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8-Iso-prostaglandin F2α as a biomarker of type 2 low airway inflammation and remodeling in adult asthma

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ABSTRACT

Background: Although 8-iso-prostaglandin F2a has been proposed as a potential biomarker for oxidative stress in airway diseases, its specific role in asthma remains poorly understood.
Objective: To evaluate the diagnostic potential of 8-iso-prostaglandin F2a in assessing airway inflammation, airway remodeling, airway hyperresponsiveness, and oxidative stress in asthma.
Methods: Blood and urine concentrations of 8-iso-prostaglandin F2a were quantified using liquid chromatography–tandem mass spectrometry in 128 adults with asthma who had maintained antiasthma medications. Their correlations with clinical data, sputum cell counts, lung function parameters, and serum markers of epithelial/neutrophil activity and airway remodeling were then analyzed.
Results: The urinary 8-iso-prostaglandin F2a concentrations were significantly higher in patients with noneosinophilic asthma than in those with eosinophilic asthma ($P < .05$). The area under the curve was 0.678, indicating moderate diagnostic accuracy for noneosinophilic asthma. There were significant correlations with neutrophilic inflammation markers and airway remodeling markers (all $P < .05$). Negative correlations were observed with forced expiratory volume in 1 second (%), forced expiratory volume in 1 second/forced vital capacity, forced expiratory flow at 25% to 75% of forced vital capacity, and serum club cell protein 16 levels (all $P < .05$). High 8-iso-prostaglandin F2a concentrations were also noted in obese and smoking subgroups (all $P < .05$). However, the serum 8-iso-prostaglandin F2a concentrations were not correlated with these asthma-related parameters.
Conclusion: Urinary 8-iso-prostaglandin F2a concentrations are a potential biomarker for phenotyping severe asthma, particularly noneosinophilic asthma, offering oxidative stress-induced epithelial inflammation/remodeling as an additional target in asthma management.

Introduction

Asthma, characterized by chronic airway inflammation and variable expiratory airflow limitation, usually responds to treatment with inhaled corticosteroids (ICS) and/or β_2 agonists.[1] Nevertheless, a subset of patients continue to exhibit persistent airway inflammation despite treatment with ICS–long-acting β_2 agonist (LABA) combinations, leading to ongoing symptoms, exacerbations, and potential airway remodeling and fixed obstruction.[2] This underscores the need for new biomarkers to predict the persistence of inflammation and lung function decline in patients, despite optimal standard-of-care controller therapy.

Eicosanoids are biologically active lipids, including prostaglandins, leukotrienes, thromboxanes, and other inflammation and immune response mediators. They are important in the pathophysiologic mechanisms underlying asthma and allergic diseases.[3] Recent research has underscored the utility of noninvasive urinary eicosanoid metabolite profiling for molecular phenotyping in asthma, paving the way for individualized treatment strategies.[4] Among these, 8-iso-prostaglandin F2α (8-iso-PGF2α), predominant among F2-isoprostanes, has gained attention as a potential biomarker for oxidative stress in asthma.[5,6] Increased levels of 8-iso-PGF2α in serum, urine, and sputum are associated with airway inflammation and airway hyperresponsiveness in asthma, indicating its potential association with the type and severity of inflammatory airway diseases.[6-10]

However, the specific role of 8-iso-PGF2α in asthma remains poorly understood. Thus, we hypothesized that 8-iso-PGF2α could be a valuable biomarker for phenotyping asthma severity and

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inflammatory profiles. To explore this hypothesis, 8-iso-PGF2α concentrations in blood and urine samples from patients with asthma undergoing ICS/LABA combination therapy were analyzed, potentially guiding more effective management strategies.

Methods

Study Design and Participants

This cross-sectional observational study was conducted in a cohort of adult patients with asthma who had maintained ICS/LABA combinations according to asthma severity before participation. Overall, 128 patients were diagnosed with asthma on the basis of typical symptoms, such as shortness of breath, wheezing, chest discomfort, cough, and airway reversibility (an increase in forced expiratory volume in 1 second [FEV_1] of at least 12% and 200 mL after bronchodilator [BD] use) or airway hyperresponsiveness (≤16 mg/mL methacholine causing 20% decrease in FEV_1) were enrolled in this study. Blood and urine samples were collected from patients during their visits to the clinics for asthma management, without being limited to specific situations or conditions. This study also included a control group of 18 healthy individuals without a history of asthma or other chronic illnesses. Participants who had received systemic corticosteroids in the 30 days before enrollment or had a history of biologic therapy or chronic diseases, including chronic obstructive pulmonary disease, malignant diseases, or cystic fibrosis, were not included in the study.

The institutional review board of Ajou University Hospital approved this research (Reference numbers: AJIRB-BMR-SUR-15-498). All participants provided written consent before being included in this study.

Data Collection

Clinicodemographic data were collected, including age, sex, body mass index (BMI), and history of smoking. The levels of fractional exhaled nitric oxide and serum IgE were obtained using the NIOX system (Aerocrine AB, Solna, Sweden) and the ImmunoCAP system (Thermo Fisher Scientific, Waltham, Massachusetts), respectively. Sputum was obtained through the inhalation of hypertonic saline and subsequently treated with dithiothreitol for dispersion. Spirometry was used to assess lung function, measuring the forced vital capacity (FVC), FEV_1, the ratio of FEV_1 to FVC, and the midrange expiratory flow (forced expiratory flow at 25% to 75% of FVC [$FEF_{25\%-75\%}$]). Moreover, BD responsiveness was determined by calculating the percentage increase in FEV_1 values before and after BD application. Asthma symptom control and patients' life quality were assessed using the Asthma Control Questionnaire (ACQ)-6, Asthma Control Test (ACT), and Asthma Quality of Life Questionnaire (AQLQ) scores.

Variable Definitions

Patients who underwent sputum analysis were classified into 4 groups, determined on the basis of the levels of eosinophils and neutrophils in their sputum, which were consistent with previously established criteria.[11-14] The eosinophilic group had at least 3% eosinophils and less than65% neutrophils; the mixed granulocytic group, with at least 3% eosinophils and at least 65% neutrophils; the neutrophilic group, with less than3% eosinophils and at least 65% neutrophils; and the paucigranulocytic group, having neither. Patients with at least3% eosinophils and less than65% neutrophils were identified as having eosinophilic asthma; all others were categorized as having noneosinophilic asthma.

Participants with asthma were categorized into high-urinary 8-iso-PGF2α (≥1645.3 pg/mg creatinine, n = 54) and low-urinary 8-iso-PGF2α (< 1645.3 pg/mg creatinine, n = 74) groups on the basis of the cutoff point derived from the control's mean and an addition of 2 SDs. In addition, the participants were grouped on the basis of the ACQ-6, ACT, and AQLQ scores, with cutoff points set at 0.75, 20, and 6.0, respectively, as previously described.[15-17] High and low ACQ-6 scores indicated poor and better asthma control, respectively. In contrast, low ACT and AQLQ scores indicated poor asthma control. Further stratifications were made according to the smoking status as follows: smokers (current or past smokers with a history of ≥5 pack-years) and nonsmokers. Obesity was defined as having a BMI of at least30 kg/m^2, and overweight ranged between 25 and 29.9 kg/m^2.

