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Abstract

Objective: Asthma and chronic obstructive pulmonary disease (COPD) are airflow limitation diseases with similar clinical manifestations but different pathophysiologic mechanisms. To implement the appropriate treatment, it is important to distinguish between asthma and COPD which sometimes might result difficult in clinical practice. We evaluated biomarkers to distinguish between asthma and COPD. *Methods:* Blood eosinophil counts and fractional exhaled nitric oxide (FeNO) levels were analyzed. Serum periostin, interleukin-25 (IL-25), and immunoglobulin E (IgE) concentrations were compared between patients with asthma (n=60), including atopic-asthma (n=30) and non-atopic asthma (n=30), and patients with COPD (n=30). *Results:* Significantly higher peripheral blood eosinophil counts ($p<0.001$), FeNO levels ($p<0.001$), and total serum IgE ($P=0.003$) concentrations, but not serum periostin ($p=0.584$) or serum IL-25 ($p=0.085$) concentrations, were detected in patients with asthma compared to patients with COPD. Serum periostin and IgE concentrations were increased in patients with atopic-asthma compared with those with non-atopic asthma and COPD ($p<0.05$). The FeNO levels were significantly correlated with the peripheral blood eosinophil counts ($r=0.430$,

p=0.001) and serum IL-25 concentrations ($r=0.338$, $p=0.009$) in patients with asthma. The serum periostin concentrations were also correlated with the serum IgE concentrations ($r=0.375$, $p=0.003$) and FeNO levels ($r=0.291$, $p=0.024$) in patients with asthma. Asthma patients were effectively differentiated from COPD patients based on the FeNO levels ($p<0.001$) and peripheral blood eosinophil counts ($p<0.001$). *Conclusions:* FeNO levels and peripheral blood eosinophil counts were useful biomarkers for distinguishing between patients with asthma and COPD. Serum periostin and IgE concentrations could be biomarkers for atopic asthma.

Introduction

Asthma is a common respiratory disease characterized by reversible airway obstruction, airway hyperresponsiveness, and chronic airway inflammation, especially with eosinophils. Asthma is mainly caused by sensitizing agents and results from Th2 inflammation characterized by interleukin (IL)-5, IL-13, mast cells, and eosinophils dominant in the airway [1-3]. Asthma has clinical and molecular heterogeneity, and classical phenotypes, such as atopic and non-atopic asthma, which complicates diagnostic evaluations and affects therapeutic responsiveness. Patients with atopic asthma are sensitized by environmental allergens such as mites and pollen, and have specific IgE against these allergens, whereas patients with non-atopic asthma do not have such specific IgE [4]. Chronic obstructive pulmonary disease (COPD) is a common preventable disease that is characterized by persistent respiratory symptoms and airflow limitation due to airway and /or alveolar abnormalities usually caused by overexposure to noxious particles or gases. COPD results from Th1 inflammation characterized by neutrophils and macrophages dominant in the airway [5, 6]. Both asthma and COPD are associated with

airflow limitation, but their pathophysiologic mechanisms differ. Typical asthma symptoms include episodic and fluctuating dyspnea, wheezing, and cough, especially at night. On the other hand, COPD is prevalent in the elderly and closely related with smoking. The symptoms of COPD are relatively continuous, with dyspnea on exertion. Because both diseases often exhibit similar clinical manifestations, such as dyspnea, cough, and wheezing, it is sometimes difficult to distinguish between them clinically [3, 5, 7]. Therefore, the discovery of biomarkers to facilitate the differential diagnosis between asthma and COPD based on pathophysiologic mechanisms would be useful.

In asthma, biomarkers are used to explore the underlying pathogenesis, disease phenotypes, and to identify subgroups of patients who preferentially respond to treatments [8, 9]. Sputum eosinophils and elevated fractional exhaled nitric oxide (FeNO) levels identify patients with asthma highly responsive to inhaled corticosteroid. Biomarkers may also identify a phenotype of severe asthma with a greater response to novel treatments targeting eosinophilic type 2 inflammation, such as blood eosinophils, or serum immunoglobulin E (IgE) and periostin concentrations. These biomarkers could be useful for identifying responders to anti-IL-5 monoclonal antibody (mAb), anti-IgE mAb, and anti-IL-13 mAb therapy [10-12].

