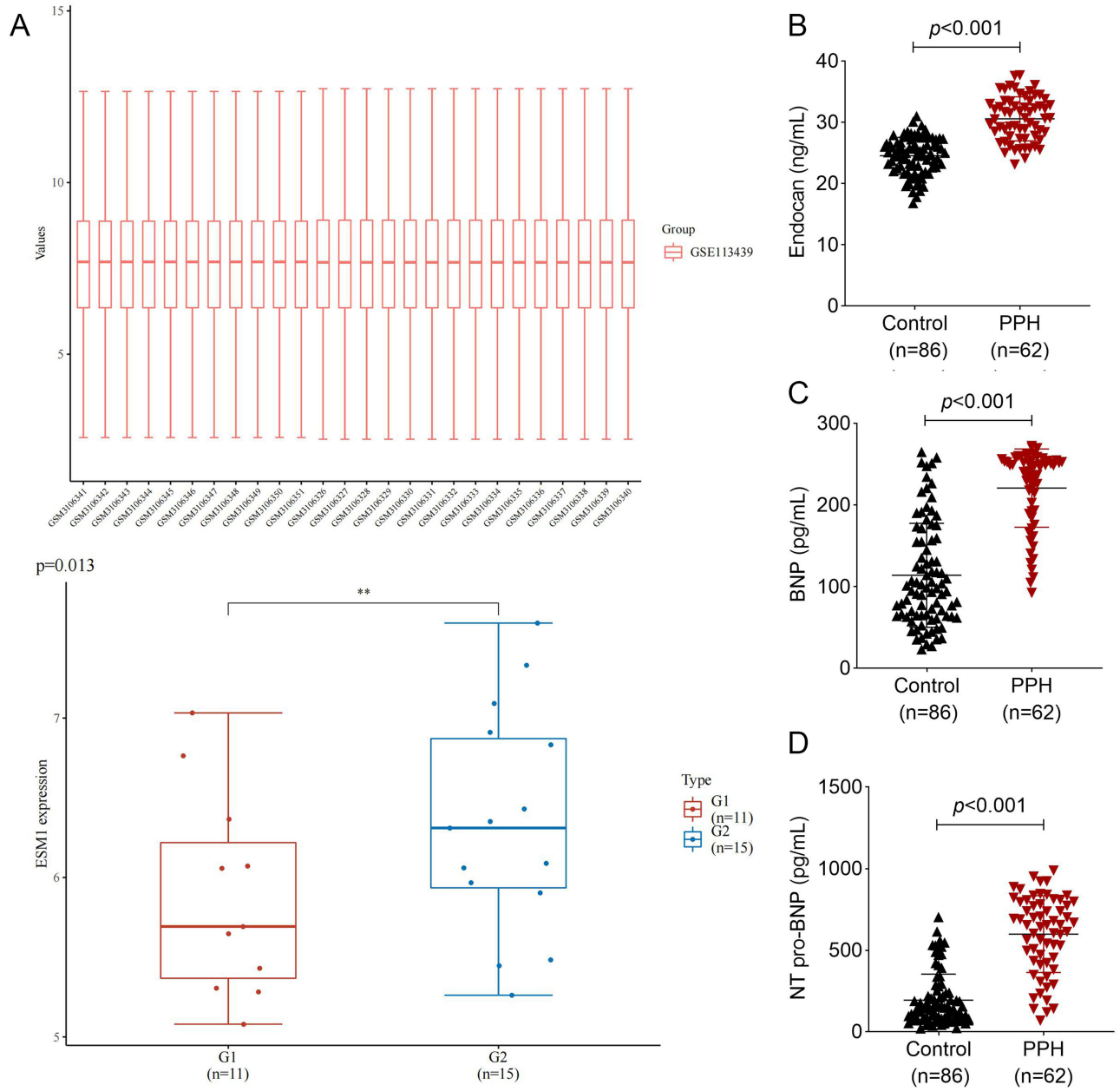



**Figure 1** EMS1, endocan, BNP, and NT pro-BNP are upregulated in the PPH patients.(**A**) Analysis of differential expression of the ESM1 gene in GSE113439 datasets. (n = 11). (**B**–**D**) The concentrations of endocan, BNP, and NT pro-BNP in the serum of the control group (n = 86) and patients with PPH (n = 62) were detected by ELISA. ***p*<0.01 vs G1 group.

