

## SERUM YKL-40, PERIOSTIN, AND EOSINOPHIL-DERIVED NEUROTOXIN AS COMPLEMENTARY BIOMARKERS FOR ASSESSING DISEASE SEVERITY IN ADULTS WITH BRONCHIAL ASTHMA: A RETROSPECTIVE OBSERVATIONAL STUDY

SERUMSKI YKL-40, PERIOSTIN I NEUROTOKSIN POREKLOM IZ EOZINOFILA KAO KOMPLEMENTARNI BIOMARKERI ZA PROCENU TEŽINE BOLESTI KOD ODRASLIH OSOBA SA BRONHIJALNOM ASTMOM: RETROSPEKTIVNA OPSERVACIONA STUDIJA

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### Summary

**Background:** Bronchial asthma is a heterogeneous inflammatory airway disease, and conventional clinical and inflammatory indicators may not fully reflect its biological complexity or severity. YKL-40, periostin, and eosinophil-derived neurotoxin (EDN) represent distinct aspects of airway remodeling, type 2 inflammation, and eosinophil activation, respectively. This study investigated their individual and combined clinical value for assessing disease severity in adults with bronchial asthma.

**Methods:** This single-center retrospective observational study included 82 adults with bronchial asthma, comprising 23 patients with mild, 39 with moderate, and 20 with severe asthma. Serum YKL-40, periostin, and EDN concentrations were measured using enzyme-linked immunosorbent assays. Clinical characteristics, Asthma Control Test (ACT) scores, pulmonary function parameters, fractional exhaled nitric oxide (FeNO), peripheral blood eosinophils, and total immunoglobulin E were collected. Correlation and logistic regression analyses were performed to evaluate associations between serum biomarkers and asthma severity. Receiver operating characteristic analysis was used to assess the ability of individual biomarkers and their combined model to distinguish severe from non-severe asthma.

**Results:** Serum YKL-40, periostin, and EDN levels increased progressively from mild to moderate and severe

### Kratak sadržaj

**Uvod:** Cilj je bio da se ispita odnos između nivoa proteina sličnom oligomernom matriks proteinu hrskavice (Cartilage Oligomeric Matrix Protein Like 26 – CORS26), gena 14 stimulisanog TNF-om (TSG-14) u serumu i dijabetičke retinopatije (DR).

**Metode:** U jednocentričnu studiju preseka uključeno je 276 pacijenata sa dijabetes melitusom tipa 2 (T2DM), hospitalizovanih u periodu od aprila 2023. do aprila 2025. godine. Na osnovu dijagnostičkih nalaza, pacijenti su podeljeni u grupu sa DR (156 pacijenata) i grupu bez DR (120 pacijenata). U okviru DR grupe izdvojene su podgrupe: neproliferativna DR (NPDR, 98 ispitanika) i proliferativna DR (PDR, 58 pacijenata). Veza između parametara glikolipidnog metabolizma, TSG-14 i CORS26 je analizirana Pearsonovom metodom. Korišćena je logistička regresiona analiza za ispitivanje faktora koji utiču na pojavu DR kod osoba sa dijabetesom tipa 2. Dijagnostička vrednost serumskih TSG-14 i CORS26 za DR kod pacijenata sa T2DM je procenjena pomoću ROC krivih.

**Rezultati:** Opšti podaci za obe grupe pacijenata su bili slični. Nivo HDL-C bio je niži u DR grupi u poređenju sa grupom bez DR, dok su nivoi serumskih TSG-14, CORS26, ukupnog holesterola (TC), triglicerida (TG), LDL-C i HOMA-IR bili viši u DR grupi (sve  $P < 0,05$ ). Serumski nivoi TSG-14 i CORS26 bili su viši u PDR grupi u odnosu na NPDR grupu (oba  $P < 0,05$ ). TSG-14 i CORS26

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asthma (all  $P$  for trend  $<0.001$ ). YKL-40 showed the strongest inverse correlation with percentage predicted forced expiratory volume in one second ( $FEV_1$ ) ( $r=-0.60$ ,  $P<0.001$ ), whereas periostin was closely correlated with FeNO ( $r=0.57$ ,  $P<0.001$ ) and peripheral eosinophil count ( $r=0.53$ ,  $P<0.001$ ). EDN showed the strongest correlations with eosinophil count ( $r=0.66$ ,  $P<0.001$ ), eosinophil percentage ( $r=0.63$ ,  $P<0.001$ ), and FeNO ( $r=0.60$ ,  $P<0.001$ ). In multivariable logistic regression, lower percentage predicted  $FEV_1$  and higher YKL-40, periostin, and EDN concentrations remained independently associated with severe asthma. The areas under the receiver operating characteristic curve for YKL-40, periostin, and EDN were 0.801, 0.776, and 0.818, respectively. Their combined model achieved an area under the curve of 0.899 (95% CI, 0.811–0.951), with 85.0% sensitivity and 83.9% specificity. Bootstrap internal validation yielded an optimism-corrected area under the curve of 0.897.

**Conclusions:** Serum YKL-40, periostin, and EDN are associated with distinct but complementary clinical and inflammatory features of adult asthma. Their concentrations increase with disease severity, and combined assessment provides greater discrimination of severe asthma than individual biomarkers. These circulating biomarkers may serve as complementary tools for biological assessment and risk stratification in adults with bronchial asthma.

