Allergy Asthma Immunol Res. 2019 May;11(3):394-405
https://doi.org/10.4168/aair.2019.11.3.394
pISSN 2092-7355·eISSN 2092-7363

Allergy, Asthma & Immunology Research AAIR

Original Article

Serum Levels of Eosinophil-Derived Neurotoxin: A Biomarker for Asthma Severity in Adult Asthmatics

Youngsoo Lee[1], **Ji-Ho Lee**[2], **Eun-Mi Yang**[1], **EunMi Kwon**[3], **Chang-Gyu Jung**[4], **Su-Chin Kim**[1], **Youngwoo Choi**[1], **You Sook Cho**[5], **Chang-Keun Kim**[3], **Hae-Sim Park**[1*]

[1]Department of Allergy and Clinical Immunology, Ajou University School of Medicine, Suwon, Korea
[2]Department of Pulmonology and Allergy, Yonsei University Wonju College of Medicine, Wonju, Korea
[3]Asthma and Allergy Center, Department of Pediatrics, Inje University Sanggye Paik Hospital, Seoul, Korea
[4]Department of Allergy, Keimyung University School of Medicine, Daegu, Korea
[5]Department of Allergy and Clinical Immunology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Received: Dec 28, 2018
Revised: Jan 15, 2019
Accepted: Jan 18, 2019

Correspondence to
Hae-Sim Park, MD, PhD
Department of Allergy and Clinical Immunology, Ajou University School of Medicine, 164, Worldcup-ro, Yeongtong-gu, Suwon 16499, Korea.
Tel: +82-31-219-5150
Fax: +82-31-219-5154
E-mail: hspark@ajou.ac.kr

ORCID iDs
Youngsoo Lee
https://orcid.org/0000-0001-8918-9353
Ji-Ho Lee
https://orcid.org/0000-0001-8744-156X
Eun-Mi Yang
https://orcid.org/0000-0001-8371-3513
EunMi Kwon
https://orcid.org/0000-0003-3871-8379

ABSTRACT

Purpose: Eosinophilic inflammation is a key component of severe asthma (SA). However, there has been no reliable serum biomarker for the eosinophilic inflammation of SA. We hypothesized that serum eosinophil-derived neurotoxin (EDN) could predict the eosinophilic inflammation of SA in adult asthmatics.
Methods: Severe asthmatics (n = 235), nonsevere asthmatics (n = 898), and healthy controls (n = 125) were enrolled from Ajou University Hospital, South Korea. The serum levels of EDN and periostin were measured by enzyme-linked immunosorbent assay and compared between severe and nonsevere asthmatics. Their associations with total eosinophil count (TEC) and clinical parameters were evaluated; clinical validation of the K-EDN kit for the measurement of serum EDN was evaluated.
Results: Severe asthmatics were older and had longer disease duration with significantly lower levels of forced expiratory volume in 1 second and methacholine PC20 than nonsevere asthmatics. Significant differences were found in TEC or sputum eosinophil count (%) between the groups. The serum levels of EDN and periostin were significantly higher in severe asthmatics than in nonsevere asthmatics and in healthy controls (all $P < 0.05$). Although significant correlations were found between serum EDN levels measured by the 2 kits ($\rho = 0.545$, $P < 0.0001$), higher correlation coefficients between serum EDN levels measured by the K-EDN kit and TEC were higher ($\rho = 0.358$, $P < 0.0001$) than those between serum EDN levels measured by the MBL kit and TEC ($\rho = 0.319$, $P < 0.0001$) or serum periostin level ($\rho = 0.222$, $P < 0.0001$). Multivariate regression analysis demonstrated that serum EDN levels measured by the K-EDN kit predicted the phenotype of SA ($P = 0.003$), while 2 other biomarkers did not.
Conclusions: The serum EDN level may be a useful biomarker for assessing asthma severity in adult asthmatics.

Keywords: Eosinophil-derived neurotoxin; asthma; biomarkers

Chang-Gyu Jung
https://orcid.org/0000-0001-9163-9253
Su-Chin Kim
https://orcid.org/0000-0002-8890-6143
Youngwoo Choi
https://orcid.org/0000-0002-8384-9557
You Sook Cho
https://orcid.org/0000-0001-8767-2667
Chang-Keun Kim
https://orcid.org/0000-0003-4119-2849
Hae-Sim Park
https://orcid.org/0000-0003-2614-0303

Disclosure
There are no financial or other issues that might lead to conflict of interest.

INTRODUCTION

Asthma is a chronic inflammatory disease of multifactorial etiologies that affects 300 million people worldwide. Initially, asthma was considered typical eosinophilic airway inflammation initiated by allergic sensitization, which results in airway hyperresponsiveness (AHR) and acute bronchoconstriction. Among asthmatic patients, severe asthma (SA) is responsible for more than 50% of the medical expense of asthma, even though it accounts for 5%-10% of entire asthma patients. Severe asthmatics are suffering from frequent exacerbations that contribute to progressive lung function decline and increasing burden of medical cost.[1]

The eosinophilic phenotype of asthma is related to type 2 cytokines such as interleukin (IL)-4, IL-5, and IL-13. Corticosteroid (CS) is a key anti-inflammatory agent to control eosinophilic inflammation, and biologics targeting type 2 inflammation are effective in SA with high total eosinophil count (TEC).[2,3] There have been traditional biomarkers used to monitor eosinophilic inflammation in asthma, including TEC, sputum eosinophil count (%), serum periostin level, and fractional excretion of nitric oxide (FeNO) concentration.[4] Not only are sputum eosinophil count and FeNO concentration relatively hard to yield and variable by CS therapy, but also the studies of TEC predicting sputum eosinophil counts in eosinophilic asthma have shown conflicting results.[5,10] To date, TEC has been suggested to be a biomarker for predicting favorable responses to anti-IL-5 antibody treatment in SA.[11,13] Since there has been no serum biomarker available that can effectively reflect eosinophilic inflammation in SA, the need to identify an appropriate biomarker is continuously emerging. While periostin has been known to be related to type 2 airway inflammation in asthma, several studies have reported eosinophil-derived neurotoxin (EDN), a degranulation protein released from eosinophils, is deemed to represent eosinophil activation in childhood asthma and atopic dermatitis, and its value as a serum biomarker has not yet been evaluated in adult severe asthmatics.[14,15]

We hypothesized that serum EDN could predict SA. This study aimed to analyze the association of serum EDN with eosinophil-related clinical parameters, including TEC, and serum periostin level in patients with SA as compared to those with nonsevere asthma (NSA). It also aimed to validate the new enzyme-linked immunosorbent assay (ELISA) kit developed in this country.