In this study, in order to identify biomarkers that distinguish between asthma and COPD, we evaluated several biomarkers in the peripheral blood and airway in patients with clinically typical asthma or clinically typical COPD.

Methods

Study subjects

The study included 30 patients with atopic asthma (22 women and 8 men; age, 39.8 ± 2.9 years), 30 patients with non-atopic asthma (24 women and 6 men; age, 62.5 ± 3.3 years), and 30 patients with COPD (2 women and 28 men; age, 71.0 ± 1.6 years). They were enrolled by a pulmonologist at the outpatient clinic of the Department of Respiratory Medicine, Kawasaki Medical School. Asthma patients were non-smokers, aged ≥ 18 years, and diagnosed based on a clinical history of episodic cough, wheezing, shortness of breath especially at night, as well as a positive bronchodilator test ($>12\%$, and $>200\text{mL}$), according to the Global Initiative for Asthma guidelines [3]. Patients with atopic asthma exhibited specific serum IgE against house dust mites, with a class score of at least 3 of 6 (CLEIA; $1.80 \leq$, MBL, Nagano, Japan). Those with non-atopic asthma had no specific IgE against common inhaled allergens (grass, tree, weed pollen, animal, mite, and mold allergens) in the serum. Exclusion criteria of asthma included atopic dermatitis or interstitial pneumonia. Subjects were oral or inhaled corticosteroid-naïve, with a new diagnosis of mild-moderate persistent asthma [3], and subjects having a respiratory tract infection within the previous 8 weeks were excluded. COPD patients aged >40 years with a smoking history of >10 pack-years were diagnosed according to the Global Initiative for COPD criterion, characterized by persistent airway limitation (post-bronchodilator forced expiratory volume in 1 s to forced vital capacity ratio of <0.70) [5]. Exclusion criteria included a history of asthma (episodic wheezing or shortness of breath at night), atopic dermatitis, or interstitial pneumonia. Subjects were also excluded if they were current smokers, had a respiratory tract infection within 8 weeks. All subjects were oral or inhaled corticosteroid-naïve. All patients

provided written informed consent to participate in the study, and the human ethics review committee of Kawasaki Medical School approved the study protocol.

Measurement of systemic biomarkers

Blood eosinophil counts, and serum concentrations of total IgE and specific IgE against common inhaled allergens, IL-25, IL-33, thymic stromal lymphopoietin (TSLP), ST2, and periostin were determined. Serum samples for biomarker analysis were stored at -80°C until analysis. Serum concentrations of IL-25, IL-33, TSLP, ST2, and periostin were measured by enzyme-linked immunosorbent assay using the respective ELISA kits (IL-25: Abnova, Walnut, CA, USA, IL-33, TSLP: R&D Systems, Minneapolis, MN, USA, ST2: MBL, Nagoya, Japan, Periostin: Aviscera Bioscience, Santa Clara, CA, USA). The detection limits were 1.9 pg/mL (IL-25), 3.1 pg/mL (IL-33), 3.4 pg/mL (TSLP), 32 pg/mL (ST2), and 2 ng/mL (periostin). Concentrations below the detection limits were assumed to be zero for the purpose of statistical analysis.

Measurement of FeNO

FeNO levels were measured by an online electrochemical NO analyzer (NIOX MINO Analyzer, Aerocrine AB, Solna, Sweden), as previously described [13]. Patients were instructed to inhale NO-free air to total lung capacity and immediately exhale fully into the device at a flow rate of 50 mL/s; the mean of three values (<10% variation) was reported.

Statistical analysis

Characteristics of patients are presented as median values (25%-75%). Other data are expressed as mean $\pm$ standard error. The Kruskal-Wallis test was used to compare values of different groups. Inter-group comparisons were examined by the Mann-Whitney U test. Nonparametric Spearman's correlation was used for analyses of correlations between two continuous variables. To investigate the optimum threshold, receiver-operating curves were prepared. Differences with probability values of less than 0.05 were considered significant. Statistical analysis was performed with Prism 6 software (GraphPad Software, La Jolla, CA, USA).