biomarkers (all p<0.001): endocan (AUC=0.895, 95% CI:0.845–0.946), kynurenine (0.760, 0.684–0.836), kynurenate (0.759, 0.683–0.836), anthranilate (0.826, 0.756–0.896), and quinolinate (0.767, 0.690–0.844) (Figure 4A–E). Figure 4F shows the diagnostic value of these indicators taken together (AUC: 0.935, *p*<0.001; 95% CI: 0.896–0.974), with baseline and prediction lines as references. To sum up, these results indicated the high diagnostic accuracy of endocan and IDO-dependent tryptophan metabolites (kynurenine, kynurenate, anthranilate, and quinolinate) in PPH, supporting that the potential of endocan and IDO-dependent tryptophan metabolites as effective biomarkers for the diagnosis of PPH.


https://doi.org/10.2147/IJWH.S527345

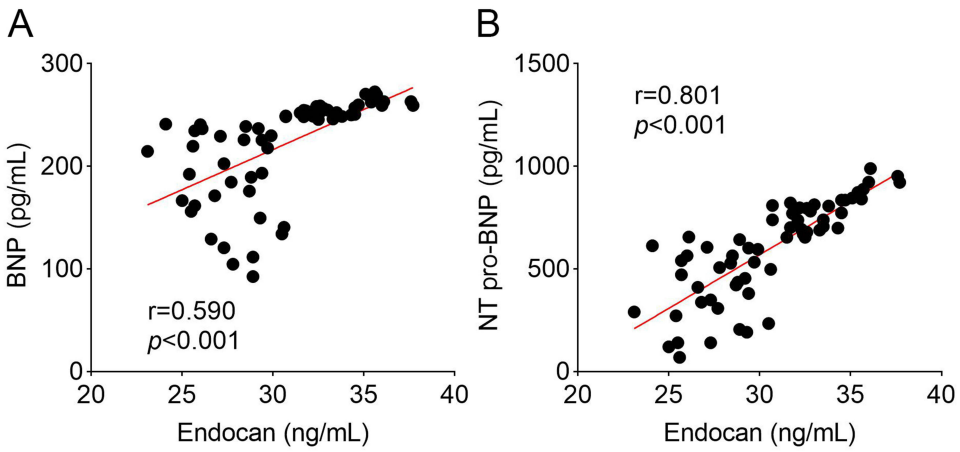



**Figure 2** Endocan expression is positively correlated with BNP and NT pro-BNP. (**A**–**B**) The correlation between endocan level and BNP and NT pro-BNP was analyzed by the Pearson correlation coefficient.

