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ORIGINAL RESEARCH

# Difference of Serum Cytokine Profile in Allergic Asthma Patients According to Disease Severity

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**Purpose:** Allergic asthma is a heterogeneous disease with complex underlying mechanisms. Cytokines are key mediators in immune system and potential indicators of disease status. The aim of this study is to compare the difference of serum cytokine profile in allergic asthma patients with different disease severity and explore candidate biomarkers for disease monitoring and targeting therapeutic agents.

**Patients and Methods:** A total of 40 allergic asthmatics (mild, n=22; moderate-to-severe, n=18) were included in this study. Serum samples, lung function and exhaled nitric oxide data were collected from each subject. A Meso Scale Discovery (MSD) electrochemiluminescence platform was applied to access serum levels of 33 cytokines. Serum cytokine profile was compared between mild and moderate-to-severe allergic asthmatics, and the correlation between serum cytokine levels, lung function and exhaled nitric oxide were analyzed.

**Results:** Moderate-to-severe allergic asthmatics displayed higher levels of eotaxin-1, eotaxin-2, MCP-1, MCP-2, MCP-3, YKL-40 and lower IL-23, IL-31 and TRAIL in serum in comparison with mild allergic asthmatics. Serum YKL-40, eotaxin-1 and MCP-1 had the best ability to discriminate mild and moderate-to-severe allergic asthmatics, with an AUC of 0.833, 0.811 and 0.760. Serum IP-10 was positively correlated with FeNO levels, while FnNO displayed a strong positive correlation with serum IL-25.

**Conclusion:** Compared with mild allergic asthmatics, significant increase in serum eotaxin-1, eotaxin-2, MCP-1, MCP-2, MCP-3, YKL-40 and decrease in serum IL-23, IL-31 and TRAIL was noted in moderate-to-severe allergic asthmatics. YKL-40, eotaxin-1 and MCP-1 might be candidate biomarkers in reflecting severity in allergic asthma patients.

**Keywords:** allergic asthma, cytokine, YKL-40, eotaxin, MCP

## Introduction

Asthma is one of the most rapidly growing disorder among chronic respiratory diseases, affecting people from childhood to old age. Despite adequate medication administered under the guidance of Global Initiative for Asthma (GINA), 5–10% of asthmatics remain only partially controlled or even uncontrolled and require further add-on therapy such as oral cortisone or novel biologic medication, and they suffer from increased mortality, increased healthcare costs and reduced quality of life.[1] Severe asthma is, therefore, a nonnegligible burden for patients, their families, and the healthcare system.

Allergic asthma, defined as asthma associated with sensitization to environmental allergens, is the most common and most well-studied subtype of asthma, accounting for 60–80% of the whole asthma population.[2,3] It has long been evident that allergic asthma is a prototypical Type 2 inflammation-driven disease, which is characterized based on release of type 2 cytokines (such as IL-4, IL-5 and IL-13) by T-helper cell type 2 (Th2) cells or innate lymphoid cells-type 2 (ILC2), followed by IgE class switching, eosinophil recruitment, and airway hyperresponsiveness (AHR). Thus, the majority of

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existing biological therapies are T2-targeted, like anti-IgE (omalizumab), anti-IL-5/IL-5R (mepolizumab, benralizumab) and anti-IL-4R (dupilumab). Unfortunately, although the clinical outcome of those biologics is favorable overall, some patients could not get a satisfactory response, indicating that solely targeting T2-related cytokine might not be enough. From previous studies we could come to the conclusion that other Th subsets, eosinophils, neutrophils, basophils, mast cells, macrophages also function at certain stage during its pathogenesis.[4] Therefore, it is crucial to take them into consideration when it comes to optimize treatment strategy for allergic asthma.

Cytokines play a key role in the immunopathogenesis of asthma and are potent candidates in reflecting ongoing pathophysiological changes among patients and monitoring disease advancing. The cytokine network might have a difference in mild and moderate-to-severe allergic asthma patients and lead to different clinical outcome. In light of better understanding of the underlying mechanism, improving treatment regimens and decreasing disease burden, it is essential to investigate whether patients in different severity confer different cytokine expression.[5]

In the present study, we hypothesized that serum cytokine profile had a difference between mild and moderate-to-severe allergic asthmatics, and that these differences were associated with clinical heterogeneity. Thus, we quantified the level of 33 cytokines in serum samples from allergic asthma patients with different disease severity using Meso Scale Discovery (MSD) electrochemiluminescence platform, and analyzed the correlations between serum cytokine concentration, lung function and fractional exhaled nitric oxide levels.

# Materials and Methods

## Subjects

The study was approved by the ethic committee of Ruijin Hospital (No.2019-YK061) and was conducted in accordance with the Declaration of Helsinki. A total of 40 allergic asthma patients from outpatient clinic in Shanghai were enrolled in this study. Inclusion criteria included a diagnosis of asthma by physician according to the diagnosis criteria of the Global Strategy for Asthma Management and Prevention guidelines[6] for at least one year, and a positive result of serum allergen-specific IgE (>0.35kUA/L) to at least one allergen tested and a total IgE >60kU/L. Exclusion criteria included poor medication compliance, history of cancer, autoimmune diseases and other respiratory or cardiovascular diseases. Patients were divided into two groups: those taking step 1 and step 2 medication and did not have any exacerbation in the past year were selected as mild group, while patients taking step 4 or step 5 medication routinely and had 2 or more exacerbations which needed urgent-care visits in the past year were categorized as moderate-to-severe group. To better display the difference between patients with different disease severity, patients taking step 3 medication were not included. At time of visit, all patients were in stable state of asthma, had no infection, no asthma exacerbation, no oral cortisone use or change in medication in the past month. Venous blood samples, ACT score and spirometry data was collected on all subjects according to ATS/European Respiratory Society (ERS) guidelines[7] at the same day. All patients were informed about the purpose of the study and provided a written consent.

