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# Interplay of clinical biomarkers in allergic asthma diagnosis and severity: A case-control study

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**Abstract**

Given asthma's large phenotypic diversity, the study was aimed to use specific biomarkers to characterize Allergic asthma (AA) and its severity. Blood was collected from 42 healthy controls (HCs) and 96 patients with AA. Biomarkers related to blood cell number and function: total leukocyte count (TLCs), neutrophil, lymphocyte, monocyte, eosinophil, basophil, neutrophil-to-lymphocyte ratio (NLR), immunoglobulin E (IgE), tryptase and eosinophilic cationic protein (ECP) as well as remodelling biomarkers (Matrix metalloproteinase (MMP-9), (MMP-16), Fibroblast growth factor (FGF-18) and (FGF-23) and alpha-skeletal muscle actin-1 (ACTa-1) were measured. Significant differences were observed in hematological parameters with higher levels of total leukocytes, eosinophil, and basophil counts in the AA group compared to HCs. The disease group also had significantly higher levels of several serum biomarkers (IgE, TPs, ECP, MMP-9, MMP-16, FGF-18, FGF-23, and ACTa-1) compared to HC. Forced expiratory volume 1 (FEV1) and forced vital capacity (FVC) had a strong negative correlation with ECP, IgE, and ACTa-1. FEV1 was negatively correlated with MMP-16 and tryptase. Patients with AA have higher levels of several biomarkers, such as MMP-9, MMP-16, FGF-18, FGF-23, IgE, tryptase, and ACTa-1. In addition, IgE, tryptase, ACTa-1, and MMP-16 are related to lung function impairment in AA. This indicates that measuring multiple biomarkers may be of value in the future when diagnosing and monitoring AA.

**Keywords:** Allergic asthma, Biomarkers, Diagnosis, Remodelling and severity

## 1. Introduction

Asthma can be classified into various phenotypes and endotypes. Allergic asthma (AA) is one of the common asthma phenotypes. The most common symptoms of AA are coughing, wheezing, chest tightness, and mucus production. AA treatment is complex [1] and biomarkers and pulmonary function variables such as forced expiratory volume 1 (FEV1) and forced vital capacity (FVC) are often used when diagnosing and monitoring AA.

Eosinophil cationic protein (ECP) is one of the biomarkers sometimes used to monitor AA. ECP has many biological functions, including its cytotoxic function. ECP is vital in tissue repair processes because of its ability to activate mucus secretion in the airways [2]. ECP can be detected in different biological fluids and is used as a clinical tool in asthma [3]. Another inflammatory marker is the neutrophil-to-lymphocyte ratio (NLR) which often is increased in asthma [4].

Matrix metalloproteinases (MMPs) are a family of enzymes that have a role in degrading extracellular matrix proteins and impact many lung diseases including AA [5]. MMP-9 has been reported to be elevated in the serum and sputum of asthmatic patients. Furthermore, MMP-16 is also thought to contribute to the pathophysiology of AA.

Airway remodelling in AA. is caused by chronic inflammation, leading to fibroblast cell growth, smooth muscle thickening, and mucous cell metaplasia [6]. Fibroblast growth factor (FGF), released by the fibroblast cells, is a mitogenic factor that drives remodelling. FGF-18 and FGF-23 are part of the FGF family, linked to airway inflammation and respiratory diseases [7, 8].

Immunoglobulin E (IgE) is primarily responsible for immune defence against parasitic infections but also plays a crucial role in allergic disorders such as AA and allergic rhinitis [9]. The binding of IgE to the FcεR1 receptor on the mast cell triggers the mast cell to degranulate and increases tryptase [10]. Tryptase (TPs), is an enzyme found in mast cell granules that contributes to acute airway inflammation, leading to an increase in airway mucus secretion. Additionally, it contributes to the remodelling and fibrosis of lung tissue [11]. The degranulation of mast cells is also enhanced by muscle contraction. The contraction

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is associated with alpha-skeletal muscle actin-1 (ACTa-1). The latter is present in the lung biopsy of severe asthmatic patients who respond to anti-IgE immunotherapy [12].

Personalized medicine is used as a targeted therapy approach for treating different diseases. Managing AA through personalized medicine in the future may require finding effective biomarkers [13, 14]. The study aimed to evaluate the level of different biomarkers in patients with AA and healthy controls (HCs) and to study the association between biomarkers and lung function in AA.