Measurement of Urine and Serum 8-Iso-Prostaglandin F2α Concentrations

Urine and serum samples were rigorously analyzed following a comprehensive protocol. These samples were initially stored at −70° Cand subsequently thawed. Liquid chromatography–tandem mass spectrometry was performed to accurately quantify and identify various metabolites. The chromatographic separation of these metabolites was performed using a Hypersil GOLD column (2.1 mm × 100 mm, 1.9 μm) (Thermo Fisher Scientific, San Jose, California). Subsequently, the compounds were analyzed using an API5500 Triple Quadrupole mass spectrometer (AB Sciex, Framingham, Massachusetts). A deuterated internal standard, 8-iso-PGF2α-d4 (Cayman Chemical Company, Ann Arbor, Michigan), was used specifically to calibrate the mass spectrometer and accurately quantify 8-iso-PGF2α. In addition, urinary creatinine levels were quantified using 1,3-dimethyl-2-imidazolidinone (Sigma-Aldrich, St. Louis, Missouri) as the internal standard, facilitating the normalization of biomarker concentrations. Urinary 8-iso-PGF2α concentrations were categorized into low and high on the basis of the cutoff point of 1645.3 pg/mg creatinine.

Measurement of Other Serum Biomarkers

Various laboratory variables, including serum biomarkers associated with neutrophils and epithelial-derived airway remodeling, were also assessed. Specifically, serum levels of neutrophil-related biomarkers (eg, myeloperoxidase [MPO] and monocyte chemoattractant protein [MCP]-1) and epithelial-derived airway remodeling biomarkers (ie, matrix metalloproteinase [MMP]-9, tissue inhibitor of metalloproteinase [TIMP]-1, and transforming growth factor [TGF]-β1) were measured using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, Minnesota). In addition, serum concentrations of club cell protein 16 (CC16), a biomarker indicative of anti-inflammatory and antioxidant function in asthma and other respiratory diseases, were quantified using a standardized enzyme-linked immunosorbent assay kit (BioVendor GmbH, Heidelberg, Germany).

Statistical Analyses

For normally distributed variables, results were expressed as the mean with SD, and for nonnormally distributed data, the median, along with the IQR, was reported. We used the 2-sample *t* test or Mann-Whitney *U* test to analyze continuous data, and the χ^2 test was performed to assess categorical data. The comparison of BMI was analyzed using Welch's *t* test. To determine correlations among the variables, we used either Spearman's or Pearson's correlation coefficients, following the guidelines previously suggested for categorizing correlation strengths: weak (0.1-0.3), moderate (0.3-0.5), and strong (0.5-1.0).[18] The utility of the receiver operating characteristic curve was harnessed to determine the prognostic utility of urinary 8-iso-PGF2α levels in predicting noneosinophilic asthma. Given the exploratory nature of this study, no adjustments were made for multiple comparisons. The statistical analyses for this study were conducted

Table 1
Clinical Characteristics of the Study Participants

Study group	Patients with asthma				*P* value	
	Overall (n = 128)	High urinary 8-iso-PGF2α group (n = 54)	Low urinary 8-iso-PGF2α group (n = 74)	Healthy controls (n = 18)	high vs low 8-iso-PGF2α	patients with asthma vs control
Age (y)	50.0 ± 14.8	52.8 ± 15.4	48.0 ± 14.0	44.8 ± 10.6	.074	.154
Female sex (%)	83 (64.8)	35 (64.8)	48 (64.9)	13 (72.2)	.995	.606
Body mass index (kg/m^2)	23.9 ± 3.7	24.8 ± 4.5	23.2 ± 2.8	24.3 ± 3.1	.022	.631
≥30 kg/m^2	8 (6.3)	7 (13.0)	1 (1.4)	0 (0.0)		
25-29.9 kg/m^2	33 (25.7)	15 (27.8)	18 (24.3)	7 (38.9)		
< 25 kg/m^2	87 (68.0)	32 (59.2)	55 (74.3)	11 (61.1)		
Smoking history (pack-years)	12.5 (4.3-21.8)	20.0 (8.8-27.0)	5.0 (2.3-20.0)	0 (0.0)	.073	NA
Current or former (≥5 pack-years)	16 (12.5)	10 (18.5)	6 (8.1)	0 (0.0)		
Never	112 (87.5)	44 (81.5)	68 (91.9)	0 (0.0)		
Serum IgE level (kU/mL)	122.5 (35.0-399.8)	116.5 (44.0-384.0)	123.5 (34.0-406.0)	18.0 (7.0-37.5)	.514	<.001
Blood eosinophil count (/μL)	224.4 (127.4-405.4)	245.4 (135.1-476.0)	209.0 (116.4-416.6)	152.7 (77.0-191.4)	.368	<.001
FeNO (ppb)	31.7 (11.5-41.5)	23.5 (14.0-44.0)	17.0 (9.0-40.0)	20.5 (16.0-28.0)	.116	.030
Sputum eosinophils (%), n = 79	8.0 (0.0-52.0) (n = 79)	1.0 (0.0-21.0) (n = 36)	18.0 (0.0-60.8) (n = 43)	NA	.042	NA
Sputum neutrophils (%), n = 79	77.0 (36.0-91.0) (n = 79)	83.0 (48.5-91.5) (n = 36)	63.0 (16.3-90.8) (n = 43)	NA	.092	NA

Abbreviations: 8-iso-PGF2α, 8-iso-prostaglandin F2α; FeNO, fractional exhaled nitric oxide; NA, not available; ppb, parts per billion.
NOTE. Data are presented as number (No.) and percentage (%), mean (±SD), or median with IQR. For categorical variables, the χ^2 test was used, whereas the *t* test or the Mann-Whitney *U* test was applied for continuous variables. Participants with asthma were divided into high urinary 8-iso-PGF2α (≥1645.3 pg/mg creatinine, n = 54) and low urinary 8-iso-PGF2α (< 1645.3 pg/mg creatinine, n = 74) groups on the basis of the cutoff point derived from the control's mean and an addition of 2 SD.

using IBM Statistical Package for the Social Sciences statistics for Windows, version 25.0 (IBM Corporation, Armonk, New York) and the R statistical software (version 3.6.1) (R Core Team, Vienna, Austria). Statistical significance was set at *P* less than .05.

Results

Patient Characteristics

Overall, 8 (6.3%) and 33 (25.8%) patients were obese and overweight, respectively. Furthermore, 16 (12.5%) patients had a history of smoking greater than or equal to5 pack-years. In total, 79 patients could produce induced sputum; among them, 30 (38.0%), 16 (20.3%), 30 (38.0%), and 3 (3.8%) patients had eosinophilic, mixed, neutrophilic, and paucigranulocytic asthma phenotypes, respectively. Overall, 54 and 74 patients were classified into the high and low urinary 8-iso-PGF2α groups, respectively. Table 1 presents comparisons of the clinical characteristics between the 2 groups. In addition, comparisons of serum markers of neutrophil activity and airway remodeling, along with lung function parameters, are provided in eTable 1 and eFigure 1.

Associations Between Urinary 8-Iso-Prostaglandin F2α Concentration and Airway Inflammation

The urinary 8-iso-PGF2α concentrations were significantly higher in the noneosinophilic asthma subgroup than in the eosinophilic asthma subgroup ($P < .05$) (Fig 1A). When assessing the discriminatory capability of urinary 8-iso-PGF2α for noneosinophilic asthma, our receiver operating characteristic analyses revealed an overall model predictive capacity with an area under the curve of 0.678 (Fig 1B).