MATERIALS AND METHODS

Study populations

A total of 1,133 Korean adult asthmatic patients, including 898 severe and 235 nonsevere asthmatics, were enrolled in the study. The study populations included 125 normal controls (NCs) without known allergic diseases. Allergy specialists diagnosed asthma according to the Global Initiative for Asthma guideline. The subjects were classified into the SA and NSA groups by the International European Respiratory Society/American Thoracic Society guidelines.[16] Any patient who had known to have underlying respiratory diseases other than asthma was excluded. All participants provided written informed consent before participating in this study, and Ajou University Institutional Review Board approval was obtained (AJIRB-GEN-SMP-13-108).

The demographic characteristics of the study subjects were collected, including age, sex, smoking history, and the duration of asthma. Atopy was defined as at least 1 positive reaction

to a skin prick test of 55 common inhalant allergens (Bencard Co., Brentford, UK), in which a positive reaction was defined as the ratio of the mean diameter of wheal of the allergen to histamine was 1 or higher. Chronic rhinosinusitis and nasal polyps were diagnosed with paranasal sinus radiography, computed tomography, and rhinoscopic findings. Pulmonary function test was completed as previously described.[17] Methacholine bronchial challenge test was performed using double doses of Provocholine® (methacholine chloride USP, Methapharm Inc., Brantford, Canada) from 1 to 16 mg/mL. Serum total immunoglobulin E (IgE), serum specific IgE, and serum eosinophil cationic protein (ECP) levels were measured by the UniCAP® system (ThermoFisher Scientific, Waltham, MA, USA). Serum samples were collected at the initial visit and frozen at −70°C; on initial evaluation, they were thawed immediately before use. Sputum eosinophil and neutrophil counts were expressed as the percentage of the cells among nonsquamous cells in the samples.

Measurement of serum EDN and serum periostin

The serum levels of EDN were measured using 2 different ELISA kits, following each manufacturer's protocol. The one is a commercial kit that has already been used for the measurement of EDN (MBL International, Woburn, MA, USA), and the other is a newly developed kit named the K-EDN kit (SKIMS-BIO Co., Seoul, Korea). Their measurements were taken as previously described.[18] As another biomarker for eosinophilic inflammation, serum periostin levels were measured simultaneously using ELISA (Shino-Test, Kanagawa, Japan) as previously described.[19]

Statistical analysis

The Mann-Whitney *U* test and the χ^2 test were used to compare the unpaired continuous and dichotomous variables. Spearman rank correlation analysis defined correlations among the continuous variables. Logistic regression analyses identified the contributions of continuous and dichotomous variables to SA. All statistical analyses were performed by using IBM SPSS software version 18.0 (IBM Corp., Armonk, NY, USA). *P* values of < 0.05 were considered statistically significant.

RESULTS

Demographic and clinical characteristics

Demographic and clinical parameters of the study subjects were compared (**Table 1**). Severe asthmatics were older (46.01 ± 13.93 *vs.* 42.69 ± 14.63 years, $P = 0.001$) and had more frequent smoking history (57.4% *vs.* 45.9%, $P = 0.023$) and longer asthma duration (8.04 ± 6.61 *vs.* 5.72 ± 10.56 years, $P < 0.0001$) compared to nonsevere asthmatics. They had higher prevalences of upper airway comorbidities such as chronic rhinosinusitis (50.0% *vs.* 35.0%, $P = 0.008$) and nasal polyps (41.7% *vs.* 28.0%, $P = 0.007$). However, no difference was noted in the proportion of female sex or atopy between severe and nonsevere asthmatics (62.55% *vs.* 61.87%, $P = 0.848$; 53.33% *vs.* 54.48%, $P = 0.885$, respectively). Severe asthmatics had lower levels of baseline forced expiratory volume in 1 second (FEV1; 71.38% ± 21.81% *vs.* 89.75% ± 20.29%, $P < 0.001$) and lower methacholine PC20 levels (7.37 ± 12.15 *vs.* 10.79 ± 20.09 mg/mL, $P = 0.042$). While log-transformed serum total IgE level was not significantly different (2.27 ± 0.55 *vs.* 2.20 ± 0.64, $P = 0.248$), log-transformed TEC was measured higher in severe asthmatics (2.53 ± 0.44 *vs.* 2.39 ± 0.47, $P = 0.002$). Sputum eosinophil count was identified higher in severe asthmatics (26.95% ± 35.59% *vs.* 22.11% ± 33.15%, $P = 0.031$), whereas sputum neutrophil count was not different between the groups (56.62% ± 35.29% *vs.* 58.90%

https://doi.org/10.4168/aair.2019.11.3.394

Table 1. Comparisons of clinical characteristics and eosinophil biomarkers between severe and nonsevere asthmatics