## 2. Materials and methods

### 2.1. Study design and participants

This case-control study was carried out between April 2021 and March 2022 in Sulaymaniyah, Erbil, and Kirkuk Asthma Diagnostic Centres in northern Iraq. It comprised 138 participants, divided into 96 AA (46 males/ 50 females) with a mean age of (44.0 ± 1.530) and 42 HCs (22 males/20 females) with a mean age (38.1 ±2.112). The diagnosis of AA patients was made by a respiratory physician based on the Global Initiative for Asthma (GINA) guideline [15]. The diagnostic procedure included clinical history, laboratory tests including IgE, pulmonary function tests, and having at least two respiratory symptoms: cough, sneeze, and dyspnoea. The patients were excluded if they had other chronic diseases, respiratory disorders or had been taking oral corticosteroids four weeks prior to the study inclusion.

The ethical committee of the College of Medicine/University of Sulaimani, approved the current study (Rs23476, 25th March 2021). Prior to sample collection, each participant was required to submit a consent form. If the patient had disabilities or couldn't complete the form, it was obtained from their legal companions.

### 2.2. Sample and data collection

Ten millilitres of blood was drawn from each participant, it was put into an EDTA tube for cell differential count and serum separation tubes (SSTs) for serological tests. SST was left for 30 minutes to let clot the blood; then it was centrifuged for 10 minutes at 3000 rpm. The serum samples were stored at -70 $^{o}$C.

A data management system in the Asthma Diagnostic Centres containing patient information was used to fill out a questionnaire form. This included the patient's medical history, clinical data, disease duration, and demographics.

### 2.3. Pulmonary function tests and laboratory markers determination

Computerized spirometry (Jaeger, Germany) was used to assess pulmonary function thin (FEV1 and FVC). The differential count was done using the hematological analyzer (Sysmex XT-2000i, Japan). IgE was analysed with Cobas e411 (Roche Diagnostics, Switzerland cat.No 04827031190). Special enzyme-linked immunosorbent assay (ELISA) kits were used to evaluate all other biomarkers (TPs cat.No E0950Hu), (ECP cat.No E1391Hu), (MMP-9 cat.No.E0936Hu), (MMP-16 cat.No E5644Hu), (FGF18 cat.No 5287Hu), (FGF23 cat.No E0059Hu) and (ACTa-1 cat.No E2972Hu), according to the manufacturer's instructions (BT Lab, China).

### 2.4. Statistical analysis

The normality tests (Shapiro-Wilk, Kolmogorov-Smirnov, De-Agostino) in GraphPad 9 Prism (GraphPad Software, USA) were performed to know if the data were normally distributed. As the data have passed normality tests, we applied parametric tests for all data. Data were presented as mean ± standard error of the mean (SEM). T-test was used for comparison of the two groups. The Pearson correlation test was used to assess the correlation between the variables. The receiver operating characteristic (ROC) curve was used to test the parameters that distinguished AA from HCs**.** A ROC curve in MedCalc 20 (MedCalc Software, Belgium) provided values for area under the curve (AUC), cut-off, specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV). To predict the severity of AA, we utilized multivariate binary logistic regression and multiple linear regression in SPSS 29 (IBM, USA). A statistical significance was assumed if the p-value was less than 0.05.

## 3. Results

### 3.1. Comparison of demographics and clinical characteristics between HCs and Allergic asthma patients.

There was no difference in gender distribution between the asthma and HC groups, but the asthmatics group had a higher mean age and BMI (Table 1).

### 3.2. Comparison of pulmonary function tests and laboratory biomarkers between HCs and Allergic asthma patients

FEV1/FVC and FEV1 were significantly lower in the asthma than in the control group (Table 2). Total leukocyte, eosinophil, and basophil counts were significantly higher in the asthma than in the HC group (Table 2). There were no significant group differences regarding neutrophils, lymphocytes, NLR and monocytes of the serum biomarkers, the asthma group had significantly higher levels of IgE, TPS, ECP, MMP-9, MMP-16, FGF18, FGF23, and

**Table 1.** Comparison of demographics and clinical characteristics between HCs and AA patients.

| Parameters | HCs M ± SEM | AA M ± SEM | P-value |
|---|---|---|---|
| N | 42 | 96 | - |
| Gender (m/f) | 22/20 | 46/50 | 0.712 |
| Age (Years) | 38.10 ±2.112 | 43.97 ± 1.530 | 0.032 |
| BMI | 25.80 ± 0.526 | 27.86 ± 0.478 | 0.01 |
| Smoking (Yes/No) | NA | 26 (27.08%)/70 (72.92%) | - |

The comparison between groups was done via independent t-test in SPSS. Data were presented as mean (M) ± standard error of mean (SEM), p-value < 0.05 was considered as statistically significant. Abbreviations: BMI: body mass index, f: female, M: mean, m: male, SEM: standard error of the mean.