A significant association was found between urinary 8-iso-PGF2α concentrations and serum levels of MPO (Spearman ρ = 0.350, $P < .001$) and MCP-1 (Spearman ρ = 0.315, $P < .001$) (Fig 2A,B). Individuals with higher urinary 8-iso-PGF2α concentrations exhibited substantially increased serum MPO and MCP-1 levels compared with those with lower urinary 8-iso-PGF2α concentrations (all $P < .001$) (eFig 1A,B).

Sputum eosinophils were significantly lower in the high urinary 8-iso-PGF2α group than in the low urinary 8-iso-PGF2α group (1.0 [0.0-21.0] vs 18.0 [0.0-60.8], $P < .05$); however, the difference in blood eosinophil count between 2 groups was not significant (Table 1).

Associations Between Urinary 8-Iso-Prostaglandin F2α Concentration and Airway Remodeling

Urinary 8-iso-PGF2α concentrations were positively correlated with serum levels of MMP-9 (Spearman ρ = 0.254, $P < .005$), TIMP-1 (Spearman ρ = 0.196, $P < .05$), and TGF-β1 (Spearman ρ = 0.321, $P < .001$) (Fig 2C-E). Serum MMP-9, TIMP-1, and TGF-β1 levels were significantly higher in the high urinary 8-iso-PGF2α group than in the low urinary 8-iso-PGF2α group (all $P < .05$) (eFig 1C-E).

Meanwhile, urinary 8-iso-PGF2α concentrations were negatively correlated with FEV_1 (%) (Spearman ρ = −0.211, $P < .05$), FVC (%) (Spearman ρ = −0.156, $P = .079$), FEV_1/FVC (Spearman ρ = −0.200, $P < .05$), and $FEF_{25\%-75\%}$ (Spearman ρ = −0.252, $P < .005$) (Fig 3A-D). The high urinary 8-iso-PGF2α group exhibited significantly lower FEV_1 (%), FVC (%), FEV_1/FVC, and $FEF_{25\%-75\%}$ values than the low urinary 8-iso-PGF2α group (all $P < .05$) (eFig 1G-J).

Associations Between Urinary 8-Iso-Prostaglandin F2α Concentration and Airway Reversibility

Urinary 8-iso-PGF2α concentrations were positively correlated with post-BD FEV_1 change (%) (Spearman ρ = 0.192, $P < .05$) (Fig 3E). The post-BD FEV_1 change (%) was significantly higher in the high urinary 8-iso-PGF2α group than in the low urinary 8-iso-PGF2α group ($P < .05$) (eFig 1K).

Associations Between Urinary 8-Iso-Prostaglandin F2α Concentration and Asthma Symptoms

Patients with high ACQ-6 scores (≥0.75) had significantly higher urinary 8-iso-PGF2α concentrations than those with low scores ($P < .05$) (Fig 4A). Furthermore, patients with low ACT and AQLQ scores (<20 and <6.0, respectively) had significantly higher urinary concentrations of 8-iso-PGF2α than those with high scores (all $P < .05$) (Fig 4B,C).

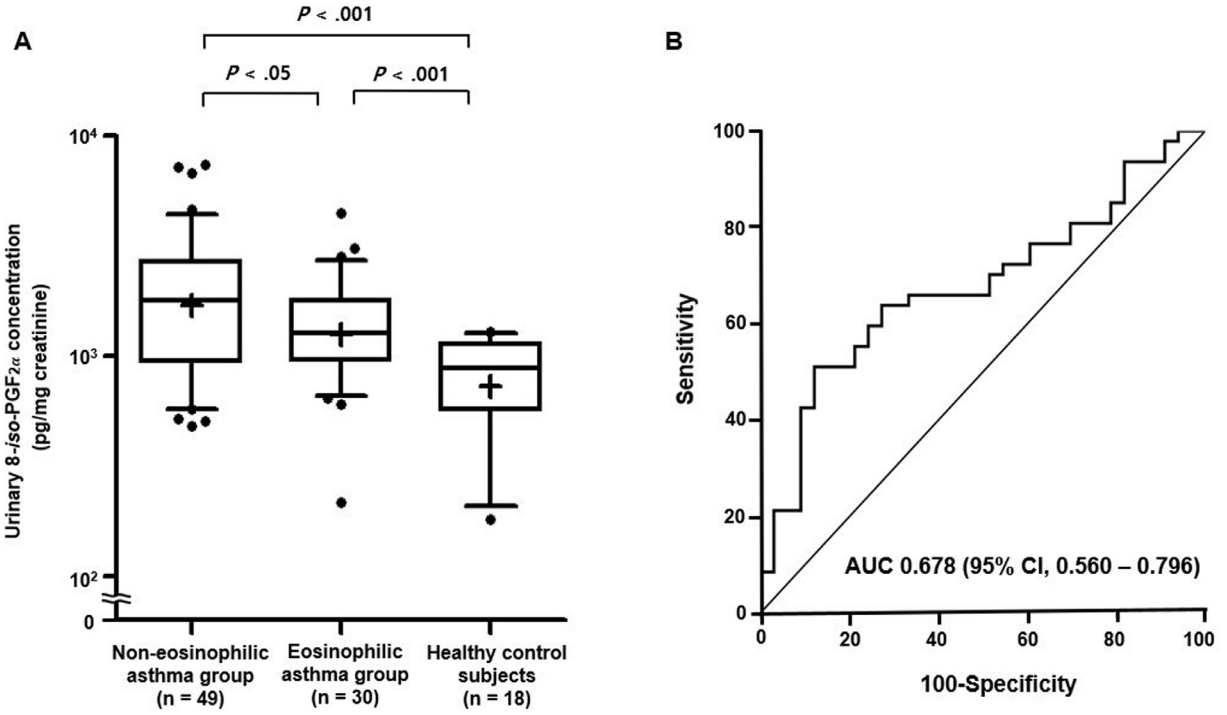

Figure 1. (A) Comparisons of urinary 8-iso-PGF2α concentrations among noneosinophilic and eosinophilic asthma groups and healthy control participants. (B) Receiver operating characteristic curve analysis for distinguishing noneosinophilic asthma in patients with asthma. AUC, area under the curve; PGF2α, prostaglandin F2α.

Urinary 8-Iso-Prostaglandin F2α Concentration as an Indicator of Oxidative Stress

Urinary 8-iso-PGF2α concentrations were significantly higher in the obese subgroup than in the nonobese subgroup ($P < .05$) (Fig 5A). They were higher in smokers than in nonsmokers; however, the difference was not significant ($P = .066$) (Fig 5B). A weak positive correlation was observed between urinary 8-iso-PGF2α concentrations and aging ($r = 0.117$, $P < .05$) (Fig 5C). In contrast, negative correlations were noted between the urinary 8-iso-PGF2α concentrations