Variables	Severe asthmatics (n = 235)	Non severe asthmatics (n = 898)	*P* value
Age (yr)	46.01 ± 13.93	42.69 ± 14.63	0.001
Sex (female, %)	62.6	61.9	0.848
Atopy (%)	54.4	54.9	0.885
Smoking history (current and ex-smoker, %)	57.4	45.9	0.023
Duration of asthma (yr)	8.04 ± 6.61	5.72 ± 10.56	< 0.0001
Chronic rhinosinusitis (%)	50.0	35.0	0.008
Nasal polyp (%)	41.7	28.0	0.007
Log (baseline FEV1)	1.83 ± 0.15	1.94 ± 0.12	< 0.0001
Log (methacholine PC20)	0.40 ± 0.67	0.53 ± 0.73	0.042
Log (total IgE)	2.27 ± 0.55	2.20 ± 0.64	0.248
Log (total eosinophil count)	2.53 ± 0.44	2.39 ± 0.47	0.002
Sputum eosinophil (%)	26.95 ± 35.59	22.11 ± 33.15	0.031
Sputum neutrophil (%)	56.62 ± 35.29	58.90 ± 33.84	0.558
Serum ECP (μg/L)	44.52 ± 45.95	31.99 ± 38.46	< 0.0001
Serum EDN (MBL) (ng/mL)	69.08 ± 42.40	58.46 ± 35.56	0.034
Serum EDN (K-EDN) (ng/mL)	157.75 ± 128.18	109.02 ± 104.12	0.001
Serum periostin (ng/mL)	91.56 ± 41.21	77.72 ± 38.56	0.001

Each value is presented as mean ± standard deviation or %.
FEV1, forced expiratory volume in 1 second; PC20, provocation concentration causing a 20% fall in FEV1; Log (total IgE), log-transformed serum total immunoglobulin E levels; Log (total eosinophil count), log-transformed total eosinophil count; ELISA, enzyme-linked immunosorbent assay; EDN, eosinophil-derived neurotoxin; Serum EDN (MBL), serum EDN levels measured by ELISA kit of MBL; Serum EDN (K-EDN), serum EDN levels measured by ELISA kit of K-EDN.

± 33.84%, $P = 0.558$). Both serum ECP level (44.52 ± 45.95 μg/L *vs.* 31.99 ± 38.46 μg/L, $P < 0.0001$) and serum periostin level (91.56 ± 41.21 ng/mL *vs.* 77.72 ± 38.56 ng/mL, $P = 0.001$) were measured higher in severe asthmatics than in nonsevere asthmatics.

Comparison of serum EDN levels measured by the 2 different ELISA kits

Serum EDN levels measured by both MBL and K-EDN kits were higher in severe asthmatics (69.08 ± 42.40 *vs.* 58.46 ± 35.56 ng/mL, $P = 0.034$; 157.75 ± 128.18 *vs.* 109.02 ± 104.12 ng/mL, $P = 0.001$, respectively), while no difference was found between nonsevere asthmatics and NCs ($P = 0.55$) as shown in **Fig. 1**. Although a significant correlation was found between serum EDN levels measured by the 2 ELISA kits ($\rho = 0.545$; $P < 0.0001$), the mean serum EDN level was found to be higher by the K-EDN kit than by the MBL kit in all study subjects. Serum EDN levels measured by the MBL kit showed a significant difference between severe and nonsevere asthmatics, but they were not so significant as serum EDN levels measured by the K-EDN kit ($P = 0.034$ and $P = 0.001$, respectively; **Fig. 1**). The mean age of NCs was 51.1 years, which was older than that of severe or nonsevere asthmatics. Analysis of covariance was performed to identify the contribution of age to the serum EDN levels measured by the K-EDN kit, but no significant impact was observed ($P = 0.104$).

Association of serum EDN with clinical parameters including TEC

When correlations between serum EDN levels measured by the 2 kits and TEC were evaluated, serum EDN levels measured by the K-EDN kit showed a higher correlation coefficient ($\rho = 0.358$, $P < 0.001$, **Fig. 2A**) than that measured by the MBL kit ($\rho = 0.319$, $P < 0.001$, **Fig. 2B**). Serum periostin levels were less correlated to TEC than serum EDN levels measured by the 2 kits ($\rho = 0.222$, $P < 0.0001$, **Fig. 2C**). Significant correlations were found between serum EDN levels measured by the 2 kits and serum periostin levels ($\rho = 0.304$, $P < 0.0001$ for serum EDN levels measured by K-EDN kit; $\rho = 0.302$, $P < 0.0001$ for serum EDN levels measured by MBL kit), but less correlated to TEC. Serum EDN levels measured by the 2 kits were significantly correlated ($\rho = 0.545$, $P < 0.0001$). Serum ECP levels were correlated to