ACTa-1), than in the HC (Table 2).

### 3.3. Correlation among the variables

Correlation analyses were conducted to evaluate how the serum biomarker levels relate to lung function. In patients with AA, ECP, IgE, and ACTa-1 were negatively correlated with FEV1/FVC. Similarly, FEV1 showed a significant negative correlation with IgE. Regarding ECP, it was strongly correlated with ACTa-1, FGF-18 and MMP-16 (Table 3).

### 3.4. Multiple linear regression of some laboratory markers predicts PFTs in asthmatic patients.

The study analyzed the independent variables (TLCs,

**Table 2.** Comparison of PFTs and laboratory biomarkers between HCs and AA patients.

| Parameters | HCs M ± SEM | AA M ± SEM | P-value (Odds ratio) |
|---|---|---|---|
| FEV1/FVC | 107.05 ± 1.668 | 67.04 ± 1.144 | <0.001 |
| FEV1 | 97.46 ±1.394 | 63.11 ±1.282 | <0.001 |
| TLC | 7.02 ± 0.201 | 8.32 ± 0.226 | 0.001 |
| Neutrophil | 3.93 ± 0.166 | 4.48 ± 0.195 | 0.083 |
| Lymphocyte | 2.49 ± 0.130 | 4.05 ± 1.623 | 0.527 |
| NLR | 1.837 ± 0.160 | 2.175 ± 0.211 | 0.315 |
| Monocyte | 0.42 ± 0.022 | 0.90 ± 0.560 | 0.568 |
| Eosinophil | 0.09 ± 0.011 | 0.91 ± 0.041 | <0.001 |
| Basophil | 0.07 ± 0.004 | 0.09 ± 0.008 | 0.027 |
| IgE | 41.52 ±6.753 | 221.00 ±12.981 | <0.001 |
| ECP | 10.73 ±0.633 | 46.03 ± 2.119 | <0.001 |
| FGF-18 | 286.43 ± 45.395 | 1748.31 ± 113.457 | <0.001 |
| FGF-23 | 50.42 ±8.371 | 249.08 ±66.131 | 0.050 |
| MMP-16 | 28.70 ±4.213 | 654.30 ±58.262 | <0.001 |
| MMP-9 | 321.47 ±18.361 | 1624.77 ± 200.456 | <0.001 |
| TPS | 4.77 ±0.831 | 208.61 ±41.761 | 0.002 |
| ACTa-1 | 8.71 ±1.666 | 26.37 ±1.588 | <0.001 |

The comparison between groups was done via an independent t-test in SPSS 29. Data were presented as mean (M) ± standard error of mean (SEM), and p-value < 0.05 was considered as statistically significant. Abbreviations: ACTa-1: alpha-skeletal muscle actin-1, ECP: eosinophil cationic protein, FEV: forced expiratory volume, FVC: forced vital capacity, FGF: Fibroblast growth factor, IgE: immunoglobulin E, MMP: Matrix metalloproteinase, M: mean, NLR: neutrophil-to-lymphocyte, SEM: standard error of mean, TLC: total leukocyte count, TPS: tryptase.