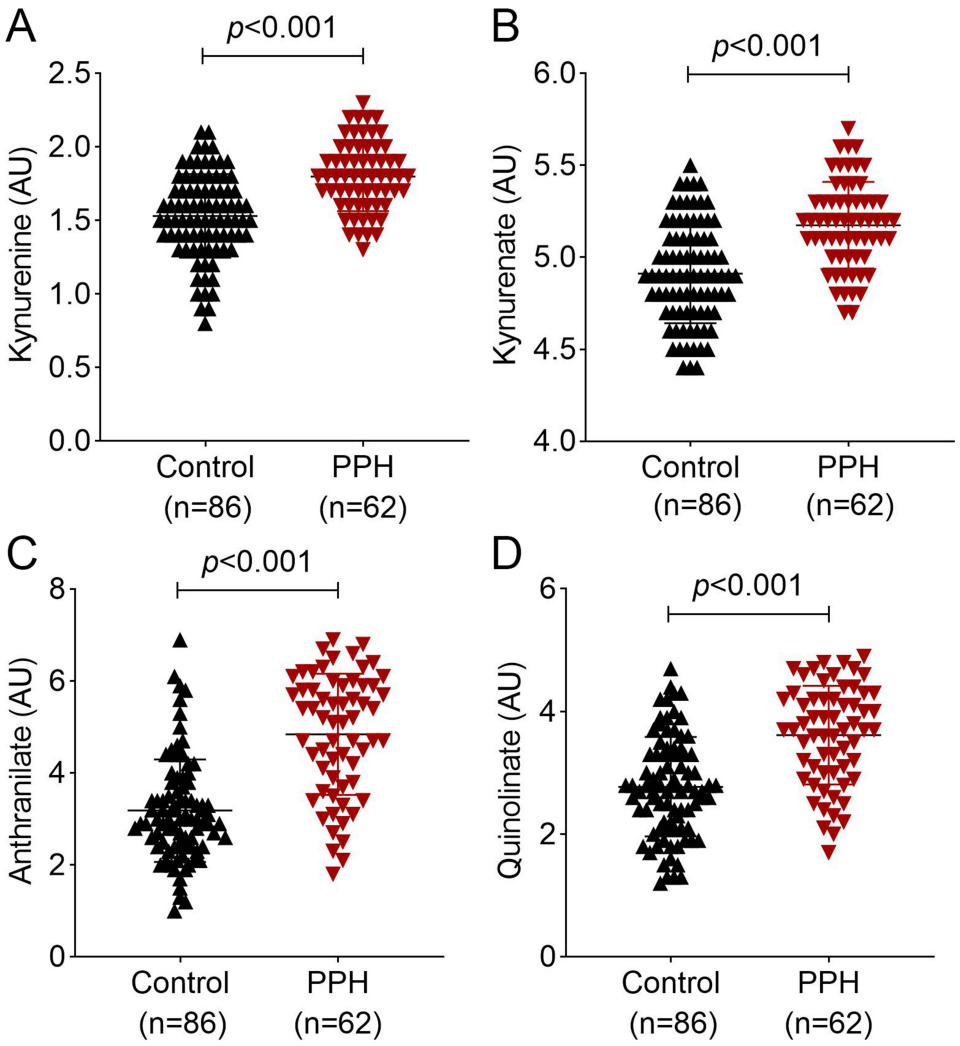



**Figure 3** The levels of IDO-dependent tryptophan metabolites are elevated in the serum of PPH patients.(**A**–**D**) The concentrations of kynurenine, kynurenate, anthranilate, and quinolinate in the serum of the control group (n = 86) and patients with PPH (n = 62) were measured by HPLC-MS/MS.

**Table 6** ROC Curve Analysis

| Project | Sensitivity | Specificity | AUC | 95% CI | P value | Cut off | Youden |
|---|---|---|---|---|---|---|---|
| Endocan | 0.73 | 0.94 | 0.90 | 0.85–0.95 | <0.001 | 28.30 | 0.67 |
| Kynurenine | 0.74 | 0.66 | 0.76 | 0.68–0.84 | <0.001 | 1.65 | 0.41 |
| Kynurenate | 0.73 | 0.70 | 0.76 | 0.68–0.84 | <0.001 | 5.05 | 0.42 |
| Anthranilate | 0.73 | 0.83 | 0.83 | 0.76–0.90 | <0.001 | 4.05 | 0.55 |
| Quinolinate | 0.63 | 0.80 | 0.77 | 0.69–0.84 | <0.001 | 3.45 | 0.43 |
| Combine | 0.81 | 0.95 | 0.94 | 0.90–0.97 | <0.001 | 0.51 | 0.76 |

## Discussion

Pregnancy-induced significant hemodynamic changes can predispose women to cardiovascular complications, including PH.[23,24] Additionally, changes in hormone levels and inflammatory responses may also induce or worsen PH.[25] PPH, a severe condition with significant morbidity, lacks well-established biomarkers for early diagnosis and risk stratification. In this study, we identified endocan and IDO-dependent tryptophan metabolites as potential diagnostic biomarkers for PPH, demonstrating their significant elevation in affected patients and strong correlation with disease severity.

Several diagnostic and prognostic biomarkers for PH have been identified, notably that BNP and NT pro-BNP are recognized clinical serological biomarkers for PH and cardiac function. They are widely used in risk stratification of PH, pulmonary hemodynamic correlation, prognosis, and prediction of mortality.[26] In our work, we studied the clinical

