

Staphylococcal enterotoxin IgE sensitization in late-onset severe eosinophilic asthma in the elderly

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Summary

Background Asthma in the elderly (aged ≥ 65 years old) is a significant concern with high morbidity, but the pathophysiology remains unclear particularly in late-onset asthma. Recent studies suggest staphylococcal enterotoxin IgE (SE-IgE) sensitization to be a risk factor for asthma in general populations; however, the associations have not been examined in late-onset elderly asthma.

Objective We aimed to examine the associations of SE-IgE sensitization with late-onset asthma in the elderly, using a database of elderly asthma cohort study.

Methods A total of 249 elderly patients with asthma and 98 controls were analysed. At baseline, patients were assessed for demographics, atopy, induced sputum profiles and comorbidities including chronic rhinosinusitis (CRS). Serum total IgE and SE-IgE levels were measured. Asthma severity was assessed on the basis of asthma outcomes during a 12-month follow-up period.

Results At baseline, serum SE-IgE concentrations were significantly higher in patients with asthma than in controls [median 0.16 (interquartile range 0.04–0.53) vs. 0.10 (0.01–0.19), $P < 0.001$]. Elderly asthma patients with high SE-IgE levels had specific characteristics of having more severe asthma, sputum eosinophilia and CRS, compared to those with lower SE-IgE levels. In multivariate logistic regression analyses, the associations between serum SE-IgE concentrations and severe asthma were significant, independently of covariables [SE-IgE-high (≥ 0.35 kU/L) vs. negative (< 0.10 kU/L) group: odds ratio 7.47, 95% confidence interval 1.86–30.03, $P = 0.005$]. Multiple correspondence analyses also showed that high serum SE-IgE level had close relationships with severe asthma, CRS and sputum eosinophilia together.

Conclusions and Clinical Relevance This is the first report on the significant associations of SE-IgE sensitization with late-onset asthma in the elderly, particularly severe eosinophilic asthma with CRS comorbidity. Our findings indicate a potential implication of SE in the high morbidity burden of elderly asthma and suggest clues to the pathogenesis of severe late-onset eosinophilic asthma in the elderly.

Keywords aged, asthma, enterotoxins, eosinophils, immunoglobulin E, nasal polyps, *Staphylococcus aureus*

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Introduction

Recent evidence indicates that asthma is a significant disease in the elderly. The prevalence of asthma in the elderly is higher than was previously thought, and their morbidity and mortality are higher than those of younger patients [1]. Considering the rapid ageing of the global population [2], the burden of asthma in the elderly is expected to rise significantly. However, the pathophysiology of asthma in the elderly is not clear. Clinical observations suggest that a considerable proportion is adult or late-onset disease [1] and that it is a heterogeneous condition resulting from complex interactions with environmental exposures, microbial antigens or comorbidities [3]. In the literature, several risk factors have been suggested for asthma in the elderly, such as smoking and obesity [4, 5]. However, there is still little data on its pathophysiology.

Staphylococcus aureus is a human commensal microorganism that is responsible for infectious diseases. However, evidence is also accumulating that the superantigenic properties of staphylococcal enterotoxin (SE) play a role in the pathophysiology of allergic airway diseases including asthma [6–10]. In recent studies, SE-specific IgE (SE-IgE) sensitization was suggested as a major risk factor for severe asthma in adults [11]. It was also demonstrated that SE-IgE sensitization was an independent risk factor for adult asthma in general populations [12, 13]. Interestingly, SE-IgE sensitization rate increased or remained high in the elderly, whereas atopy rate significantly decreased with ageing [12, 13]. As asthma in the elderly was frequently non-atopic [1], we hypothesized that the pathophysiology of asthma in the elderly could be mediated by a strong IgE-stimulant like SE more than inhalant allergen. To our knowledge, the associations between SE-IgE sensitization and elderly asthma have not been examined in details, particularly in relation to late-onset asthma.

Here, we aimed to examine the associations of SE-IgE sensitization with late-onset asthma in the elderly, using the database of an elderly asthma cohort study.

Materials and methods

Study design

We conducted a case-control study of elderly patients with asthma and non-asthmatic controls (aged ≥ 65 years old) in 2013. Elderly patients with asthma were recruited from a previously reported cohort study [14]. Briefly, the cohort was established as a prospective observational multicentre study with planned 5-year follow-up (2009–2013). The cohort consisted of 1031 elderly patients with asthma recruited from nine referral

hospitals in Korea. The diagnosis of asthma was made by allergy physicians, based on clinical history and the documented presence of a reversible airway obstruction [a positive bronchodilator response or $\geq 12\%$ and 200 mL improvement in forced expiratory volume in 1 s (FEV1) after anti-asthmatic treatments]. Of the cohort participants, this study included the patients recruited at two institutions [Seoul National University Hospital (SNUH) and Seoul National University Bundang Hospital (SNUBH)] as they underwent specific assessments for chronic rhinosinusitis (CRS) and nasal polyps (NP); CRS and NP are known to be associated with asthma, particularly by interaction with SE-IgE [15]. Finally, 249 elderly patients with asthma from 370 patients recruited at SNUH and SNUBH were included in this study; the reasons for exclusion were no serum collection ($n = 59$), several comorbidities which could affect respiratory symptoms or serum IgE levels ($n = 19$), and no follow-up ($n = 43$). The flow chart of patient inclusion is presented in Fig. S1.