**Table 3.** Correlation among various variables in patients with AA.

| | | FEV1/FVC | FEV1 | FGF-18 | MMP-16 | ACTa-1 | TPS | ECP | FGF-23 | MMP-9 | IgE | NLR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FEV1/FVC | R | | 0.791** | -0.310** | -0.391** | -0.452** | -.362** | -0.598** | -.133 | -.275** | -0.495** | .018 |
| | P-value | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| FEV1 | R | | | -.271** | -0.419** | -.399** | -.159 | -0.535** | -.084 | -.239** | -0.578** | -.006 |
| | P-value | | | 0.001 | <0.001 | <0.001 | 0.063 | <0.001 | 0.325 | 0.005 | <0.001 | 0.941 |
| FGF-18 | R | | | | .340** | 0.736** | .136 | 0.729** | .006 | .073 | .080 | .063 |
| | P-value | | | | .000 | .000 | .113 | 0.000 | .945 | .395 | .352 | .465 |
| MMP-16 | R | | | | | 0.445** | .175* | 0.542** | .010 | .139 | .306** | .074 |
| | P-value | | | | | <0.001 | 0.040 | <0.001 | 0.903 | 0.104 | <0.001 | 0.390 |
| ACTa-1 | R | | | | | | .222** | 0.888** | .035 | .140 | .158 | .147 |
| | P-value | | | | | | 0.009 | <0.001 | 0.682 | 0.100 | 0.064 | 0.085 |
| TPS | R | | | | | | | .288** | .054 | .080 | .181* | -.053 |
| | P-value | | | | | | | 0.001 | 0.530 | 0.353 | 0.034 | 0.539 |
| ECP | R | | | | | | | | .057 | .199* | .278** | .170* |
| | P-value | | | | | | | | 0.509 | 0.019 | 0.001 | 0.047 |
| FGF-23 | R | | | | | | | | | .041 | .181* | .010 |
| | P-value | | | | | | | | | 0.633 | 0.034 | 0.911 |
| MMP-9 | R | | | | | | | | | | .232** | .017 |
| | P-value | | | | | | | | | | 0.006 | 0.847 |
| IgE | R | | | | | | | | | | | .023 |
| | P-value | | | | | | | | | | | 0.793 |

A Pearson correlation was done by SPSS 27. Probability (p)-value less than 0.05 was considered significant. Abbreviations: ACTa-1: alpha-skeletal muscle actin-1, ECP: eosinophil cationic protein, FEV: forced expiratory volume, FVC: forced vital capacity, FGF: Fibroblast growth factor, IgE: immunoglobulin E, MMP: Matrix metalloproteinase, M: mean, NLR: neutrophil-to-lymphocyte, R: correlation coefficient, TPS: tryptase.

Lymphocyte, neutrophil, eosinophil, basophil, NLR, IgE, TPs, ECP, MMP-9, MMP-16, FGF-18, FGF-23, and ACTA-1) to establish their relationship with the dependent variables, FEV1/FVC and FEV1. According to Table 3, TPS and IgE serve as a predictor for decreasing FEV1/FVC (B=-0.009) and FEV1 (B= -.020), respectively. The regression equation only included significant contributing variables (Table 4).

### 3.5. Evaluating biomarkers' diagnostic value in asthma patients using ROC curve.

Candidate biomarkers for distinguishing between AA and controls were evaluated by constructing an ROC curve (Figure 1, Table 5). Determining optimal cut-off values and distinguishing the true state of subjects are key functions of Area Under Curve (AUC) in assessing diagnostic ability.

ECP (AUC value: 0.999) was found to be the most reliable biomarker for differentiation when comparing the AA group to the HC group, followed by MMP16 (AUC value: 0.998), TPS (AUC value: 0.996), and FGF18 (AUC value: 0.981). Each of them had cut-off values of 21.30, 150.89, 21.14, and 0.981, respectively. Nevertheless, MMP-9, IgE, FGF23, and ACTa-1 were recognized as usual biomarkers for detecting AA. NLR (AUC value: 0.568) was found to be an inadequate biomarker, with a cut-off value of 1.937 for distinguishing asthma patients from HCs.

## 4. Discussion

Asthma is a heterogeneous disease, which makes it challenging to treat. Instead of examining the pathological mechanisms, disease severity is predicted using various biomarkers [16]. The current study examined previous and novel biomarkers in AA patients to identify markers that aid in categorizing the disease and personalizing patient treatment.

In the current study, the group with AA patients had higher levels of total leukocyte count than the control group. This finding aligns with the result of [17], who found that increased leukocyte levels in asthmatic patients correlated with longer hospitalization periods and the need for mechanical ventilation. The inflammatory response of AA, which is mediated by cytokines and chemokines, results in

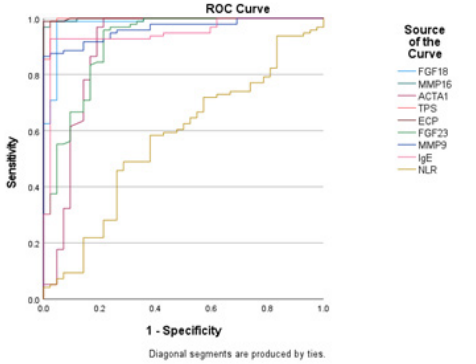


**Fig. 1.** The ROC curve analysis was plotted using MedCalc 20. Abbreviations: ACTa-1: alpha-skeletal muscle actin-1, AUC: area under the curve, ECP: eosinophil cationic protein, FGF: Fibroblast growth factor, IgE: immunoglobulin E, MMP: Matrix metalloproteinase, M: mean, NLR: neutrophil-to-lymphocyte ratio, TPS: tryptase.