**Keywords:** bronchial asthma, YKL-40, periostin, eosinophil-derived neurotoxin, disease severity, biomarkers

## Introduction

Bronchial asthma is a common chronic inflammatory airway disease characterized by variable respiratory symptoms, reversible airflow limitation, and persistent airway inflammation (1, 2). It affects more than 300 million individuals worldwide and continues to impose a substantial burden on healthcare systems because of recurrent exacerbations, impaired quality of life, and increased medical expenditures (3, 4). Despite remarkable advances in pharmacological management, asthma remains a highly heterogeneous disease with considerable variations in inflammatory phenotypes, disease severity, and therapeutic responses among individual patients (5). Early identification of patients at increased risk of severe disease is therefore essential for optimizing treatment strategies, preventing exacerbations, and improving long-term clinical outcomes (6).

Current assessment of asthma severity mainly relies on symptom evaluation, pulmonary function testing, exacerbation history, and the intensity of treatment required to achieve disease control, as recommended by the Global Initiative for Asthma (GINA) (7). Clinical tools such as the Asthma Control Test (ACT), forced expiratory volume in one second ( $FEV_1$ ), fractional exhaled nitric oxide (FeNO), peripheral blood eosinophil count, and serum immunoglobulin E (IgE) are widely used in routine practice. However, each of these indicators has in-

herent limitations (8). Pulmonary function parameters do not always accurately reflect ongoing airway inflammation, while ACT scores are influenced by patients' subjective perceptions. Similarly, FeNO, eosinophil counts, and IgE levels primarily represent specific aspects of type 2 inflammation and may be affected by corticosteroid therapy, smoking status, allergic comorbidities, and other clinical factors. Consequently, reliance on a single biomarker is often insufficient to comprehensively characterize disease severity in adult asthma.

Recent advances in translational respiratory medicine have highlighted the potential value of circulating biomarkers in reflecting the complex biological processes underlying asthma. Among these, YKL-40, also known as chitinase-3-like protein 1 (CHI3L1), has attracted increasing attention because of its involvement in chronic inflammation, tissue remodeling, extracellular matrix deposition, and airway structural changes (9). Elevated circulating YKL-40 concentrations have been associated with persistent airflow limitation, airway remodeling, and poor asthma control in several clinical studies, suggesting that this protein may reflect pathological processes beyond eosinophilic inflammation (10, 11).

**Ključne reči:** gen 14 stimulisan TNF-om (TSG-14), protein sličan oligomernom matriks proteinu hrskavice 26 (CORS26), dijabetes tipa 2, dijabetička retinopatija

u serumu su pokazali negativnu povezanost sa HDL-C i pozitivnu korelaciju sa TC, TG, LDL-C i HOMA-IR (sve  $P<0.001$ ). Logistička regresiona analiza je pokazala da su sledeći faktori rizika povezani sa pojavom DR kod pacijenata sa dijabetesom tipa 2: trajanje dijabetesa, TC, TG, LDL-C, HOMA-IR, TSG-14 i CORS26 (sve  $P<0.05$ ). Površine ispod ROC krive (AUC) za serumske TSG-14 i CORS26 u dijagnostici DR kod pacijenata sa T2DM iznosile su 0,833, 0,832 i 0,918. AUC kombinovane dijagnostike bio je veći nego za TSG-14 ili CORS26 pojedinačno ( $Z=2,811$ ;  $2,827$ ;  $P=0,018$ ;  $0,015$ ).

**Zaključak:** Nivoi TSG-14 i CORS26 u serumu su usko povezani sa pojavom dijabetičke retinopatije kod pacijenata sa dijabetesom tipa 2. Njihova kombinovana detekcija ima određenu kliničku vrednost u dijagnostici DR kod ovih pacijenata.

Periostin is another promising biomarker that has been extensively investigated in type 2-high asthma (12). As a matricellular protein induced primarily by interleukin (IL)-4 and IL-13, periostin par-

ticipates in extracellular matrix remodeling, epithelial-mesenchymal interactions, subepithelial fibrosis, and eosinophilic airway inflammation. Previous studies have demonstrated significant associations between circulating periostin concentrations and eosinophilic inflammation, airway remodeling, and responsiveness to biologic therapies targeting type 2 cytokines (13, 14). Nevertheless, periostin alone cannot fully represent the biological heterogeneity of asthma, particularly in patients exhibiting mixed inflammatory phenotypes.

Eosinophil-derived neurotoxin (EDN), a granule protein released by activated eosinophils during degranulation, has emerged as a sensitive indicator of eosinophil activation rather than eosinophil quantity alone (15). Compared with peripheral blood eosinophil counts, serum EDN may more directly reflect eosinophil functional activity and ongoing airway inflammation. Elevated EDN concentrations have been associated with uncontrolled asthma, acute exacerbations, and increased airway inflammation, suggesting that EDN may provide complementary information beyond conventional hematological parameters (16).

Although YKL-40, periostin, and EDN have each been investigated individually in asthma, existing studies have predominantly focused on single biomarkers, and relatively few have evaluated their combined clinical utility in assessing disease severity among adult patients with bronchial asthma. Because these three biomarkers represent distinct yet interconnected biological pathways—including airway remodeling, type 2 inflammation, and eosinophil activation—their combined assessment may provide a more comprehensive evaluation of disease severity than any individual marker alone. Furthermore, evidence regarding the relationships between these circulating biomarkers and conventional clinical indicators, including pulmonary function, symptom control, FeNO, and peripheral eosinophilia, remains limited.

Therefore, the present retrospective observational study aimed to investigate the clinical significance of serum YKL-40, periostin, and EDN levels in adult patients with bronchial asthma. Specifically, we compared serum biomarker levels among patients with different asthma severity, evaluated their associations with pulmonary function, ACT scores, FeNO, peripheral blood eosinophil counts, and serum IgE concentrations, and explored their diagnostic performance individually and in combination for identifying severe asthma. We hypothesized that the combined measurement of these complementary biomarkers would provide a more comprehensive assessment of disease severity and offer additional clinical value for risk stratification in adult bronchial asthma.