Results

Characteristics of the patients and biomarkers, including blood examination data, are shown in Table 1. Patients with asthma were younger than patients with COPD, but the difference was not significant ($p=0.1$). While women comprised the majority of patients with asthma (46/60, 76.7%), men comprised the majority of patients with COPD (28/30, 93.3%). COPD patients had decreased lung function compared with asthma patients ($p<0.001$). Peripheral blood eosinophil counts ($p<0.001$), FeNO levels ($p<0.001$), and total serum IgE concentrations ($P=0.003$), but not serum periostin ($p=0.584$) and IL-25 ($p=0.085$) concentrations, were significantly higher in patients with bronchial asthma than in patients with COPD (Fig. 1). Serum concentrations of IL-33, TSLP, and ST2 were under the detection limit in patients with asthma and COPD (data not shown). To examine the contribution of atopic status in asthma, atopic and non-atopic asthma were analyzed separately. Peripheral blood eosinophil counts and FeNO levels were also higher in patients with atopic and non-atopic asthma than in patients with COPD ($p<0.001$, Fig. 2). Interestingly, serum periostin and IgE concentrations were increased in patients with atopic

asthma compared to those with non-atopic asthma and COPD ($p < 0.05$, Fig. 2). The FeNO levels were significantly correlated with the peripheral blood eosinophil counts ($r = 0.430$, $p = 0.001$) and serum IL-25 concentrations ($r = 0.338$, $p = 0.009$) in patients with asthma. Furthermore, the serum periostin concentrations were also correlated with the total serum IgE concentrations ($r = 0.375$, $p = 0.003$) and FeNO levels ($r = 0.291$, $p = 0.024$) in patients with asthma (Fig. 3). Next, a receiver-operating curve was prepared to calculate the optimum peripheral blood eosinophil counts (cutoff: $200/\mu\text{L}$, area under the curve [AUC]: 0.811, $p < 0.001$, 78.5 % sensitivity and 73.3% specificity, Fig. 4), FeNO levels (cutoff: 30.1ppb, AUC: 0.943, $p < 0.001$, 88.3% sensitivity and 83.3% specificity, Fig. 4), and serum IgE concentrations (cutoff: 136 IU/mL, AUC: 0.706, $p = 0.003$, 60.0% sensitivity and 62.5% specificity, Fig. 4) to distinguish asthma patients from COPD patients (Fig. 4).

Discussion

In the present study, we evaluated several biomarkers to investigate their ability to distinguish patients with asthma and COPD. Peripheral blood eosinophil counts and FeNO levels were significantly higher in patients with both atopic and non-atopic asthma than in patients with COPD. Serum periostin and total IgE concentrations were significantly higher in patients with atopic asthma, but not non-atopic asthma, compared to patients with COPD. The FeNO levels, in particular, could effectively differentiate patients with asthma from those with COPD.

FeNO is used as a marker for Th2-mediated airway inflammation in asthma [14]. FeNO is induced in the bronchial epithelial cells in response to IL-4 and IL-13, which are produced

locally by the Th2 cells, mast cells, and eosinophils [15]. Therefore, an increased FeNO level indicates the involvement of these cytokine mechanisms in the pathophysiology of eosinophilic airway inflammation. The majority of asthma is associated with eosinophilic inflammation [16]. Previous studies demonstrated that increased FeNO levels are associated with eosinophilia in the blood and sputum [17, 18]. FeNO is a potentially valuable aid in asthma diagnosis [19]. In the present study, we found increased FeNO levels and peripheral blood eosinophil counts in patients with both atopic and non-atopic asthma compared to patients with COPD ($p < 0.001$, Fig. 2), and FeNO levels significantly correlated with the peripheral blood eosinophil counts in patients with asthma ($r = 0.430$, $p = 0.001$, Fig. 3), including atopic ($r = 0.467$, $p = 0.009$, data not shown) and non-atopic asthma ($r = 0.377$, $p = 0.04$, data not shown). Instead of FeNO, peripheral blood eosinophil counts could be a useful biomarker to clinically differentiate asthma from COPD without overlapping asthma.