As a control group, non-asthmatic elderly people were recruited using bulletin board advertisement. The absence of asthma was confirmed by both questionnaires and methacholine challenge test (MCT). Questionnaires included the items 'Have you ever had asthma?' and 'Have you ever had wheezing or whistling in your chest?' Controls were recruited if they gave negative responses to both questions and also had a negative MCT (PC20 > 16 mg/mL by a five-breath dosimeter method [16]).

To focus on relationships between asthma and SE-IgE levels, several comorbid conditions that could affect lower respiratory tract symptoms or serum IgE levels were excluded. The exclusion criteria were applied to all of patients with asthma and controls and were congestive heart failure, Churg–Strauss syndrome, allergic bronchopulmonary aspergillosis, chest X-ray abnormality, eczema, malignancy, recent anti-IgE monoclonal antibody treatment and recent systemic corticosteroid therapy for conditions other than asthma or rhinosinusitis. Comorbid medical conditions were checked by questions of the following type: 'Have you ever been diagnosed with or treated for (specific disease) by a physician' or 'Have you been treated with (specific medication) by a physician within the last year?' Chest X-ray abnormality included bronchiectasis, emphysema, tuberculosis or other grossly abnormal parenchymal lesions.

All interviews were performed by research nurses experienced in geriatric assessment. All assessments were performed at the participating institutions. All participants were fully informed about the protocols and provided a written statement of informed consent. The study protocol was approved by the institutional review board of all participating institutions.

Baseline assessment

All participants underwent a baseline assessment at recruitment. Demographic factors and clinical history were assessed by self-administered questionnaires. Late-onset asthma was defined as development of asthma after the age of 40 years [3]. Body mass index (BMI) was calculated by measuring weight and height and using the formula $BMI = \text{weight/height squared}$. Diabetes mellitus and gastroesophageal reflux disease were evaluated as comorbidities using the structured questionnaires mentioned above.

Chronic rhinosinusitis was determined by the persistence of two or more of the following symptoms for ≥ 3 months during the last year: nasal discharge, nasal obstruction, facial pain and/or anosmia [17]. Subjects who had any of these symptoms underwent nasal endoscopy for NPs. NPs were also deemed positive if the subject had a history of NP surgery. Thus, the subjects were classified as no CRS, CRS without NP (CRSsNP) or CRS with NP (CRSwNP).

Atopy was assessed by skin prick tests for 55 common inhalant allergens in Korea. Atopy was defined positive if the subject had a positive skin response to any of the tested allergens (defined by an allergen-induced wheal 3 mm or greater than the negative control) [18]. They were instructed to withhold antihistamines and over-the-counter drugs for common colds for 5 days before skin prick tests. Predicted percentage values for FEV₁ (FEV_{1%}_{pred}) and forced vital capacity (FVC%_{pred}) were obtained using the methods of Morris [19]. Peripheral blood eosinophil counts were measured using an automated haematology analyzer (XE-2100; Sysmex, Kobe, Japan), and blood eosinophilia was defined by ≥ 450 cells/ μL [20].

In patients with asthma, induced sputum tests were performed as previously described [21] and were successful in 186 patients (success rate: 74.7%). Briefly, after the basal FEV₁ measurement, patients were pretreated with 200 μg of salbutamol aerosol inhalation. Sputum was induced by 4.5% hypertonic saline inhalation using an ultrasonic nebulizer with output set at 4.5 mL/min (Omron Co., Tokyo, Japan) for 5 min, up to four times (for a total of 20 min or until their FEV₁ decreased by $\geq 10\%$). After each hypertonic saline inhalation, they were asked to blow the nose, rinse the mouths and encouraged to expectorate sputum into a sterile Petri dish plate. Sputum was processed within 4 h of induction. The equal volume of 0.01 M dithioerythritol (DTE) was added to sputum samples and gently mixed. They were filtered with 100- μm cell strainer or mesh on 15-mL tube and centrifuged at 400–800 g for 10 min at 4°C. Cell pellets were then resuspended in phosphate-buffered saline to a volume equal to the original sputum plus DTE. Total cell counting was car-

ried out using a haemocytometer, and cell concentrations were then adjusted to 1.0×10^6 cells/mL. Cytospins were prepared by adding 60 μL of this cell suspension to Shandon II cytocentrifuge cups and spun for 5 min at 42 g. Slides were stained with Diff Quick solution (Sysmex Co.). Leucocytes, bronchial epithelial cells and squamous cells were counted. To determine cell differentiation, 300 nucleated cells per slide were counted and these counts were expressed as percentages of intact round nucleated cells, excluding squamous epithelial cells. Sputum samples containing more than 20% squamous epithelial cells were not analysed. Finally, sputum eosinophilia was defined by $\geq 3\%$ [22]. They withheld theophylline and montelukast for 2 days and inhaled corticosteroids for 3 days before induced sputum analyses.