## Fractional Exhaled Nitric Oxide Measurement

Fractional exhaled nitric oxide measurement was performed prior to spirometry test using Sunvou-CA2122 system (Sunvou, China) as previously described.[8] Patients were required to avoid smoking, exercising and consuming nitrate-rich foods (such as sausage, broccoli, lettuce, celery and cabbage) one hour before testing. Fractional exhaled nitric oxide (FeNO) and nasal nitric oxide (FnNO) levels were measured under the guidance of ATS guidelines for online NO measurement in adults.[9] Alveolar nitric oxide (CaNO) was accessed using a simplified estimation method based on fractional concentration of exhaled NO at 50 and 200mL/s.[10] Any exhalation that did not meet the ATS requirements was automatically rejected by the Sunvou system.

## Total IgE and Specific IgE Detection

Serum total IgE and allergen-specific IgE to five common inhaled allergens in Shanghai (mold, dust mite, cat dander, dog dander and cockroach) were measured using the Phadia 250 fluorescence enzyme immunoassay system (Phadia, Sweden) according to the manufacturer's instructions. Serum allergen-specific IgE levels>0.35 kUA/L were considered positive.

## Meso Scale Discovery Assay

Serum cytokine levels including Th1-related cytokines (IFN-γ, IP-10, TNF-β, IL-18), Th2-related cytokines (IL-4, IL-5, IL-6, IL-13, IL-17E, IL-31, IL-33, TARC, TSLP), Th9-related cytokine IL-9, Th17-related cytokines (IL-17A, IL-21, IL-22, IL-23), Treg-related cytokine IL-10, monocyte-related cytokines (IL-27, IL-1β, MCP-1, MCP-2, MCP-3, MCP-4), eosinophil-related cytokines (eotaxin-1, eotaxin-2, eotaxin-3, GM-CSF) and airway remodeling-related cytokines (VEGF-A, SDF-1α, YKL-40, TRAIL) were measured by MSD using U-PLEX Biomarker Group 1 (hu) Assays (MSD, USA) according to the instructions given by the manufacturer.

## Statistical Analysis

Statistical analyses were performed in SPSS 25.0 software package (IBM Corp. Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad software, San Diego, CA, USA). Data was presented as mean ± SD for quantitative data and as number and percentage for categorical data. Comparisons between different groups were made by independent samples *t*-test for quantitative statistics and chi-square test for categorical statistics. Receiver operating characteristic (ROC) curves were analyzed to identify candidate biomarkers to distinguish mild and moderate-to-severe allergic asthma patients and to provide their optimal cut-off values. Correlation analysis between quantitative variables was done using Spearman's correlations. A P-value<0.05 was considered as statistically significant.

# Results

## Patient Characteristics

The study consisted of 40 patients diagnosed with allergic asthma (mild, n=22; moderate-to-severe, n=18). Clinical characteristics of all subjects are summarized in Table 1. In general, patients in mild group were younger in those in moderate-to-severe group ((32.3±9.6) years vs (44.0±15.0) years, $P$=0.005), and their lung function (including FVC%, FEV1%, FEV1/FVC%, MEF75%, MEF50% but not MEF25%) along with ACT score were significantly better (for FVC %, FEV1% and MEF75%, $P$<0.001; for FEV1/FVC% and ACT score, $P$<0.01; for MEF50%, $P$=0.013). Mold sensitization was more common in moderate-to-severe group ($P$=0.025) while patients in mild group were mostly sensitized to dust mite. There was no significant difference regarding BMI, IgE, smoking status or gender distribution between two groups.

## Difference of Serum Cytokine Profile Between Mild and Moderate-to-Severe Allergic Asthma Patients

We measured serum concentration of 33 cytokines in all subjects, including Th1-related cytokines (IFN-γ, IP-10, TNF-β, IL-18), Th2-related cytokines (IL-4, IL-5, IL-6, IL-13, IL-17E, IL-31, IL-33, TARC, TSLP), Th9-related cytokine IL-9, Th17-related cytokines (IL-17A, IL-21, IL-22, IL-23), Treg-related cytokine IL-10, monocyte-related cytokines (IL-27, IL-1β, MCP-1, MCP-2, MCP-3, MCP-4), eosinophil-related cytokines (eotaxin-1, eotaxin-2, eotaxin-3, GM-CSF) and airway remodeling-related cytokines (VEGF-A, SDF-1α, YKL-40, TRAIL) using Meso Scale Discovery method. The overall results are displayed in Table 2, and cytokines with significant difference between mild and moderate-to-severe group are demonstrated in Figure 1. In general, levels of Th2-related cytokines in serum were higher in moderate-to-severe allergic asthmatics, among which IL-31 was an exception ($P$=0.004). Among Th17-related cytokines, a significant increase of serum IL-23 level was observed in mild allergic asthmatics ($P$=0.008). Serum eotaxin-1, eotaxin-2, MCP-1, MCP-2, MCP-3, YKL-40 levels were significantly higher in moderate-to-severe group ($P$=0.001,0.017,0.003,0.018,0.036,0.022), while serum TRAIL concentration was significantly lower than that in mild group ($P$=0.045).