Periostin, a matricellular protein secreted from bronchial epithelial cells, is induced by IL-4 and IL-13 [20]. Periostin gene expression in airway epithelial cells is associated with an asthma phenotype driven by increased expression of IL-5 and IL-13, with high serum IgE concentrations, systemic and lung eosinophilia, increased thickness of the basement membrane, and responsiveness to corticosteroids [21, 22]. A recent study demonstrated that serum periostin concentrations were positively associated with FeNO levels, blood eosinophil counts, and serum IgE concentrations in obstructive airway disease [23]. In the present study, we found that the serum periostin concentration was positively correlated with the serum IgE concentrations and FeNO levels in patients with asthma. Those biomarkers could be associated with Th2 cytokine, IL-4, and IL-13.

Increased serum periostin levels are reported in patients with atopic dermatitis and interstitial pneumonia [24]. Patients with atopic dermatitis and interstitial pneumonia were excluded in the present study. Whether serum periostin levels are increased in patients with asthma remains controversial. One study reported that serum periostin levels were significantly higher in Japanese patients with asthma than healthy subjects (average: 92.8 versus 39.1 ng/mL) [25]. Another series reported that serum periostin levels in adults of a predominantly Caucasian population without asthma or COPD were similar to those in adults with asthma (average: 51.2 ng/mL), but the authors noted a trend towards higher serum periostin levels in Asian participants [26]. In the present study, serum periostin levels were similar between patients with non-atopic asthma and those with COPD. Interestingly we found higher levels of serum periostin in Japanese asthma patients with high levels of house dust mite-specific IgE. Ethnicity or the allergen itself might contribute to the increase in serum periostin levels. Further studies are required to clarify the reasons for the differences in serum periostin levels.

IL-25 induces type 2 innate lymphoid cells and Th2 cells to produce IL-4, IL-5, and IL-13, and plays a critical role in airway eosinophilia, mucus overproduction, and airway remodeling in mouse models of allergic airway disease [27, 28]. Cheng et al reported that IL-25 might contribute to the pathogenesis of Th2-high allergic asthma in adults. They demonstrated that both the plasma concentration and mRNA expression of IL-25 were elevated in a subset of asthma patients with enhanced airway hyperresponsiveness, an increased number of eosinophils in the circulation and airway, and higher expression of Th2 cytokines [29]. In the present study, we found that serum IL-25 concentrations were positively correlated with FeNO levels in

patients with asthma ($r=0.338$, $p=0.009$, Fig. 3), but not COPD ($r=-0.095$, $p=0.619$, data not shown). IL-25 might contribute to the eosinophilic airway inflammation in patients with asthma.

This study has several limitations. The study population was small, but clinically typical patients with atopic and non-atopic asthma, or COPD were selected to find the biomarker to differentiate those diseases. Patients that were clearly sensitized to house dust mites, one of the most common allergens, were used as the atopic asthma subgroup. To exclude patients with overlapping asthma and COPD in this study, we recruited only non-smoking asthma patients, and COPD patients with a suspected history of episodic asthma like symptoms were excluded. Furthermore, COPD patients that were current smokers were excluded, because active smoking is associated with decreased FeNO and serum periostin levels [26, 30]. There were some significant differences in the characteristics of the asthma and COPD patients, such as age and sex. Peripheral blood eosinophil counts and serum periostin levels are not associated with age or sex [26, 31]. Olin et al. demonstrated that individuals more than 64 years of age had higher FeNO levels than those 35-44 years of age [32]. Another study reported that mean FeNO levels were slightly higher in men than those in women, but were not affected by age [33]: COPD patients were older than the asthma patients, and included more men (COPD, 93%: asthma, 23%). Although these factors might influence the biomarkers analyzed in this study, the effect on the results obtained in this study is small. Elderly asthma patients, especially those with non-atopic asthma share considerably more similar symptoms with COPD patients. The age of the COPD patients was similar to that of patients with non-atopic asthma, but there were significant differences in the peripheral blood eosinophil counts and FeNO levels. It may be possible to

distinguish between asthma and COPD in patients with different background factors in the clinic based on peripheral blood eosinophil counts and FeNO levels.

Conclusion

In conclusion, FeNO levels and peripheral blood eosinophil counts were useful biomarkers for distinguishing between patients with asthma and COPD. Clinicians should consider the existence of bronchial asthma in patients with obstructive airway disease if the number of eosinophils in the peripheral blood is greater than 200/ μ L. Further studies are required to identify biomarkers of subjects with overlapping asthma and COPD.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Authors' contributions: SK, MI, and MO designed the study. MA, YO, YK and SK performed the experiments. MI, RS and SK wrote the paper.