## Materials and Methods

### *Study design and participants*

This single-center retrospective observational study included adult patients with bronchial asthma who were treated at the Department of Respiratory and Critical Care Medicine of Zhuhai Hospital of Integrated Traditional Chinese and Western Medicine between January 2023 and December 2025. Patients were consecutively screened through the electronic medical record system. The diagnosis of asthma was established by respiratory physicians on the basis of a compatible history of variable respiratory symptoms and documented variable expiratory airflow limitation.

The inclusion criteria were as follows: (1) age  $\geq 18$  years; (2) a confirmed diagnosis of bronchial asthma; (3) availability of complete demographic, clinical, treatment, and pulmonary function data; (4) availability of serum samples collected during the same clinical assessment period; and (5) sufficient stored serum for measurement of YKL-40, periostin, and eosinophil-derived neurotoxin (EDN). Patients were excluded if they had chronic obstructive pulmonary disease, asthma–chronic obstructive pulmonary disease overlap, bronchiectasis, interstitial lung disease, active pulmonary tuberculosis, acute respiratory infection at the time of blood sampling, autoimmune disease, active malignancy, severe hepatic or renal dysfunction, pregnancy, or incomplete key clinical data.

A total of 82 eligible patients were included. According to asthma severity, 23 patients were classified as having mild asthma, 39 as having moderate asthma, and 20 as having severe asthma.

### *Assessment of asthma severity*

Asthma severity was retrospectively assessed according to the intensity of maintenance treatment required to achieve and maintain symptom control and prevent exacerbations. Mild asthma was defined as asthma that could be controlled with low-intensity treatment, including as-needed low-dose inhaled corticosteroid (ICS)–formoterol or regular low-dose ICS therapy. Moderate asthma was defined as asthma that required Step 3 or Step 4 treatment, generally consisting of low- or medium-dose ICS combined with a long-acting  $\beta 2$ -agonist (LABA), to maintain adequate control. Severe asthma was defined as asthma that remained uncontrolled despite adherence to maximal optimized high-dose ICS–LABA therapy and management of contributory factors, or asthma that worsened when high-dose treatment was reduced.

Severity classification was independently reviewed by two respiratory physicians using main-

tenance medication intensity, symptom-control records, exacerbation history, inhaler technique, treatment adherence, and relevant comorbidities. Disagreements were resolved through consultation with a third senior respiratory physician.

#### *Clinical and laboratory data collection*

Demographic and clinical data were extracted from the electronic medical records, including age, sex, body mass index, smoking history, duration of asthma, allergic rhinitis, previous exacerbations, hospitalizations, maintenance medications, oral corticosteroid use, and biologic therapy. Asthma control was evaluated using the Asthma Control Test (ACT). The number of exacerbations requiring systemic corticosteroids and asthma-related hospitalizations during the preceding 12 months was also recorded.

Laboratory variables included white blood cell count, neutrophil count, eosinophil count, eosinophil percentage, C-reactive protein, and total immunoglobulin E. Fractional exhaled nitric oxide (FeNO) was measured according to the standardized operating procedures of the hospital. Pulmonary function parameters included forced expiratory volume in one second ( $FEV_1$ ), percentage predicted  $FEV_1$ , forced vital capacity (FVC), and the  $FEV_1$ /FVC ratio. Pulmonary function testing was performed using a calibrated spirometer in accordance with standard clinical procedures.

#### *Serum biomarker measurements*

Fasting peripheral venous blood samples were collected during the initial clinical assessment. Patients with an acute respiratory infection at sampling were excluded. The records did not consistently permit classification of every sample as having been obtained during a stable interval versus close to a recent exacerbation; this limitation is now acknowledged. Maintenance inhaled or oral corticosteroid therapy was not withheld for research purposes. Whenever possible, sampling preceded any major adjustment of maintenance therapy, and treatment exposure at the time of sampling was extracted from the medical record. Blood samples were collected into serum separator tubes and allowed to clot at room temperature for approximately 30 min. Samples were processed within 2 h of collection and centrifuged at  $3,000 \times g$  for 10 min at room temperature. The separated serum was aliquoted into sterile polypropylene tubes and stored at  $-80^\circ\text{C}$  until analysis. Repeated freeze-thaw cycles were avoided.

Serum YKL-40 concentrations were measured using a commercially available Human YKL-40 enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, Thermo Fisher Scientific, Vienna, Austria;

Catalog No. BMS2322). The manufacturer-reported analytical measurement range was 78–5,000 pg/mL, and the analytical sensitivity was 10.83 pg/mL. Serum samples were diluted 1:50 before analysis. Concentrations read from the calibration curve (pg/mL) were multiplied by the dilution factor and divided by 1,000 to report the final serum concentrations in ng/mL. The reported intra-assay and inter-assay coefficients of variation were 2.3% and 7.2%, respectively. All YKL-40 values and unit conversions were rechecked using this procedure.

Serum periostin concentrations were determined using a Human Periostin Rapid ELISA Kit (Invitrogen, Thermo Fisher Scientific; Catalog No. EELR025). The analytical measurement range was 3.12–200 ng/mL, with an analytical sensitivity of 1.52 ng/mL. The intra-assay and inter-assay coefficients of variation were both  $<10\%$ .