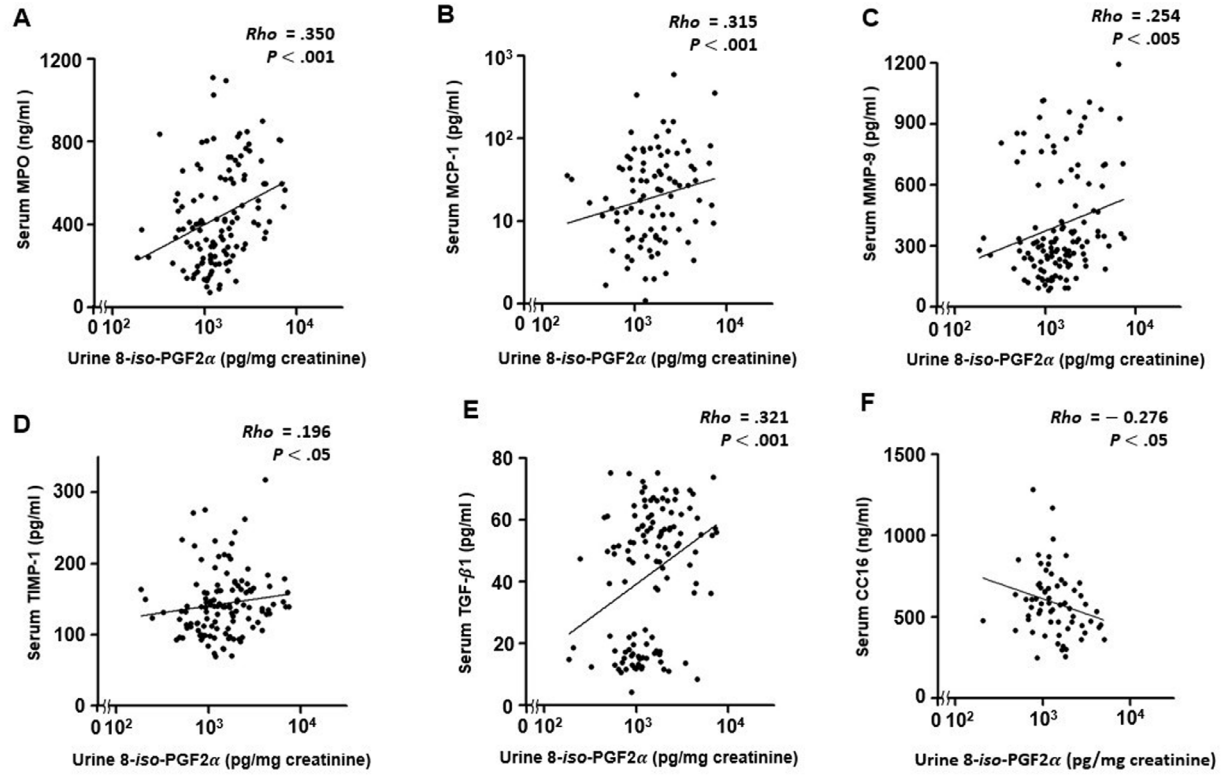

Figure 2. Spearman correlations of urinary 8-iso-PGF2α concentrations with serum markers associated with neutrophilic inflammation (A, B), epithelial-derived airway remodeling (C-E), and antioxidant property (F) in patients with asthma. 8-iso-PGF2α, 8-iso-prostaglandin F2α; CC16, club cell protein 16; MMP-9, matrix metalloproteinase; MPO, myeloperoxidase; TGF-β1, transforming growth factor β1; TIMP-1, tissue inhibitor of metalloproteinase-1.

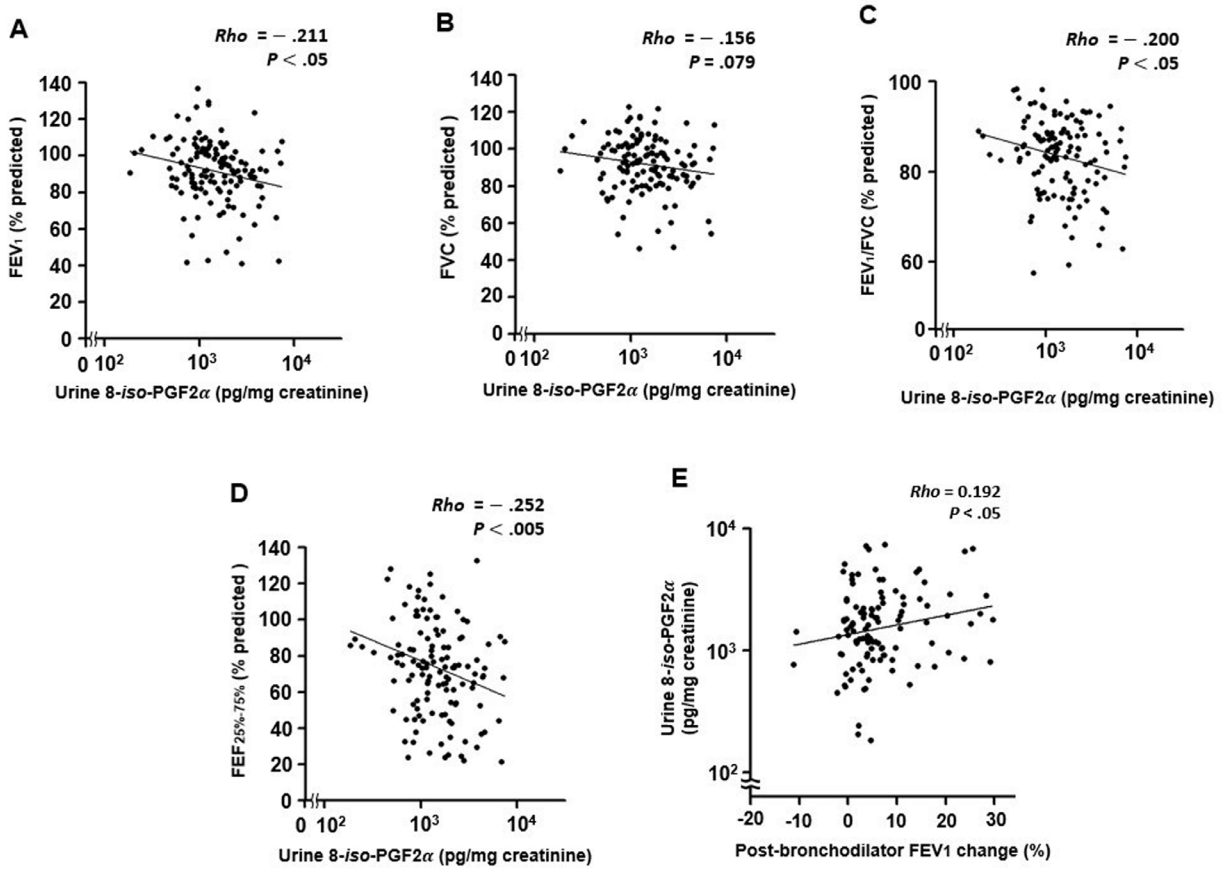

Figure 3. Spearman correlations of urinary 8-iso-PGF2α concentrations with lung function parameters (A-D) and airway reversibility (E) in patients with asthma. 8-iso-PGF2α, 8-iso-prostaglandin F2α; $FEF_{25\%-75\%}$, forced expiratory flow at 25% to 75% of FVC; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity.