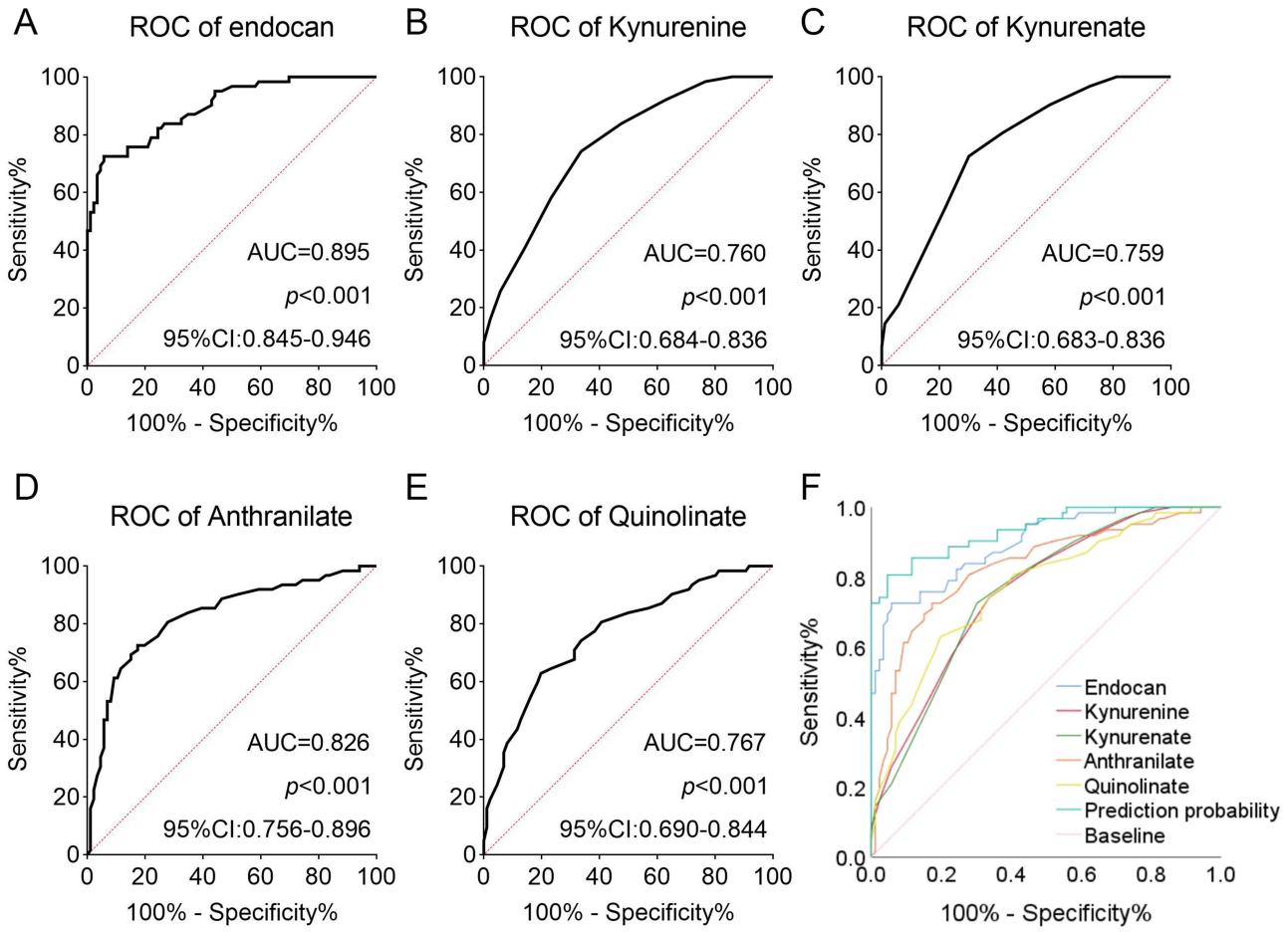



**Figure 4** Endocan and IDO-dependent tryptophan metabolites may be effective diagnostic biomarkers of PPH.(**A**–**F**) The diagnostic value of endocan and IDO-dependent tryptophan metabolites for PPH was evaluated by the ROC curve.


https://doi.org/10.2147/IJWH.S527345

features of 86 healthy people and 62 patients with PPH and observed that BNP and NT pro-BNP were upregulated in the PPH group. In addition, sPAP, endocan and tryptophan metabolites were all increased in the PPH group and were significantly correlated with upregulated sPAP, suggesting that the association of these factors with PPH disease. Meanwhile, endocan, kynurenine, and anthranilate also were independent risk factors for patients with PPH development, implicating their involvement in disease development. In addition, the strong association between these biomarkers and disease severity, as indicated by NYHA classification and sPAP levels, further highlights their clinical utility in risk stratification and monitoring.