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Table1: Characteristics of the study patients

	Asthma (n=60)	AA (n=30)	NAA (n=30)	COPD (n=30)	p value
Age (years)	47.5 (34.3-67.8)	39.0 (25.0-52.3) ^b	67.0 (46.5-79.3)	70.0(64.8-78.3)	p<0.001
Sex (male, %)	14 (23.3) ^b	8 (26.7) ^b	6 (20.0) ^b	28(93.3)	p<0.001
Smoking (pack years)	0 ^b	0 ^b	0 ^b	50.0(40.0-63.0)	p<0.001
FEV1/FVC (%)	73.5(64.5-81.3) ^b	80.1(64.7-84.0) ^b	72.1 (64.5-75.9) ^b	56.3(47.8-65.4)	p<0.001
FeNO (ppb)	64.3(36.4-93.8) ^b	73.5(46.4-92.4) ^b	52.1(33.8-123.3) ^b	15.7(10.8-24.3)	p<0.001
Eosinophil count (/μL) ^a	326.0(231.8-445.3) ^b	332.0(239.3-422.5) ^b	322.0(187.5-506.3) ^b	144.5(92.8-211.3)	p<0.001
IgE (IU/mL)	220.0(65.0-666.8) ^b	661.5(334.5-1073.0) ^b	75.5(39.8-137.8)	58.5(22.3-186.5)	p<0.001
IL-25 (pg/mL)	34.6(13.6-58.0)	27.2(0-51.0)	43.7(13.9-70.6)	16.8(0-42.7)	p=0.11
Periostin (ng/mL)	73.4(50.0-284.6)	126.4(64.3-1615.0) ^b	54.2(34.6-91.3)	71.3(49.9-153.8)	p=0.002

^a Peripheral blood eosinophil count. AA, atopic asthma; NAA, non-atopic asthma; COPD,

chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; FeNO, fractional exhaled nitric oxide. Data represent median (25%-75%).^b Indicates a significant difference compared COPD patients ($p < 0.05$). Significance was evaluated by the Kruskal-Wallis test.

Figure legends

Fig. 1

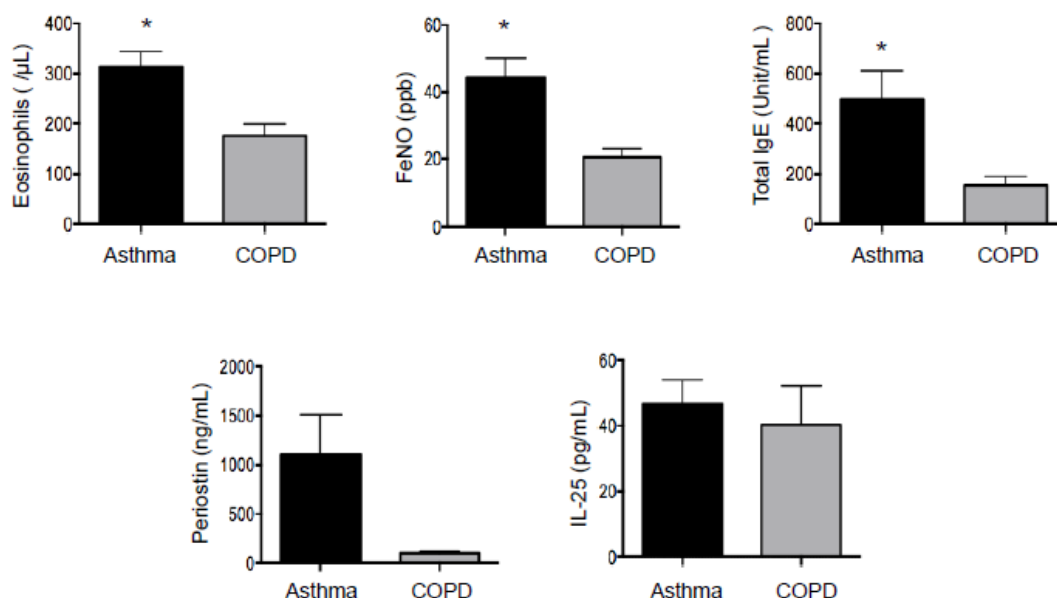


Figure 1. Comparison of biomarkers between patients with asthma and chronic obstructive pulmonary disease (COPD). Eosinophils, peripheral blood eosinophil counts; FeNO, fractional exhaled nitric oxide. Inter-group comparisons were assessed using the Mann-Whitney U test. * $p < 0.05$ compared with COPD.