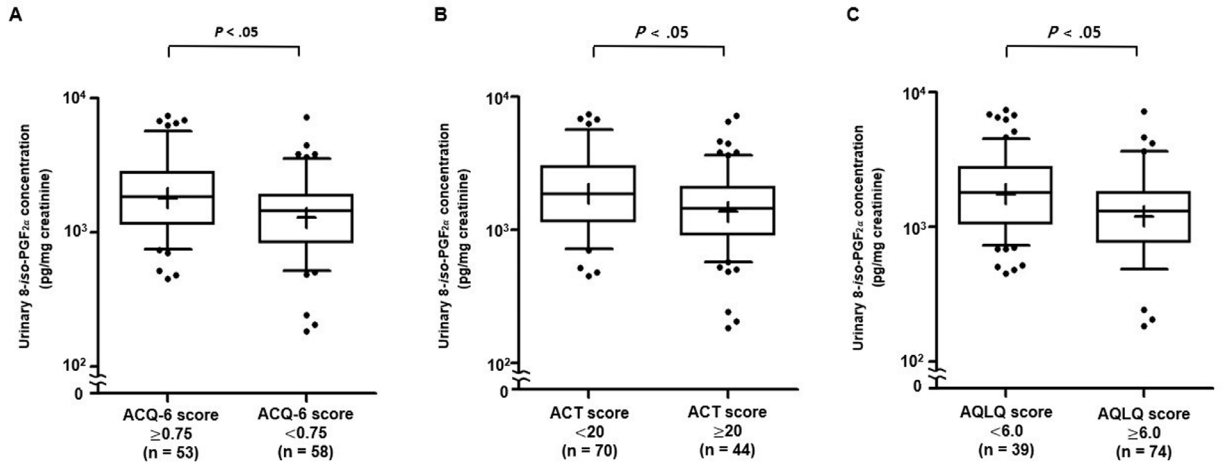

Figure 4. Comparative analysis of urinary 8-iso-PGF2α concentrations according to asthma symptom scores in 114 patients with asthma by ACQ-6 (A), ACT (B), and AQLQ (C). 8-iso-PGF2α, 8-iso-prostaglandin F2α; ACQ-6, asthma control questionnaire-6; ACT, asthma control test; AQLQ, asthma quality of life questionnaire.

and serum CC16 levels (Spearman ρ = −0.276, $P < .05$) (Fig 2F). Individuals with higher urinary 8-iso-PGF2α concentrations had significantly reduced serum CC16 levels compared with those with lower urinary 8-iso-PGF2α concentrations ($P < .05$) (eFig 1F).

Serum 8-Iso-Prostaglandin F2α Concentrations

Patients with asthma exhibited significantly elevated serum 8-iso-PGF2α concentrations compared with healthy controls (0.46 [0.28-0.76] vs 0.22 [0.14-0.31] ng/mL, $P < .05$) (eFig 2A). Serum and urinary 8-iso-PGF2α concentrations correlated significantly (Spearman ρ = 0.260, $P < .05$) (eFig 2B). The serum 8-iso-PGF2α concentration was higher in the obese subgroup than in the nonobese subgroup ($P < .05$) (Fig 5D). However, no significant associations were observed between serum 8-iso-PGF2α concentrations and other asthma-related parameters.

Discussion

This study found that higher urinary 8-iso-PGF2α concentrations are associated with noneosinophilic airway inflammation and worse

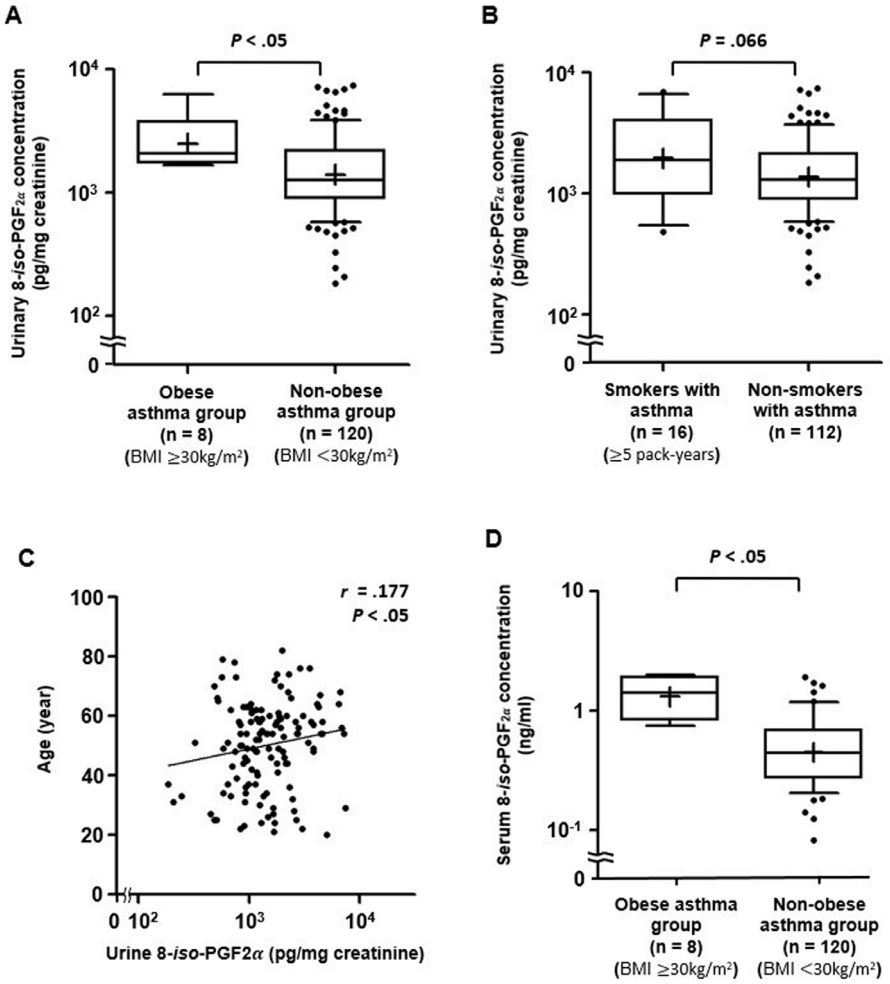

Figure 5. (A, D) Comparison of urine and serum concentrations between obese and nonobese asthma groups. (B) Comparison of urinary 8-iso-PGF2α concentrations between smokers and nonsmokers asthma groups. (C) Pearson correlations of urinary 8-iso-PGF2α concentrations with age in patients with asthma. 8-iso-PGF2α, 8-iso-prostaglandin F2α; BMI, body mass index.

airway remodeling, as evidenced by their correlations with serum type 2 (T2) low markers (MPO and MCP-1) and lung function parameters. High urinary 8-iso-PGF2α concentration is associated with airway remodeling markers (TIMP-1 and TGF-β1) and poorer symptom control scores. Furthermore, higher urinary 8-iso-PGF2α concentrations are observed in patients with asthma having obesity and smoking history, suggesting their potential as indicators of noneosinophilic asthma (oxidative stress-mediated airway inflammation and remodeling) in adults. These findings support additional treatment strategies beyond standard ICS/LABA therapy that need to be developed to effectively address the persistent airway inflammation and remodeling for patients with asthma with higher urinary 8-iso-PGF2α concentrations. This study's findings provide a comprehensive insight into the diagnostic potential of urinary 8-iso-PGF2α as a biomarker in adult asthma, particularly in the context of noneosinophilic asthma refractory to conventional anti-inflammatory medications.

This study presents the clinical utility of urinary 8-iso-PGF2α as a potentially promising biomarker for airway inflammation, remodeling, and reversibility associated with oxidative stress in asthma. One of the most notable results is the higher levels of urinary 8-iso-PGF2α in the noneosinophilic asthma subgroup than in the eosinophilic asthma subgroup. Currently, no accepted biomarkers exist for this phenotype. Although several biomarkers, including interleukin-17, neutrophil extracellular traps, YKL-40, and S100 calcium-binding protein A9, have been suggested, their clinical applicability remains constrained.[14,19] Our result is distinct from that of previous research as it uniquely identifies a biomarker in the urine of patients with asthma undergoing ICS/LABA therapies in real-world practice. The complexity of noneosinophilic asthma makes it challenging to define a single biomarker that could be universally applied. Our results contribute to a better understanding of underlying mechanisms and identifying reliable biomarkers to effectively manage noneosinophilic asthma.