**Table 4.** Multiple linear regression of some laboratory markers predicts PFT in patients with AA.

| Dependent Variables | Independent Variables | B | p-value | Tolerance | VIF |
|---|---|---|---|---|---|
| TPS | FEV1/FVC | -.009 | <0.0001 | 1.000 | 1.000 |
| IgE | FEV1 | -.020 | 0.047 | 1.000 | 1.000 |

We used multivariate regression in SPSS 29 to see how independent variables (WBC, Lymphocyte, neutrophil, eosinophil, basophil, NLR, FGF-18, MMP-16, ACTa-1, TPS, ECP, FGF-23, MMP-9, and IgE) were associated with FEV1/FVC or FEV1. The stepwise method was applied to exclude variables not associated with Dependent Variables. A p-value of less than 0.05 was considered statistically significant. Abbreviations: ACTa-1: alpha-skeletal muscle actin-1, B: regression coefficient ECP: eosinophil cationic protein, FEV: forced expiratory volume, FVC: forced vital capacity, FGF: Fibroblast growth factor, IgE: immunoglobulin E, MMP: Matrix metalloproteinase, M: mean, NLR: neutrophil-to-lymphocyte, TPS: tryptase, VIF: variance inflation factor.

**Table 5.** ROC curve to assess diagnostic value of biomarkers in patients with AA.

| Biomarkers | AUC | P-value | Cut-off |
|---|---|---|---|
| FGF18 | 0.981 | <0.001 | 845.01 |
| MMP16 | 0.998 | <0.001 | 150.89 |
| ACTA1 | 0.892 | <0.001 | 10.99 |
| TPS | 0.996 | <0.001 | 21.14 |
| ECP | 0.999 | <0.001 | 21.30 |
| FGF23 | 0.913 | <0.001 | 83.29 |
| MMP9 | 0.962 | <0.001 | 643.14 |
| IgE | 0.945 | <0.001 | 97.92 |
| NLR | 0.568 | 0.200 | 1.937 |

The ROC curve analysis was carried out using SPSS 29. Statistical significance was established with a p-value less than 0.05. Abbreviations: ACTa-1: alpha-skeletal muscle actin-1, AUC: area under the curve, ECP: eosinophil cationic protein, FGF: Fibroblast growth factor, IgE: immunoglobulin E, MMP: Matrix metalloproteinase, M: mean, NLR: neutrophil-to-lymphocyte ratio, TPS: tryptase.

an increase in the total number of leukocytes.

The level of eosinophil, basophil, and IgE increases in allergic inflammation [18]. Similarly to Price's findings, the current research discovered an increase in eosinophil levels associated with asthma exacerbation [19]. The damage to airway epithelial cells can be caused by eosinophils through an increase in free radicals [20]. Anti-IL-5 immunotherapy (e.g. mepolizumab) reduces the occurrence of asthma attacks in individuals with asthma by decreasing eosinophil count [21]. T-helper 2 (Th2) lymphocytes play a role in AA and release IL-5, IL-4, and IL-13 [22]. Eosinophil maturation, proliferation, and activation are induced by IL-5 [23].

Eosinophil mediators play a role in asthma pathology, with ECP being one of them and causing inflammatory complications in AA. ROC curve analysis in this study confirmed ECP's potential as a diagnostic marker for the disease. Its diagnostic value is limited because of the increase in numerous other respiratory disorders [24]. This study has established that ECP exacerbates asthma, and shows a negative correlation with PFTs (FEV1 and FEV1/FVC).

B-lymphocyte is stimulated to produce IgE by IL-4 and IL-13, released by Th2 and basophil upon allergen exposure [25]. Identifying AA relies on detecting elevated levels of IgE. In the current study, an elevation in IgE was observed, which was associated with a decrease in FEV1 levels. Like our study, other studies have found an increase in IgE levels and its correlation with the severity of asthma [26, 27]. Binding IgE to FcεR1 on basophil and mast cells leads to a release of various mediators, including histamine and TPS [18]. This study suggests that the cause of elevated basophil levels may be related to elevated IgE levels, as confirmed by the correlation found in the current results. The tryptase of the present research was elevated in AA patients and linked with FEV1/FVC depression and disease severity. Gao et al., study found that tryptase is associated with the severity of childhood asthma which is consistent with this finding [28]. Furthermore, the ROC curve in this study demonstrated the ability of IgE and TPS to distinguish individuals with AA from those without HCs.

