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Impact of *Staphylococcus aureus* and superantigens on asthma severity and lung functions in late-onset asthmatics

후기 발병 천식 환자에서 포도상 구균과 초항원의 천식 중증도 및 폐기능에 미치는 영향

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Abstract

Impact of *Staphylococcus aureus* and superantigens on asthma severity and lung functions in late-onset asthmatics

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Introduction: *Staphylococcus aureus* (SA) is a frequent colonizer, mainly found in human skin and upper airways. Recent studies suggest that SA is known to be associated with asthma and its severity. However, the long-term impact of Staphylococcal enterotoxin IgE (SE-IgE), and the associations of SA nasal colonization with SE-IgE sensitization in adults with late-onset asthma remain unclear. Moreover, the effects of Spls on airway inflammation and epithelial integrity in airway epithelium *in vitro* experiments have not yet been studied. Thus, we aimed to test the hypothesis that SA including SE and Spls is associated with asthma severity and lung functions in late-onset asthmatics.

Methods: First, a total of 223 late-onset elderly asthmatics and 89 controls were analyzed. Patients were assessed for demographics, history of chronic rhinosinusitis (CRS), asthma duration, acute exacerbation frequency, and lung function at baseline and then were prospectively followed up for 2 years. Serum total IgE and SE-IgE

levels were measured at baseline, and their associations with clinical outcomes were examined. Airflow obstruction was defined as FEV1/FVC ratio<0.7 at baseline and fixed airflow obstruction (FAO) was defined as FEV1/FVC ratio<0.7 over the 2-year follow-up. Second, a total of 181 late-onset asthmatics were prospectively enrolled. We analyzed their demographic factors, asthma severity, treatment status, acute exacerbation, inflammation, and lung function. FAO was defined as FEV1/FVC ratio<0.7 at 18-24 months after enrollment. Nasal swabs were taken to evaluate SA colonization, and SE-IgE levels were measured in serum. Subjects were classified into 4 groups according to SA colonization and SE sensitization status. Then, clinical correlations of SE-IgE and SA colonization were analyzed. Finally, when Spls were exposed to airway epithelium, the changes in airway epithelial cells were examined by immunofluorescence, western blots, and mRNA expression.

Results: In the first study, the prevalence of airflow obstruction was 29.1% at baseline. Patients with airflow obstruction were significantly more likely to be male, and have a positive smoking history, comorbid CRS, and higher levels of SE-IgE than those without airflow obstruction. Multivariate logistic regression analysis showed that airflow obstruction was significantly associated with current smoking and SE-IgE sensitization at baseline. After the 2-year follow-up, baseline SE-IgE sensitization was consistently related to FAO. Meanwhile, the number of exacerbations per year was significantly correlated with SE-IgE levels. In the second study, patients with SA/SE (+/+) were more likely to be males and current smokers, and had significantly higher serum total IgE levels, treatment levels, and worse lung function, compared to SA/SE (–/–). In multivariate logistic regression, SA/SE (+/+)

demostrated consistent associations with total IgE levels and asthma treatment severity at baseline, and with eosinophilic inflammation and FAO at follow-ups. In the third study, SplA and SplD reduced the epithelial integrity of BEAS-2B cells, and SplD induced elevated Cystatin SN in A549 cells, and primary human nasal epithelial cells.

Conclusion: SE-IgE sensitization was significantly associated with frequent asthma exacerbations and FAO in late-onset elderly asthmatics, and the intranasal presence of SA may have synergistic effects with SE-IgE sensitization on asthma severity. Moreover, SplD may play a notable role in disrupting airway epithelial integrity and inducing eosinophilic airway inflammation. These findings warrant further investigation of the direct and mediating effects of SA on asthma pathogenesis and its severity.

Keywords: asthma, late-onset, *Staphylococcus aureus*, enterotoxins, severity

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Introduction

Atopic asthma is mainly associated with early-onset asthma, while late-onset asthma often presents as a non-atopic phenotype.[1] However, late-onset asthma is frequently non-atopic, but involves eosinophilic inflammation.[2,3] Recent studies have suggested that *Staphylococcus aureus* (SA) may induce type 2 airway inflammation.[4, 5, 6]

SA is a normal human flora and frequent colonizer. SA is a species of gram-positive cocci, and facultative aerobic-anaerobic bacterium, mainly found in human skin and upper airways.[7] It can secrete superantigens such as Staphylococcal enterotoxins (SEs) or pro-allergic proteins like serine protease-like proteins (Spls), which are known to amplify or develop allergic sensitization and type 2 inflammation. About 20-25% of the general population are persistent SA colonizers and 60% are intermittent carriers of SA.[8, 9, 10] A meta-analysis reported that the mean prevalence of nasal colonization of SA was about 30% in five different geographic locations.[11] More than 70% of SA can secrete exotoxin (e.g., Toxic shock syndrome toxin, TSST-1) and Staphylococcal enterotoxins (SEs) such as SEA, SEB, SEC, SED, SEE, and SEG, which are known as potent T cell superantigens. These superantigens directly interact with T cells and class II major histocompatibility complex molecules (MHC class II) to induce polyclonal T cell proliferation and activation. Activated T cells can secrete type 2 cytokines such as IL-4, IL-5, and IL-13, which lead to eosinophilic inflammation and polyclonal IgE formations.[5] They play a crucial role as a disease modifier in upper and lower airway diseases.

Recent studies have reported that SE-IgE sensitization is associated with airway diseases such as allergic rhinitis, chronic rhinosinusitis (CRS), and asthma.[8, 12] Particularly in asthma, SE-IgE sensitization is associated with the disease onset and

severity. In the community-based population, serum SE-IgE concentration was significantly associated with asthma.[13, 14] A recent meta-analysis corroborated the relationship between asthma and SE sensitization.[15] A European cohort study showed that SE sensitization was related to asthma severity and acute exacerbation, use of oral corticosteroids, and lower lung functions.[16] Additionally, our previous study found that SE-IgE was specifically linked to eosinophilic asthma and its severity in late-onset elderly asthmatics.[3] SA nasal colonization is also associated with asthma prevalence in adults, and is related to asthma and its severity in the younger general population, and adolescents. [11, 17, 18] Moreover, Spls derived from SA induced specific IgE formation and Th2 immune response in asthma patients and IL-33-dependent Th2 inflammation in airway epithelium.[19, 20]

Asthma is a heterogeneous disease which involves chronic airway inflammation and presents with variable expiratory airflow limitation, and it can be particularly more complex in older adults.[21] The variable airflow limitation can develop into persistent airflow limitation or fixed airflow obstruction (FAO) in some patients. FAO is specifically found in about 55-60% of severe or difficult-to-treat adult asthmatics.[22, 23, 24] Generally, FAO in asthma has been mainly considered a consequence of airway remodeling due to persistent airway inflammation. Pulmonary function test is commonly used to assess physiologic airway remodeling in clinical practice.[25] Meanwhile, although the definition of asthma onset age varies throughout literature, it is believed that asthma phenotypes are classified according to age of onset.[26] Recent studies have found that late-onset asthma is less atopic than early-onset or childhood-onset asthma, and is more highly associated with CRS with nasal polyps (CRSwNP) and severe asthma.[2,27,28] Recently, late-onset asthma has increased along

with an aging society. Therefore, understanding the mechanisms of late-onset asthma is important in individualized management in diagnosing and treating asthma.

However, the effects of SE sensitization, and SA nasal colonization on airway inflammation and FAO related to remodeling in patients with late-onset asthma have not yet been studied. Also, the effects of Spls on epithelial integrity and inflammation in airway epithelial cells *in vitro* experiments have not yet been conducted. Considering the cross-sectional associations between SE-IgE sensitization and lower lung functions, we first hypothesized that SE-IgE sensitization is associated with FAO. Second, we hypothesized that the accompanying SA nasal colonization and SE-IgE sensitization in late-onset asthma contributes to asthma severity and lung functions in an interactive fashion. Third, Spls may decrease epithelial integrity and directly influence inflammation in airway epithelial cells. To this end, we took the following approaches.

First, we aimed to examine the longitudinal association of SE-IgE sensitization with FAO in late-onset elderly asthmatics. Second, we evaluated whether there are synergistic impacts of SA nasal colonization and SE sensitization on asthma severity and lung functions in late-onset asthmatics. Finally, we examined the *in vitro* effects of Spls on airway epithelial cells including decrease of epithelial integrity and airway inflammation.

Materials and Methods

2.1. Impact of SE-IgE sensitization in late-onset asthmatics

First, we aimed to examine longitudinal associations of SE-IgE sensitization with FAO and clinical outcomes of asthma in late-onset elderly asthmatics based on a prospective cohort design.

Study population

This prospective cohort study analyzed data from late-onset elderly asthmatics and non-asthmatic controls (onset age ≥ 40 years and baseline age ≥ 65 years). Elderly asthmatics were recruited from a previously reported cohort study.[29] The cohort study was a prospective observational multicenter study with a 5-year follow-up (2009-2013) and asthmatics were recruited from nine referral hospitals in South Korea. Among the participants in the cohort, this study included patients recruited from the Seoul National University Hospital who had been evaluated for CRS, NP, and follow-up pulmonary function outcomes.

Asthma was diagnosed by allergists, based on the clinical history and positive bronchodilator responsiveness (BDR) in a pulmonary function test at baseline or after anti-asthmatic treatment. Comorbidities were confirmed using the following question; "Have you ever been diagnosed with or treated by a doctor?"

The same exclusion criteria were applied to both asthma patients and controls. Subjects were excluded if they had any comorbidities that could affect respiratory symptoms or serum IgE levels, including eosinophilic granulomatosis with polyangiitis, allergic bronchopulmonary aspergillosis, atopic dermatitis, recent treatment with anti-IgE monoclonal antibody or corticosteroid, congestive heart

failure, and malignancy plus cases with concomitant diseases. Also, those without serum collection or follow-up were excluded. The same exclusion criteria were applied to both asthma patients and controls, and the final sample included 223 elderly patients with asthma.

Non-asthmatic elderly controls were recruited through bulletin board advertisements. The absence of asthma was confirmed based on a questionnaire, which asked if they had previously had asthma or wheezing, and methacholine challenge test (MCT). Subjects with negative results both for the questionnaire and MCT (PC20>16 mg/ml) by a five-breath dosimeter method were recruited as controls.[30] Informed consent was obtained from all participants and the study protocol was approved by the institutional review boards of all participating institutions.

Baseline assessment

Demographic information of participants such as age, sex, smoking history, body mass index (BMI), and disease duration were collected using a structured questionnaire. Smoking status was defined as follows. A never smoker was a non-smoker or who smoked less than 5 pack-year over the lifetime, and a former smoker smoked more than 100 cigarettes over the lifetime and had stopped smoking for more than 6 months. A current smoker had smoked 100 or more cigarettes over the lifetime and was currently smoking or had quit smoking for less than 6 months. BMI was calculated by dividing weight (kg) by the square of height (m^2).

CRS was operationalized as a condition in which two or more of the following symptoms persisted for more than 12 weeks over the past year; nasal discharge/obstruction, anosmia, and/or facial pain. [31] The NP was evaluated through

rhinoscopy and a history of NP surgery. Based on this, subjects were classified into three categories: no CRS, CRS without NP (CRSsNP), and CRS with NP (CRSwNP). The predicted forced expiratory volume in 1 second (FEV1) and forced volume capacity (FVC) were measured with about 3-month intervals based on the methods of Morris. [32] Atopy was defined based on a positive skin test result, meaning that the allergen-induced wheal is 3 mm or greater than the negative control in tests performed with 55 common allergens.