For the 9 cytokines that differ significantly among two groups, a correlation matrix between their levels and all 33 cytokines tested is displayed in Figure 2. Besides correlated with each other ($P$<0.001), IL-23 and IL-31 was also positively correlated with IL-17A, IL-25 (all $P$<0.001), GM-CSF, TSLP, IL-21, IL-33 (all $P$<0.01), IL-9 (all $P$<0.05), and negatively correlated with eotaxin-2 ($P$<0.01), eotaxin-1 (IL-23, $P$<0.05; IL-31, $P$<0.01) and YKL-40 ($P$<0.05). MCP-1 displayed a positive correlation with IL-13, VEGF-A, eotaxin-1, eotaxin-3, TARC, TNF-β, MCP-2, MCP-3, MCP-4 (all $P$<0.001), IL-18, eotaxin-2, YKL-40 (all $P$<0.01), IL-4, IL-5 (all $P$<0.05). MCP-2 was correlated positively

**Table 1** Baseline Characteristics of Patients with Mild and Moderate-to-Severe Allergic Asthma

| | Mild | Moderate-to-Severe | *P* value |
|---|---|---|---|
| N | 22 | 18 | |
| Male: Female | 9:13 | 7:11 | 0.897 |
| Age, years | 32.3±9.6 | 44.0±15.0 | 0.005** |
| BMI, kg/m$^2$ | 23.2±3.3 | 23.4±4.5 | 0.849 |
| Smoking status, current: past: never | 0:1:21 | 0:1:17 | 0.884 |
| FVC, %predicted | 98.77±10.36 | 78.03±18.44 | <0.001*** |
| FEV1, %predicted | 95.36±12.53 | 63.64±25.06 | <0.001*** |
| FEV1/FVC, %predicted | 96.91±11.52 | 79.20±22.75 | 0.007** |
| MEF75, %predicted | 91.49±19.44 | 51.18±33.13 | <0.001*** |
| MEF50, %predicted | 79.55±19.67 | 49.06±40.87 | 0.013* |
| MEF25, %predicted | 70.55±28.06 | 46.05±43.35 | 0.078 |
| ACT score | 22.72±1.81 | 17.53±4.45 | 0.002** |
| IgE, kU/L | 761.9±1164.7 | 473.2±834.7 | 0.501 |
| Allergen, positive result (number, %) | | | |
| Mold | 2, 9.1% | 7, 38.9% | 0.025* |
| Dust mite | 21, 95.4% | 11, 61.1% | 0.007** |
| Cat dander | 7, 31.8% | 2, 11.1% | 0.119 |
| Dog dander | 7, 31.8% | 4, 22.2% | 0.499 |
| Cockroach | 5, 22.7% | 1, 5.6% | 0.130 |

**Notes**: Data are presented as mean ± standard deviation or number, percentage. *$P$<0.05; **$P$<0.01; ***$P$<0.001.
**Abbreviations**: BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in the first second; MEF, maximal expiratory flow; ACT, asthma control test; IgE, immunoglobulin E.

with IL-13, eotaxin-1, MCP-1, MCP-4, TARC, TNF-β, VEGF-A (all $P$<0.001), IL-18 (all $P$<0.01), IL-5, eotaxin-2, eotaxin-3, MCP-3, IP-10 (all $P$<0.05). YKL-40 showed a negative correlation with IL-17A, IL-21, TRAIL (all $P$<0.01), IL-23, IL-25, IL-31 (all $P$<0.05), and a positive correlation with eotaxin-1 ($P$<0.001), eotaxin-2, VEGF-A, MCP-1, MCP-3 (all $P$<0.01).

To find a candidate serum biomarker in distinguishing mild and moderate-to-severe allergic asthmatics, ROC analyses were performed, and main results are summarized in Table 3 and Figure 3. The area under the curves (AUCs) of YKL-40, eotaxin-1, MCP-1, IL-23, IL-31, eotaxin-2, MCP-2, MCP-3 were 0.833, 0.811, 0.760, 0.754, 0.749, 0.725, 0.720 and 0.707, respectively. The best cut-off points obtained from ROC curve analysis with the greatest likelihood ratios for YKL-40, eotaxin-1, MCP-1, IL-23, IL-31, eotaxin-2, MCP-2, MCP-3 were 33,753, 134.1, 142.9, 1.035, 11.78, 1210, 43.06 and 5.847 pg/mL, respectively. TRAIL was excluded from the result as it did not reach an AUC of 0.7.

## Correlations Between Serum Cytokine Levels and Lung Function

We further explored the relationship between serum cytokine levels and lung function of allergic asthmatics. Main results were shown in Table 4, and a correlation matrix was displayed in Figure 4. Serum eotaxin-1 level was negatively correlated with FEV1%, FVC%, FEV1/FVC%, MEF75%, MEF50% and MEF25% ($P$=0.003, 0.003, 0.012, 0.006, 0.013, 0.017, respectively). Eotaxin-2 was negatively correlated with FEV1%, FEV1/FVC%, MEF75%, MEF50%, MEF25% ($P$=0.010, 0.007, 0.008, 0.003, 0.006, respectively). YKL-40 displayed a negative correlation with FEV1%, FVC%, MEF75%, MEF50% and MEF25% ($P$=0.003, 0.001, 0.016, 0.022, 0.026, respectively). Serum TARC concentration was inversely correlated with FEV1% ($P$=0.023) and FVC% ($P$=0.019). Among the cytokines tested, IL-21 was the only one positively correlated with FEV1% ($P$=0.049). Serum IL-23 and IL-31 was positively correlated with FEV1/FVC% ($P$=0.015, 0.034, respectively).