Asthma outcomes

The patients with asthma were followed up, and their adherence was checked according to the original cohort protocols. They were treated by allergy physicians according to Global Initiative for Asthma (GINA) guidelines [23] and were instructed to visit at 3-month intervals regularly or visit urgently if exacerbated. Medication adherence and inhaler technique were checked by research nurses at every visit, to maintain good adherence and correct techniques; patients were asked to respond to by a questionnaire item 'How often did you take your inhalers on days when you are not having asthma symptoms?' (all of the time, most of the time, some of the time, occasionally or never) [24] and were repeatedly educated to ensure their medication adherence to be 'all of the time' or 'most of the time'. They were also asked to bring their inhalers to check dose counters and inhaler techniques, to ensure their proper usage. Formal education was followed whenever they were determined to have difficulties in using inhalers.

Asthma 'exacerbation' was defined according to the official American Thoracic Society/European Respiratory Society Statement: (1) use of systemic corticosteroids or an increase from the stable maintenance dose for at least 3 days due to an asthma attack (defined as 'systemic corticosteroid rescue therapy') or (2) hospitalization or an emergency room visit requiring systemic corticosteroids for asthma [25]. The present analysis included asthma outcomes during the 12 months after the baseline assessment. The difference between the best and worst FEV_{1%}_{pred} values during the 12-month period was defined as 'FEV_{1%}_{pred} variability'. Medication details were also collected. On the basis of the asthma outcomes during the 12-month follow-up period, severe asthma was defined as the disease which requires the GINA steps 4–5 medications (high-dose inhaled

corticosteroid and long-acting beta2-agonist or leukotriene modifier/theophylline) or systemic corticosteroid therapy for $\geq 50\%$ of the year to maintain control or to remain uncontrolled despite the high-intensity treatment [26].

Serum IgE assessment

Baseline sera were analysed for total IgE and SE-IgE levels, using an ImmunoCAP 250 analyzer (Thermo Fisher, Uppsala, Sweden) according to the manufacturer's instructions. SE-IgE levels were measured using staphylococcal enterotoxin mix (SEA, SEC and TSST-1; Thermo Fisher), as described previously [13]. SE-IgE sensitization was classified into three groups: negative (< 0.10 kU/L), moderate (0.10 – 0.35 kU/L) and high (≥ 0.35 kU/L).

Statistical analyses

Descriptive data are presented as the means $\pm$ standard deviation, medians [interquartile range (IQR)] or percentages. Groups were compared by *t*-test, Mann–Whitney *U*-test, chi-squared test, one-way ANOVA or Kruskal–Wallis test. Bivariate correlations for nonparametric variables were tested by Spearman's test. Correlations between sputum eosinophil percentage and other variables were examined using general linear models, with adjustment for demographic factors (age, gender, BMI and smoking status) and clinical variables with $P < 0.10$ in univariate tests. Multivariate logistic regression was used to investigate relationships between severe asthma and other variables; confounders included demographic factors (age, gender, BMI and smoking status) and variables for which P was < 0.10 in univariate tests (Table S1). As sputum eosinophil percentage, blood eosinophil counts and serum SE-IgE levels were not normally distributed ($W < 0.001$, Shapiro–Wilk *W* test), they were log-transformed in the statistical analyses. Finally, multiple correspondence analyses were performed to present the pattern of relationships between asthma severity, SE-IgE sensitization and related factors (CRS and sputum eosinophilia). All statistical analyses were carried out using STATA 12.0 (Stata Corporation, College Station, TX, USA). All statistical tests were two sided, and $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics

The baseline characteristics are presented in Table 1. Mean age at asthma onset was 64.3 years (range: 29–83 years), and none had a history of childhood-onset

asthma. The proportion of late-onset asthma (onset after the age of 40 years [3]) was 98.8%, and mean duration of asthma was 7.5 ± 6.8 years. There was no significant difference in the distribution of age, gender, smoking status and serum total IgE between elderly patients and controls. Serum SE-IgE levels were significantly higher in elderly patients compared to controls [median 0.16 (IQR 0.04–0.53) vs. 0.10 (0.01–0.19), $P < 0.001$].

Clinical correlations of SE-IgE sensitization in elderly asthma

The comparison of elderly patients with asthma according to serum SE-IgE levels is shown in Table 2. Compared to the SE-IgE-negative group (< 0.10 kU/L), the SE-IgE-high group (≥ 0.35 kU/L) had significantly more CRS (CRSsNP 48.8% vs. 35.1% and CRSwNP 23.2% vs. 4.3%, $P < 0.001$), higher blood eosinophil counts (median 296 vs. 128 cells/ μ L, $P < 0.001$), higher total IgE levels (median 290.2 vs. 24.5 kU/L, $P < 0.001$) and a higher rate of sputum eosinophilia (87.7% vs. 34.4%, $P < 0.001$).