Medication adherence was checked by the research coordinator at each visit to maintain compliance and correct use of inhalers. Study participants were asked how often they used inhalers on days with and without asthma symptoms, and they were asked to bring their inhalers to examine the appropriateness of inhaler usage and dose counter. They received a formal training whenever researcher coordinators found that participants had difficulty using their inhalers.

Outcome assessment

Asthma duration was defined as the time between the initial asthma diagnosis and the baseline assessment. Late-onset asthma was defined as asthma diagnosed at or after the age of 40 years. After the baseline assessment, asthma outcome was prospectively evaluated. Acute exacerbation (AE) of asthma was determined 1) when the corticosteroid was increased for at least 3 days after the maintenance dose, or when a subject who had not been previously treated with systemic corticosteroids was treated with corticosteroids for at least 3 days, or 2) when a subject was hospitalized or visited the emergency room for the corticosteroid treatment due to an asthma attack. The annual frequency of AE was based on the last 1 year of data.

A pulmonary function indicator of pre-bronchodilator (BD) FEV1/FVC<0.7 at baseline was used to determine airflow obstruction. Pulmonary function tests were conducted as a part of routine clinical practice and the test results data were collected sequentially. FAO was identified if the best pre-BD FEV1/FVC ratio during the 2-year follow-up was less than 0.7.

Serum IgE measurement

Serum total IgE and SE-IgE were measured using an immunoCAP 250 analyzer (ThermoFisher, Uppsala, Sweden). To measure the level of SE-IgE, staphylococcal enterotoxin mix was used (SEA, SEC, TSST-1; Thermo Fisher).[14] SE-IgE sensitization was defined as serum SE-IgE ≥ 0.35 kU/L.

Statistical analyses

Descriptive data were expressed as means ± standard deviations, medians (interquartile ranges), or percentages. Group comparisons were performed using t-test, chi-squared test, and one-way ANOVA and correlations among nonparametric variables were examined using Spearman correlation analysis. Univariate logistic regression analyses were performed to examine the risk factors for airflow obstruction and FAO, and multivariate logistic regression analyses were performed after adjusting for confounding factors. All statistical tests, performed using Stata 15.1 (Stata Corporation, College Station, TX, USA), were two sided, and a *P-value* of < 0.05 was considered statistically significant.

2.2. Synergistic impact of SA nasal colonization and SE-IgE

sensitization in late-onset asthmatics

Next, we aimed to examine the clinical associations of SA colonization and SE-IgE sensitization with asthma severity through the prospective study design.

Study population

This study analyzed the 181 patients from late-onset asthmatics (onset age ≥ 40 years) who were prospectively recruited from two institutions in Seoul, South Korea, between 2018 March and 2021 April.[28] Demographic factors such as age, sex, smoking history, weight, height, disease duration, and comorbidities were collected through structured questionnaires. Asthma was diagnosed by asthma specialists, based on clinical history and positive bronchodilator responsiveness in a lung function test at baseline or after anti-inflammatory treatments. Subjects were classified into 4 groups according to SA nasal colonization and SE sensitization. Among them, the reference group was defined as the SA/SE (–/–).

Definition of parameters and outcomes

Asthma duration was defined as the time between the initial asthma diagnosis and the enrollment of the study. Recent AE of asthma was defined as 1) when a patient who has not previously used systemic corticosteroid needs treatment with systemic corticosteroid for at least 3 days, or if a patient who is using a systemic corticosteroid maintenance dose needs an increase of at least 3 days, or 2) an emergency room visit or hospitalization for corticosteroid treatment due to asthma attack in last year. Atopy defined based on a positive skin test result, meaning the allergen-induced wheal is 3 mm or greater than the negative control in a test performed with 54 common

allergens. The predicted FEV1 and FVC, and fractional exhaled nitric oxide (FeNO) was measured at baseline. After 18-24 months of study enrollment, FeNO and lung function data were retrospectively confirmed through medical records. Pre-bronchodilator (BD) FEV1/FVC<0.7 at baseline was used to determined airflow obstruction, and the best pre-BD FEV1/FVC<0.7 at follow-up was defined as FAO. Pulmonary function test followed the American Thoracic Society/European Respiratory Society recommendations.[33] The patients were excluded if they had interstitial lung disease, pulmonary tuberculosis, congestive heart failure, and malignancy. Informed consent was obtained from all subjects, and the study protocol was approved by the institutional review board.

SA culture and measurement of serum IgE

Nasal swabs were obtained from the anterior nares using BBL CultureSwab and Collection & Transport System swabs. Specimens were incubated using Mannitol salt agar (MSA, Synergy Innovation, Seongnam, Korea) for 24 hours at 37 °C. Colony that turned yellow in MSA was transferred using broth and cultured in Blood agar plate (BAP, KoMed Ltd. Co., Seongnam, Korea) for 24 hours at 37 °C. Colony showing beta-hemolysis in BAP was selected and incubated in Tryptic soy broth (Synergy Innovation, Seongnam, Korea) for 24 hours at 37 °C. Finally, the SA strain was confirmed by 16S-ribosomal RNA sequences. A positive result for culture was defined as SA nasal colonization. Serum total IgE and SE-IgE (SEA, SEB, TSST-1) were measured using immunoCAP 250 analyzer (ThermoFisher, Uppsala, Sweden). SE sensitization was defined as the total sum of each serum SE-IgE titer (SEA, SEB, TSST-1) ≥ 0.35 kU/L.

Statistical analyses

Descriptive data were expressed as means ± standard deviations, medians (interquartile ranges), or percentages. Categorical variables were analyzed using chi-square tests, and continuous variables were analyzed using t-tests. Univariate and multivariate logistic regression analyses were performed to examine the associated factors for SA and SE after adjusting for confounding factors. All statistical analyses were performed using Stata 15.1 (Stata Corporation, College Station, TX, USA), and *P-value* of <0.05 were considered statistically significant.

2.3. Effects of Spls on airway epithelial cells

Next, we aimed to investigate whether Spls from SA induce eosinophilic inflammation in the airway epithelium. Second, we aimed to determine if Spls induces decreased integrity of airway epithelial cells to examine whether it affects the decrease in epithelial cell integrity.

Culture of cell lines and primary cells

To examine airway inflammation, human bronchial epithelial cell lines BEAS-2B (CRL-9609, American Type Culture Collection, Manassas, VA) were grown as monolayers in serum-free growth media (Bronchial epithelial cell growth media, BEGM, SingleQuots, Lonza, USA) at 37°C and supplemented with BEGM. A549 (CCL-185, American Type Culture Collection, Manassas, VA) were grown in media (RPMI 1640, Gibco, MA, USA) at supplemented 10% fetal bovine serum (FBS, Gibco, MA, USA) and 1% antibiotics (Penicillin-streptomycin, WELGENE,

Gyeongsan-si, Korea).

Both cells were cultured until they grew to $1x10^5$ cells in 12-transwell under standard culture conditions. In addition, primary human nasal epithelial cells (HNEpC, PromoCell, Heidelberg, Germany) were cultured in PneumaCult-Ex Plus medium (STEMCELL Technologies, BC, Canada), and used late expansion phase of air-liquid interface culture. Other culture conditions were the same as those of cell lines culture.

Additionally, BEAS-2B cells were cultured until they grew to $1x10^5$/ml cells in the confocal dish to examine tight junction injury of airway epithelium, and the rest of culture conditions were the same as above. Then, BEAS-2B cells were cultured until they grew to $1x10^5$/ml cells in a total of 6 wells for immunoblotting.

mRNA isolation and Quantitative real-time polymerase chain reaction

BEAS-2B cells, A549 cells and HNEpC cells were used as the airway epithelial cells. A total $1.5x10^5$ cells (BEAS2B cells, A549 cells, and HNEpC) per well were seeded on 12 well plates. SEB (10 ug/ml, S4881, Sigma-Aldrich, MO, USA), and SplA/D (1 and 10 ug/ml, CSB-EP642055FLF-B, Cusabio, TX, USA) were all simultaneously treated in the airway epithelial cells. Total RNA was extracted using TRIzol Reagent (Life technologies, CA, USA) and messenger RNA (mRNA) was reverse transcribed to produce cDNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Basel, Switzerland) with anchored-oligo (dT) 18 primers according to the manufacturer's protocol.

Afterwards, the quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed with 96-wells white plate using a LightCycler 480 SYBR

Green I Master (Roche, Basel, Switzerland). The qRT-PCR was performed after 24 hours (in all types of cells). The qRT-PCR was amplified through 45 cycles of 95°C for 10 seconds, 55°C for 35 seconds, and 72°C for 35 seconds. Target mRNA levels were quantified using specific primers and probes for C-C Motif Chemokine Ligand 11 (CCL-11, Limited in HNEpC), Interleukin-33 (IL-33, Limited in HNEpC), and Cystatin SN (CST1). β-actin gene was used as a housekeeping gene for normalization and the expression of each gene was presented as fold against β-actin.

Immunofluorescence staining of tight junctions

To confirm the effects of Spls on the airway epithelium, BEAS-2B cells were stimulated for 24 hours with SplA/D (5 ug/ml), washed with phosphate buffered saline (PBS), and fixed with 2% paraformaldehyde for 10 min at 37°C. Fixed samples were permeabilized with 0.1% Triton X-100 in PBS for 8 minutes. For immunofluorescence (IF) staining, primary antibodies against Zo-1 (1:200, Invitrogen, MA, USA) were used for overnight incubation at 4°C, and primary antibodies against E-cadherin (Cell Signaling, MA, USA) 1:200 was incubated for 2 hours at room temperature. Then, secondary antibodies against Zo-1 (1:500, Invitrogen, MA, USA), and E-cadherin (1:500, Cell Signaling, MA, USA) were treated at room temperature for IF.

Western blot

Western blot was performed for Zo-1, E-cadherin, and fibronectin. The $3x10^5$ per well (BEAS-2B) cells were stimulated with transforming growth factor beta (TGF-β, 1 ng/ml), SEB (10 ug/ml), and SplA/D (20 ug/ml). Then cells were harvested after

48 hours, and protein was extracted. Protein was separated by sodium dodecyl sulphate/polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Amersham BioSciences, Amersham, UK).

Blots were blocked in 5% skim milk in Tris-buffered saline (TBS), pH 7.4 before being incubated in primary antibodies in 1% bovine serum albumin (BSA) in TBS. Blots were rinsed and washed three times for 10 minutes in TBS with 0.1% Tween 20 Detergent (TBST). Then, blots were incubated with horseradish peroxidase (HRP) conjugated secondary antibodies (anti-rabbit and anti-mouse, Invitrogen, MA, USA) in TBST, and rinsed and washed in TBST. Protein bands were detected using Amersham ECL Western Blotting Detection Reagents (GE Healthcare, IL, USA). Primary antibodies were rabbit polyclonal anti-fibronectin (1:3000, Abcam, Cambridge, UK), mouse monoclonal anti-E-cadherin (1:2000, Santa Cruz, TX, USA), and mouse monoclonal anti-Zo-1 (1:500, Santa Cruz, TX, USA), followed by anti-rabbit antibodies (Fibronectin 1:5000, Abcam, Cambridge, UK) as secondary antibodies. As loading control, blots were stained overnight with Glyaldehyde 3-phosphate dehydrogenase (GAPDH, 1:500, Santa Cruz, TX, USA), and stained with secondary anti-mouse antibodies (1: 5000, Santa Cruz, TX, USA) at room temperature for 1 hour.