https://doi.org/10.4168/aair.2019.11.3.394

Allergy, Asthma & Immunology Research AAIR

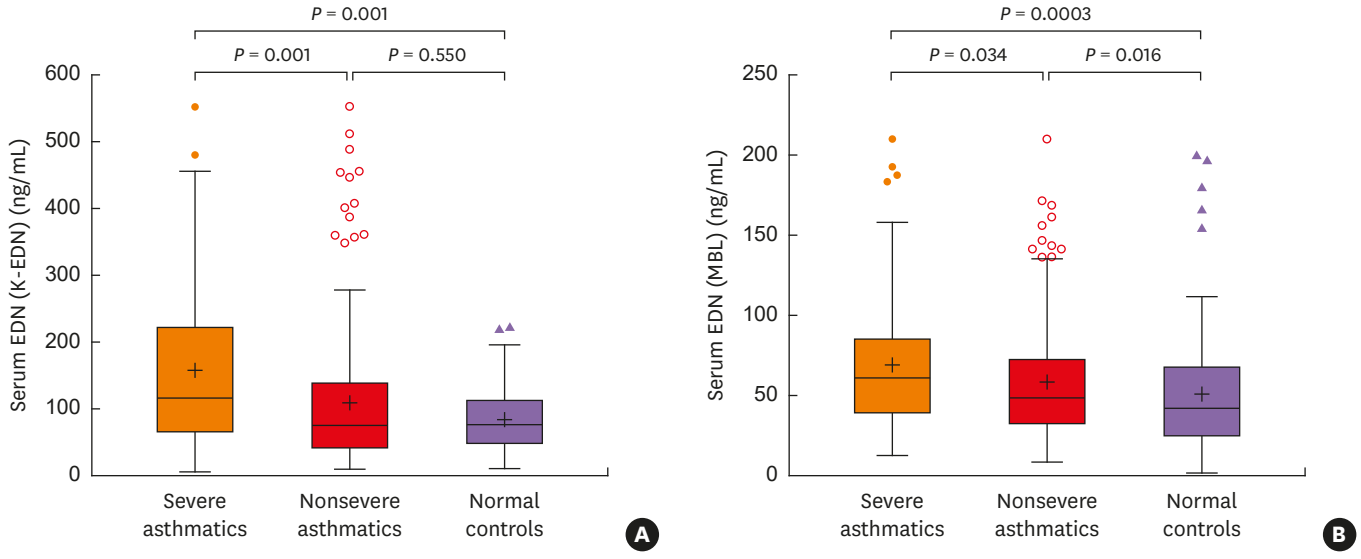

Fig. 1. (A) Comparison of serum EDN levels measured by the K-EDN kit among severe asthmatics, nonsevere asthmatics, and NCs. (B) Comparison of serum EDN levels measured by the MBL kit between severe asthmatics, nonsevere asthmatics, and NCs.
EDN, eosinophil-derived neurotoxin; NC, normal control.

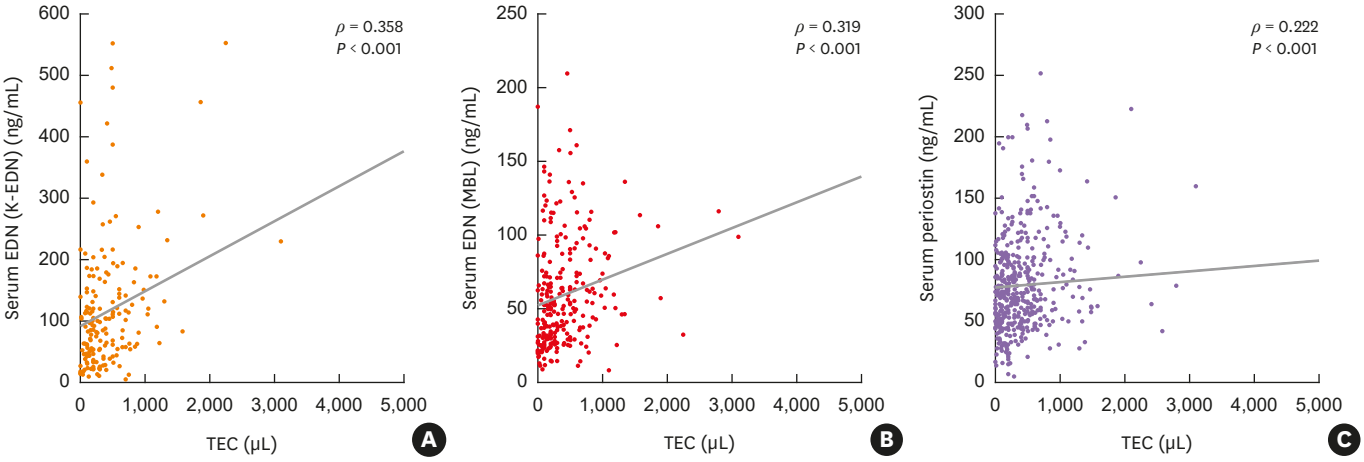

Fig. 2. Correlation between the eosinophilic biomarkers in total asthmatics. (A) TEC and serum EDN levels measured by the K-EDN kit. (B). TEC and serum EDN levels measured by the MBL kit. (C) TEC and serum periostin levels.
EDN, eosinophil-derived neurotoxin; TEC, total eosinophil count.

TEC ($\rho = 0.302$, $P < 0.0001$); they were less correlated to TEC than serum EDN levels were. There was no significant correlation between ECP levels and serum periostin levels ($\rho = 0.083$ and $P = 0.083$, respectively). Either serum ECP, EDN levels measured by the 2 kits or periostin levels were not correlated to sputum eosinophil count.

Serum EDN level as a biomarker of SA

The receiver operating characteristic curves of serum EDN levels measured by the 2 kits were obtained to predict the phenotype of SA. Serum EDN levels measured by the K-EDN kit showed a higher area under the curve (AUC) value with statistical significance (AUC value: 0.632, $P = 0.002$ at the cutoff value of 85.97 ng/mL with 60.3% of sensitivity and 56.6% of specificity, **Fig. 3A**) than those measured by the MBL kit (AUC value: 0.576, $P = 0.034$ at the cutoff value of 53.22 ng/mL with 60.7% of sensitivity and 56.2% of specificity, **Fig. 3B**). In