Urinary 8-iso-PGF2α concentrations were significantly correlated with markers of neutrophilic inflammation (serum MPO and MCP-1 levels and sputum neutrophilia) and airway remodeling (serum MMP-9, TIMP-1, and TGF-β1 levels) in this study. Moreover, they were negatively correlated with lung function parameters (FEV_1, FVC, FEV_1/FVC, and $FEF_{25\%-75\%}$). Furthermore, 8-iso-PGF2α, a marker of oxidative stress, has been recognized for its role in cellular damage and inflammation in the airways, particularly in asthma.[6,9,20] The MPO, predominantly found in neutrophils, is an enzyme critical for defending against pathogens, such as bacteria, using hydrogen peroxide to produce potent oxidants that destroy these pathogens.[21] The oxidative stress generated in this process can damage epithelial and smooth muscle cells.[22] The MCP-1, a cytokine that attracts leukocytes to the site of inflammation, also acts on epithelial and smooth muscle cells, thereby augmenting the inflammatory response.[23] In epithelial cells, it can induce cell migration and proliferation, thereby facilitating inflammation-associated tissue remodeling.[22] Overall, increased urinary 8-iso-PGF2α levels may represent neutrophilic inflammation

in adult asthma, contributing to treatment insensitivity and asthma severity.

The MMP-9, TIMP-1, and TGF-β1 play pivotal roles in airway remodeling. The MMP-9, a matrix metalloproteinase crucial in the degradation of the extracellular matrix, contributes to airway remodeling by facilitating cell migration and proliferation.[24] The TIMP-1 plays a pivotal role in airway remodeling by balancing extracellular matrix synthesis and breakdown.[25] Beyond their well-acknowledged roles in structural alterations of the airways, MMP-9 and TIMP-1 have significant implications for inflammatory processes.[26] The TGF-β1, a growth factor that promotes fibrosis and cell proliferation, is involved in altering airway structure by inducing smooth muscle cell proliferation and fibrosis.[27] Meanwhile, CC16 plays a role in asthma by exerting anti-inflammatory properties and inhibiting airway remodeling, thereby helping to reduce the inflammatory response in response to oxidative stress.[17,28] This study found increased levels of MMP-9, TIMP-1, and TGF-β1; however, decreased CC16 levels were observed in patients with higher levels of urinary 8-iso-PGF2α. Positive correlations were noted between 8-iso-PGF2α and MMP-9/TIMP-1/TGF-β1. The role of MMP-9 is implicated in neutrophilic activation in patients with T2-low asthma.[29] These findings indicate that 8-iso-PGF2α is implicated in the interconnected mechanisms of airway inflammation, remodeling, and oxidative stress—mediated by MMP-9 and TIMP-1. New interventions targeting these pathways may provide strategies that mitigate disease progression and improve clinical outcomes.

Elevated levels of 8-iso-PGF2α were observed in the obese and smoking subgroups of this study, suggesting their potential as an indicator of oxidative stress in asthma. These results support previous findings that link higher serum and urinary levels of 8-iso-PGF2α with advanced age, obesity, and a history of smoking.[6,10] However, considering that high-density lipoprotein cholesterol plays a significant role in mitigating oxidative stress,[30] it becomes necessary for our analysis to account for high-density lipoprotein cholesterol levels as a confounding factor in future studies. Excessive accumulation of adipose tissue promotes inflammatory responses and reactive oxygen species production, potentially increasing 8-iso-PGF2α levels. The chemicals in cigarette smoke also promote reactive oxygen species production, leading to elevated 8-iso-PGF2α levels. Consequently, smoking cessation and proper weight control are fundamental strategies in asthma management. Furthermore, oxidative stress is closely related to aging and can contribute to the development of various aging-related diseases through cellular damage. Therefore, this individualized and comprehensive approach is necessary for asthma management, especially in older adults with asthma.

Asthma is characterized by a complex interplay of various inflammatory pathways, in which eicosanoids play a crucial role in mediating inflammatory responses.[3] 8-iso-PGF2α is recognized as an F2-isoprostane eicosanoid, indicative of oxidative stress levels. In contrast to other eicosanoids that are formed enzymatically (eg, leukotrienes, prostaglandins, and thromboxanes), F2-isoprostanes, including 8-iso-PGF2α are produced through nonenzymatic, free radical-driven peroxidation of arachidonic acid. This unique formation pathway means that 8-iso-PGF2α may be a specific metabolomic marker of T2-low airway inflammation in asthma, contrary to recent data showing elevated urinary leukotriene E4 and prostaglandin D2 metabolites in T2-high severe asthma and aspirin-exacerbated respiratory disease.[4] By integrating 8-iso-PGF2α into the broader context of the eicosanoid network in asthma, our research can enhance the understanding of the mechanism of airway inflammation, potentially guiding the development of more effective management strategies. In this study, serum 8-iso-PGF2α concentrations were revealed to have a significant relationship with obesity comparable to those of a previous study reporting that obese asthma is characterized by T2-low airway inflammation and poor clinical outcomes.[31] In addition, the short lifespan of eicosanoids and their rapid excretion by the kidneys result in fluctuating and low serum levels, making it challenging to establish consistent associations with various variables. The wide distribution of serum 8-iso-PGF2α concentrations observed in our study may indicate not only significant variability in oxidative stress among individuals but also the inherent difficulties in measuring such eicosanoids. The measurement of urinary metabolite levels represents the degree of systemic inflammation; however, urine collection is uncomfortable compared with serum collection in clinical practice. Therefore, more sensitive and specific assays for detecting serum 8-iso-PGF2α concentrations should be developed to accurately quantify their low and fluctuating levels.

This study had some limitations, particularly the limited sample size. Despite this, our cohort involved patients with well-characterized asthma under standard-of-care treatment in real-world clinical practice. Another limitation was the cross-sectional design. This observational study contributes to the existing literature by proposing a novel metabolomic biomarker in urine for the potential detection of T2-low/neutrophilic asthma. Future research should involve larger, more diverse, and longitudinal cohorts to validate our findings and strengthen the evidence on the discriminatory capability of urinary 8-iso-PGF2α concentration for classifying noneosinophilic asthma. In addition, our classification of correlation strengths is on the basis of general guidelines, and interpretations of these terms may vary depending on the specific criteria applied.

In conclusion, urinary 8-iso-PGF2α is a potentially promising biomarker for noneosinophilic and not well-controlled asthma. These results may contribute to achieving personalized asthma management by aiding patients' stratification and guiding therapeutic decisions.

Disclosures

The authors have no conflicts of interest to report.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.anai.2024.04.007.