In Spearman's tests, serum SE-IgE level showed positive correlations with total IgE level ($r = 0.825$, $P < 0.001$), blood eosinophil count ($r = 0.373$, $P < 0.001$) and sputum eosinophil percentage ($r = 0.584$, $P < 0.001$) but not with sputum neutrophil percentage ($r = -0.100$, $P = 0.310$). The correlation between sputum eosinophil percentage and blood eosinophil count was also significant ($r = 0.502$, $P < 0.001$). The significant correlation of serum SE-IgE level with sputum eosinophil percentage remained independent of confounders in the multivariate analysis [coefficient estimate 0.29, 95% confidence interval (CI) 0.07–0.52, $P = 0.010$; Table 3].

Associations between SE-IgE sensitization and severe asthma

Severe asthma was more frequent in the SE-IgE-high group (53.7% vs. 13.8%, $P < 0.001$; Table 2). SE-IgE-high group showed more FEV1%_{pred} variability ($21.9 \pm 10.5\%$ vs. $14.5 \pm 9.0\%$, $P < 0.001$), more frequent asthma exacerbation (2.0 ± 1.8 vs. 0.3 ± 0.8 , $P < 0.001$) and more frequent systemic corticosteroid rescue therapy (2.2 ± 2.0 vs. 0.5 ± 1.0 , $P < 0.001$), compared to the SE-IgE-negative group.

Multivariate logistic regression analyses were performed to test whether the associations between SE-IgE sensitization and asthma severity were independent of confounders. SE-IgE sensitization showed significant associations with severe asthma in a dose-dependent manner [SE-IgE-high vs. SE-IgE-negative group: odds ratio (OR) 7.47, 95% CI 1.86–30.03, $P = 0.005$; Table 4]. Atopy was not related to severe asthma in the

Table 1. Baseline characteristics of study participants

	Elderly control (<i>n</i> = 98)	Elderly asthma (<i>n</i> = 249)	<i>P</i> values
Female (%)	68.4	66.3	0.708
Age (years)	70.6 ± 4.8	71.5 ± 5.1	0.168
Asthma onset age (years)		64.3 ± 8.7	
BMI (kg/m ²)	23.6 ± 3.6	24.4 ± 3.3	0.057
Smoking status (%)			
Never	72.5	74.7	0.480
Former	20.4	15.7	
Current	7.1	9.6	
DM (%)	7.1	12.9	0.130
GERD (%)	12.2	20.4	0.078
CRS (%)			< 0.001
No CRS	82.7	41.4	
CRSsNP	17.3	45.8	
CRSwNP	0.0	12.9	
Atopy (%)	13.4	42.5	< 0.001
Pre-BD FEV1% _{pred}	117.0 ± 23.6	85.1 ± 19.5	< 0.001
Pre-BD FVC% _{pred}	95.0 ± 16.1	86.7 ± 18.0	< 0.001
Pre-BD FEV1/FVC%	84.8 ± 8.9	75.3 ± 12.5	< 0.001
Blood eosinophils (/μL)	129 (78–213)	200 (108–350)	< 0.001
Blood eosinophilia (≥ 450 cells/μL, %)	4.9	16.2	< 0.001
Sputum eosinophilia (≥ 3%), (%)		58.6	
Serum total IgE (kU/L)	91.5 (28.9–218.2)	83.3 (31.4–220.0)	0.934
Serum SE-IgE (kU/L)	0.10 (0.01–0.19)	0.16 (0.04–0.53)	< 0.001
SE-IgE sensitization (%)			
Negative (< 0.10 kU/L)	48.0	37.8	0.008
Moderate (0.10–0.35 kU/L)	35.7	29.3	
High (> 0.35 kU/L)	16.3	32.9	

BMI, body mass index; DM, diabetes mellitus; GERD, gastroesophageal reflux disease; CRS, chronic rhinosinusitis; NP, nasal polyp; pre-BD, pre-bronchodilator; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; SE, staphylococcal enterotoxin; IQR, interquartile range.

Results were presented as percentage, mean ± standard deviation, median (interquartile range) or otherwise specified.

P values were determined by chi-squared tests, *t*-tests or Mann–Whitney *U*-tests.

univariate test (*P* = 0.800) and did not affect the relationship between SE-sensitization and severe asthma when included as a confounder in the multivariate analyses (specific data not shown).

As patients with severe asthma had significantly more sputum eosinophils compared to non-severe asthma patients (Table S1), we repeated the multivariate logistic regression analyses in a subgroup of 186 patients with asthma for which induced sputum data were available. The association with severe asthma remained significant when sputum eosinophilia was added as a confounder (SE-IgE-high vs. SE-IgE-negative group: OR 5.01, 95% CI 1.32–19.04, *P* = 0.018; Table S2).