Serum eosinophil-derived neurotoxin (EDN) concentrations were measured using a Human Eosinophil-Derived Neurotoxin ELISA Kit (Creative Diagnostics, Shirley, NY, USA; Catalog No. DEIASL093). The assay had a detection range of 0.625–40 ng/mL and an analytical sensitivity of 0.375 ng/mL. The intra-assay and inter-assay coefficients of variation were  $<8\%$  and  $<10\%$ , respectively. Samples with EDN concentrations exceeding the upper limit of the analytical range were appropriately diluted according to the manufacturer's instructions and reassayed.

Before analysis, frozen serum aliquots were thawed at room temperature and gently mixed. All assays were performed in accordance with the respective manufacturers' instructions. Samples were analyzed in duplicate, and the mean value of the duplicate measurements was used for statistical analysis. Samples for each biomarker were analyzed using the same reagent lot whenever possible to minimize inter-assay variability. A calibration curve generated from the standards supplied with each kit was established independently for each microplate. Duplicate measurements showing a coefficient of variation  $>15\%$  were reassayed. Laboratory personnel performing the assays were blinded to patients' clinical characteristics, pulmonary function results, and asthma severity classifications.

#### *Study outcomes*

The primary outcome was the association of serum YKL-40, periostin, and EDN levels with asthma severity. Secondary outcomes included the relationships between these biomarkers and ACT score, pulmonary function parameters, FeNO, peripheral blood eosinophil count, and total immunoglobulin E. The ability of each biomarker, individually and in combination, to distinguish severe from non-severe

asthma was also evaluated. For binary analyses, patients with mild or moderate asthma were combined into the non-severe asthma group, whereas patients with severe asthma formed the severe asthma group.

#### *Statistical analysis*

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 26.0 (IBM, Armonk, NY, USA). Continuous variables with a normal distribution were expressed as mean  $\pm$  standard deviation and compared using one-way analysis of variance; when the omnibus test was significant, Tukey's honestly significant difference test was used for pairwise comparisons. Non-normally distributed variables were presented as median and interquartile range and compared using the Kruskal–Wallis test, followed, when significant, by Dunn's pairwise test with Bonferroni adjustment. Categorical variables were expressed as numbers and percentages and compared using the chi-square test or Fisher's exact test, as appropriate. For ordered asthma-severity groups, linear polynomial contrasts were used for normally distributed variables and the Jonckheere–Terpstra test for non-normally distributed variables to calculate P values for trend.

Spearman correlation analysis was used to evaluate associations between serum biomarkers and clinical indicators. Univariable logistic regression was initially performed to identify factors associated with severe asthma. Candidate predictors were selected on the basis of  $P < 0.10$  in univariable analysis and prespecified clinical relevance. To limit model complexity, the final multivariable model retained percentage predicted  $FEV_1$  and FeNO as conventional clinical measures together with the three biomarkers of primary interest. Collinearity was assessed using variance inflation factors and tolerance values (maximum VIF, 2.18; minimum tolerance, 0.459). Odds ratios and 95% confidence intervals (CI) were reported. Given the 20 severe-asthma events and five predictors, this model remained vulnerable to overfitting and was interpreted as exploratory.

Receiver operating characteristic curves were constructed to evaluate the discriminatory performance of YKL-40, periostin, EDN, and their combined model for classifying severe versus non-severe asthma. The area under the curve (AUC), 95% CI, optimal cutoff value, sensitivity, and specificity were calculated. The combined biomarker model was a separate logistic regression containing only YKL-40, periostin, and EDN and was distinct from the five-predictor explanatory model reported in Table V. Its equation was:  $\text{logit}(P_{\text{severe}}) = -5.93 + 0.028 \times \text{YKL-40 (ng/mL)} + 0.034 \times \text{periostin (ng/mL)} + 0.032 \times \text{EDN (ng/mL)}$ , where  $P_{\text{severe}} = 1/(1 + e^{-\text{logit}})$ . AUCs were compared using the De-

Long method, and pairwise P values are reported in Table VI. Internal validation of the combined model was performed using 1,000 bootstrap resamples. A two-sided P value  $< 0.05$  was considered statistically significant.

## **Results**

### *Patient characteristics according to asthma severity*

A total of 82 adult patients with bronchial asthma were included in the final analysis, comprising 23 patients with mild asthma, 39 with moderate asthma, and 20 with severe asthma. The demographic and clinical characteristics of the three groups are summarized in Table I. No statistically significant differences were observed among the groups with respect to sex distribution, body mass index, smoking history, or prevalence of allergic rhinitis (all  $P > 0.05$ ).

Patients with severe asthma were older and had a longer duration of asthma than those with mild or moderate asthma. The number of exacerbations requiring systemic corticosteroids during the preceding 12 months and the proportion of patients with asthma-related hospitalization increased progressively across the mild, moderate, and severe groups. ACT scores decreased significantly with increasing asthma severity. As expected, the use of medium- or high-dose inhaled corticosteroid–long-acting  $\beta_2$ -agonist therapy, maintenance oral corticosteroids, and biologic agents was more frequent among patients with severe asthma.

### *Pulmonary function and conventional inflammatory indicators*

Pulmonary function and conventional inflammatory markers differed significantly according to asthma severity (Table II). Percentage predicted  $FEV_1$  and the  $FEV_1/FVC$  ratio declined progressively from the mild to the severe asthma group. Patients with severe asthma had significantly higher FeNO levels, peripheral blood eosinophil counts, eosinophil percentages, serum total immunoglobulin E, and C-reactive protein concentrations than those with mild asthma.