Endocan has a wide range of biological functions in endothelial cells and is suggested as a marker of endothelial dysfunction, pathological angiogenesis, and inflammation. These changes gradually lead to hemodynamic changes and elevated pulmonary artery pressure and resistance in patients, such as cardiovascular diseases, and immune diseases.[27,28] It is worth noting that Alberto et al have proven that endocan levels were positively correlated with sPAP in systemic sclerosis.[12] In addition, Zhao et al demonstrated that endocan levels were increased in endothelium-interstitial transition (EndMT), and targeting endocan can suppress EndMT and inflammatory response in PH, thereby attenuating PH,[29,30] suggesting that endocan has a certain function in the diagnosis of PH. The upregulation of ESM1 (endocan-encoding gene) in PH patients from the GEO dataset reinforces the clinical relevance of endocan in PH-related conditions. Moreover, endocan expression was positively correlated with BNP and NT pro-BNP, both well-established biomarkers of PH. This correlation supports the notion that endothelial injury and inflammatory activation contribute to PPH progression. These results demonstrate endocan's potential as a diagnostic biomarker for PPH.

IDO is an enzyme that degrades tryptophan into the metabolite kynurenine and a metabolic pathway that has been extensively studied, especially as a potential biomarker. For instance, this metabolic pathway could be a potential clinical biomarker for cancer, depression, neurodegeneration, coronary artery disease, and autoimmune diseases.[31–34] Of note, Simpson et al[35] revealed that upregulation of the kynurenine pathway occurs early in PH, mainly in the lungs, suggesting that it may be a candidate biomarker for PH. Nagy et al also confirmed that kynurenine was significantly elevated and strongly associated with PH. However, the IDO level was not changed in the PH group.[36] Besides, kynurenine pathway metabolism is closely related to decreased right ventricular systolic and diastolic function, and right ventricular dilation during exercise, indicating that metabolites can be used as biomarkers of right ventricular function in PH.[37] However, the study of IDO-dependent tryptophan metabolites in PPH has not been reported. In the present work, the elevated levels of IDO-dependent tryptophan metabolites suggest enhanced tryptophan catabolism in PPH, possibly due to immune and inflammatory activation. The increased concentrations of these metabolites may reflect oxidative stress and vascular remodeling, which are key pathological features of PH. Finally, we proved that IDO-dependent tryptophan metabolites and endocan have high diagnostic values for PPH, especially combined detection of the two can improve diagnostic accuracy. This underscores their potential as non-invasive diagnostic tools for early PPH detection.

Despite these promising findings, our study has several limitations. First, while the current sample size meets the minimum requirements for our statistical analyses, we agree this warrants further investigation. In future studies, we plan to expand the patient cohort through multicenter collaboration to validate these preliminary findings. Second, this was a cross-sectional study, and longitudinal data are required to establish causal relationships between biomarker levels and PPH progression. Third, although we demonstrated significant correlations between biomarker levels and disease severity, the clinical utility of these markers remains to be established. Meanwhile, further mechanistic studies are required to elucidate the exact biological roles of these biomarkers in PPH development.

In summary, we found that the contents of IDO-dependent tryptophan metabolites and endocan were associated with high diagnostic accuracy of PPH, suggesting that combined IDO-dependent tryptophan metabolites and endocan could be used as diagnostic biomarkers of PPH. These findings hold significant implications for improving the management of PPH patients. Specifically, the identification of these biomarkers provides a promising approach for early detection and risk stratification, enabling timely intervention in high-risk individuals. Given their strong correlation with disease severity, these biomarkers may facilitate more precise monitoring of disease progression and therapeutic response. Moreover, their association with endothelial dysfunction and inflammatory pathways suggests potential therapeutic targets, paving the way for personalized treatment strategies. Future studies should validate these biomarkers in larger,

multicenter cohorts and explore their utility in guiding targeted therapies, ultimately improving clinical outcomes for PPH patients.

## Data Sharing Statement

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

## Ethical Approval

Our research was approved by the Ethics Committee of Changzhou Maternal and Child Health Hospital. Written informed consent was obtained from all patients. This study was performed in line with the principles of the Declaration of Helsinki. All methods of this study were carried out following relevant reporting guidelines and regulations.

## Consent for Publication

Written informed consent was obtained from all patients.

## Funding

This study was supported by Research Project of Changzhou Maternal and Child Health Hospital(YJ202404); Clinical Research Project of Nanjing Medical University Changzhou Medical Center (CMCC202205).

## Disclosure

The authors have no relevant financial or non-financial interests to disclose for this work.

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