Multiple correspondence analyses were performed to demonstrate the relationships between severe asthma, CRS, sputum eosinophilia and serum SE-IgE level (Fig. 1); severe asthma was located near high SE-IgE levels, CRSwNP and sputum eosinophilia. However, non-severe asthma was situated close to moderate or negative SE-IgE levels, no CRS and sputum non-eosinophilia. These results confirmed the significant

relationships between asthma severity, SE-IgE sensitization, CRS and sputum eosinophilia, which were shown in multiple logistic regression analyses (Tables 4 and S2).

These analyses described above were replicated within the subgroup of late-onset asthma patients (*n* = 246; 98.8% of total asthmatics) and pointed to the same conclusions (specific data not shown).

Discussion

The present study demonstrated that SE-IgE sensitization was significantly related to asthma in the elderly. In particular, it was closely related to late-onset severe eosinophilic asthma, comorbid with CRSwNP. Our findings indicate a potential implication of SE in the high morbidity burden of asthma in the elderly and also suggest important clues to the pathogenesis of late-onset severe eosinophilic asthma in the aged group.

The pathogenesis of late-onset asthma in the elderly remains elusive, despite the characteristics have been reported in the literature since the 1970s. Lee and

Table 2. Comparison of 249 elderly patients with asthma according to serum staphylococcal enterotoxin-specific IgE levels

	Negative SE-IgE group (< 0.10 kU/L; $n = 94$)	Moderate SE-IgE group (0.10 – 0.35 kU/L; $n = 73$)	High SE-IgE group (≥ 0.35 kU/L; $n = 82$)	<i>P</i> value
Baseline characteristics				
SE-IgE (kU/L)	0.03 (0.01–0.06)	0.17 (0.13–0.24)	0.92 (0.54–1.47)	< 0.001
Female (%)	75.5	67.1	54.9	0.015
Age (years)	71.3 ± 5.0	71.8 ± 5.3	71.4 ± 5.2	0.842
Asthma onset age (years)	64.1 ± 9.7	64.5 ± 8.0	63.9 ± 8.1	0.895
BMI (kg/m^2)	24.9 ± 3.4	24.5 ± 3.2	23.8 ± 3.2	0.774
Smoking (%)				
Never	80.9	74.0	68.3	0.448
Former	11.7	16.4	19.5	
Current	7.4	9.6	12.2	
DM (%)	8.5	15.1	15.9	0.278
GERD (%)	19.5	23.4	18.9	0.778
CRS (%)				
No CRS	60.6	48.0	28.0	< 0.001
CRSsNP	35.1	39.7	48.8	
CRSwNP	4.3	12.3	23.2	
Atopy (%)	26.4	52.9	47.6	0.014
Blood eosinophils ($/\mu\text{L}$)	128 (89–230)	175 (86–325)	296 (184–436)	< 0.001
Blood eosinophilia (≥ 450 cells/ μL , %)	8.2	16.0	25.0	0.026
Serum total IgE (kU/L)	24.5 (9.6–61.5)	92.9 (54.4–176.1)	290.2 (133.1–620.1)	< 0.001
Sputum neutrophils (%), $n = 186$	26.0 (12.7–30.0)	26 (9.7–31.3)	15.3 (8.5–36.8)	0.599
Sputum eosinophils (%), $n = 186$	1.3 (0.3–4.3)	2.5 (1.0–5.0)	9.7 (5.5–18.3)	< 0.001
Sputum eosinophilia ($\geq 3\%$), (%), $n = 186$	34.4	46.2	87.7	< 0.001
Baseline FEV1% _{pred}	98.1 ± 24.7	98.4 ± 26.6	83.9 ± 21.4	< 0.001
Clinical observations during the study period				
FEV1% _{pred} variability	14.5 ± 9.0	16.5 ± 8.9	21.9 ± 10.8	< 0.001
Exacerbation frequency [median (mean $\pm$ SD)]	0 (0.4 $\pm$ 0.8)	0 (0.8 $\pm$ 1.3)	2 (2.0 $\pm$ 1.8)	< 0.001
OCS rescue frequency [median (mean $\pm$ SD)]	0 (0.5 $\pm$ 1.0)	0 (0.9 $\pm$ 1.4)	2 (2.2 $\pm$ 2.0)	< 0.001
Mean ICS dose (μg , fluticasone equivalent/day)	522.2 ± 281.6	586.7 ± 235.2	733.5 ± 256.0	< 0.001
Regular OCS use (%)	7.3	12.0	19.7	0.089
Severe asthma (%)	13.8	28.8	53.7	< 0.001

SE, staphylococcal enterotoxin; BMI, body mass index; DM, diabetes mellitus; GERD, gastroesophageal reflux disease; CRS, chronic rhinosinusitis; NP, nasal polyp; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; SD, standard deviation; OCS, oral corticosteroid; IQR, interquartile range.