Although total white blood cell and neutrophil counts tended to be higher in the severe asthma group, the between-group differences were not statistically significant. These findings indicated that increasing asthma severity was accompanied by worsening airflow limitation and greater systemic and type 2 inflammatory activity.

**Table I** Demographic and clinical characteristics of patients according to asthma severity.

| Variable                                   | Mild asthma (n=23) | Moderate asthma (n=39) | Severe asthma (n=20) | P value |
|--------------------------------------------|--------------------|------------------------|----------------------|---------|
| Age, years                                 | 42.3±11.6          | 47.8±12.4              | 53.6±11.7            | 0.006   |
| Male sex, n (%)                            | 10 (43.5)          | 16 (41.0)              | 9 (45.0)             | 0.952   |
| Body mass index, kg/m <sup>2</sup>         | 23.8±3.1           | 24.6±3.4               | 25.0±3.5             | 0.493   |
| Current or former smoker, n (%)            | 5 (21.7)           | 10 (25.6)              | 6 (30.0)             | 0.829   |
| Asthma duration, years                     | 5.0 (3.0–8.0)      | 8.0 (5.0–12.0)         | 12.0 (8.0–17.0)      | <0.001  |
| Allergic rhinitis, n (%)                   | 12 (52.2)          | 23 (59.0)              | 13 (65.0)            | 0.702   |
| Exacerbations in the previous year, n      | 0 (0–1)            | 1 (0–2)                | 3 (2–4)              | <0.001  |
| Asthma-related hospitalization, n (%)      | 1 (4.3)            | 7 (17.9)               | 9 (45.0)             | 0.003   |
| ACT score                                  | 22.1±2.0           | 18.3±2.8               | 14.1±3.0             | <0.001  |
| Medium-/high-dose ICS–LABA, n (%)          | 0 (0.0)            | 31 (79.5)              | 20 (100.0)           | <0.001  |
| Maintenance oral corticosteroid use, n (%) | 0 (0.0)            | 2 (5.1)                | 6 (30.0)             | 0.003   |
| Biologic therapy, n (%)                    | 0 (0.0)            | 1 (2.6)                | 4 (20.0)             | 0.012   |

Data are presented as mean ± standard deviation, median (interquartile range), or number (percentage). ACT, Asthma Control Test; ICS, inhaled corticosteroid; LABA, long-acting  $\beta$ 2-agonist.

**Table II** Pulmonary function and conventional laboratory indicators according to asthma severity.

| Variable                                    | Mild asthma (n=23) | Moderate asthma (n=39) | Severe asthma (n=20) | P value |
|---------------------------------------------|--------------------|------------------------|----------------------|---------|
| FEV <sub>1</sub> , L                        | 2.82±0.61          | 2.29±0.58              | 1.72±0.51            | <0.001  |
| FEV <sub>1</sub> , % predicted              | 88.4±9.7           | 72.6±11.5              | 55.6±12.1            | <0.001  |
| FVC, L                                      | 3.55±0.72          | 3.29±0.69              | 2.98±0.66            | 0.018   |
| FEV <sub>1</sub> /FVC, %                    | 78.9±6.4           | 69.8±8.1               | 58.2±9.3             | <0.001  |
| FeNO, ppb                                   | 24.0 (17.0–35.0)   | 41.0 (29.0–57.0)       | 61.0 (44.0–84.0)     | <0.001  |
| White blood cell count, ×10 <sup>9</sup> /L | 6.7±1.5            | 7.1±1.8                | 7.5±1.9              | 0.271   |
| Neutrophil count, ×10 <sup>9</sup> /L       | 3.9±1.2            | 4.3±1.5                | 4.7±1.6              | 0.128   |
| Eosinophil count, ×10 <sup>9</sup> /L       | 0.18 (0.11–0.28)   | 0.35 (0.21–0.52)       | 0.56 (0.34–0.79)     | <0.001  |
| Eosinophil percentage, %                    | 2.8 (1.8–4.1)      | 5.1 (3.2–7.4)          | 7.6 (4.9–10.2)       | <0.001  |
| Total IgE, IU/mL                            | 104 (61–186)       | 196 (112–326)          | 306 (171–495)        | <0.001  |
| C-reactive protein, mg/L                    | 1.6 (0.9–2.8)      | 2.9 (1.5–4.7)          | 5.0 (2.7–8.1)        | <0.001  |

FEV<sub>1</sub>, forced expiratory volume in one second; FVC, forced vital capacity; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E.

**Table III** Serum biomarker levels according to asthma severity.

| Biomarker        | Mild asthma (n=23) | Moderate asthma (n=39) | Severe asthma (n=20) | P value | P for trend |
|------------------|--------------------|------------------------|----------------------|---------|-------------|
| YKL-40, ng/mL    | 36.8 (28.4–47.5)   | 58.6 (45.2–72.9)       | 89.8 (69.4–115.6)    | <0.001  | <0.001      |
| Periostin, ng/mL | 42.5±11.3          | 57.8±14.6              | 72.9±16.8            | <0.001  | <0.001      |
| EDN, ng/mL       | 22.7 (16.1–31.4)   | 39.5 (28.7–52.6)       | 63.7 (46.2–84.5)     | <0.001  | <0.001      |

EDN, eosinophil-derived neurotoxin.