Measurement of outcomes

CST1 was measured in BEAS-2B cells and A549 cells after 24 hours, followed by treatment with β-actin, SEB (10 ug/ml), and SplA/D (1 and 10 ug/ml). HNEpC were treated with β-actin, SEB (10 ug/ml), SplA (10 ug/ml), and SplD (1 and 10 ug/ml). CCL-11, IL-33, and CST1 known as markers related to eosinophilic inflammation

were measured after 24 hours.

BEAS-2B cells were treated with SplA/D (5 ug/ml) and IF staining was performed after 24 hours to examine changes in Zo-1 (Green), and E-cadherin (Red), which are molecules related to epithelial integrity. In addition, to examine the quantitative change of junction molecules in decrease integrity of airway epithelial cells, BEAS-2B cells were treated with TGF-β, SEB, and SplA/D (20 ug/ml). After 48 hours, immunoblotting was performed for fibronectin, Zo-1, and E-cadherin.

Statistical analyses

Data were analyzed using GraphPad Prism 9.5.0 (La Jolla, CA, USA). Statistical differences between control and treatment groups were evaluated using Mann-Whitney U test. *P*-values were considered significantly different less than 0.05.

Results

3.1. Impact of SE-IgE sensitization in late-onset asthmatics

Baseline characteristics

Baseline characteristics of 223 late-onset elderly asthmatics and 89 controls at the time of cohort enrollment are summarized in Table 1. The baseline proportion of airflow obstruction among the elderly asthmatics was about 29.1%. The mean ages of those with and without airflow obstruction were 71.4±5.0 and 70.9±4.9 years, respectively. Also, those with airflow obstruction were significantly more likely to be male, use bronchodilators, and have a positive smoking history, CRS, heightened levels of SE-IgE and SE sensitization than their counterparts. However, there were no significant differences between the two groups in terms of atopy, blood eosinophils, mean inhaled corticosteroid dose, and use of LTRA.

Association between SE-IgE sensitization and airflow obstruction

Univariate logistic regression analysis showed that former (OR = 4.01 [95% CI = 1.84-8.72]) and current smoking (OR = 4.90 [95% CI = 1.98-12.12]) were significantly and substantially associated with airflow obstruction (Table 2). Also, being male (OR = 3.65 [95% CI = 2.0-6.77]), low FEV1 (<80%, OR = 3.58 [95% CI = 1.91-6.7]), use of LABA (OR = 3.44, [95% CI = 1.14-10.34]), and SE sensitization (OR = 2.59 [95% CI = 1.42-4.72]) were significantly associated with airflow obstruction. Multivariate logistic regression was performed to examine the risk factors for airflow obstruction in elderly asthmatics (Table 3). Current smoking was a significant risk factor for airflow obstruction (OR = 3.50 [95% CI = 1.19-10.30]). Additionally, SE sensitization was significantly associated with airflow obstruction

after adjusting for smoking status as well as age, sex, and CRS. However, atopy, blood eosinophilia, and asthma medications were not significantly related to airflow obstruction after adjusting for the same confounding factors. Additionally, the SE-sensitized group had significantly lower baseline FEV1/FVC ratio than the SE-negative group (Table 4).

Clinical characteristics of asthmatics regarding FAO after the 2-year follow-up

After the 2-year follow-up, the proportion of asthmatics with FEV1/FVC ratio <0.7 was 22.4%. The proportion of those with consistently low FEV1/FVC ratio (<0.7) was 17.5% while the proportion of those with worsened airflow obstruction compared to baseline was 4.9% (Figure 1). However, the proportion of low FEV1 (<80%) decreased by more than 50% over the 2-year follow-up (Figure 2). Table 5 shows the clinical characteristics of the subjects with and without FAO based on the results of the lung function test after the 2-year follow-up. Though only marginally significant ($0.05 < p < 0.10$), subjects with FAO were older and had lower BMI than those without FAO. In addition, those with FAO were significantly more likely to be male and have a positive smoking history and a longer asthma duration than their counterparts. The rate of SE sensitization and total IgE level were significantly higher in the FAO group than the non-FAO group. The lung function of the FAO group was significantly lower than the non-FAO group, but there were no significant differences in terms of atopy, eosinophilic inflammations, and the number of AEs per year.

Relationship between SE sensitization and FAO in elderly asthmatics after the 2-year

follow-up

A univariate logistic regression analysis showed that the subjects with FAO after the 2-year follow-up were more likely to be male and have a longer asthma duration, a positive smoking history, CRSwNP, and greater SE sensitization than those without FAO (Table 6). A multivariate logistic regression analysis (Table 7) showed that asthma duration and former smoking were only marginally significantly ($0.05<p<0.10$) associated with FAO. However, current smoking and SE sensitization were consistently significantly associated with FAO (Model 1 in Table 7). Additionally, when FAO was regressed on CRS and SE sensitization, the association between FAO and CRSwNP was non-significant, while the association between FAO and SE sensitization was consistently significant (Model 2 in Table 7). Both baseline and follow-up FEV1/FVC ratios were significantly lower in the group continuously sensitized to SE in univariate (data not shown) and multivariate logistic regression analyses (Table 8). Meanwhile, the number of AEs per year showed a significant correlation with the level of SE-IgE ($r = 0.43$ for asthmatics without FAO; $r = 0.40$ for asthmatics with FAO, both correlations significant with $p < 0.01$, Figure 3). In never smokers, SE-IgE and the frequency of asthma exacerbations was significantly correlated in asthma patients without FAO, but it was non-significant in asthma patients with FAO (Figure 4).

3.2. Synergistic impact of SA nasal colonization and SE-IgE sensitization in late-onset asthmatics

Baseline characteristics of study population

In a total of 181 patients with late-onset asthma, SA nasal colonization was positive in 38.1% (Table 9). The SA (+) group was more likely to be older, male, smokers, and have higher total IgE levels than the SA (-) group. It was also associated with Global Initiative for Asthma (GINA) step 5 including biologics, and lower lung functions at baseline. Meanwhile, the SE (+) group showed significant associations with high total IgE and GINA step 5, the use of biologics compared to the SE (-) group (Table 10). In the four groups classified according to SA and SE, the proportions of SA/SE (–/–), SA/SE (+/–), SA/SE (–/+), and SA/SE (+/+) were 33.7%, 15.5%, 28.2%, and 22.6%, respectively (Figure 5). Compared to the reference group [SA/SE (–/–)], the SA/SE (+/–) group was related to older onset age, male sex, current smoking, and lower lung functions, and the SA/SE (–/+) group showed significantly higher total IgE and SE-IgE compared to the reference group (Table 11 and 12). Moreover, the SA/SE (+/+) was significantly associated with male sex, current smoking, high total IgE, GINA step 5 and the use of biologics, and the FEV1/FVC ratio at baseline for this group was also the lowest.

Associated factors for SA colonization and SE sensitization

Compared to the reference group in univariate and multivariate logistic regression analysis after adjusted for age, sex, smoking, and BMI, the SA/SE (+/–) showed a significant association with FeNO at follow-up, and the SA/SE (–/+) consistently showed a correlation with total IgE, and FEV1/FVC ratio<0.7 at follow-up (Table 13). Meanwhile, the SA/SE (+/+) showed a continuous association with high total IgE, GINA step 5, the use of biologics, the FeNO and FEV1/FVC ratio<0.7 at follow-up, but the FeNO, and FEV1/FVC ratio<0.7 at baseline were not significant after

adjusted for confounding factors (Figure 6A-D).

3.3. Effects of Spls on airway epithelial cells

Decrease in integrity of airway epithelial cells

In BEAS-2B cells were treated with SplA/D 5 ug/ml, IF staining showed a decrease in Zo-1 and E-cadherin compared to the control (Figure 7A). Meanwhile, when BEAS-2B cells were treated with SplA/D 20 ug/ml on western blot, fibronectin increased in SplA, but not in SplD compared to the control. Zo-1 was decreased compared to the control in both SplA and SplD, but more so in SplA. However, E-cadherin was similar compared to the control (Figure 7B-D).

Airway inflammation of epithelial cells

When BEAS-2B cells were treated with SplA/D, CST1 did not increase regardless of the concentration of Spls (Figure 8A). CST1 significantly increased in a dose-dependent manner compared to the control when A549 cells were stimulated with SplD (Figure 8B). In a case of HNEpC, CCL-11 was not increased in HNEpC treated by SplA/D, but IL-33 and CST1 were significantly increased at SplD 10 ug/ml (Figure 8C-E).

Discussion

4.1. Impact of SE-IgE sensitization in late-onset asthmatics

In the first study, we found the risk factors for FAO in late-onset elderly asthma patients. Elderly asthmatics had higher rates of CRS and SE sensitization than controls. Among the elderly asthmatics at baseline, we detected a significant association between current smoking and airflow obstruction, and SE-IgE was also significantly associated with airflow obstruction. In addition, pulmonary function tests over the 2-year follow-up showed that subjects with persistent or worsened airflow obstruction were significantly more likely to be male and have a smoking history, longer asthma duration, greater SE sensitization, and lower lung function compared to their counterparts. Multivariate logistic regression analysis also showed that FAO was significantly linked to current smoking and SE sensitization, and the number of AEs per year were positively correlated with the levels of SE-IgE. In never smokers, the number of AEs per year was consistently and significantly correlated with the levels of SE-IgE. According to our findings, SE-IgE is suggested to be a biomarker predicting FAO and SE-IgE sensitization is a potential risk factor for FAO and asthma severity in elderly asthmatics.

Smoking is a well-known major risk factor for the decline of lung function, accelerating the negative effects of asthma. Further, it increases the chance of airway inflammation in asthmatics, impairing asthma controls and compromising the corticosteroid treatment responsiveness.[34, 35] Additionally, smoking status is associated with sensitization to SEs in both the general population and asthmatics, particularly in current smokers.[13, 36] Thus, in asthma patients with a positive smoking history, smoking itself not only affects airway

inflammation and causes poor asthma controls, but also increases the risk of SE sensitization. This may eventually contribute to increased asthma severity and decreased lung function. FAO accompanied by airway remodeling may be attributable to smoking and SE-IgE.

It is worth noting the functional features of SE-IgE reported in previous studies. Mucosal tissue polyclonal IgE from 14 NP subjects, but not serum, was able to activate mast cell on exposure to inhalant allergen and SEB. [5] In atopic dermatitis patients with SE sensitization, basophils from peripheral blood induced histamine secretion upon exposure to SE. [37] Further, SEs may increase the chance of eosinophil survival by inhibiting eosinophil apoptosis and activate macrophages by inducing the secretion of cytokines like IL-8 and IL-12.[38, 39]

In terms of exacerbations, Tanaka found that SEA-IgE was closely associated with asthma control status and high level of FeNO. [40] Also, Sintobin's longitudinal cohort study reported that SE-IgE was related to an increased risk of asthma severity and AE.[41] As in previous studies, a notable finding of this study was the significant correlation between the level of SE-IgE and the number of AEs per year. These findings suggest that SE-IgE is a risk factor for increased asthma severity. It was revealed that superantigens such as SEs and TSST-1 were particularly immunogenic and intensified eosinophilic inflammation by releasing excessive cytokines including Th2 cytokines, which induced mast cell and basophils activation when SE-IgE was present.[5, 42] Ultimately, these can lead to inflammation aggravation and increased asthma severity as well as frequent exacerbations, accelerating the lung function decline. Therefore, we infer that persistent inflammation and repeated exacerbations may contribute to airway remodeling through both intrinsic and

extrinsic factors such as decreased epithelial integrity, goblet cell hyperplasia, and mechanical injury, which can subsequently lead to FAO.