Results were presented as percentage, mean $\pm$ standard deviation, median (interquartile range) or otherwise specified.

P values were determined by chi-squared tests, one-way ANOVA tests or Kruskal–Wallis tests.

Stretton [27] described 15 cases of elderly patients who developed asthma after the age of 60 years. Interestingly, these cases were frequently eosinophilic (12/15) but non-atopic (11/15), which is similar to our findings. However, the reason for airway eosinophilic inflammation remains to be clarified in the elderly. In the present study, sputum eosinophilia was frequent (58.6%) in elderly patients with asthma, but did not significantly correlate with atopic status ($P = 0.227$). Rather, serum SE-IgE level had significant correlations with sputum eosinophilia. Thus, our findings lead to the speculation that SE-IgE sensitization may be involved in the pathogenesis of eosinophilic airway inflammation in late-onset elderly patients with asthma.

We also found significant relationships between SE-IgE sensitization and severe asthma in the elderly, confirming two recent reports from younger adult patients

with asthma. Kowalski *et al.* [28] found threefold higher levels of SE-IgE in patients with severe asthma ($n = 109$, mean age: 50.9 years) compared to patients with non-severe asthma ($n = 101$, mean age 38.4 years). We also previously demonstrated that SE-IgE sensitization was significantly associated with severe asthma in younger adults ($n = 166$, mean age: 46.5 years), independently of atopy and total IgE [11]. However, it has never been examined which severe asthma patients are related to SE-IgE sensitization; those two studies did not evaluate asthma onset age, sputum profiles or comorbidities. Thus, the novelty of the present study is that it does not only extend the relationships of SE-IgE sensitization with asthma severity into the elderly population, but also specifically defines its associations with severe late-onset eosinophilic asthma comorbid with sinusitis.

Table 3. General linear models for sputum eosinophil percentage among 186 elderly patients with asthma

For log-sputum eosinophil percentage	<i>P</i> values	Coef.	Std. err.	<i>z</i>	95% CI
Age (years)	0.698	−0.01	0.10	−0.39	−0.02–0.02
Male gender	0.681	0.06	0.14	0.41	−0.21–0.32
BMI (kg/m ²)	0.979	0.01	0.02	0.03	−0.04–0.04
Former smoker	0.847	−0.03	0.16	−0.19	−0.35–0.29
Current smoker	0.908	0.02	0.19	0.12	−0.35–0.40
CRSsNP	0.047	0.24	0.12	1.99	0.00–0.47
CRSwNP	0.075	0.29	0.16	1.78	−0.03–0.62
Atopy	0.854	−0.02	0.10	−0.18	−0.22–0.19
Log-blood eosinophils (/μL)	0.001	0.47	0.15	3.19	0.18–0.76
Log-total IgE (kU/L)	0.460	−0.09	0.13	−0.74	−0.35–0.16
Log-SE-IgE (kU/L)	0.010	0.29	0.11	2.59	0.07–0.52

CI, confidence interval; BMI, body mass index; CRS, chronic rhinosinusitis; NP, nasal polyp; SE, staphylococcal enterotoxin.

P values were determined by general linear models with adjustment for age, gender, BMI, smoking status, CRS, atopy, log-blood eosinophil, log-total IgE and log-SE-IgE.

Table 4. Multivariate logistic regression analyses for asthma severity among 249 elderly patients with asthma

Severe asthma (<i>n</i> = 78) vs. non-severe asthma (<i>n</i> = 171)	Adjusted OR	95% CI	<i>P</i> values
Age (years)	1.04	0.95–1.14	0.370
Male gender	0.17	0.04–0.66	0.011
BMI (kg/m ²)	0.98	0.86–1.12	0.754
Smoking status			
Never	Reference		
Former	1.09	0.22–5.35	0.912
Current	6.02	1.03–35.30	0.047
GERD	8.24	2.74–24.76	< 0.001
CRS			
No CRS	Reference		
CRSsNP	2.49	0.93–6.65	0.069
CRSwNP	7.41	1.82–30.19	0.005
Blood eosinophilia (≥ 450/μL)	2.52	1.38–12.81	0.012
Total IgE ≥ 100 kU/L	1.14	0.36–3.60	0.828
SE-IgE sensitization			
Negative (< 0.10 kU/L)	Reference		
Moderate (0.10–0.35 kU/L)	1.82	0.52–6.39	0.348
High (≥ 0.35 kU/L)	7.47	1.86–30.03	0.005

OR, odds ratio; CI, confidence interval; BMI, body mass index; GERD, gastroesophageal reflux disease; CRS, chronic rhinosinusitis; NP, nasal polyp.

P values were determined by multivariate logistic regression tests with adjustment for age, gender, BMI, smoking, GERD, CRS, blood eosinophilia (≥ 450 vs. < 450 cells/μL), total IgE (≥ 100 vs. < 100 kU/L) and SE-IgE sensitization status.