References

1. GINA Report, Global Strategy for Asthma Management and Prevention; 2023 GINA main report. Available at: https://ginasthma.org/. Accessed April 24, 2024.
2. Porsbjerg C, Melén E, Lehtimäki L, Shaw D. Asthma. *Lancet*. 2023;401(10379):858–873.
3. Sokolowska M, Rovati GE, Diamant Z, Untersmayr E, Schwarze J, Lukasik Z, et al. Current perspective on eicosanoids in asthma and allergic diseases: EAACI Task Force consensus report, part I. *Allergy*. 2021;76(1):114–130.
4. Kolmert J, Gómez C, Balgoma D, Sjödin M, Bood J, Konradsen JR, et al. Urinary leukotriene E4 and prostaglandin D2 metabolites increase in adult and childhood severe asthma characterized by type 2 inflammation. A clinical observational study. *Am J Respir Crit Care Med*. 2021;203(1):37–53.
5. Sahiner UM, Birben E, Erzurum S, Sackesen C, Kalayci O. Oxidative stress in asthma. *World Allergy Organ J*. 2011;4(10):151–158.
6. Basu S. Bioactive eicosanoids: role of prostaglandin F 2α and F 2-isoprostanes in inflammation and oxidative stress related pathology. *Mol Cells*. 2010;30(5):383–391.
7. Milne GL, Yin H, Hardy KD, Davies SS, Roberts 2nd LJ. Isoprostane generation and function. *Chem Rev*. 2011;111(10):5973–5996.
8. Jonasson S, Hjoberg J, Hedenstierna G, Basu S. Allergen-induced formation of F2-isoprostanes in a murine asthma model identifies oxidative stress in acute airway inflammation in vivo. *Prostaglandins Leukot Essent Fatty Acids*. 2009;80(1):1–7.

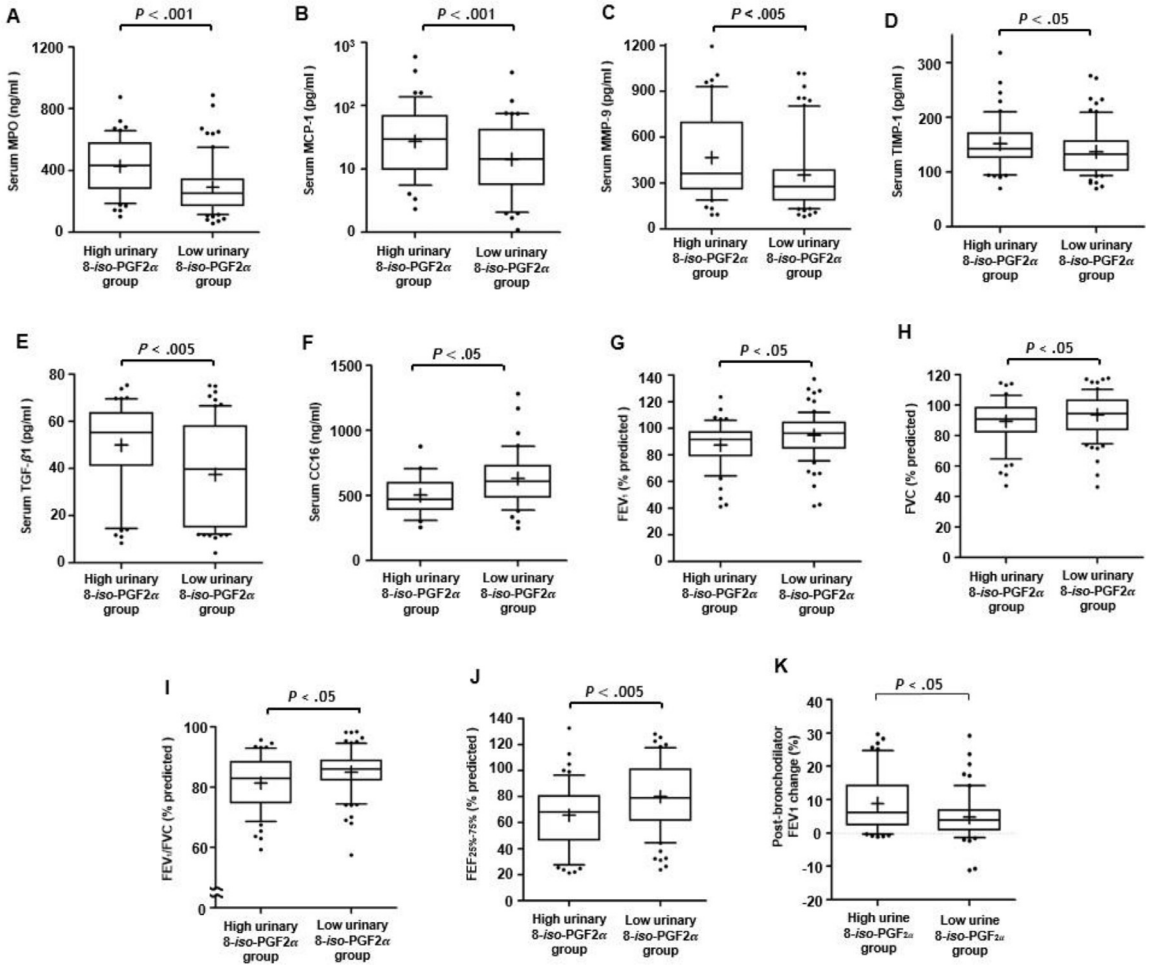
9. Wood LG, Garg ML, Simpson JL, Mori TA, Croft KD, Wark PA, et al. Induced sputum 8-isoprostane concentrations in inflammatory airway diseases. *Am J Respir Crit Care Med*. 2005;171(5):426–430.
10. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers—smoking as a cause of oxidative damage. *N Engl J Med*. 1995;332(18):1198–1203.
11. Abdo M, Uddin M, Goldmann T, Marwitz S, Bahmer T, Holz O, et al. Raised sputum extracellular DNA confers lung function impairment and poor symptom control in an exacerbation-susceptible phenotype of neutrophilic asthma. *Respir Res*. 2021;22(1):167.
12. Svenningsen S, Nair P. Asthma endotypes and an overview of targeted therapy for asthma. *Front Med.*. 2017;4:158.
13. Wan R, Jiang J, Hu C, Chen X, Chen C, Zhao B, et al. Neutrophil extracellular traps amplify neutrophil recruitment and inflammation in neutrophilic asthma by stimulating the airway epithelial cells to activate the TLR4/NF-κB pathway and secrete chemokines. *Aging (Albany NY)*. 2020;12(17):16820.
14. Quoc QL, Choi Y, Thi Bich TC, Yang EM, Shin YS, Park HS. S100A9 in adult asthmatic patients: a biomarker for neutrophilic asthma. *Exp Mol Med*. 2021;53(7):1170–1179.
15. Juniper E, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J*. 1999;14(4):902–907.
16. Sá-Sousa A, Amaral R, Morais-Almeida M, Araújo L, Azevedo LF, Bugalho-Almeida A, et al. Asthma control in the Portuguese National Asthma Survey. *Rev Port Pneumol (2006)*. 2015;21(4):209–213.
17. Louis G, Pétré B, Schleich F, Nekoee Zahrei H, Donneau AF, Henket M, et al. Predictors of change in asthma-related quality of life: a longitudinal real-life study in adult asthmatics. *Qual Life Res*. 2023;32(5):1507–1520.
18. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. Academic Press; 2013.
19. Lee Y, Quoc QL, Park HS. Biomarkers for severe asthma: lessons from longitudinal cohort studies. *Allergy Asthma Immunol Res*. 2021;13(3):375.
20. Dworski R, Roberts 2nd LJ, Murray JJ, Morrow JD, Hartert TV, Sheller JR. Assessment of oxidant stress in allergic asthma by measurement of the major urinary metabolite of F2-isoprostane, 15-F2t-IsoP (8-iso-PGF2α). *Clin Exp Allergy*. 2001;31(3):387–390.
21. Monteseirín J, Bonilla I, Camacho J, Conde J, Sobrino F. Elevated secretion of myeloperoxidase by neutrophils from asthmatic patients: the effect of immunotherapy. *J Allergy Clin Immunol*. 2001;107(4):623–626.
22. Dickerhof N, Huang J, Min E, Michaëlsson E, Lindstedt EL, Pearson JF, et al. Myeloperoxidase inhibition decreases morbidity and oxidative stress in mice with cystic fibrosis-like lung inflammation. *Free Radic Biol Med*. 2020;152:91–99.
23. Sousa A, Lane SJ, Nakhosteen JA, Yoshimura T, Lee TH, Poston RN. Increased expression of the monocyte chemoattractant protein-1 in bronchial tissue from asthmatic Participants. *Am J Respir Cell Mol Biol*. 1994;10(2):142.
24. Barbaro MPF, Spanevello A, Palladino GP, Salerno FG, Lacedonia D, Carpagnano GE. Exhaled matrix metalloproteinase-9 (MMP-9) in different biological phenotypes of asthma. *Eur J Intern Med*. 2014;25(1):92–96.
25. Kim SH. Roles of tissue inhibitor of metalloproteinase-1 in severe asthma. *Allergy Asthma Immunol Res*. 2023;15(4):416.
26. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuna JM, Perez-Romero BA, Guerrero-Rodriguez JF, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci*. 2020;21(24):9739.
27. Halwani R, Al-Muhsen S, Al-Jahdali H, Hamid Q. Role of transforming growth factor −β in airway remodeling in asthma. *Am J Respir Cell Mol Biol*. 2011;44(2):127–133.
28. Goudarzi H, Kimura H, Kimura H, Makita H, Takimoto-Sato M, Abe Y, et al. Association of serum CC16 levels with eosinophilic inflammation and respiratory dysfunction in severe asthma. *Respir Med*. 2023;206: 107089.
29. Quoc QL, Cao TBT, Moon JY, Jang JH, Shin YS, Choi Y, et al. Contribution of monocyte and macrophage extracellular traps to neutrophilic airway inflammation in severe asthma. *Allergol Int*. 2024;73(1):81–93.
30. Vazzana A, Ganci A, Cefalu AB, Lattanzio S, Noto D, Santoro N, et al. Enhanced lipid peroxidation and platelet activation as potential contributors to increased cardiovascular risk in the low-HDL phenotype. *J Am Heart Assoc*. 2013;2(2): e000063.
31. Fitzpatrick AM, Chipps BE, Holguin F, Woodruff PG. T2-"low" asthma: overview and management strategies. *J Allergy Clin Immunol Pract*. 2020;8(2):452–463.