There have been a few studies showing that SE sensitization may be involved in chronic obstructive pulmonary disease (COPD), rather than asthma. Lymphocytes and neutrophils in bronchoalveolar lavage, CD8+ lymphocytes and granulocytes in lung tissue, as well as IL-13 mRNA expression in lung and goblet cell hyperplasia in airway wall were increased with the combined exposure to smoking and SEB in mice model.[43] Additionally, SE-IgE was significantly higher in smoker subjects with both stable and exacerbated COPD compared to healthy control and smoker subjects without COPD.[44] Moreover, SE-IgE decreased significantly as FEV1 improved in hospitalized subjects due to exacerbated COPD. Although further studies are needed, the contribution of SE-IgE in COPD to airway inflammation and airway remodeling is suggested. However, there has been no study directly examining the longitudinal association between asthma-COPD overlap (ACO) and SE-IgE. Jointly considering our findings and previously demonstrated associations in asthma and COPD, SE-IgE is suggested to be a biomarker for FAO, and involved in airway inflammation and airway remodeling, contributing to FAO and the ACO phenotype.

Our results showed that SE sensitization was closely associated with airflow obstruction at baseline and persistent or worsened airflow obstruction after the 2-year follow-up. We also showed significant correlations between SE sensitization and frequent AEs, in line with previous studies. Therefore, we speculate that asthmatics with high SE-IgE are more likely to experience severe asthma symptoms, poor asthma control, and frequent acute exacerbation. This may contribute to airway remodeling due to persistent airway inflammation, frequent bronchoconstriction, and mechanical injury. In sum, SE

sensitization might be a potential risk factor for FAO accompanied by airway remodeling in elderly asthmatics.

Besides SE sensitization, Staphylococcal protein A secreted by SA can act as a B cell superantigen, degranulating mast cells by binding them to V_H3-positive IgE. SA can stimulate Innate Lymphoid Cells-2 (ILC-2) by secreting alarmins such as IL-25, IL-33, and thymic stromal lymphopoietin from the epithelium.[7] Subsequently, IL-5 released from ILC-2 or Th2 cells activates eosinophilic inflammation, followed by a series of reactions resulting in substances like extracellular eosinophilic traps, galectin 10, and Charcot-Leyden-Crystals, inducing persistent inflammation of the airway epithelium. These responses may eventually lead to FAO accompanied by airway remodeling by worsening respiratory epithelial inflammation and undermining epithelial integrity.[45, 46] However, evidence from human studies is still lacking and further *in vivo* and mechanistic studies are needed on the interrelations between SA, including SE-IgE, and respiratory epithelium. This study is the first follow-up study on the associations between SE sensitization and FAO in elderly patients with asthma. A previous study showed that the rate of SE sensitization was significantly higher in smoking asthmatics compared to never smokers, and it was associated with eosinophilic inflammation and asthma severity.[14, 47] Consistent with these findings, the present study demonstrated that SE sensitization was associated with smoking and frequent exacerbation. Further, it was closely related to airflow obstruction and FAO in conjunction with airway remodeling. FAO accompanied by airway remodeling accelerates the deterioration of lung function and increases morbidity. However, proactive prevention methods or symptomatic treatment approaches for FAO are not yet readily available due to our lack of understanding of its underlying

pathophysiology.[48] At the same time, the prevalence of elderly asthma has recently begun to increase, and it is considered to be a different disease entity characterized by more severe symptoms, worse control, and lower quality of life than non-elderly asthma.[49] For the development of novel treatment strategies, a mechanistic understanding of the causes of FAO, especially in elderly asthmatics, is required. To this end, our study examined the relationship between SE-IgE sensitization and FAO, offering a new insight on the associations between SE-sensitization and airway remodeling in elderly asthmatics.

However, there are several limitations in the present study. First, the subjects were patients who visited the tertiary referral hospital and they were likely to have relatively high severity. Second, the definition of FAO was not based on post-BD values but on the best pre-BD FEV/FVC ratio over the 2-year follow-up. In the present study, lung function was measured sequentially as a part of routine clinical practice in which post-BD response was often not assessed. Nonetheless, the participants were sequentially assessed for pre-BD at the tertiary referral hospital, and the best pre-BD value might be a reasonable approximation of post-BD value. Third, demographic information such as race and geographic location was not considered in this study. Fourth, type 2 biomarkers such as sputum eosinophil counts, or fractional exhaled nitric oxide levels were not analyzed. Future studies need to address these limitations for the enhanced internal and external validity. Additionally, studies with longer-term follow-up are needed to further elucidate the relationship of SE sensitization with airway remodeling and FAO.

4.2. Synergistic impact of SA nasal colonization and SE-IgE

sensitization in late-onset asthmatics

In the second study, we prospectively recruited late-onset asthmatic patients and examined the prevalence of SA nasal colonization and the clinical characteristics associated with SA nasal colonization and SE sensitization. SA nasal colonization was positive in 38.1% and related to males and current smoking. In the subgroup analysis, the SA/SE (+/+) had higher total IgE, SE-IgE, GINA step 5, the use of biologics, and FeNO at follow-up than other groups, and showed a significant association with FAO. According to our findings, SA colonization combined with SE sensitization may have synergistic effects on asthma severity including FAO in late-onset asthma.

To our knowledge, this is the first study to explore the synergistic effects of SA nasal colonization and SE-IgE sensitization in late-onset asthmatics. SA nasal colonization has associations with asthma and allergic rhinitis, and is related to asthma and its severity in the younger general population, and adolescents.[12,17,18] Moreover, SE sensitization has associations with asthma prevalence in the general population and eosinophilic asthma and its severity in late-onset asthmatics.[3,14] However, very few studies evaluated interactive effects of SA colonization and SE-IgE sensitization on clinical outcomes. In a high school-based cohort study in Norway, 50.2% of students with SE-IgE sensitization had SA carriage.[50] Those with SE-IgE sensitization and SA carriage showed a significant association with the poly-sensitization of inhalant allergens compared to the SE non-sensitization or SA non-carriage group (OR 1.65, 95% CI 1.19-2.31). Meanwhile, SA colonization and SEA-IgE in NP tissue homogenates increased in parallel in NP and comorbid asthma or aspirin sensitivity, but all colonization rates were higher than those of

SEA-IgE.[51] In a community-based population in Korea, SA carriage and SE sensitization was significantly associated with total IgE and eosinophilic inflammation, and showed a synergistic effect on the prevalence of cough and sputum symptoms compared to SA non-carriage without SE sensitization.[52] However, no studies to date reported their clinical associations in late-onset adult asthmatics.

In our study, SA colonization was more likely among males and current smokers, and SA colonization in combination with SE sensitization showed the associations with total IgE and treatment levels in a dose-dependent manner. Moreover, the SA/SE (+/+) was related to eosinophilic airway inflammation, and FAO, compared to the reference.

Exactly what role of SA colonization combined with SE sensitization plays in asthma pathogenesis is unknown, but there are several studies suggesting that it may contribute to asthma severity. SE sensitization induces eosinophilic inflammation through polyclonal T-cell proliferation and activation by releasing type 2 cytokines, and is related to polyclonal IgE formation.[3,5] Additionally, Spls can induce type 2 inflammations, and IgE formations.[53, 19] SA facilitates alternative macrophage polarization to M2, which could inhibit Th1 and Th17 responses and prevent clearance of SA. Additionally, SA-derived exotoxins promote type 2 inflammation.[4] Deficiencies in bacterial clearance and phagocytosis in severe asthma support intramucosal persistence of SA.[54] Moreover, smoking itself is also associated with SA colonization and SE sensitization.[18, 55]

Considering previous studies, it is possible that inflammation due to chronic airway disease, as well as smoking, may alter immunological functions or susceptibility to SA of mucosa. We suppose that patients with SA colonization may have a longer exposure to SA and a relatively greater chance of being exposed to superantigens, Spls, and exotoxins from SA.

These substances may affect the airway epithelium, contributing to decreased epithelial integrity and increased airway inflammation. These may finally lead to frequent asthma exacerbations and increased airway inflammation, which contributes to clinical severity, such as requiring high level of asthma treatment, airway inflammation, and lowering lung function.

However, there are several limitations. First, our findings cannot determine a causal relationship between SA colonization and SE sensitization, because the antecedent of the relationship is unclear. Second, the longitudinal association of SA and SE with systemic type 2 marker could not be confirmed. Third, we could not identify regional and racial differences.

4.3. Effects of Spls on airway epithelial cells

This study showed that when airway epithelium was stimulated with SplA and SplD, tight junction injury of airway epithelium was estimated from IF. Also, western blots results showed an increase in fibronectin and a decrease in Zo-1, but no significant changes in E-cadherin. Meanwhile, airway inflammation was not clearly identified in BEAS-2B cells when airway epithelium was stimulated with SplA and SplD, but type 2 inflammation markers including CST1 were elevated at SplD 10 ug/ml in A549 cells and HNEpC. Considering these findings, Spls may induce tight junction injury and airway inflammation including CST1, contributing to decrease in integrity of airway epithelial cells.

Spl-IgE was identified in most asthma patients, and Th2 cytokine was induced in peripheral T cells by Spls stimulation. SplD inhalation in mice also caused Th2 cytokine

and eosinophilic infiltration in the lung without an adjuvant trigger.[20] In addition, type 2 inflammation was induced when stimulated with SplD in allergic asthma murine model.[19] In terms of CST1 related to eosinophilic inflammation, CST1 could amplify eosinophilic and type 2 inflammation in NP, which may contribute to the severity and postoperative recurrence of CRSwNP. Moreover, it was reported that CST1 in epithelium in NP can contribute to eosinophilic inflammation through a positive-feedback loop with TSLP, IL-33, and type 2 cytokine.[56] Meanwhile, TSLP was increased and Claudin-1 was degraded by German cockroach extract (GCE) treatment in A549 cells, and the GCE-induced mouse model also showed the same results.[57] Additionally, SEB reduced the integrity of nasal polyp epithelial cells by stimulating Toll-like receptor-2 in CRSwNP.[58] These results suggest that the several allergens can induce the loss of airway epithelial cell integrity.

However, no study has yet investigated the association of Spls with decreased integrity including tight junction injury and airway inflammation, especially CST1 in airway epithelial cells. Therefore, the strength of this study is that it is the first study to examine whether SplA and SplD cause a damage of epithelial integrity, and whether SplD induces type 2 inflammation, including CST1 in airway epithelial cells. To date, it is known that exposure of Spls to the airway epithelium induces an increase in IL-33 and type 2 cytokines.[19] We speculate that this will increase secretion of alarmins like TSLP and the substances such as CST1 related to type 2 inflammation, which may eventually contribute to exacerbating airway inflammation. In addition, our findings showed that when Spls were exposed to airway epithelial cells, tight junction injury occurred in IF staining, and western blots, decreasing the integrity of epithelial cells. Thus, we speculate that exposure of Spls to the airway epithelium can exacerbate the inflammatory response. If this is

repeated, ultimately, epithelial integrity will decrease, and goblet cell hyperplasia may occur. Alternatively, exposure of Spls to the airway epithelium may cause a decrease in epithelial integrity, which in turn may contribute to exacerbation of allergens-induced inflammation. Therefore, we suggest that increased airway inflammation and epithelial cell damage may lead to increased AE and FAO including airway remodeling, contributing to the severity of asthma.