We found close relationships between SE-IgE sensitization and severe late-onset eosinophilic asthma comorbid with CRS in the elderly. Interestingly, this

phenotype has repeatedly been described in recent studies of younger adult patients. A late-onset eosinophilic asthma phenotype (mean age at onset 32.6 years) was observed in cluster analyses of UK refractory patients with asthma in secondary care [29]. In the Netherlands, Amelink et al. [30] reported associations between severe adult-onset asthma (mean age at onset 40.2 years) and nasal polyposis, sputum eosinophil count, exhaled nitric oxide, blood neutrophil count and absence of atopy. van Veen et al. [31] also reported difficult-to-treat asthma characterized by persistent sputum eosinophilia, non-atopy and extensive sinus disease. These findings are supported by more recent unsupervised phenotyping studies using the Severe Asthma Research Program cohort, where a specific cluster characterized by older age, later onset, less atopy, more sinus disease, more eosinophilia and more severe asthma was described [32]. Thus, our findings demonstrated here indicate that this phenotype is clinically significant also in the elderly patients, and further suggest for the first time that SE-IgE sensitization is involved in the pathogenesis of this asthma phenotype. To prove this, our findings warrant replication in different population samples.

Biological plausibility of our findings is supported by several lines of evidences. First, on the eosinophilic association; *S. aureus* or staphylococcal enterotoxin B (SEB) has properties to directly activate human eosinophils [33, 34] and also augment eosinophil survival via activating human airway epithelial cells [35]. It can also induce immune tolerance breakdown to allergen [36], Th2 polarization [37] and polyclonal IgE formation including SE-IgE which is functional [38]. Second, the associations with asthma and severity are supported by following experimental evidences; SEB induces airway inflammation and hyperresponsiveness in animal models [39], induces corticosteroid resistance [40] and inhibits regulatory T cell functions in humans [41]. Moreover, *S. aureus* may directly induce epithelial cell-derived cytokines such as TSLP and IL-33 from human nasal polyp tissues and BEAS-2B cells, which could implicate the mechanistic roles of *S. aureus* in steroid-resistant type 2 inflammation [42].

The inter-relationships of SE-IgE with late-onset asthma and CRS are supported by observational studies. CRS had a significant association with adult-onset asthma in European general populations, irrespective of nasal allergy [43]. SE-IgE sensitization was also significantly related to adult-onset asthma independently of rhinitis symptom in Korean community populations, although CRS was not studied then [13]. As *S. aureus* is a major colonizer in the nasal cavity of patients with CRS and/or NP [44], the relationships of SE-IgE and late-onset asthma could be confounded by comorbid CRS. However, in the literature reported so far, the relationships of SE-IgE with asthma were independent of

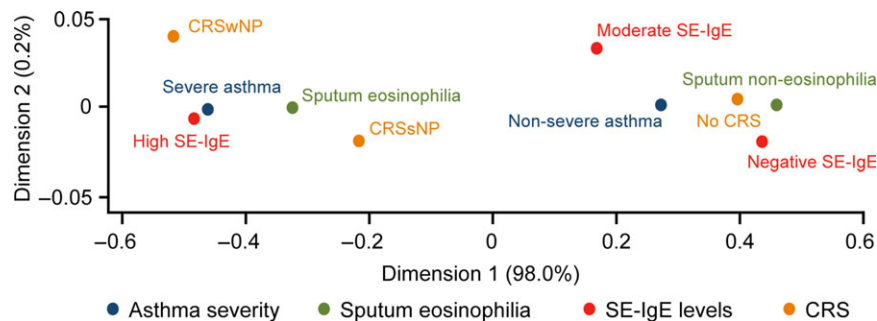


Fig. 1. Multiple correspondence analyses plot for the inter-relationships between severe asthma, CRS, sputum eosinophilia and serum SE-IgE levels among 186 elderly patients with asthma. Severe asthma is situated close to high SE-IgE (≥ 0.35 kU/L), CRSwNP and sputum eosinophilia ($\geq 3\%$). CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; SE-IgE, staphylococcal enterotoxin-specific IgE.

rhinitis, CRS or NP comorbidity [12, 13, 28]. Thus, we hypothesize that SE is a mechanistic link between asthma and CRS, either as a direct cause or disease modifier for late-onset asthma.

The abundance of *S. aureus* has been demonstrated in the upper airways, including intramucosal growth and intracellular survival [45, 46]. Direct evidence is still lacking on the presence of *S. aureus* within the lower airways. However, an inability to cope with *S. aureus* colonization in the lower airways by impaired macrophages has been demonstrated in uncontrolled asthma [47], and *S. aureus* may still develop a considerable impact on the mucosal immunity. SE-IgE was detectable in about 50% of biopsies from severe asthmatics (P. Howarth, Southampton, personal communication), and *S. aureus* biofilms were observed in the bronchoalveolar lavage fluids of these patients.