## Correlations Between Serum Cytokine Levels and FeNO, CaNO and FnNO Levels

We next analyzed correlations between FeNO, CaNO and FnNO levels and serum cytokine levels. Main results are summarized in Table 5, and a correlation matrix between cytokine levels and FeNO, CaNO and FnNO levels is

**Table 2** Serum Cytokine Levels in Mild and Moderate-to-Severe Allergic Asthma Patients

| | Mild (pg/mL) | Moderate-to-Severe (pg/mL) | *P* value |
|---|---|---|---|
| Th1-related cytokines | | | |
| IFN-γ | 15.0327±18.5494 | 9.9081±5.4423 | 0.265 |
| IP-10 | 219.5332±81.9197 | 179.8225±67.2624 | 0.107 |
| TNF-β | 1.3760±1.1251 | 1.6338±0.6957 | 0.402 |
| IL-18 | 382.3738±180.2698 | 437.5303±237.5416 | 0.409 |
| Th2-related cytokines | | | |
| IL-4 | 0.0552±0.1760 | 0.0715±0.0508 | 0.168 |
| IL-5 | 0.8976±0.4461 | 1.0189±0.6217 | 0.477 |
| IL-6 | 1.9240±3.6287 | 10.6227±37.7049 | 0.343 |
| IL-13 | 2.9740±1.4682 | 3.6162±0.9953 | 0.115 |
| IL-17E | 1.4924±0.8692 | 1.2052±0.7952 | 0.300 |
| IL-31** | 13.3371±6.2233 | 7.9754±3.7165 | 0.004** |
| IL-33 | 0.8603±0.5922 | 0.5982±0.2588 | 0.071 |
| TARC | 179.4743±325.5475 | 248.3678±154.6949 | 0.415 |
| TSLP | 1.4226±0.5462 | 1.3881±0.4468 | 0.831 |
| Th9-related cytokine | | | |
| IL-9 | 0.5086±0.5584 | 0.3077±0.2093 | 0.157 |
| Th17-related cytokines | | | |
| IL-17A | 3.8704±2.6369 | 3.0720±1.6177 | 0.269 |
| IL-21 | 67.1995±146.7237 | 3.1749±1.8464 | 0.053 |
| IL-22 | 0.7479±0.9150 | 0.6558±0.3282 | 0.687 |
| IL-23** | 2.3996±1.7093 | 0.9643±0.9247 | 0.008** |
| Treg-related cytokine | | | |
| IL-10 | 0.4019±0.1935 | 0.4314±0.1231 | 0.579 |
| Monocyte-related cytokines | | | |
| IL-27 | 650.1278±315.4457 | 759.9686±469.0485 | 0.383 |
| IL-1β | 3.9250±16.9320 | 4.6160±18.2887 | 0.902 |
| MCP-1** | 134.0964±59.9890 | 206.7393±83.5712 | 0.003** |
| MCP-2* | 38.4753±13.8157 | 49.2572±13.6278 | 0.018* |
| MCP-3* | 4.7400±1.7576 | 6.7599±3.9217 | 0.036* |
| MCP-4 | 99.7898±109.3274 | 133.5642±70.0432 | 0.264 |
| Eosinophil-related cytokines | | | |
| Eotaxin-1*** | 101.8066±149.2978 | 191.8802±83.5456 | <0.001*** |
| Eotaxin-2* | 1203.4756±901.7847 | 1938.4275±936.9545 | 0.017* |
| Eotaxin-3 | 84.7753±133.9955 | 37.7562±20.2448 | 0.119 |
| GM-CSF | 0.0733±0.0430 | 0.0911±0.0837 | 0.391 |
| Airway remodeling-related cytokines | | | |
| VEGF-A | 74.0250±74.6739 | 111.3350±61.3555 | 0.097 |
| SDF-1α | 842.2624±263.7370 | 893.5355±255.9779 | 0.539 |
| YKL-40* | 23,210.1859±15,116.1899 | 51,974.8527±54,283.5695 | 0.022* |
| TRAIL* | 134.3080±37.1827 | 110.7160±34.0288 | 0.045* |

**Notes**: Data are presented as mean ± standard deviation. *$P$<0.05; **$P$<0.01; ***$P$<0.001.

**Abbreviations**: Th, T helper cell; IFN-γ, interferon-γ; IP-10, interferon-γ-induced protein 10; TNF-β, tumor necrosis factor-β; IL, interleukin; TARC, thymus and activation-regulated chemokine; TSLP, thymic stromal lymphopoietin; MCP, monocyte chemoattract protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; VEGF-A, vascular endothelial growth factor-A; SDF-1α, stromal derived factor-1α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

displayed in Figure 5. FeNO showed a positive correlation with serum IP-10 ($P$=0.042) while CaNO levels were positively correlated with IL-4 and IL-1β ($P$=0.031, 0.018, respectively). FnNO levels were positively correlated with serum IL-4, IL-25, IL-31, IL-9, GM-CSF and negatively correlated with serum eotaxin-2 ($P$=0.042, 0.009, 0.047, 0.030, 0.037, 0.040, respectively).