However, there are several limitations. First, decreased epithelial integrity was confirmed in both SplA and SplD by IF staining and western blots, but the results for fibronectin were not consistent. Thus, if there is a difference in the biological functions of SplA and SplD, further exploration is needed, and additional experiments with different concentrations of SplD in BEAS-2B cells and using primary epithelial cells will be needed. Second, since airway inflammation by SplA are not as pronounced compared to SplD, further experiments at different concentrations of SplA are needed. Third, the effect on epithelial integrity was not examined when Spls were exposed in A549 cells or primary cells. Additional experiments in epithelial cells will be needed in the future. Finally, this study is limited to *in vitro* experiments, so the cascade of reactions caused by interactions within the immune system of *in vivo* is not reflected. Thus, further *in vivo* studies including animal models and human samples are required.

4.4. Integrated Interpretations

The present studies demonstrate that SA and substances secreted by SA can affect asthma severity and FAO, possibly leading to airway remodeling. It is possible to speculate their interrelationship through our results. First, in both the first and second studies, SE-IgE

sensitization showed associations with total IgE, treatment levels, and FAO. This could result in an increase in total IgE as SE sensitization eventually induces polyclonal T-cell activation and IgE formation. Additionally, increased IgE and type 2 inflammation due to SE sensitization may contribute to asthma severity, including high treatment levels and FAO. Second, *in vitro* studies showed Spls exposure to epithelium reduced epithelial integrity and induced airway inflammation. It can be assumed that SA-derived Spls induce a decrease in airway epithelial cell integrity and increase the risk of SE sensitization. This suggests that substances secreted by SA, such as SE or Spls, contribute to the development of asthma severity including airway inflammation and FAO. Third, in the SA positive group, airway eosinophilic inflammation was associated with follow-up regardless of SE sensitization. This is because the SA positive group has a relatively higher risk of exposure to nasal epithelium to substances like SEs or Spls secreted by SA, and it is possible that the various immune responses caused by these substances acted as a driving factor for airway eosinophil inflammation during follow-up more so than baseline.

Considering the results of our studies, the SA positive group was comparatively more closely related to male sex and smoking history than the SA negative group and showed an association with airway eosinophilic inflammation during follow-ups. This suggests that males have a relatively high smoking history, and that smoking affects nasal microbiome changes, immunological functions, or susceptibility to SA of mucosa, and increases the risk of SA nasal colonization. Compared to the SE negative group in both the first and second studies, high total IgE, treatment levels, and the risk of FAO during follow-up increased for the SE positive group. In addition, in the case of SA/SE (+/+), through high total IgE, treatment severity level, airway eosinophilic inflammation, and FAO,

eventually, the SA/SE (+/+) group showed clinically synergistic effects compared to the SA or SE positive groups alone in late-onset asthmatics.

Meanwhile, *in vitro* experiments confirmed that Spls can reduce epithelial cell integrity and induce airway inflammation when exposed to airway epithelial cells. From these results, we estimate that patients with SA nasal colonization are more likely to be exposed to various substances, including SEs and Spls, compared to the SA negative group. Among those with SA nasal colonization, we speculate that exposure of airway epithelial cells to Spls particularly causes a decrease in epithelial cell integrity, contributing to an increase in the risk of SE sensitization, thereby contributing to an increase in asthma severity and progression to FAO.

Therefore, we assume that the more SA or/and SE are positive, the greater the potential for increased asthma severity and airway remodeling. Thus, we need to preemptively confirm the presence of SA and SE when evaluating asthma patients, particularly in late-onset asthma, and consider it a clinical indicator for active monitoring and treatment to prevent future deterioration. Longitudinal data analysis and *in vivo* studies on the association between SA and late-onset asthma are additionally needed in the future.

Conclusions

The increasing evidence suggests that SA is correlated with late-onset asthma. First, SE-IgE sensitization was significantly associated with FAO in late-onset elderly asthma patients independent of other known risk factors for FAO such as asthma duration or smoking history. Thus, SE-IgE could have a pathogenic role in the development of persistent airflow obstruction in late-onset elderly asthmatics and is deemed a biomarker of FAO. Second, SA nasal colonization combined with SE sensitization was related to asthma severity compared to other groups, in terms of treatment levels, airway inflammation, and FAO compared to other groups. Thus, intranasal presence of SA may have synergistic effects with SE-IgE sensitization on asthma severity and worsening. Third, pro-allergic proteins like Spls were associated with decrease in integrity of airway epithelial cells and airway inflammations at *in vitro* experiments. These findings warrant further investigation of the direct and mediating roles of SA including SE-IgE, SA nasal colonization, and Spls on asthma severity and FAO.

Tables

Table 1. Baseline characteristics of the study population

At baseline	Control	Asthma without FEV1/FVC<0.70	Asthma with FEV1/FVC<0.70	*P* value
Subjects, n	89	158	65	
Age (years)	70.4±4.7	71.4±5.0	70.9±4.9	0.363
Female sex (%)	66.3	75.9	26.2	<0.001
Smoking (%)				
Never	71.9	83.5	53.8	<0.001
Former	21.4	10.1	26.2	
Current	6.7	6.3	20.0	
BMI (kg/m^2)	23.7±3.5	24.6±3.2	23.7±3.0	0.067
CRS				
No CRS	84.3	49.4	35.4	<0.001

CRSsNP	15.7	38.0	49.2	
CRSwNP	0.0	12.6	15.4	
Asthma duration (year)	NA	7 (3-11)	6 (2-11)	0.788
Atopy (%)	NA	32.2	42.4	0.166
Blood eosinophils (/mm^3)	NA	201 (110-384)	209 (169-418)	0.245
Total IgE (kU/L)	91.0 (28.9-218.2)	76.1 (28.1-175.5)	143.9 (36.7-348.8)	0.160
SE-IgE (kU/L)	0.10 (0.00-0.18)	0.13 (0.04-0.45)	0.34 (0.09-0.98)	<0.001
SE sensitization (%)	14.6	27.2	49.2	<0.001
Baseline FEV1% pred. (%)	NA	92.1±19.4	78.5±17.0	<0.001
Baseline FEV1/FVC ratio	NA	0.76±0.07	0.65±0.08	<0.001
ICS dose (ug, fluticasone equivalent/day)	NA	588.8±269.0	669.6±270.1	0.06
LABA (%)	NA	74.4	90.9	0.021
LAMA (%)	NA	10.8	23.7	0.049
LTRA (%)	NA	61.7	56.8	0.566

Notes. BMI; body mass index, CRS; chronic rhinosinusitis, CRSsNP; CRS without nasal polyp, CRSwNP; CRS with nasal polyp, IgE; Immunoglobulin E, SE-IgE; Staphylococcal enterotoxin-specific IgE, ICS; inhaled corticosteroids, LABA; long-acting beta2-agonists, LAMA; long-acting muscarinic antagonists, LTRA; leukotriene receptor antagonist, NA; not available

Data are presented as means ± standard deviations, medians (interquartile ranges), or percentages.

Table 2. Factors associated with airflow obstruction in elderly asthmatics: Univariate logistic regression analysis

Baseline FEV1/FVC ratio≥0.70 (n=158) vs. <0.70 (n=65) in asthma	Crude OR (95% CI)	*P* value
Age (years)	0.98 (0.93-1.04)	0.592
Male sex	3.68 (2.00-6.77)	<0.001
BMI (kg/m^2)	0.91 (0.83-1.00)	0.061
Smoking		
Never	Reference	
Former	4.01 (1.84-8.72)	<0.001
Current	4.90 (1.98-12.12)	0.001
CRS		
No CRS	Reference	
CRSsNP	1.81 (0.96-3.41)	0.066
CRSwNP	1.70 (0.70-4.13)	0.245
Atopy	1.55 (0.83-2.87)	0.168

Asthma duration (years)	1.02 (0.98-1.06)	0.322
Blood eosinophilia (≥300/mm^3)	1.18 (0.39-3.55)	0.770
Total IgE (≥100 kU/L)	1.72 (0.96-3.07)	0.070
SE sensitization (≥ 0.35 kU/L)	2.59 (1.42-4.72)	0.002
Baseline FEV1% pred. (<80%)	3.58 (1.91-6.7)	<0.001
ICS dose	1.00 (0.99-1.00)	0.061
LABA	3.44 (1.14-10.34)	0.028
LAMA	2.56 (0.98-6.68)	0.054
LTRA	0.82 (0.41-1.64)	0.567

Notes. FEV1; forced expiratory volume in 1 sec, FVC; forced volume capacity, OR; odds ratio, CI; confidence interval, BMI; body mass index, CRS; chronic rhinosinusitis, CRSsNP; CRS without nasal polyp, CRSwNP; CRS with nasal polyp, IgE; Immunoglobulin E, SE; Staphylococcal enterotoxin, ICS; inhaled corticosteroids, LABA; long-acting beta2-agonists, LAMA; long-acting muscarinic antagonists, LTRA; leukotriene receptor antagonist

Table 3. Factors associated with airflow obstruction in elderly asthmatics: Multivariate logistic regression analysis

Baseline FEV1/FVC≥0.70 (n=158) vs. <0.70 (n=65) in asthma	Adjusted OR (95% CI)	*P* value
Age (years)	0.97 (0.91-1.03)	0.313
Male sex	1.95 (0.85-4.49)	0.117
Smoking		
Never	Reference	
Former	2.52 (0.94-6.74)	0.065
Current	3.50 (1.19-10.30)	0.023
CRS		
No CRS	Reference	
CRSsNP	1.58 (0.78-3.19)	0.204
CRSwNP	1.19 (0.42-3.39)	0.740
Asthma duration (years)	1.01 (0.97-1.06)	0.607
Atopy	1.33 (0.67-2.64)	0.420

Blood eosinophilia (≥300/mm^3)	1.26 (0.31-5.04)	0.746
Total IgE (≥100 kU/L)	1.12 (0.56-2.25)	0.739
SE sensitization (≥ 0.35 kU/L)	2.05 (1.05-4.01)	0.036
Baseline FEV1% pred. (<80%)	2.64 (1.34-5.22)	0.005
Mean ICS dose	1.00 (0.99-1.00)	0.323
LABA	2.76 (0.87-8.71)	0.084
LAMA	1.63 (0.56-4.75)	0.372
LTRA	0.85 (0.4-1.81)	0.677

Notes. FEV1; forced expiratory volume in 1 sec, FVC; forced volume capacity, OR; odds ratio, CI; confidence interval, CRS; chronic rhinosinusitis, CRSsNP; CRS without nasal polyp, CRSwNP; CRS with nasal polyp, SE; Staphylococcal enterotoxin, ICS; inhaled corticosteroids, LABA; long-acting beta2-agonists, LAMA; long-acting muscarinic antagonists, LTRA; leukotriene receptor antagonist

Adjusted for age, sex, smoking, and CRS

Table 4. Changes in lung functions depending on SE sensitization in elderly asthmatics

Variables	SE-negative group	SE-sensitized group	*P* value
Proportion (%)	65.5	34.5	
Baseline lung functions			
Pre-BD FEV1 (%)	90.56±19.0	83.92±20.34	0.019
Pre-BD FEV1/FVC ratio	0.74±0.09	0.71±0.08	0.013
Follow-up lung functions			
Pre-BD FEV1 (%)	97.31±18.44	93.27±17.33	0.124
Pre-BD FEV1/FVC ratio	0.78±0.09	0.74±0.08	0.001

Notes. Pre-BD; pre-bronchodilator, FEV1; forced expiratory volume in 1 sec, FVC; forced volume capacity, SE; Staphylococcal enterotoxin

Data are presented as means ± standard deviations, or percentages.