**Table IV** Spearman correlations of serum biomarkers with clinical and laboratory indicators.

| Variable                           | YKL-40, r | P value | Periostin, r | P value | EDN, r | P value |
|------------------------------------|-----------|---------|--------------|---------|--------|---------|
| ACT score                          | −0.57     | <0.001  | −0.44        | <0.001  | −0.53  | <0.001  |
| FEV <sub>1</sub> , % predicted     | −0.60     | <0.001  | −0.38        | <0.001  | −0.47  | <0.001  |
| FEV <sub>1</sub> /FVC ratio        | −0.52     | <0.001  | −0.30        | 0.006   | −0.40  | <0.001  |
| FeNO                               | 0.36      | <0.001  | 0.57         | <0.001  | 0.60   | <0.001  |
| Eosinophil count                   | 0.30      | 0.006   | 0.53         | <0.001  | 0.66   | <0.001  |
| Eosinophil percentage              | 0.28      | 0.011   | 0.50         | <0.001  | 0.63   | <0.001  |
| Total IgE                          | 0.23      | 0.038   | 0.46         | <0.001  | 0.41   | <0.001  |
| C-reactive protein                 | 0.49      | <0.001  | 0.27         | 0.014   | 0.34   | 0.002   |
| Asthma duration                    | 0.42      | <0.001  | 0.24         | 0.030   | 0.32   | 0.003   |
| Exacerbations in the previous year | 0.47      | <0.001  | 0.40         | <0.001  | 0.55   | <0.001  |

ACT, Asthma Control Test; FEV<sub>1</sub>, forced expiratory volume in one second; FVC, forced vital capacity; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E.

#### Serum YKL-40, periostin, and EDN levels

Serum concentrations of YKL-40, periostin, and EDN increased progressively across the three asthma severity groups (Table III). Median serum YKL-40 increased from 36.8 ng/mL in patients with mild asthma to 58.6 ng/mL in those with moderate asthma and 89.8 ng/mL in those with severe asthma. Similar graded increases were observed for periostin and EDN.

Tukey and Dunn–Bonferroni post hoc comparisons, as applicable, showed that YKL-40 and EDN differed significantly between each pair of severity groups (mild vs moderate, mild vs severe, and moderate vs severe). Periostin was significantly higher in the moderate and severe groups than in the mild group and was also higher in the severe group than in the moderate group. Ordered trend testing confirmed significant stepwise increases in all three biomarkers with increasing asthma severity (all P for trend <0.001).

#### Correlations between serum biomarkers and clinical indicators

The correlations between the three serum biomarkers and selected clinical indicators are shown in Table IV. YKL-40 was negatively correlated with ACT score, percentage predicted FEV<sub>1</sub>, and the FEV<sub>1</sub>/FVC ratio, whereas it was positively correlated with asthma duration, exacerbation frequency, and C-reactive protein.

Periostin showed moderate positive correlations with FeNO, eosinophil count, eosinophil percentage, and total immunoglobulin E. EDN was strongly correlated with peripheral eosinophil count and eosinophil percentage and was also positively correlated with FeNO and exacerbation frequency. All three biomarkers were negatively associated with ACT score and percentage predicted FEV<sub>1</sub>, although the strongest inverse association with pulmonary function was observed for YKL-40.

**Table V** Logistic regression analysis of factors associated with severe asthma.

| Variable                                              | Univariable OR (95% CI) | P value | Multivariable OR (95% CI) | P value |
|-------------------------------------------------------|-------------------------|---------|---------------------------|---------|
| Age, per 1-year increase                              | 1.06 (1.02–1.10)        | 0.006   | —                         | —       |
| Asthma duration, per 1-year increase                  | 1.08 (1.02–1.15)        | 0.008   | —                         | —       |
| ACT score, per 1-point increase                       | 0.63 (0.51–0.78)        | <0.001  | —                         | —       |
| FEV <sub>1</sub> , per 10% predicted increase         | 0.50 (0.35–0.71)        | <0.001  | 0.59 (0.39–0.89)          | 0.012   |
| FeNO, per 10-ppb increase                             | 1.25 (1.08–1.45)        | 0.003   | 1.08 (0.90–1.30)          | 0.409   |
| Eosinophil count, per 0.1×10 <sup>9</sup> /L increase | 1.29 (1.11–1.50)        | 0.001   | —                         | —       |
| YKL-40, per 10-ng/mL increase                         | 1.48 (1.20–1.82)        | <0.001  | 1.30 (1.04–1.63)          | 0.021   |
| Periostin, per 10-ng/mL increase                      | 1.66 (1.25–2.20)        | <0.001  | 1.40 (1.02–1.92)          | 0.038   |
| EDN, per 10-ng/mL increase                            | 1.58 (1.26–1.98)        | <0.001  | 1.37 (1.06–1.77)          | 0.016   |

OR, odds ratio; CI, confidence interval; ACT, Asthma Control Test; FEV<sub>1</sub>, forced expiratory volume in one second; FeNO, fractional exhaled nitric oxide; EDN, eosinophil-derived neurotoxin.

**Table VI** Discriminatory performance of serum biomarkers for classifying severe asthma.

| Marker/model             | AUC (95% CI)        | Optimal cutoff             | Sensitivity, % | Specificity, % | Youden index | DeLong P vs combined model |
|--------------------------|---------------------|----------------------------|----------------|----------------|--------------|----------------------------|
| YKL-40                   | 0.801 (0.697–0.882) | 71.8 ng/mL                 | 75.0           | 79.0           | 0.540        | 0.071                      |
| Periostin                | 0.776 (0.669–0.861) | 65.2 ng/mL                 | 70.0           | 77.4           | 0.474        | 0.018                      |
| EDN                      | 0.818 (0.716–0.895) | 50.9 ng/mL                 | 80.0           | 80.6           | 0.606        | 0.094                      |
| Combined biomarker model | 0.899 (0.811–0.951) | 0.33 predicted probability | 85.0           | 83.9           | 0.689        | —                          |

AUC, area under the receiver operating characteristic curve; CI, confidence interval; EDN, eosinophil-derived neurotoxin. DeLong P values compare each individual marker with the combined model.