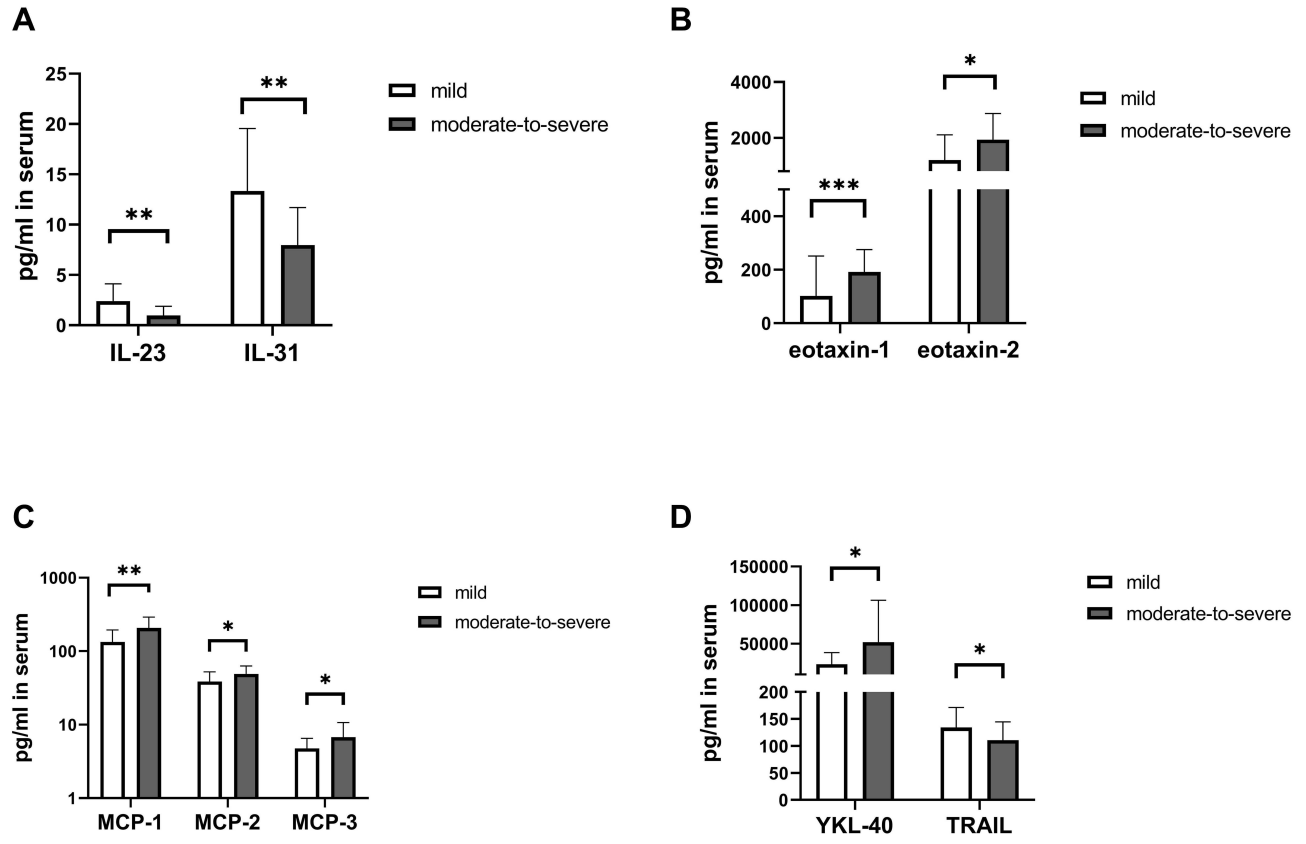


**Figure 1** Serum cytokine concentrations of the most discriminative biomarkers (**A**) IL-23 and IL-31, (**B**) eotaxin-1 and eotaxin-2, (**C**) MCP-1, MCP-2 and MCP-3, (**D**) YKL-40 and TRAIL in mild and moderate-to-severe allergic asthmatics. *$P$<0.05; **$P$<0.01; ***$P$<0.001.

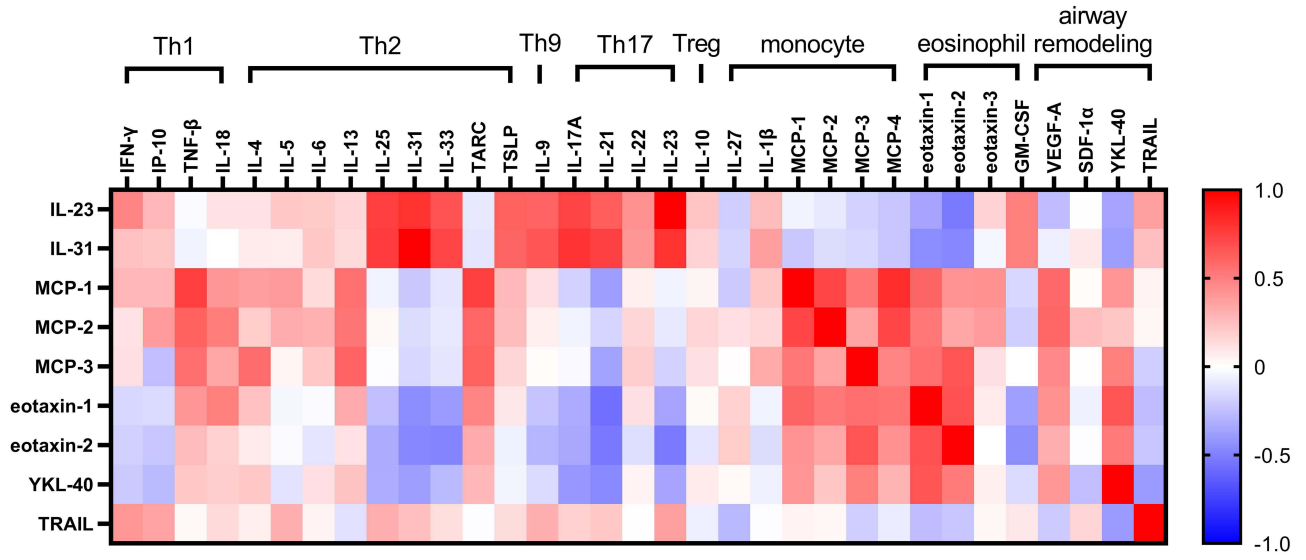


**Figure 2** Correlation matrix for 9 discriminative biomarkers with all 33 cytokines tested. Blue represented a negative correlation index while red represented a positive one. The darker the color, the higher the correlation index was.