Table 5. Clinical features among elderly asthmatics depending on the presence of fixed airflow obstruction after the 2-year follow-up

Elderly asthma	**FEV1/FVC≥0.70**	**FEV1/FVC<0.70**	***P* value**
Subjects, n (%)	173 (77.6)	50 (22.4)	
Age (years)	70.9±4.8	72.3±5.4	0.095
Female sex (%)	74.6	42.0	<0.001
Smoking (%)			<0.001
Former	12.7	22.0	
Current	4.6	30.0	
BMI (kg/m^2)	24.5±3.2	23.6±2.8	0.080
CRS (%)			0.118
CRSsNP	41.6	40.0	
CRSwNP	11.0	22.0	
Asthma duration (years)	6.6 (2.8-10.5)	10.4 (3-11.8)	0.030

Atopy (%)	33.7	37.7	0.593
Blood eosinophils (/mm^3)	201.5 (110-403)	200.0 (104-380)	0.474
Total IgE (kU/L)	75.2 (27.0-209.8)	144.3 (41.4-415.6)	0.027
SE-IgE (kU/L)	0.13 (0.04-0.46)	0.35 (0.10-1.24)	0.002
SE sensitization (%)	28.9	50.0	0.005
Best FEV1% pred. (%)	100.2±17.4	81.1±13.3	<0.001
Best FEV1/FVC ratio	0.80±0.05	0.63±0.06	<0.001
Acute exacerbation of asthma (number/year)	0 (0-2)	1 (0-2)	0.134

Notes. FEV1; forced expiratory volume in 1 sec, FVC; forced volume capacity, BMI; body mass index, CRS; chronic rhinosinusitis, CRSsNP; CRS without nasal polyp, CRSwNP; CRS with nasal polyp, IgE; Immunoglobulin E, SE-IgE; Staphylococcal enterotoxin-specific IgE

Data are presented as means ± standard deviations, medians (interquartile ranges), or percentages.

Table 6. Factors associated with fixed airflow obstruction in elderly asthmatics after the 2-year follow-up: Univariate logistic regression analysis

Best FEV1/FVC ≥0.70 (n=173) vs. <0.70 (n=50) in asthma	Crude OR (95% CI)	*P* value
Age (years)	1.05 (0.99-1.19)	0.097
Male sex	4.05 (2.10-7.81)	<0.001
Smoking		
Never	Reference	
Former	2.98 (1.28-6.92)	0.011
Current	11.17 (4.27-29.20)	<0.001
CRS		
No CRS	Reference	
CRSsNP	1.20 (0.59-2.42)	0.613
CRSwNP	2.50 (1.02-6.11)	0.045
Asthma duration (years)	1.05 (1.00-1.09)	0.040

Atopy	1.19 (0.63-2.26)	0.593
Blood eosinophilia (≥300/mm^3)	1.34 (0.49-3.65)	0.572
Total IgE (≥100 kU/L)	2.08 (1.07-4.04)	0.031
SE sensitization (≥ 0.35 kU/L)	2.46 (1.29-4.69)	0.006

Notes. FEV1; forced expiratory volume in 1 sec, FVC; forced volume capacity, OR; odds ratio, CI; confidence interval, CRS; chronic rhinosinusitis, CRSsNP; CRS without nasal polyp, CRSwNP; CRS with nasal polyp, SE; Staphylococcal enterotoxin

Table 7. SE sensitization and other factors associated with fixed airflow obstruction in elderly asthmatics after the 2-year follow-up: Multivariate logistic regression analysis

Best FEV1/FVC ratio≥0.70 (n=173) vs. <0.70 (n=50) in asthma	Adjusted OR (95% CI)	*P* value
Model 1		
Smoking		
Former	2.33 (0.92-5.88)	0.074
Current	8.90 (3.23-24.51)	<0.001
Asthma duration (years)	1.04 (0.99-1.09)	0.070
SE sensitization (≥ 0.35 kU/L)	2.40 (1.16-5.00)	0.019
Model 2		
CRS		
CRSsNP	0.99 (0.47-2.05)	0.971
CRSwNP	1.81 (0.70-4.65)	0.221
SE sensitization (≥ 0.35 kU/L)	2.24 (1.13-4.43)	0.020

Notes. FEV1; forced expiratory volume in 1 sec, FVC; forced volume capacity, OR; odds ratio, CI; confidence interval, CRSsNP; CRS without nasal polyp, CRSwNP; CRS with nasal polyp, SE; Staphylococcal enterotoxin

Model 1: Adjusted for smoking and asthma duration, Model 2: Adjusted for CRS

Table 8. Associations between SE sensitization and changes in lung functions in elderly asthmatics: Multivariate logistic regression analysis

SE-sensitized group (n=77) vs. SE-negative group (n=146) in asthma	Adjusted OR (95% CI)	*P* value
Baseline lung functions		
Pre-BD FEV1 (<80%)	1.80 (0.97-3.34)	0.063
Pre-BD FEV1/FVC ratio (<0.7)	2.13 (1.1-4.13)	0.026
Follow-up lung functions		
Pre-BD FEV1 (<80%)	1.18 (0.55-2.53)	0.663
Pre-BD FEV1/FVC ratio (<0.7)	2.36 (1.13-4.93)	0.023

Notes. Pre-BD; pre-bronchodilator, FEV1; forced expiratory volume in 1 sec, FVC; forced volume capacity, OR; odds ratio, CI; confidence interval, SE; Staphylococcal enterotoxin

Adjusted for age, sex, and smoking status

Table 9. Baseline characteristics of the study population according to SA nasal colonization

Variables	**SA negative group (N=112)**	**SA positive group (N=69)**	**Total (N=181)**	***P value***
Proportion (%)	61.9	38.1	100	
Age (years)	67.5±11.8	71.0±7.7	68.9±10.6	0.011
Asthma onset age (years)	62.8±11.2	64.8±7.8	63.6±9.8	0.187
Asthma duration (years)	5.0±6.0	6.2±6.4	5.5±6.2	0.219
Male sex (%)	64.8	91.4	75.0	<0.001
Smoking (%)				0.009
Never	37.5	16.2	29.4	
Former	54.5	70.6	60.6	
Current	8.0	13.2	10.0	
Pack years (PY)	18.2±22.0	23.8±20.1	20.3±21.4	0.088
BMI (kg/m^2)	25.0±3.2	25.6±3.2	25.2±3.2	0.262
Family history of asthma (%)	19.2	27.3	22.4	0.22
Underlying disease (%)				
Allergic rhinitis	49.5	52.2	50.6	0.732
CRS				

CRSsNP	35.5	43.5	38.6	0.311
CRSwNP	7.3	5.8	6.7	0.871
Diabetes mellitus	20.9	27.5	23.5	0.309
Hypertension	36.4	58.0	44.7	0.005
Atopy (%)	41.0	45.7	43.7	0.561
Blood Eosinophils (/mm^3)	228 (209-462)	201 (110-380)	220 (110-1120)	0.207
Serum total IgE (kU/L)	203 (69-526)	282 (153-999)	226.7 (61.6-744)	0.014
Serum total SE-IgE titer (kU/L)	0.17 (0-0.73)	0.74 (0.06-1.95)	0.29 (0-1.14)	0.087
SE sensitization (%)	31.1	51.4	68.5	0.219
GINA Step 5 (%)	17.0	33.3	23.2	0.011
The use of biologics (%)	3.6	14.5	7.7	0.008
OCS continued use (%)	5.4	8.7	6.6	0.381
Recent AE (%)	23.2	36.2	28.2	0.059
FeNO (ppb)	35.0±28.8	31.4±20.9	33.2±25.1	0.467
Pre-BD FEV1% pred. (%)	79.1±18.6	72.1±19.4	76.4±19.1	0.017
Pre-BD FEV1/FVC ratio	0.69±0.12	0.65±0.12	0.67±0.12	0.024

Notes. SA, *Staphylococcus aureus*; SE, Staphylococcal enterotoxin; BMI, body mass index; AE, acute exacerbation; OCS, oral corticosteroid; GINA, Global Initiative for Asthma; FeNO, fractional exhaled nitric oxide; BD, bronchodilator; FEV1, forced

expiratory volume in 1st second; FVC, forced vital capacity

Data are presented as mean ± standard deviation, median (interquartile range), or percentage.

Table 10. Baseline characteristics of the study population according to SE sensitization

Variables	**SE negative group (N=89)**	**SE positive group (N=92)**	**Total (N=181)**	***P value***
Proportion (%)	49.2	50.8	100	
Age (years)	68.6±10.8	69.1±10.4	68.9±10.6	0.783
Asthma onset age (years)	63.5±10.0	63.6±9.7	63.6±9.8	0.941
Asthma duration (years)	5.1±5.8	5.8±6.6	5.5±6.2	0.449
Male sex (%)	74.2	75.0	75.0	0.896
Smoking (%)				0.616
Never	29.2	29.7	29.4	
Former	62.9	58.2	60.6	
Current	7.9	12.1	10.0	
Pack years (PY)	18.8±18.4	21.7±24.0	20.3±21.4	0.367
BMI (kg/m^2)	25.6±3.1	24.9±3.2	25.2±3.2	0.167
Family history of asthma (%)	22.6	22.1	22.4	0.934
Underlying disease (%)				
Allergic rhinitis	49.4	51.7	50.6	0.767
CRS				

CRSsNP	39.8	37.4	38.6	0.824
CRSwNP	5.7	7.7	6.7	0.632
Diabetes mellitus	29.6	17.6	23.5	0.059
Hypertension	48.9	40.7	44.7	0.27
Atopy (%)	36.0	51.1	43.7	0.04
Blood Eosinophils (/mm^3)	210 (110-429)	246 (109-464)	220 (110-1120)	0.326
Serum total IgE (kU/L)	120 (46-222)	654 (245-1166)	226.7 (61.6-744)	<0.001
Serum total SE-IgE titer (kU/L)	0 (0-0.15)	1.26 (0.73-3.34)	0.29 (0-1.14)	<0.001
SA nasal colonization (%)	31.5	44.6	38.1	0.07
GINA Step 5 (%)	16.9	29.4	23.2	0.047
The use of biologics (%)	3.4	12.0	7.7	0.031
OCS continued use (%)	4.5	8.7	6.6	0.256
Recent AE (%)	29.2	27.2	28.2	0.76
FeNO (ppb)	30.1±21.5	34.6±28.9	32.5±25.7	0.342
Pre-BD FEV1% pred. (%)	76.9±18.8	76.0±19.5	76.4±19.1	0.774
Pre-BD FEV1/FVC ratio	0.69±0.12	0.66±0.12	0.67±0.12	0.109

Notes. SA, *Staphylococcus aureus*; SE, Staphylococcal enterotoxin; BMI, body mass index; CRSsNP; chronic rhinosinusitis without nasal polyp; CRSwNP, chronic rhinosinusitis with nasal polyp; AE, acute exacerbation; OCS, oral corticosteroid; GINA, Global

Initiative for Asthma; FeNO, fractional exhaled nitric oxide; BD, bronchodilator; FEV1, forced expiratory volume in 1st second; FVC, forced vital capacity

Data are presented as mean ± standard deviation, median (interquartile range), or percentage.