Fig. 2

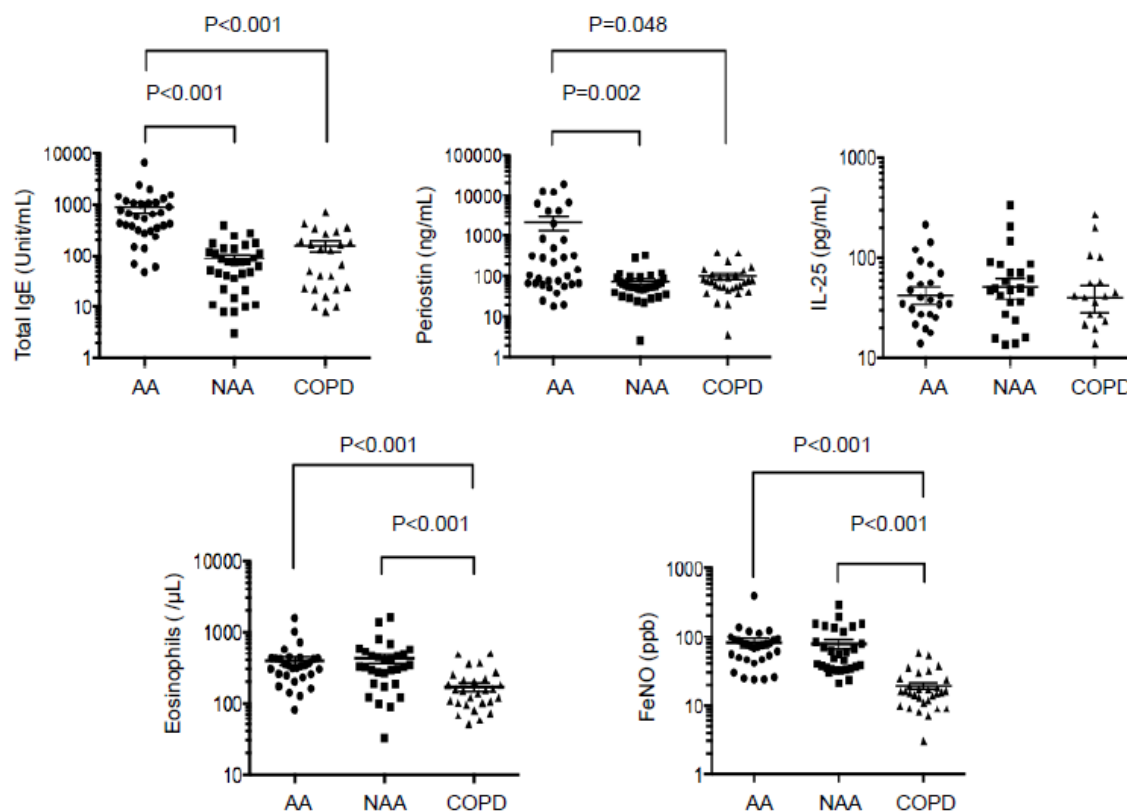


Figure 2. Concentrations of biomarkers among atopic and non-atopic asthma, and chronic obstructive pulmonary disease (COPD). Eosinophils, peripheral blood eosinophil counts; FeNO, fractional exhaled nitric oxide. The Kruskal-Wallis test was used to compare values of different groups.