https://doi.org/10.4168/aair.2019.11.3.394

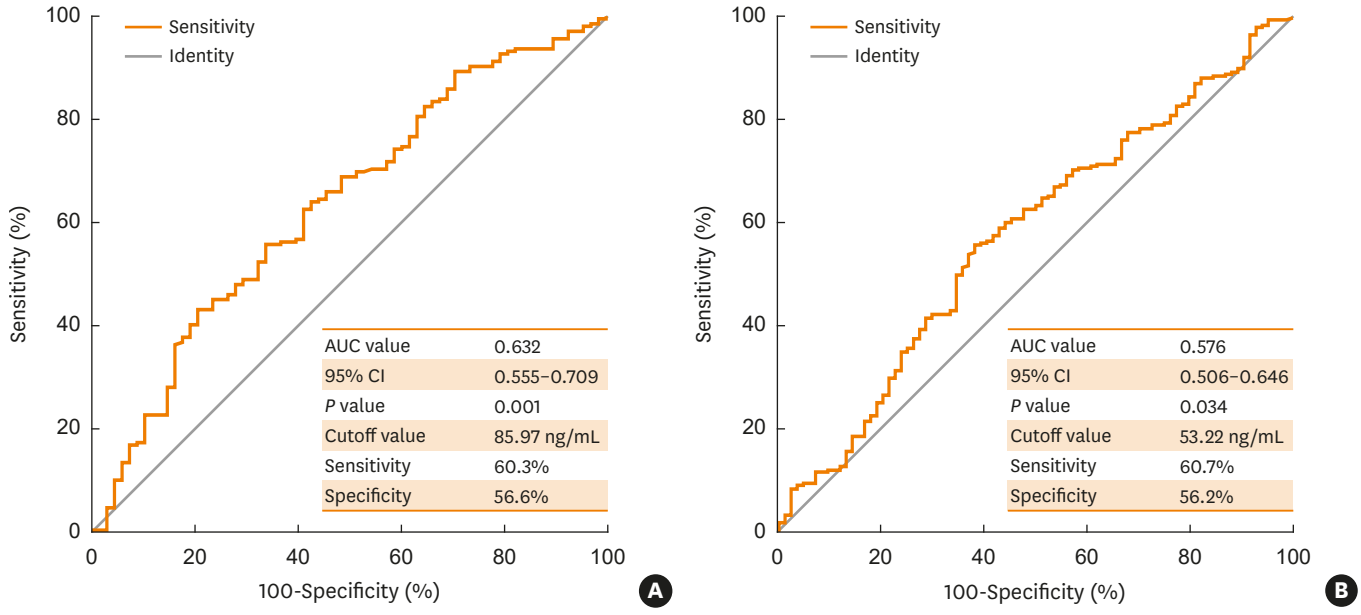

Fig. 3. (A) ROC curves for the serum EDN levels measured by the K-EDN kit in the prediction of severe asthma in the total asthmatics. (B) ROC curves for the serum EDN levels measured by the MBL kit in the prediction of severe asthma in the total asthmatics.
ROC, receiver operating characteristic; EDN, eosinophil-derived neurotoxin; AUC, area under the curve; CI, confidence interval.

addition, the predictability of each clinical parameter for SA was analyzed using univariate and multivariate logistic regression analyses (**Table 2**). Serum EDN levels measured by both K-EDN and MBL kits as well as serum ECP and periostin levels were identified to be significant parameters associated with SA in the univariate analysis (all $P < 0.05$). However, multivariate analysis showed that serum EDN levels measured by the K-EDN kit remained a significant parameter ($P = 0.003$) for predicting the phenotype of SA.

The total study subjects were divided into 2 groups (high responders and low responders) according to each cutoff value of 85.97 ng/mL for the K-EDN kit and 54.22 ng/mL for the MBL kit; their demographic and clinical characteristics were compared (data not shown). The high-responders of the K-EDN showed a higher prevalence of SA (31.5% *vs.* 18.9%, $P = 0.016$) as well as a lower log-transformed baseline FEV1 level (1.92 ± 0.11 *vs.* 1.95 ± 1.00, $P = 0.019$) compared to low-responders of the K-EDN. They also had higher log-transformed TEC and sputum eosinophil count (2.58 ± 0.46 *vs.* 2.35 ± 0.41, $P < 0.0001$; 28.59% ± 37.37% *vs.* 18.14% ± 31.79%, $P = 0.044$, respectively), and lower serum ECP (52.83 ± 46.30 μg/L *vs.* 31.65 ± 32.62

Table 2. Predictability for severe asthma by univariate and multivariate logistic regression analyses

Variable	Univariate		Multivariate	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Duration of asthma	1.016 (0.995–1.038)	0.131	-	-
Total IgE (IU/L)	1.000 (1.000–1.000)	0.694	-	-
Total eosinophil count (/uL)	1.000 (1.000–1.000)	0.116	-	-
Sputum eosinophil count (%)	1.004 (0.998–1.011)	0.207	-	-
Sputum neutrophil count (%)	0.998 (0.991–1.005)	0.586	-	-
Serum ECP (μg/L)	1.007 (1.002–1.011)	0.002	-	-
Serum periostin (ng/mL)	1.008 (1.003–1.013)	0.001	-	-
Serum EDN (MBL) (ng/mL)	1.007 (1.001–1.013)	0.025	-	-
Serum EDN (K-EDN) (ng/mL)	1.003 (1.001–1.006)	0.003	1.004 (1.002–1.007)	0.003

CI, confidence interval; IgE, immunoglobulin E; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; ELISA, enzyme-linked immunosorbent assay; Serum EDN (MBL), serum EDN levels measured by ELISA kit of MBL; Serum EDN (K-EDN), serum EDN levels measured by ELISA kit of K-EDN; OR, odds ratio.

https://doi.org/10.4168/aair.2019.11.3.394

μg/L, $P < 0.0001$) and periostin (95.88 ± 44.74 ng/mL *vs.* 73.63 ± 36.16 ng/mL, $P < 0.0001$) levels; methacholine PC20 levels and sputum neutrophil counts had no differences ($P = 0.813$ and $P = 0.960$, respectively). The high-responder group of the MBL kit had similar results of higher SA prevalence (29.8% *vs.* 17.6%, $P = 0.007$), lower log-transformed baseline FEV1 level (1.92 ± 0.12 *vs.* 1.96 ± 0.09, $P = 0.003$), higher log-transformed TEC and sputum eosinophil count (2.58 ± 0.44 *vs.* 2.32 ± 0.45, $P < 0.0001$; 28.75% ± 37.49% *vs.* 17.64% ± 30.83%, $P = 0.025$, respectively), as well as higher serum ECP and periostin level (47.97 ± 44.33 μg/L *vs.* 33.19 ± 37.38 μg/L, $P < 0.0001$; 92.83 ± 40.59 ng/mL *vs.* 74.66 ± 39.49 ng/mL, $P < 0.0001$, respectively). Additionally, they had a higher female proportion and a lower log-transformed methacholine PC20 level compared to low responders of MBL kit (55.6% *vs.* 68.4%, $P = 0.012$; 0.57 ± 0.63 *vs.* 0.77 ± 0.58, $P = 0.009$, respectively).