Table 11. Clinical characteristics of the study population depending on intranasal *S.aureus* colonization and SE sensitization

Variables	Group A	Group B	Group C	Group D	*P-value (A vs. B)*	*P-value (A vs. C)*	*P-value (A vs. D)*
	SA/SE (-/-) (N=61)	SA/SE (+/-) (N=28)	SA/SE (-/+) (N=73)	SA/SE(+/+) (N=51)			
Age (years)	67.3±12.0	71.6±6.8	67.9±11.8	70.6±8.4	0.075	0.784	0.129
Asthma onset age (years)	61.9±10.8	67.1±7.0	64.0±10.9	63.2±8.1	0.022	0.318	0.494
Asthma duration (years)	5.4±6.6	4.5±3.7	4.6±5.3	7.3±7.6	0.529	0.49	0.173
Male sex (%)	65.6	92.9	60.8	92.7	0.006	0.6	0.002
Smoking (%)					0.057	0.332	0.078
Former	59.0	71.4	49.0	70.0	0.26	0.29	0.263
Current	4.9	14.3	11.8	12.5	0.036	0.732	0.044
Pack years (PY)	16.9±18.5	23.1±17.6	19.7±25.6	24.3±21.8	0.143	0.504	0.071
BMI (kg/m^2)	25.3±3.1	26.1±3.2	24.7±3.3	25.2±3.2	0.29	0.27	0.868
Family history of asthma (%)	21.1	25.9	17.0	28.2	0.618	0.604	0.42
Underlying disease (%)							
Allergic rhinitis	45.8	57.1	54.0	48.8	0.321	0.391	0.766
CRS					0.168	0.896	0.863

CRSsNP	35.0	50.0	36.0	39.0	0.303	0.99	0.627
CRSwNP	8.3	0	6.0	9.8	0.159	0.648	0.721
Diabetes mellitus	25.0	39.3	16.0	19.5	0.171	0.248	0.518
Hypertension	38.3	71.4	34.0	48.8	0.004	0.638	0.297

Notes. SA, *Staphylococcus aureus* nasal colonization; SE, Staphylococcal enterotoxin sensitization; BMI, body mass index

Data are presented as mean ± standard deviation, median (interquartile range), or percentage.

Table 12. Clinical characteristics of the study population depending on s.aureus nasal colonization and SE sensitization

Variables	Group A	Group B	Group C	Group D	*P-value (A vs. B)*	*P-value (A vs. C)*	*P-value (A vs. D)*
	SA/SE (-/-) (N=61)	SA/SE (+/-) (N=28)	SA/SE (-/+) (N=73)	SA/SE(+/+) (N=51)			
Serum total SE-IgE titer (kU/L)	0.01 (0-0.16)	0 (0-0.14)	0.97 (0.55-2.79)	1.78 (0.89-3.5)	0.816	<0.001	<0.001
Atopy (%)	37.7	32.1	47.1	56.1	0.612	0.318	0.067
Blood eosinophils (/mm^3)	217 (108-462)	180 (110-250)	250 (100-470)	220 (120-440)	0.048	0.915	0.958
Serum total IgE (kU/L)	110 (41-228)	154 (61-210)	533 (227-1016)	754 (432-1421)	0.678	<0.001	<0.001
Recent AE (%)	24.6	39.3	21.6	34.2	0.157	0.706	0.294
OCS continued use (%)	3.3	7.1	7.8	9.8	0.414	0.285	0.173
The use of biologics (%)	1.6	7.1	5.9	19.5	0.182	0.228	0.002
GINA Step 5 (%)	13.1	25.0	21.6	39.0	0.164	0.235	0.002
FeNO at baseline (ppb)	30.3±21.8	29.0±13.4	40.4±35.0	33.2±24.9	0.793	0.206	0.642
Pre-BD FEV1% pred. at baseline	80.1±18.8	69.9±17.0	77.9±18.4	73.7±20.9	0.017	0.541	0.113
Pre-BD FEV1/FVC at baseline	0.7±0.12	0.65±0.13	0.67±0.11	0.64±0.12	0.048	0.107	0.017

Notes. SA, *Staphylococcus aureus* nasal colonization; SE, Staphylococcal enterotoxin; AE, acute exacerbation; OCS, oral corticosteroid; GINA, Global Initiative for Asthma; FeNO, fractional exhaled nitric oxide; BD, bronchodilator; FEV1, forced expiratory volume in 1st second; FVC, forced vital capacity

Data are presented as mean±standard deviation, median (interquartile range), or percentage.

Table 13. Associated factors for *S.aureus* nasal colonization and SE sensitization

Vs. Group A	Group B	Group C	Group D	*P-value (A vs. B)*	*P-value (A vs. C)*	*P-value (A vs. D)*
	Adjusted OR (CI)	Adjusted OR (CI)	Adjusted OR (CI)			
Log-eosinophils	0.8 (0.48-1.34)	1.12 (0.76-1.65)	1.3 (0.82-2.07)	0.406	0.553	0.266
Log-total IgE	1.01 (0.64-1.59)	3.29 (1.99-5.44)	6.53 (3.01-14.14)	0.974	<0.001	<0.001
Recent AE	1.98 (0.68-5.77)	0.86 (0.34-2.19)	1.57 (0.6-4.12)	0.213	0.758	0.359
The use of biologics	1.41 (0.09-21.35)	3.77 (0.31-45.76)	14.42 (1.54-134.7)	0.803	0.297	0.019
GINA Step 5	0.99 (0.26-3.77)	1.65 (0.57-4.75)	3.52 (1.23-10.07)	0.995	0.353	0.019
Log-FeNO at baseline	1.87 (0.73-4.8)	1.17 (0.61-2.27)	1.62 (0.66-3.95)	0.694	0.195	0.291
Log-FeNO at follow-up	4.59 (1.6-13.19)	1.4 (0.69-2.83)	2.37 (1.1-5.47)	0.005	0.352	0.042
FEV1<80% at baseline	1.31 (0.44-3.87)	0.86 (0.4-1.84)	1.14 (0.47-2.75)	0.622	0.693	0.779

FEV1<80% at follow-up	0.75 (0.26-2.17)	0.79 (0.34-1.85)	1.52 (0.6-3.88)	0.591	0.583	0.38
FEV1/FVC<0.7 at baseline	1.18 (0.4-3.51)	1.92 (0.8-4.63)	1.65 (0.64-4.28)	0.76	0.145	0.304
FEV1/FVC<0.7 at follow-up	1.7 (0.58-4.97)	3.6 (1.45-8.97)	3.24 (1.21-8.65)	0.333	0.006	0.019

Notes. SA, *Staphylococcus aureus* nasal colonization; SE, Staphylococcal enterotoxin; AE, acute exacerbation; OCS, oral corticosteroid; GINA, Global Initiative for Asthma; FeNO, fractional exhaled nitric oxide; BD, bronchodilator; FEV1, forced expiratory volume in 1st second; FVC, forced vital capacity

Adjusted for age, sex, smoking, and BMI

Figures

Figure 1. Changes of FEV1/FVC ratio over the 2-year follow-up

FEV1; forced expiratory volume in 1 sec, FVC; forced volume capacity

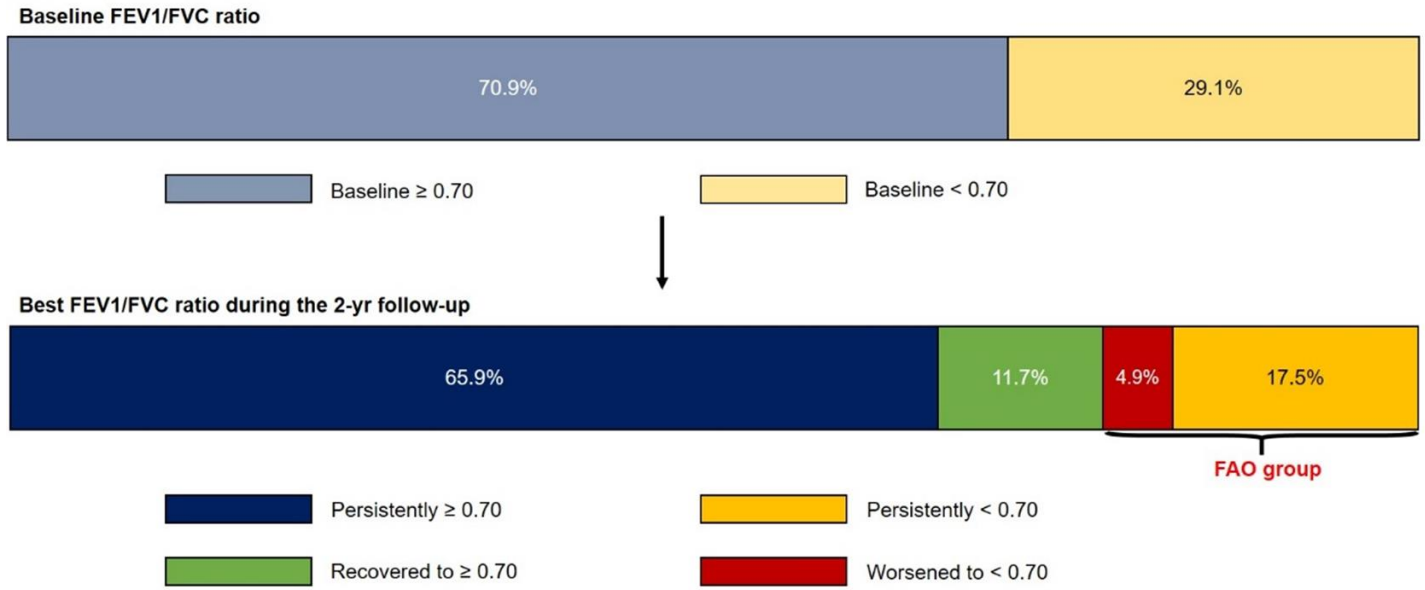


Figure 2. Changes of FEV1 (%) over the 2-year follow-up

FEV1; forced expiratory volume in 1 sec

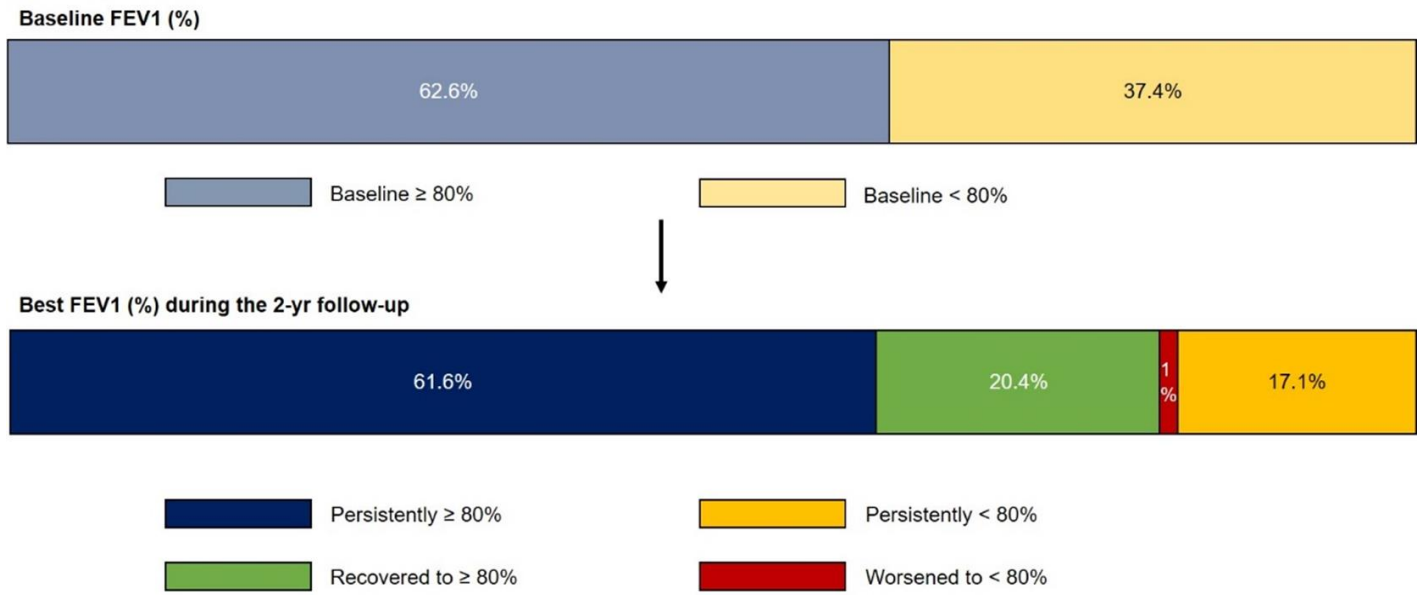


Figure 3. Correlations between the frequency of annual asthma exacerbations and level of SE-IgE depending on the presence of fixed airflow obstruction