*Factors independently associated with severe asthma*

For logistic regression analysis, patients with mild and moderate asthma were combined into the non-severe asthma group (n=62), whereas the 20 patients with severe asthma formed the severe asthma group. In univariable analysis, older age, longer asthma duration, lower ACT score, lower percentage predicted FEV<sub>1</sub>, higher FeNO, higher eosinophil count, and increased serum YKL-40, periostin, and EDN levels were associated with severe asthma.

To limit model complexity, the final multivariable model included percentage predicted FEV<sub>1</sub>, FeNO, YKL-40, periostin, and EDN, selected as prespecified conventional and biomarker measures central to the study question. Collinearity diagnostics did not indicate problematic redundancy (maximum VIF, 2.18; minimum tolerance, 0.459). After

adjustment, lower percentage predicted FEV<sub>1</sub> and higher serum concentrations of YKL-40, periostin, and EDN remained independently associated with severe asthma. FeNO did not retain statistical significance after the three serum biomarkers were included (Table V). Because only 20 severe-asthma events were available for five predictors, these estimates should be considered exploratory and potentially overfit despite the restricted predictor set.

*Discriminatory performance for classifying severe asthma*

The discriminatory performance of the individual serum biomarkers and their combined model for classifying severe versus non-severe asthma is presented in Table VI. YKL-40, periostin, and EDN each demonstrated moderate discrimination, with AUCs

of 0.801, 0.776, and 0.818, respectively. EDN had the highest AUC among the individual biomarkers.

The combined model incorporating YKL-40, periostin, and EDN achieved an AUC of 0.899, with 85.0% sensitivity and 83.9% specificity at the sample-derived optimal cutoff. The combined-model AUC was significantly higher than that of periostin alone and numerically higher than those of YKL-40 and EDN; the corresponding DeLong P values are shown in *Table VI*. Bootstrap internal validation yielded an optimism-corrected AUC of 0.897. Nevertheless, because the cutoff and performance estimates were derived from only 20 severe cases in a single-center cohort, they should not be treated as clinically validated thresholds.

In pairwise DeLong comparisons, the combined model versus YKL-40, periostin, and EDN yielded  $P=0.071$ ,  $P=0.018$ , and  $P=0.094$ , respectively.

## Discussion

In the present study, we evaluated the clinical significance of serum YKL-40, periostin, and eosinophil-derived neurotoxin (EDN) in adult patients with bronchial asthma of varying severity. The principal findings were as follows. First, circulating concentrations of all three biomarkers increased progressively from mild to moderate and severe asthma. Second, these biomarkers showed distinct but complementary relationships with clinical indicators. YKL-40 was most strongly associated with impaired pulmonary function, poor symptom control, and systemic inflammation; periostin was closely related to type 2 inflammatory indicators, including FeNO, eosinophilia, and total IgE; and EDN showed the strongest correlations with eosinophil activation and exacerbation frequency. Third, lower percentage predicted FEV<sub>1</sub> and higher serum YKL-40, periostin, and EDN levels remained independently associated with severe asthma in multivariable analysis. Finally, the combined biomarker model provided better discrimination of severe asthma than any individual biomarker alone. Collectively, these findings suggest that simultaneous assessment of airway remodeling, type 2 inflammation, and eosinophil activation may improve the biological characterization of asthma severity.

Asthma severity reflects a complex interaction among persistent airway inflammation, structural remodeling, variable airflow limitation, treatment responsiveness, and exacerbation susceptibility (17). Conventional clinical indicators remain essential for asthma assessment, but they describe only selected dimensions of the disease. Symptom scores may be affected by subjective perception, whereas pulmonary function can remain relatively preserved despite active airway inflammation. Similarly, blood

eosinophil counts, FeNO, and total IgE primarily reflect type 2 inflammatory activity and may be influenced by corticosteroid exposure, smoking, allergic disease, and recent exacerbations. The graded increase in YKL-40, periostin, and EDN observed in this study supports the concept that biomarker combinations representing different biological pathways may provide information not fully captured by conventional measurements alone.

YKL-40 is a chitinase-like glycoprotein secreted by macrophages, neutrophils, epithelial cells, and airway smooth muscle cells (10). Although it lacks enzymatic chitinase activity, it participates in inflammatory cell recruitment, extracellular matrix remodeling, tissue fibrosis, and cellular proliferation. In asthma, increased YKL-40 expression has been linked to subepithelial fibrosis, airway wall thickening, persistent airflow obstruction, and reduced treatment responsiveness. In the current study, YKL-40 showed the strongest inverse correlation with percentage predicted FEV<sub>1</sub> among the three biomarkers and was also associated with a lower ACT score, longer disease duration, higher C-reactive protein, and more frequent exacerbations. These findings are biologically plausible because YKL-40 may reflect both chronic inflammatory burden and structural airway changes. Unlike eosinophil-oriented biomarkers, YKL-40 may therefore capture a broader and more persistent component of asthma pathophysiology, including remodeling-related airflow limitation.