DISCUSSION

Eosinophils are key cells in the airway inflammation of SA and associated with persistent elevation of type 2 inflammatory markers. Since increased expression of periostin in serum/tissue has been suggested to be a biomarker for type 2 inflammation in adult asthmatic patients,[20] our previous study demonstrated that adult asthmatics with SA had significantly higher serum periostin levels, indicating that serum periostin is a biomarker for type 2 inflammation in adult asthmatics.[4] However, the present study showed that the serum periostin level was not correlated with TEC, whereas TEC is a marker for determining anti-IL-5 antibody treatment for SA patients. It is necessary to establish a useful serum biomarker for assessing the degree of type-2 airway inflammation in the management of SA.[21,22] There have been a few studies suggesting the serum EDN level to be a biomarker for asthma severity in childhood.[23] This is the first study to suggest that the serum EDN level is a useful serum biomarker for assessing the severity of SA in adult asthmatics. The serum EDN level was significantly higher in patients with SA than in those with NSA; the significant correlation coefficient was noted between TEC and serum EDN measured by the K-EDN kit compared to those by the MBL kit, and between TEC and serum periostin/ECP levels. In addition, multivariate analysis showed that serum EDN levels measured by the K-EDN kit remained a single significant parameter for predicting SA.

Recent large-scale studies have led to a better understanding of the characteristics of SA. They proved distinct demographic/clinical characteristics consistently relevant to SA; older age, possible associations with female sex and smoking, frequent exacerbations, low baseline lung function, atopy rate, need for high doses of inhaled or systemic CS, and higher prevalence of upper and lower respiratory comorbidities.[24-26] We confirmed that the characteristics of severe asthmatics in this study were consistent with these findings. However, no differences were noted in the female sex and atopy. It also showed that lower lung function and increased AHR are key features of SA. Moreover, it was noted that eosinophilic inflammation was another key feature of SA in this study. Serum EDN levels measured by the K-EDN and MBL kits as well as TEC, sputum eosinophil count, serum periostin, and serum ECP levels were significantly higher in severe asthmatics than in nonsevere asthmatics. These findings confirmed that eosinophilia in blood/airway secretion is the key feature of SA in adult asthmatics. TEC and sputum eosinophilia (>3%) have been used to define eosinophilic asthma.[27-29] A previous study demonstrated that adult asthmatics with more than 300/μL of TEC had the highest tendency of asthma exacerbation among cutoff values of 200 to 400/μL.[30] Still, another study demonstrated that TEC was rarely

https://doi.org/10.4168/aair.2019.11.3.394

correlated with eosinophilia in bronchoalveolar lavage (BAL) fluid or endobronchial biopsies in patients with SA.[31] It is not practical to differentiate phenotypes using these parameters, since the phenotypes vary widely among patients and depend on clinical severity and medications used.[26,32,33] Due to these clinical characteristics of SA, clinically applicable serum biomarkers that reflect eosinophilic inflammation are necessary to keep severe asthmatics from exacerbation and lung function decline.

It is essential to distinguish phenotypes between eosinophilic and noneosinophilic asthmatics because the more severe eosinophilic inflammation, the higher risk of asthma exacerbation which develops SA. Effective control of eosinophilic inflammation has proved beneficial to reduce asthma exacerbation.[34-37] As the phenotype of eosinophilic asthma has been determined by sputum eosinophil counts, sputum induction carries a risk of asthma exacerbation, especially in severe asthmatics, and it is not always successful because of its variability.[38] In addition, lab facilities and expertise are required to analyze sputum samples. For this reason, a serum biomarker, which is stable and reproducible to represent type 2 inflammation, is considered useful as it is relatively safe and easy-to-access in real clinical practice. Among eosinophil-derived molecules, EDN is known to be relatively cheap and stable to measure. Elevated EDN levels were found in serum, urine, and other body fluids in patients with eosinophil-associated diseases.[39] The serum EDN level was the only marker for differentiating childhood asthmatics with acute exacerbation from those in stable status, showing a greater correlation with the severity of asthma when compared to serum ECP levels or TEC.[23] In addition, it was correlated with the degree of persistent airflow limitation in allergic asthmatics.[40] Our previous study investigated that peripheral blood eosinophils and serum EDN levels were elevated in SA, which was attributed to increased eosinophil extracellular traps that promoted the degranulation of eosinophils. A significnat correlation was found between extracellular trap-contating eosinophils and EDN levels.[41] We found that the serum EDN level was significantly higher in severe asthmatics than in nonsevere asthmatics; especially, serum EDN level measured by the K-EDN kit are more significantly elevated in severe asthmatics than those measured by the MBL kit. We also observed that serum EDN levels measured by the K-EDN kit was significantly elevated in severe asthmatics compared to NCs, while no difference was found between nonsevere asthmatics and NCs or between total asthmatics and NCs. Additionally, serum EDN levels measured by the K-EDN kit showed a better correlation to TEC than those measured by the MBL kit or serum periostin levels. Moreover, serum EDN levels measured by the K-EDN kit could predict SA best among other clinical parameters. Periostin is known as a type 2 inflammation marker, but it is mainly released from airway epithelial cells affected by IL-13 and various stimuli, but not from eosinophils. In the present study, a relatively weak correlation was found between the serum periostin and TEC levels. In patients with SA, furthermore, periostin is more valuable in evaluating eosinophil activation status than in evaluating peripheral eosinophil count. When eosinophils are activated, they release several mediators including MBP, ECP, and EDN. To date, we cannot measure serum MBP levels using a kit. Several studies showed confounding results of serum ECP level as a biomarker for asthma and other allergic diseases.[15,42,43] Serum ECP level can be measured using commercially available kits, although their cost is expensive. In the present study, a significantly higher serum ECP level was noted in severe asthmatics than in nonsevere asthmatics; however, a lower correlation was found with TEC. ECP is also a highly charged protein with sticky quality; therefore, it is likely to adhere to the wall of a sample tube or other substances. Therefore, its level is variable from each measurement. These findings suggest that the serum EDN level can be applied as a stable serum biomarker for better assessing the severity of eosinophilic inflammation, especially in SA.