## Discussion

Allergic asthma is a heterogeneous disorder with complex immunopathology. In the present study, we portrayed differences of cytokine profile between serum samples of mild and moderate-to-severe allergic asthmatics and analyzed

**Table 3** The AUC, Cut-off, Sensitivity and Specificity Values for Mild and Moderate-to-Severe Allergic Asthma Patients

| | AUC | *P* value | Cut-off (pg/mL) | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|
| YKL-40 | 0.833 | <0.001*** | 33,753 | 72.22 (49.13–87.50) | 90.91 (72.19–98.38) |
| Eotaxin-1 | 0.811 | <0.001*** | 134.1 | 72.22 (49.13–87.50) | 72.73 (51.85–86.85) |
| MCP-1 | 0.760 | 0.005** | 142.9 | 77.78 (54.79–91.00) | 72.73 (51.85–86.85) |
| IL-23 | 0.754 | 0.015* | 1.035 | 71.43 (45.35–88.28) | 83.33 (60.78–94.16) |
| IL-31 | 0.749 | 0.014* | 11.78 | 86.67 (62.12–97.63) | 57.89 (36.28–76.86) |
| Eotaxin-2 | 0.725 | 0.016* | 1210 | 83.33 (60.78–94.16) | 54.55 (34.66–73.08) |
| MCP-2 | 0.720 | 0.018* | 43.06 | 66.67 (43.75–83.72) | 72.73 (51.85–86.85) |
| MCP-3 | 0.707 | 0.026* | 5.847 | 61.11 (38.62–79.69) | 72.73 (51.85–86.85) |

**Notes**: *$P$<0.05; **$P$<0.01; ***$P$<0.001.
**Abbreviations**: AUC, area under curve; CI, confidence interval.

the correlations between cytokine concentration, lung function parameters as well as exhaled nitric oxidate levels. Previous studies have examined the difference of cytokine profile in bronchial biopsy samples or bronchoalveolar lavage.[11,12] Indeed, those samples could best reflect the condition in airways. Nevertheless, serum sample would be more practical in clinical settings, making it a feasible alternative. To the best of our knowledge, this is the first research evaluating all those 33 cytokines simultaneously in serum samples of allergic asthma patients and analyzed their correlation with both lung function parameters and exhaled nitric oxide levels.

The present study elucidated that moderate-to-severe allergic asthmatics expressed higher levels of eotaxin-1, eotaxin-2, MCP-1, MCP-2, MCP-3, YKL-40 and lower IL-23, IL-31 and TRAIL in serum in comparison with mild allergic asthmatics. Serum eotaxin-1, eotaxin-2, YKL-40 levels were negatively correlated with lung function parameters for both main and small airways. YKL-40, eotaxin-1 and MCP-1 had the best ability to discriminate mild and moderate-to-severe allergic asthmatics, with an AUC of 0.833, 0.811 and 0.760. The concentration of serum IP-10 displayed a significantly positive correlation with FeNO levels, while CaNO levels were positively correlated with serum IL-4 and IL-1β. FnNO levels were positively correlated with serum IL-4, IL-25, IL-31, IL-9, GM-CSF and negatively correlated with serum eotaxin-2.

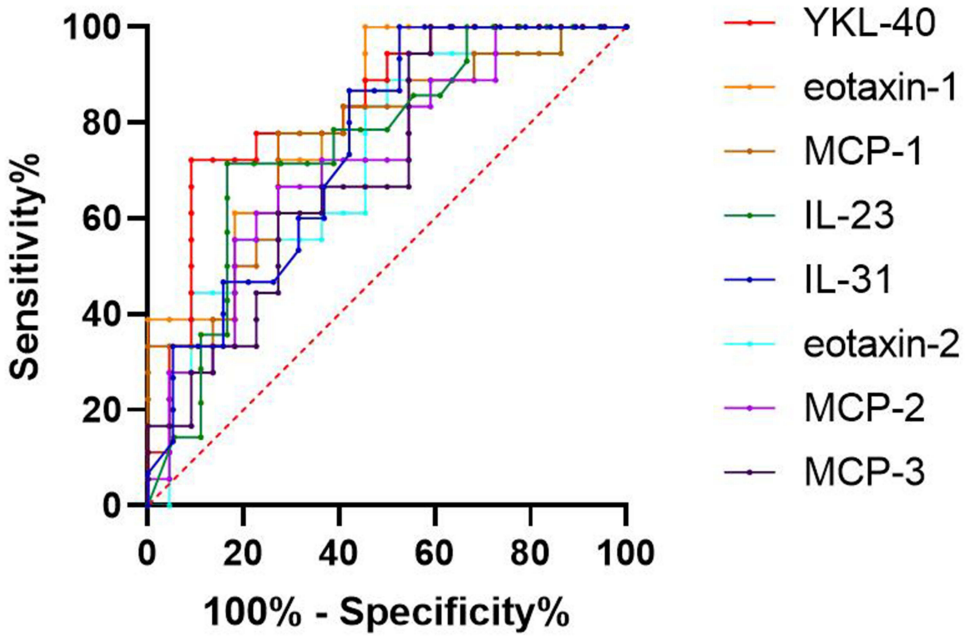


**Figure 3** Receiver operating characteristic (ROC) curves of YKL-40, eotaxin-1, MCP-1, IL-23, IL-31, eotaxin-2, MCP-2 and MCP-3 for discriminating mild and moderate-to-severe allergic asthmatics.