Based on the first year data of the entire cohort, we previously reported that exacerbation history, depression, poor adherence and poor inhaler technique were predictors for future exacerbations in elderly asthma [14]. During the first 2 years of follow-up, we further reported that smoking and lower post-bronchodilator FEV1 were predictors for future exacerbation [48]. However, these previous analyses did not include key parameters, such as asthma severity, SE-IgE sensitization, induced sputum eosinophils, blood eosinophils, CRS and NP, which were now included in the present analysis.

Several major limitations need to be considered in interpreting the present findings. First, this analysis had a correlative nature and thus could not address causal relationships of SE-IgE sensitization. Second limitation is a potential selection bias, as the participants were recruited from specialist allergy clinics at tertiary institutions with exclusion of several conditions such as heart failure or lung parenchymal diseases. As the present study only included the patients from two referral clinics (SNUH and SNUBH), another selection bias issue

may be raised; thus, we further examined serum SE-IgE levels available from 365 patients recruited at other participating institutions (Table S3). This cohort included significantly more female patients (66.3% vs. 53.2%, $P = 0.001$), with earlier age of asthma onset (64.3 ± 8.7 vs. 65.9 ± 7.1 years, $P = 0.001$), slightly more atopy and lower total IgE levels. However, we observed no significant difference in serum SE-IgE levels [patients from SNUH and SNUBH; median 0.16 (IQR 0.04–0.53) vs. patients from other institutions; 0.16 (0.04–0.57), $P = 0.603$] and SE-IgE sensitization status (percentage of SE-IgE-high group: 32.9% vs. 34.0%, $P = 0.963$) between two patients' groups. We also found the relationships between SE-IgE sensitization and late-onset asthma to be consistent in the analyses of whole available samples ($n = 711$; Table S4). Thus, the risk of aforementioned selection bias may not be significant. Considering the interactions of SE-IgE with asthma and CRSwNP [15], we believe our analyses of 249 patients characterized for CRSwNP were more appropriate for the present study purpose than whole available sample analyses without relevant information. Third, our study cohort had the characteristics of being predominantly late-onset asthma (98.8%), and thus, we could not compare with childhood-onset longstanding asthma in the elderly. Our findings on SE-IgE associations need to be interpreted in the context of late-onset disease. However, the predominance of late-onset asthma was consistent over nine different referral clinics which originally participated in our entire cohort study (about 95%; specific data not shown here). As our study participants were recruited from tertiary referral hospitals, they may have been likely to have recent-onset asthma with more severity, compared to patients with asthma in the community. Fourth, considering the heterogeneity within CRS and NP subgroups which relates to the risk of comorbid asthma [49], further studies need to characterize CRS phenotypes. In addition, the prevalence of NP may have been

underestimated in the present study, as smaller polyps (score < 2 in the Davos classification [50]) may have been missed by flexible nasal endoscopy.

Nevertheless, the present study has strength and provides important implications for further studies. This is the first report on the specific relationships of SE-IgE with late-onset severe asthma in the elderly. We analysed a well-characterized group of elderly patients with asthma, including asthma severity, comorbidity and sputum profiles, which is a new and detailed extension to previous reports on the general associations between SE-IgE and asthma severity among younger adult patients [11, 28]. Clinical relationships of SE-IgE sensitization demonstrated here may indicate SE to be a new potential therapeutic target to reduce morbidity burden of asthma in the elderly and also suggest clues to understand the pathophysiology of late-onset severe eosinophilic asthma in the elderly.

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Author contributions

Woo-Jung Song carried out the conception and design of the study, data generation, analysis, interpretation

and preparation of the manuscript. Ina Sintobin carried out the conception of the study, data analysis, interpretation and preparation of the manuscript. Kyoung-Hee Sohn, Min-Gyu Kang, Han-Ki Park, Eun-Jung Jo, Seung-Eun Lee, Min-Suk Yang carried out data generation, analysis and interpretation of the data. Sae-Hoon Kim, Hye-Kyung Park, Yong Eun Kwon, Tae-Bum Kim, Sang-Heon Kim, Byung-Jae Lee, Young-Koo Jee, Byoung Whui Choi made design of the study, data generation and critical revision of the manuscript. Heung-Woo Park carried out the conception and design of the study, data generation, analysis and critical revision of the manuscript. Yoon-Seok Chang carried out the conception and design of the study, data generation, interpretation and critical revision of the manuscript. Claus Bachert carried out the conception and design of the study, data interpretation and critical revision of the manuscript. Sang-Heon Cho carried out the conception and design of the study, data generation, analysis, interpretation and critical revision of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart for asthma patient inclusion.

Table S1. Comparison by asthma severity in 249 elderly asthma patients.

Table S2. Multivariate logistic regression analyses for severe asthma among 186 elderly asthma patients who had induced sputum data available.

Table S3. Comparison of the baseline characteristics of elderly asthma patients according to the recruiting institutions.

Table S4. Whole available cohort sample analyses for comparing staphylococcal enterotoxin IgE sensitization levels between elderly controls and late-onset asthma patients.