https://doi.org/10.4168/aair.2019.11.3.394

There are several limitations in this study. First, it is a cross-sectional study in a single tertiary center. Although the number of subjects was not small, replicated experiments in other subjects' samples are needed to enhance and consolidate the hypothesis. Secondly, it was difficult to control the medications such as inhaled/systemic CS that could affect eosinophilic inflammation status in the study subjects. Thirdly, further investigations are required to explore the exact function of EDN (difference from periostin) and other inflammatory mechanisms than eosinophilic inflammation in the pathogenesis of SA. Airway neutrophilia was documented in patients with severe exacerbations, but its role in SA remains to be elucidated.[44] A few studies demonstrated increased IL-17 levels in samples (induced sputum, BAL samples, and bronchial biopsies) of severe asthmatics.[45-47] In another study, IL-17-producing cells and IL-17-related gene expression signature were increased and orthogonal to eosinophilic inflammation in SA.[48] Some patients with SA have mixed cellular profiles (both sputum eosinophilia and neutrophilia). Our previous report demonstrated increased expression of neutrophil extracellular traps (NETs) in severe asthmatics compared to nonsevere asthmatics. NETs were found to induce eosinophil activation, suggesting a close interaction between these 2 inflammatory cells.[49] Follow-up studies are essential to evaluate the clinical significance of serum EDN, treatment response to anti-inflammatory medications, and long-term outcomes in SA.

The results of this study suggest that the serum EDN level can help identify SA in adult asthmatics. Considering that SA has heterogeneous phenotypes, further studies are needed to identify useful biomarkers for assessing subtypes and long-term clinical outcome of SA.

ACKNOWLEDGMENTS

This study was supported by a grant (HI16C0992 and HI14C2628) from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Korea. We appreciate to SKIMS-BIO Co. (Seoul, Korea) to provide the K-EDN kit to measure serum EDN levels in this study.

REFERENCES

1. Yoo KH, Ahn HR, Park JK, Kim JW, Nam GH, Hong SK, et al. Burden of respiratory disease in Korea: an observational study on allergic rhinitis, asthma, COPD, and rhinosinusitis. Allergy Asthma Immunol Res 2016;8:527-34.
PUBMED | CROSSREF
2. Castro M, Zangrilli J, Wechsler ME, Bateman ED, Brusselle GG, Bardin P, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. Lancet Respir Med 2015;3:355-66.
PUBMED | CROSSREF
3. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.
PUBMED | CROSSREF
4. Kim MA, Izuhara K, Ohta S, Ono J, Yoon MK, Ban GY, et al. Association of serum periostin with aspirin-exacerbated respiratory disease. Ann Allergy Asthma Immunol 2014;113:314-20.
PUBMED | CROSSREF
5. Pin I, Freitag AP, O'Byrne PM, Girgis-Gabardo A, Watson RM, Dolovich J, et al. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. Am Rev Respir Dis 1992;145:1265-9.
PUBMED | CROSSREF
6. Pizzichini MM, Pizzichini E, Clelland L, Efthimiadis A, Mahony J, Dolovich J, et al. Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after prednisone treatment. Am J Respir Crit Care Med 1997;155:1501-8.
PUBMED | CROSSREF

7. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. Chest 2001;119:1322-8.
PUBMED | CROSSREF
8. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. Thorax 2015;70:115-20.
PUBMED | CROSSREF
9. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. J Allergy Clin Immunol 2013;132:72-80.
PUBMED | CROSSREF
10. Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. BMC Pulm Med 2013;13:11.
PUBMED | CROSSREF
11. Goldman M, Hirsch I, Zangrilli JG, Newbold P, Xu X. The association between blood eosinophil count and benralizumab efficacy for patients with severe, uncontrolled asthma: subanalyses of the Phase III SIROCCO and CALIMA studies. Curr Med Res Opin 2017;33:1605-13.
PUBMED | CROSSREF
12. Corren J, Weinstein S, Janka L, Zangrilli J, Garin M. Phase 3 study of reslizumab in patients with poorly controlled asthma: effects across a broad range of eosinophil counts. Chest 2016;150:799-810.
PUBMED | CROSSREF
13. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. Lancet Respir Med 2016;4:549-56.
PUBMED | CROSSREF
14. Kim CK. Eosinophil-derived neurotoxin: a novel biomarker for diagnosis and monitoring of asthma. Korean J Pediatr 2013;56:8-12.
PUBMED | CROSSREF
15. Taniuchi S, Chihara J, Kojima T, Yamamoto A, Sasai M, Kobayashi Y. Serum eosinophil derived neurotoxin may reflect more strongly disease severity in childhood atopic dermatitis than eosinophil cationic protein. J Dermatol Sci 2001;26:79-82.
PUBMED | CROSSREF
16. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014;43:343-73.
PUBMED | CROSSREF
17. Kim SH, Yang EM, Lee HN, Choi GS, Ye YM, Park HS. Association of the CCR3 gene polymorphism with aspirin exacerbated respiratory disease. Respir Med 2010;104:626-32.
PUBMED | CROSSREF
18. Kim CK, Callaway Z, Park JS, Kwon E. Utility of serum eosinophil-derived neurotoxin (EDN) measurement by ELISA in young children with asthma. Allergol Int 2017;66:70-4.
PUBMED | CROSSREF
19. Masuoka M, Shiraishi H, Ohta S, Suzuki S, Arima K, Aoki S, et al. Periostin promotes chronic allergic inflammation in response to Th2 cytokines. J Clin Invest 2012;122:2590-600.
PUBMED | CROSSREF
20. Parulekar AD, Atik MA, Hanania NA. Periostin, a novel biomarker of TH2-driven asthma. Curr Opin Pulm Med 2014;20:60-5.
PUBMED | CROSSREF
21. Corren J, Lemanske RF Jr, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011;365:1088-98.
PUBMED | CROSSREF
22. Fajt ML, Gelhaus SL, Freeman B, Uvalle CE, Trudeau JB, Holguin F, et al. Prostaglandin D_2 pathway upregulation: relation to asthma severity, control, and TH2 inflammation. J Allergy Clin Immunol 2013;131:1504-12.
PUBMED | CROSSREF
23. Kim CK, Callaway Z, Fletcher R, Koh YY. Eosinophil-derived neurotoxin in childhood asthma: correlation with disease severity. J Asthma 2010;47:568-73.
PUBMED | CROSSREF