**Table 4** Correlations Between Cytokine Levels and Lung Function Parameters

| Parameter | Cytokine | Correlation Coefficient | *P* value |
|---|---|---|---|
| FEV1% | YKL-40 | −0.478 | 0.003** |
| | Eotaxin-1 | −0.472 | 0.003** |
| | Eotaxin-2 | −0.418 | 0.010* |
| | TARC | −0.372 | 0.023* |
| | MCP-3 | −0.372 | 0.023* |
| | IL-21 | 0.326 | 0.049* |
| FVC% | YKL-40 | −0.506 | 0.001** |
| | Eotaxin-1 | −0.477 | 0.003** |
| | IL-22 | −0.428 | 0.008** |
| | MCP-3 | −0.423 | 0.009** |
| | TARC | −0.385 | 0.019* |
| FEV1/FVC | Eotaxin-2 | −0.434 | 0.007** |
| | Eotaxin-1 | −0.411 | 0.012** |
| | IL-23 | 0.397 | 0.015* |
| | IL-31 | 0.350 | 0.034* |
| MEF75% | Eotaxin-1 | −0.440 | 0.006** |
| | Eotaxin-2 | −0.432 | 0.008** |
| | YKL-40 | −0.394 | 0.016* |
| MEF50% | Eotaxin-2 | −0.479 | 0.003** |
| | Eotaxin-1 | −0.404 | 0.013* |
| | YKL-40 | −0.376 | 0.022* |
| | IL-23 | 0.329 | 0.047* |
| MEF25% | Eotaxin-2 | −0.443 | 0.006** |
| | Eotaxin-1 | −0.390 | 0.017* |
| | YKL-40 | −0.366 | 0.026* |

**Notes**: Correlations between serum cytokine levels and FEV1%, FVC%, FEV1/FVC%, MEF75%, MEF50% and MEF25% were analyzed. All correlations listed above were evaluated using Spearman correlation coefficient analysis. *$P<0.05$; **$P<0.01$.
**Abbreviations**: FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; MEF, maximal expiratory flow.

The monocyte chemotactic proteins (MCPs) are considered as a subfamily of the CC chemokines, which play an important role in allergic responses via induction of mast cell activation and release of leukotriene C4 into the airway, resulting in airway hyperresponsiveness.[13] Enhanced expression of MCP-1 was observed in bronchial tissue and BAL fluids of asthmatic subjects by previous study.[14] In this study, we discovered that MCP-1, MCP-2, MCP-3 concentration is also elevated in serum of moderate-to-severe allergic asthmatics than that of mild allergic asthmatics. Furthermore,

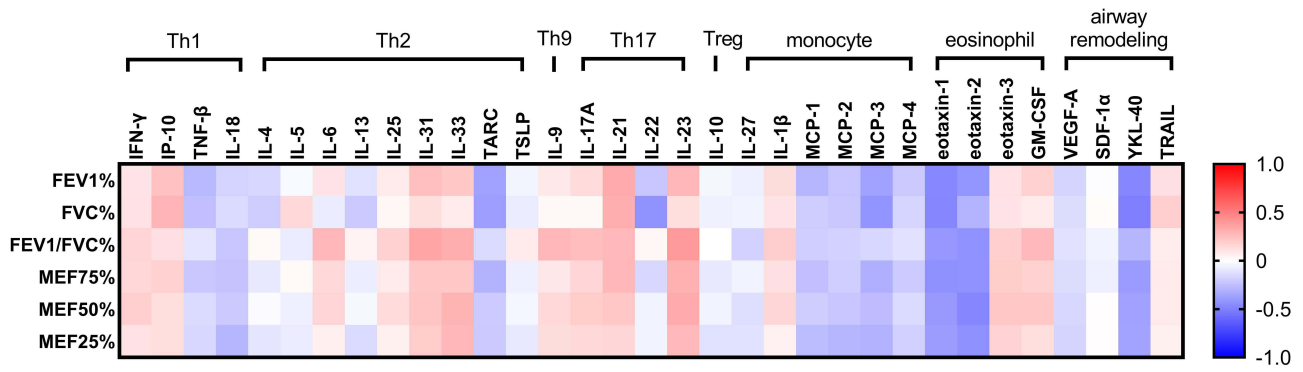


**Figure 4** Correlation matrix for lung function parameters with all 33 cytokines tested. Blue represented a negative correlation index while red represented a positive one. The darker the color, the higher the correlation index was.

**Table 5** Correlations Between Serum Cytokine Levels and Exhaled Nitric Oxide Levels

| Parameter | Cytokine | Correlation Coefficient | *P* value |
|---|---|---|---|
| FeNO | IP-10 | 0.417 | 0.042* |
| CaNO | IL-1β | 0.487 | 0.018* |
| | IL-4 | 0.450 | 0.031* |
| FnNO | IL-25 | 0.566 | 0.009** |
| | IL-9 | 0.486 | 0.030* |
| | GM-CSF | 0.469 | 0.037* |
| | Eotaxin-2 | −0.463 | 0.040* |
| | IL-4 | 0.459 | 0.042* |
| | IL-31 | 0.449 | 0.047* |

**Notes**: Correlations between serum cytokine levels and FeNO, CaNO and FnNO were analyzed. All correlations listed above were evaluated using Spearman correlation coefficient analysis. *$P<0.05$; **$P<0.01$.
**Abbreviations**: FeNO, fractional exhaled nitric oxide; CaNO, alveolar nitric oxide; FnNO, nasal nitric oxide.

serum MCP-3 was negatively correlated with FEV1% and FVC%. Taken together, serum MCP levels are promising candidates in distinguishing mild and severe asthmatics.

Members in eotaxin family, including eotaxin-1 (CCL11), eotaxin-2 (CCL24) and eotaxin-3 (CCL26), are potent eosinophil chemoattractant promoting eosinophils migration to sites of inflammation.[15] Previous study elucidated that all those three members were elevated in BAL fluids of asthmatics, and were associated with a decreased FEV1%.[16,17] Our study further discovered a significant elevation in both serum eotaxin-1 and eotaxin-2 in moderate-to-severe allergic asthmatics compared with mild group. Eotaxin-1 and eotaxin-2 displayed a negatively correlation not only with FEV1% but also with FEV1/FVC%, MEF75%, MEF50% and MEF25%, suggesting their role in both small and main airway obstruction. All these findings strongly indicates that eotaxin-1 and eotaxin-2 are related with an impaired lung function and are likely to be responsible for development of severe allergic asthma.