Periostin is an extracellular matrix protein induced predominantly by the type 2 cytokines interleukin-4 and interleukin-13 (18). It is produced by airway epithelial cells and fibroblasts and contributes to collagen deposition, epithelial–mesenchymal interactions, subepithelial fibrosis, and eosinophil recruitment. Serum periostin has been proposed as a systemic marker of type 2-high asthma and as a potential indicator of responsiveness to targeted biologic therapy. In our study, periostin increased significantly with asthma severity and demonstrated moderate correlations with FeNO, blood eosinophil counts, eosinophil percentage, and total IgE. These relationships support its role as a marker of type 2 inflammatory activity. However, the associations between periostin and pulmonary function were weaker than those observed for YKL-40. This difference may reflect the fact that periostin is more closely linked to cytokine-driven eosinophilic inflammation and extracellular matrix signaling, whereas YKL-40 may more directly reflect established structural airway injury and chronic inflammatory remodeling.

EDN is released from activated eosinophil granules and may represent eosinophil functional activity more directly than the circulating eosinophil count (19). Blood eosinophil numbers indicate the size of the eosinophil population but do not necessarily reflect the extent of degranulation or tissue-level in-

flammatory activity. In the present study, EDN had the strongest correlations with blood eosinophil count, eosinophil percentage, and FeNO and was also closely associated with exacerbation frequency and poor asthma control. These findings suggest that EDN may provide complementary information regarding active eosinophilic inflammation. The relatively strong discrimination achieved by EDN alone further supports its potential usefulness in identifying patients with more active or severe eosinophil-associated disease. Nevertheless, because eosinophil activity can be altered by systemic or inhaled corticosteroid treatment, EDN should be interpreted together with treatment history and other clinical variables.

An important finding of this study was that all three biomarkers remained independently associated with severe asthma after adjustment for pulmonary function and FeNO. This result suggests that their associations with disease severity were not explained solely by airflow limitation or conventional type 2 inflammatory measurements. The three biomarkers likely represent partially overlapping but biologically distinct domains. YKL-40 may primarily reflect chronic inflammation and airway remodeling, periostin may indicate interleukin-4/interleukin-13-driven type 2 signaling and matrix responses, and EDN may reflect active eosinophil degranulation. The combined evaluation of these processes may therefore provide a more comprehensive description of asthma severity than a single biomarker.

The combined biomarker model achieved a higher area under the receiver operating characteristic curve than the individual markers. This finding is consistent with the biological heterogeneity of asthma, in which no single pathway is likely to explain disease severity in all patients. Importantly, the combined model should not be viewed as a replacement for guideline-based severity assessment. Asthma severity remains a clinical construct determined retrospectively by the level of treatment required to achieve disease control after diagnosis, adherence, inhaler technique, and modifiable factors have been addressed. The present biomarkers may instead serve as adjunctive tools that help identify patients with a greater biological burden of remodeling and inflammation. Such information may be particularly useful when clinical symptoms, pulmonary function, and conventional inflammatory indicators provide discordant results.

From a clinical perspective, measuring YKL-40, periostin, and EDN together could potentially assist in phenotypic stratification. A patient with predominantly elevated YKL-40 and marked airflow limitation may have a stronger remodeling-related component, whereas a patient with high periostin and EDN may exhibit more pronounced type 2 and eosinophil-driven inflammation. This distinction may eventually support more individualized monitoring and therapeutic decision-making. However, the

present study was not designed to evaluate treatment response, and the proposed interpretations require confirmation in prospective studies incorporating longitudinal biomarker measurements and standardized therapeutic interventions.

This study has several strengths. The biomarkers were assessed within a single clinically characterized population and were analyzed together with symptom scores, pulmonary function, FeNO, eosinophil measurements, IgE, and exacerbation history. Asthma severity was evaluated using treatment intensity and clinical review rather than being defined exclusively by a single pulmonary function threshold. In addition, we examined both individual and combined biomarker performance and used bootstrap resampling to assess the internal stability of the combined model.

Several limitations should also be acknowledged. First, this was a single-center retrospective study with a relatively small sample size and only 20 severe-asthma events. The five-predictor multivariable model and the combined-biomarker classifier may therefore be overfit, and their coefficients, sample-derived cutoff, sensitivity, and specificity should be regarded as exploratory rather than clinically validated. Second, residual confounding cannot be excluded. Biomarker concentrations may have been affected by corticosteroid exposure, biologic therapy, obesity, smoking, allergic comorbidities, and the timing of blood collection relative to exacerbations; the retrospective records did not consistently allow all samples to be classified by proximity to an exacerbation or precise corticosteroid timing. Third, serum biomarkers were measured at a single time point; therefore, their temporal stability and responsiveness to treatment could not be evaluated. Fourth, airway samples such as induced sputum or bronchoalveolar lavage fluid were not analyzed, and direct relationships between serum concentrations and local airway inflammation could not be established. Fifth, the combined model underwent internal validation only and may overestimate performance in external populations. Finally, the cross-sectional relationship between biomarker levels and asthma severity does not establish causality.

## Conclusion

In conclusion, serum YKL-40, periostin, and EDN levels increased progressively with asthma severity and were associated with different but complementary clinical and inflammatory characteristics. Their combined assessment improved discrimination between severe and non-severe asthma within this cohort. These findings support further evaluation of the biomarkers as adjunctive tools for biological characterization and risk stratification;

larger prospective, multicenter studies with external validation are required before any cutoff or model is used in routine practice.

#### *Conflict of Interests*

The authors declared no conflict of interest.

#### *Ethical considerations*

The study protocol was approved by the Ethics Committee of Zhuhai Hospital of Integrated Traditional Chinese and Western Medicine. The approval specifically covered retrospective review of anonymized clinical data and the use of stored serum samples for biomarker measurements. Because of

the retrospective nature of the study and the use of anonymized clinical data, the requirement for written informed consent was waived by the Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

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#### **Conflict of interest statement**

All the authors declare that they have no conflict of interest in this work.

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