24. European Network for Understanding Mechanisms of Severe Asthma. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. Eur Respir J 2003;22:470-7.
PUBMED | CROSSREF

25. Dolan CM, Fraher KE, Bleecker ER, Borish L, Chipps B, Hayden ML, et al. Design and baseline characteristics of the epidemiology and natural history of asthma: Outcomes and Treatment Regimens (TENOR) study: a large cohort of patients with severe or difficult-to-treat asthma. Ann Allergy Asthma Immunol 2004;92:32-9.
PUBMED | CROSSREF

26. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. J Allergy Clin Immunol 2007;119:405-13.
PUBMED | CROSSREF

27. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. Am J Respir Crit Care Med 2000;161:475-8.
PUBMED | CROSSREF

28. Spanevello A, Confalonieri M, Sulotto F, Romano F, Balzano G, Migliori GB, et al. Induced sputum cellularity. Reference values and distribution in normal volunteers. Am J Respir Crit Care Med 2000;162:1172-4.
PUBMED | CROSSREF

29. Thomas RA, Green RH, Brightling CE, Birring SS, Parker D, Wardlaw AJ, et al. The influence of age on induced sputum differential cell counts in normal subjects. Chest 2004;126:1811-4.
PUBMED | CROSSREF

30. Tran TN, Khatry DB, Ke X, Ward CK, Gossage D. High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. Ann Allergy Asthma Immunol 2014;113:19-24.
PUBMED | CROSSREF

31. Ullmann N, Bossley CJ, Fleming L, Silvestri M, Bush A, Saglani S. Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. Allergy 2013;68:402-6.
PUBMED | CROSSREF

32. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010;181:315-23.
PUBMED | CROSSREF

33. Wu W, Bleecker E, Moore W, Busse WW, Castro M, Chung KF, et al. Unsupervised phenotyping of Severe Asthma Research Program participants using expanded lung data. J Allergy Clin Immunol 2014;133:1280-8.
PUBMED | CROSSREF

34. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med 2008;178:218-24.
PUBMED | CROSSREF

35. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.
PUBMED | CROSSREF

36. Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemière C, Pizzichini E, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. Eur Respir J 2006;27:483-94.
PUBMED | CROSSREF

37. Chlumský J, Striz I, Terl M, Vondracek J. Strategy aimed at reduction of sputum eosinophils decreases exacerbation rate in patients with asthma. J Int Med Res 2006;34:129-39.
PUBMED | CROSSREF

38. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology 2006;11:54-61.
PUBMED | CROSSREF

39. Furuta GT, Kagalwalla AF, Lee JJ, Alumkal P, Maybruck BT, Fillon S, et al. The oesophageal string test: a novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis. Gut 2013;62:1395-405.
PUBMED | CROSSREF

40. Gon Y, Ito R, Hattori T, Hiranuma H, Kumasawa F, Kozu Y, et al. Serum eosinophil-derived neurotoxin: correlation with persistent airflow limitation in adults with house-dust mite allergic asthma. Allergy Asthma Proc 2015;36:e113-20.
PUBMED | CROSSREF

41. Choi Y, Le Pham D, Lee DH, Lee SH, Kim SH, Park HS. Biological function of eosinophil extracellular traps in patients with severe eosinophilic asthma. Exp Mol Med 2018;50:104.
PUBMED | CROSSREF

42. Kim KW, Lee KE, Kim ES, Song TW, Sohn MH, Kim KE. Serum eosinophil-derived neurotoxin (EDN) in diagnosis and evaluation of severity and bronchial hyperresponsiveness in childhood asthma. Lung 2007;185:97-103.
PUBMED | CROSSREF

43. Wojnarowski C, Roithner B, Koller DY, Halmerbauer G, Gartner C, Tauber E, et al. Lack of relationship between eosinophil cationic protein and eosinophil protein X in nasal lavage and urine and the severity of childhood asthma in a 6-month follow-up study. Clin Exp Allergy 1999;29:926-32.
PUBMED | CROSSREF

44. Ordoñez CL, Shaughnessy TE, Matthay MA, Fahy JV. Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma: clinical and biologic significance. Am J Respir Crit Care Med 2000;161:1185-90.
PUBMED | CROSSREF

45. Nanzer AM, Chambers ES, Ryanna K, Richards DF, Black C, Timms PM, et al. Enhanced production of IL-17A in patients with severe asthma is inhibited by 1α,25-dihydroxyvitamin D3 in a glucocorticoid-independent fashion. J Allergy Clin Immunol 2013;132:297-304.e3.
PUBMED | CROSSREF

46. Irvin C, Zafar I, Good J, Rollins D, Christianson C, Gorska MM, et al. Increased frequency of dual-positive TH2/TH17 cells in bronchoalveolar lavage fluid characterizes a population of patients with severe asthma. J Allergy Clin Immunol 2014;134:1175-1186.e7.
PUBMED | CROSSREF

47. Bullens DM, Truyen E, Coteur L, Dilissen E, Hellings PW, Dupont LJ, et al. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? Respir Res 2006;7:135.
PUBMED | CROSSREF

48. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med 2015;7:301ra129.
PUBMED | CROSSREF

49. Pham DL, Ban GY, Kim SH, Shin YS, Ye YM, Chwae YJ, et al. Neutrophil autophagy and extracellular DNA traps contribute to airway inflammation in severe asthma. Clin Exp Allergy 2017;47:57-70.
PUBMED | CROSSREF