YKL-40, also named chitinase-3-like-1 (CHI3L1), is a chitinase-like glycoprotein related closely to airway remodeling. Previous study had shown that serum YKL-40 was elevated in asthma patients compared with healthy controls,[18] additionally, elevated serum YKL-40 levels were associated with irreversible airway obstruction and severe exacerbations.[19–21] YKL-40 is also elevated in COPD patients than Asthma-COPD Overlap patients, which may suggest its potential in distinguishing ACO patients from COPD patients.[22] In this study, we found the concentration of YKL-40 in serum of mild-to-moderate allergic asthmatics to be higher than that of mild allergic asthmatics, and was negatively correlated with FEV1%, MEF75%, MEF50% and MEF25%, indicating that patients with high serum YKL-40 might have a tendency to progress into ACO or COPD, which calls for an urgent need to take step in prevention.

IL-23 has been identified as a novel member of IL-12 family. According to previous study, IL-23-Th17 cell axis is involved not only in causing antigen-induced neutrophil recruitment into the airways but also in the enhancement of Th2 cell-mediated eosinophil recruitment, indicating a role in airway inflammation formation.[23,24] However, some other studies revealed that IL-23 performed a protective role in airway inflammation, suggesting that IL-23 may exert different

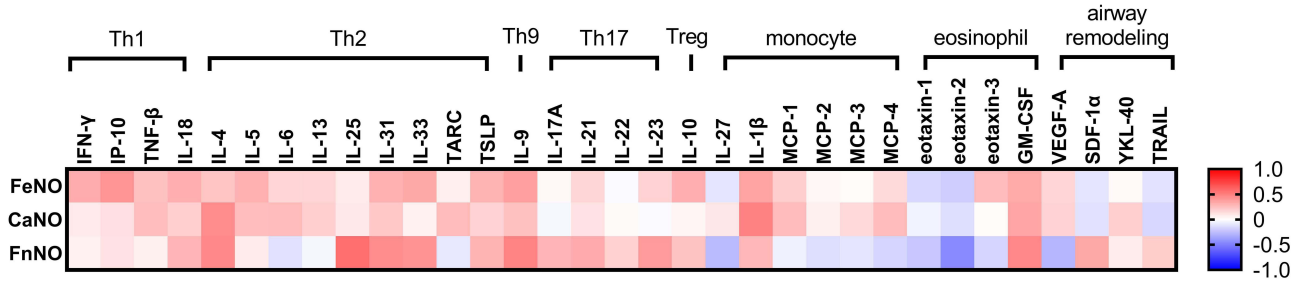


**Figure 5** Correlation matrix for FeNO, CaNO and FnNO levels with all 33 cytokines tested. Blue represented a negative correlation index while red represented a positive one. The darker the color, the higher the correlation index was.

function under different circumstances.[25,26] IL-31 is a novel T helper type 2 effector cytokine participating in allergic inflammation in the lung, gut and skin.[27] Although initial studies have found that IL-31 was elevated in serum of asthmatics in comparison with healthy controls, several animal studies suggest that it may reduce allergen-induced lung inflammation or play dual roles.[28–30] In our study, we found that serum IL-23 and IL-31 levels to be higher in mild allergic asthma patients that in moderate-to-severe ones, additionally, they were both positively correlated with FEV1/FVC%. Moreover, both serum IL-23 and IL-31 levels were positively correlated with alarmins IL-25, IL-33 and TSLP, which were indicated to be among the first events in initiating allergic inflammation.[31] Taken together, these findings suggest that IL-23 and IL-31 might play a role in the early phase of pathogenesis for allergic asthma.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of TNF family which has an important role in allergic airways inflammation and contributes to airway remodeling.[32,33] It is expressed in the airway by various type of cells, including fibroblasts, epithelial, endothelial, smooth muscle cells and eosinophils.[34] Our study demonstrated that moderate-to-severe allergic asthmatics expressed lower TRAIL than mild patients but was not correlated with lung function or NO levels. Moreover, TRAIL had poor ability in discriminating mild and moderate-to-severe allergic asthmatics in the ROC analysis. Therefore, more studies were needed to conclude the role of TRAIL in progressing disease severity.

IL-25 belongs to alarmin family, which is increased during allergen stimulation, and in turn enhance Th2-type airway inflammation.[35–37] It was also discovered that high nasal IL-25 mRNA expression was associated with higher Th2 response.[38] In this study, we observed a strong and significant association between FnNO levels and serum IL-25 levels. In combination with previous studies and our findings, we speculate that IL-25 might be one of the starters of T2-type inflammation in upper airways.

The main limitation of this study is a relatively small size. Results need to be validated in a larger cohort. Secondly, as this is a cross-sectional study, cause and effect between cytokine levels and disease severity could not be precisely concluded. Longitudinal follow-up studies will be needed to determine how these cytokines change through the course of disease and whether they lead to different prognosis. Furthermore, we only focused on serum cytokine levels, without accessing certain cell counts in blood. Nonetheless, our findings provide an integrative insight of difference in serum cytokine profile as well as their correlations with lung function and exhaled nitric oxide data in mild and moderate-to-severe asthmatics.

## Conclusion

In this study, we demonstrated significant differences in serum cytokine profile between mild and moderate-to-severe allergic asthmatics. Moderate-to-severe allergic asthmatics presented higher serum levels of MCP-1, MCP-2, MCP-3, eotaxin-1, eotaxin-2 and YKL-40 and lower serum levels of IL-23, IL-31 and TRAIL in comparison with mild allergic asthmatics. In ROC analysis, YKL-40, eotaxin-1 and MCP-1 was potent in distinguishing allergic asthmatics with different severity. Our findings highlight the potential value of these cytokines in disease monitoring and might help mechanism studying as well as targeting therapeutic agents.

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## Disclosure

The authors report no conflicts of interest in this work.

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