Supplementary Data

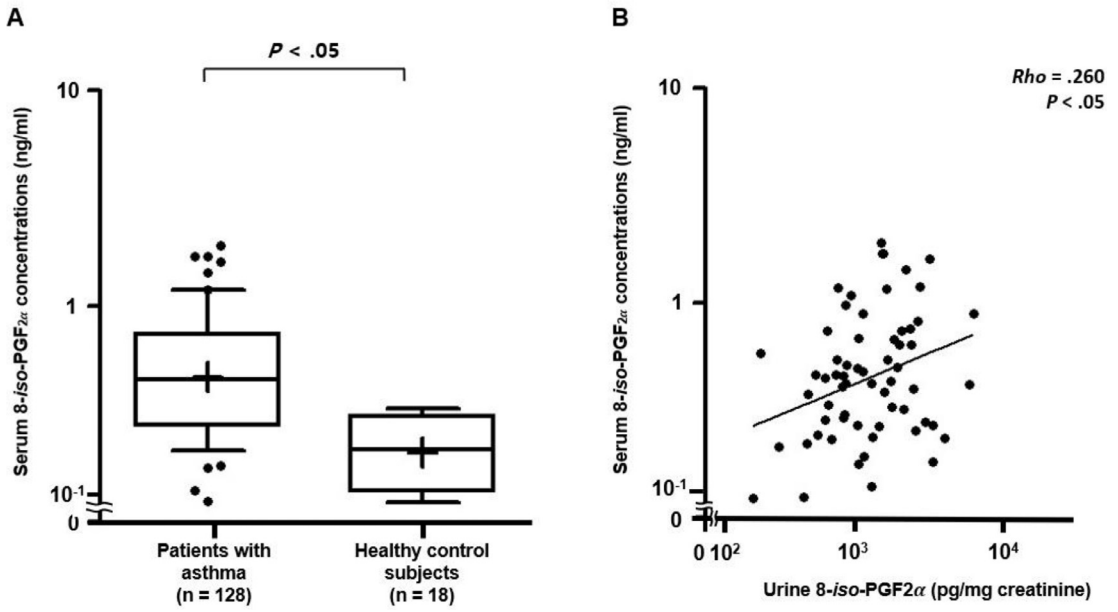
eTable 1
Comparison of Serum Markers Associated With Neutophilic Inflammation, Epithelial-Derived Airway Remodeling, Antioxidant Property, and Lung Function Parameters Between the High and Low Urinary 8-iso-Prostaglandin F2α Groups

Study group	High urine 8-iso-PGF2α level (n = 54)	Low urine 8-iso-PGF2α level (n = 74)	*P* value
Serum biomarkers			
Serum MPO (ng/mL)	541.4 (355.2-717.9)	315.5 (213.4-423.3)	<.001
Serum MCP-1 (pg/mL)	24.6 (6.5-56.9)	6.1 (0.0-30.7)	<.001
Serum MMP-9 (pg/mL)	361.8 (263.7-686.0)	277.5 (196.0-381.0)	.005
Serum TIMP-1 (pg/mL)	142.5 (127.3-170.1)	132.3 (103.7-154.6)	.039
Serum TGF-β1 (pg/mL)	55.2 (42.9-63.0)	39.8 (15.3-57.8)	.004
Serum CC16 (ng/mL)	470.8 (390.3-596.1)	608.4 (485.6-729.3)	.013
Lung function			
FEV1 (% predicted)	91.6 (80.1-96.7)	96.3 (85.5-104.1)	.030
FVC (% predicted)	90.8 (82.6-98.2)	94.3 (84.1-103.0)	.059
FEV1/FVC ratio (%)	82.9 (75.1-88.4)	86.0 (82.4-88.8)	.031
FEF25%-75% (% predicted)	68.2 (47.5-79.0)	79.0 (63.8-100.7)	.004
Postbronchodilator FEV1 change (%)	6.1 (2.3-14.3)	3.8 (0.9-6.9)	.012

Abbreviations: 8-iso-PGF2α, 8-iso-prostaglandin F2α; CC16, club cell protein 16; FEF25%-75%, forced expiratory flow at 25% to 75% of FVC; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; MMP-9, matrix metalloproteinase; MPO, myeloperoxidase; TGF-β1, transforming growth factor β1; TIMP-1, tissue inhibitor of metalloproteinase-1.



eFigure 1. Comparison of serum markers associated with neutophilic inflammation (A, B), epithelial-derived airway remodeling (C-E), antioxidant property (F), and lung function parameters (G-K) between the high and low urinary 8-iso-PGF2α groups. 8-iso-PGF2α, 8-iso-prostaglandin F2α. CC16, club cell protein 16; FEV_1, forced expiratory volume in 1 s; $FEF_{25\%-75\%}$, forced expiratory flow at 25–75% of FVC; FVC, forced vital capacity; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; MPO, myeloperoxidase; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase.



eFigure 2. Serum 8-iso-PGF2α concentrations in adult asthma. Comparisons of serum 8-iso-PGF2α concentrations between patients with asthma and healthy control participants (A), and spearman correlations between serum and urinary 8-iso-PGF2α concentrations (B). 8-iso-PGF2α, 8-iso-prostaglandin F2α.