The growth of airway smooth muscle and mucosal glands, known as hypertrophy, could be associated with fixed airway obstruction in severe asthma due to airway remodelling. Hypertrophy of airway smooth muscle mass is observed in addition to an increased number of fibroblasts, myofibroblasts, and collagen-3 deposition in the bronchial wall. To reveal airway remodelling, a bronchial biopsy is required. However, it carries a risk, as it is an invasive procedure. The diagnostic accuracy of AA can be determined by analyzing non-invasive airway remodelling biomarkers including MMP-9, MMP-16, FGF-18, and FGF-23 through blood analysis, as confirmed by ROC curve analysis in this research. The current study found that AA patients had higher levels of airway remodelling biomarkers compared to HCs. According to this study's correlation, MMP-16 was linked to higher ECP and lower FEV1. According to [29], MMP-16 was linked to the severity of asthma and inflammation. MMP-9 in this study has demonstrated a weak correlation with PFTs (FEV1 and FEV1/FVC). However other researchers found that MMP-9 could predict mild and moderate asthma, but not severe asthma, [30], similar to what this study found. In the present study, FGF-18 significantly correlated with ECP, ACTa-1, and MMP-16. A study has shown that oxidative stress is increased by FGF-18, leading to lung damage [31] may release ACTa-1 into the blood. Moreover, FGF-18 enhances oxidative stress, resulting in the up-regulation of MMPs and collagenase activity. This disrupts the lung's extracellular matrix and exacerbates respiratory complications [32].

The present research discovered a correlation between FGF-23 and IgE. Limited research has focused on FGF-23 in AA. Chronic inflammatory lung diseases like cystic fibrosis and COPD show increased levels of FGF-23, which induces lung inflammation by inhibiting vitamin D activation in the kidney and lung [31, 33]. By upregulating NADPH oxidase (NOX) and suppressing superoxide dismutase (SOD) expression, FGF-23 amplifies reactive oxygen species (ROS) production, leading to increased oxidative stress. Oxidative stress mediates the NF-kB pathway, causing human dendritic cells to upregulate IL-8 synthesis and produce more IgE, as demonstrated in previous studies.

Our results proved that the level of ACTa-1 was higher in the AA group than in HCs, which has higher diagnostic value as the ROC curve confirmed. Some results have the association of ACTa-1 with the severity of asthma. The presence of it was observed in the lung biopsy of severe asthmatic patients who respond well to anti-IgE immunotherapy (Omalizumab), [12]. Despite this, ACTa-1 is utilized as a biomarker to measure the severity of several diseases, including myopathy and hypotonia result lack of ability to move, breathe, and swallow [34, 35]. This result is the first to reveal the correlation between ACTa-1 and the depression of FEV1/FVC, which indicates the severity of the disease.

Some limitations exist in this study. First, the small sample size may affect the result's accuracy. The sample was only taken from three cities in Iraq, a larger sample from across the country would provide more accurate results. Finally, insufficient knowledge about the signs and symptoms of patients with AA has resulted in a lack of asthma score findings.

## 5. Conclusion

The biomarkers such as IgE, Tryptase, ECP, MMP-9, MMP-16, FGF-18, and FGF-23, can distinguish between AA and HCs with high diagnostic accuracy. Through depressing PFTs, IgE, TPS, and MMP-16 can predict the severity of AA. The usefulness of ACTa-1 in diagnosing and assessing the severity of AA has been brought to light in this first-of-its-kind study.

Based on these findings, physicians should refine asthma management to prevent future exacerbations by understanding the contributing factors and pathological processes driving these exacerbations.

## Interest Conflict

The authors have no conflict of interest to declare.

## Consent for publications

The author read and approved the final manuscript for publication.

## Ethics approval and consent to participate

The study was carried out by the Code of Ethics of the World Medical Association (Declaration of Helsinki) for

experiments involving human subjects. The Ethical Committee of the Sulaimani University, College of Medicine approved the research protocol (No. 77, Date 18/5/2021), and written informed consent was obtained from each subject before his enrolment in the study.

## Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Authors' contributions

Zainab Khaleel Mohammed: conceptualization, data curation, methodology, formal analysis, project administration, writing, original draft, and writing review and editing. Shukur Wasman Smail: data curation, statistical analysis. Christer Janson: Conceptualisation, formal analysis, and writing—review and editing. Kawa Amin: Conceptualisation, methodology, data curation, formal analysis, visualization, resources, software, supervision, and writing—review and editing.

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