Fig. 3

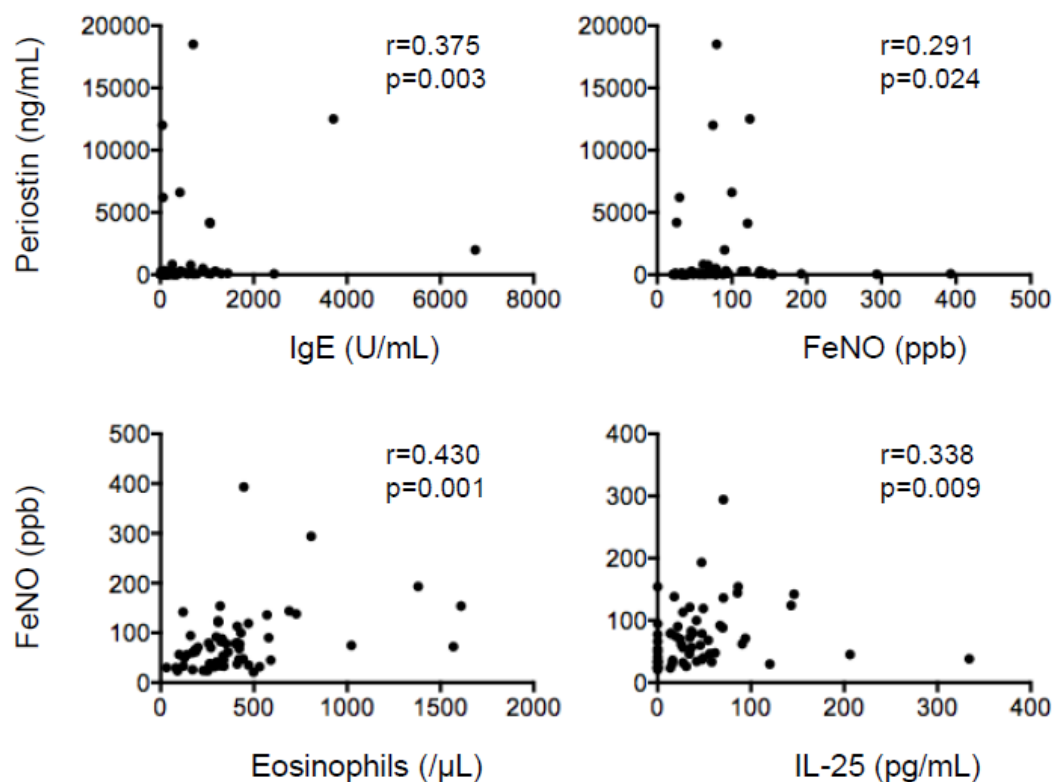


Figure 3. Correlation between concentrations of biomarkers in patients with asthma. Correlations between serum periostin and serum IgE concentrations, and FeNO levels (top). Correlation between FeNO levels and peripheral blood eosinophil counts, and serum IL-25 concentrations (bottom).

Fig. 4

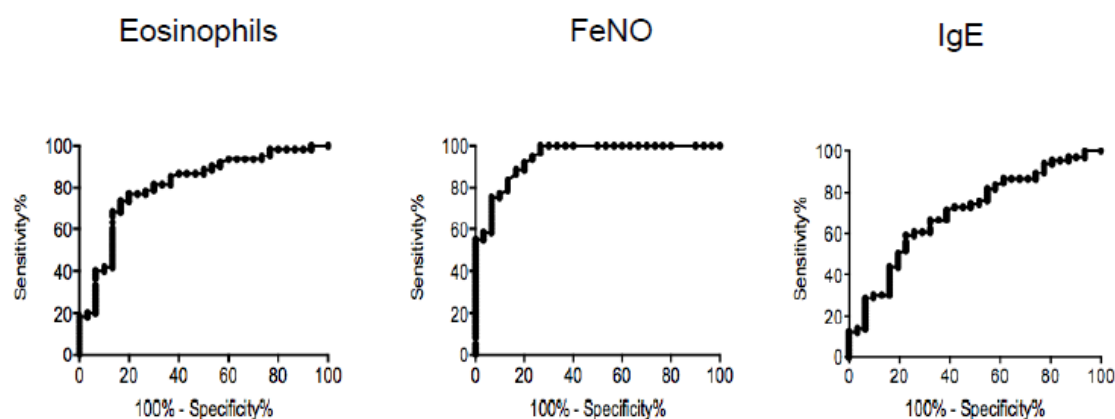


Figure 4. Sensitivity of eosinophil, FeNO levels, and IgE concentrations in patients with asthma. Receiver-operating characteristic curve analysis of the sensitivity and specificity of peripheral blood eosinophil counts, FeNO levels, and serum IgE concentrations for composite asthma. Confidence Interval: 95%. AUC, Area under the curve. Control values: COPD. FeNO, fractional exhaled nitric oxide.