(A) Asthma without FAO (n=166), r=0.434 (P<0.001)

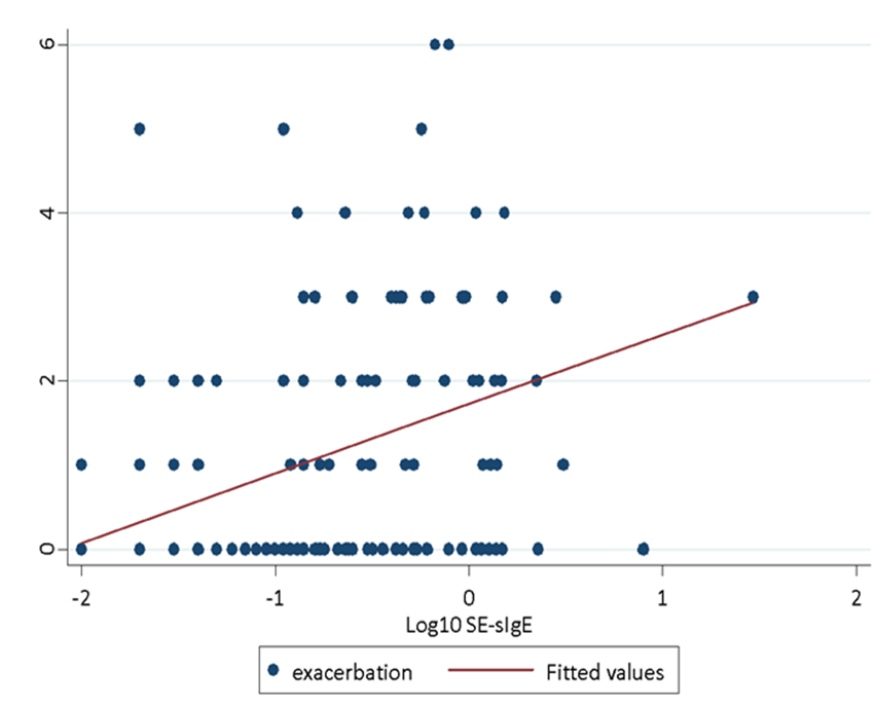


Figure 3. Correlations between the frequency of annual asthma exacerbations and level of SE-IgE depending on the presence of fixed airflow obstruction

(B) Asthma with FAO (n=48), *r*=0.404 (*P*=0.004)

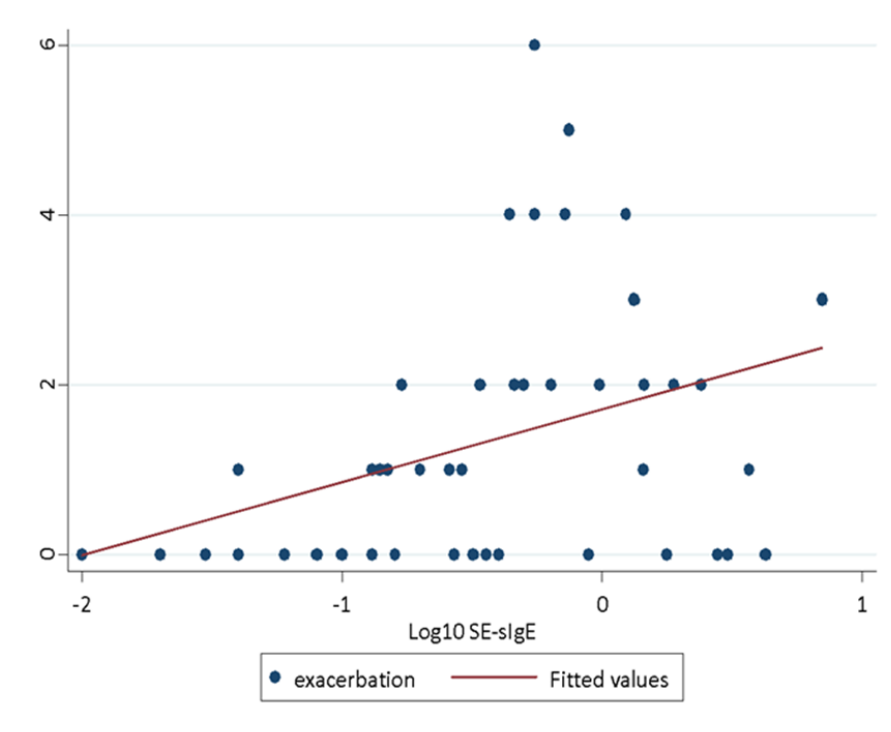


Figure 4. Correlations between the frequency of annual asthma exacerbations and level of SE-IgE depending on the presence of fixed airflow obstruction in never smokers

(A) Asthma (n=147), r=0.421 (P<0.001)

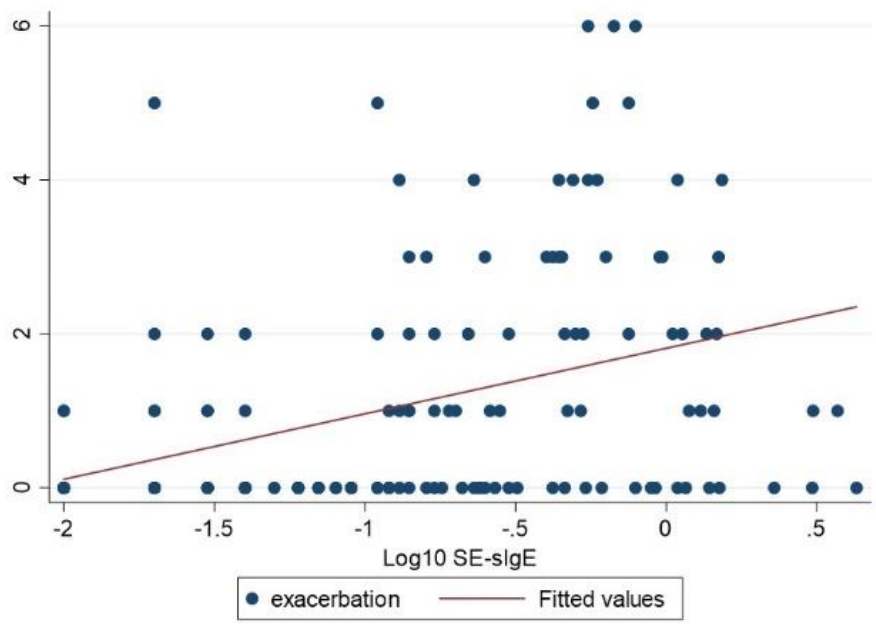


Figure 4. Correlations between the frequency of annual asthma exacerbations and level of SE-IgE depending on the presence of fixed airflow obstruction in never smokers

(B) Asthma without FAO (n=125), r=0.408 (P<0.001)

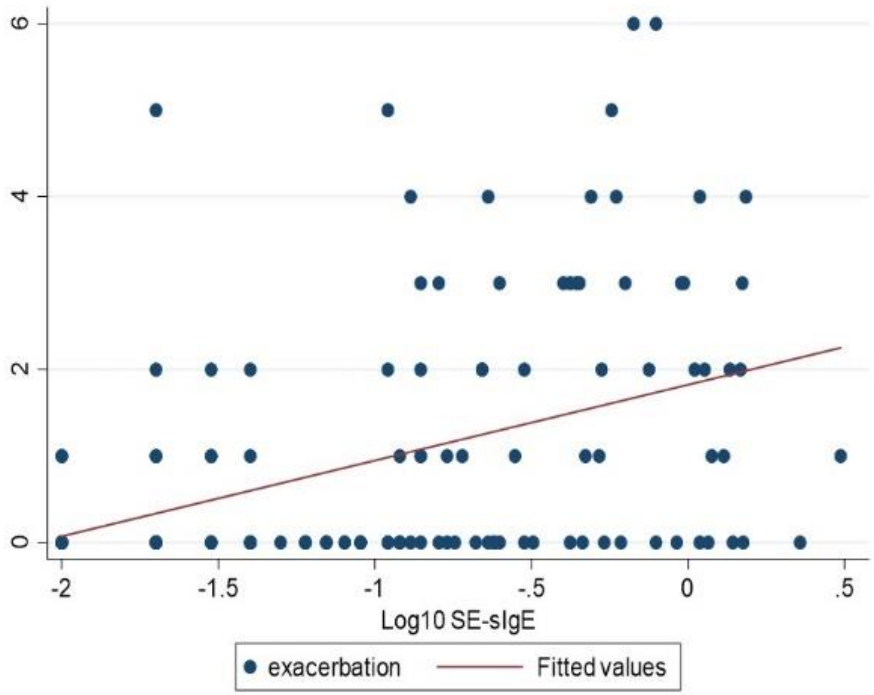


Figure 4. Correlations between the frequency of annual asthma exacerbations and level of SE-IgE depending on the presence of fixed airflow obstruction in never smokers

(C) Asthma with FAO (n=22), *r*=0.34 (*P*=0.122)

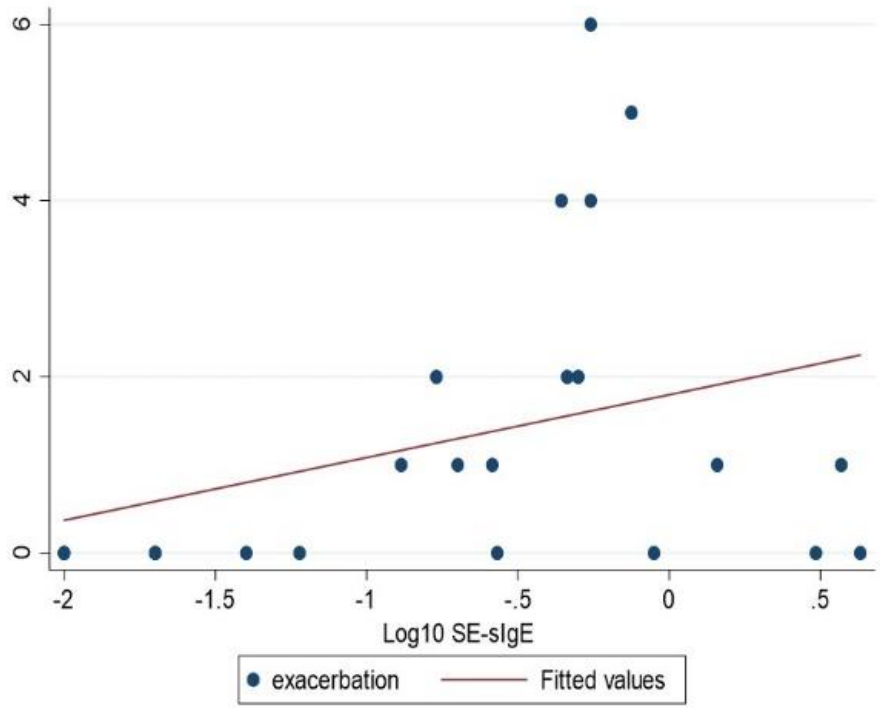


Figure 5. The proportion of the study population according to SA colonization and SE sensitization

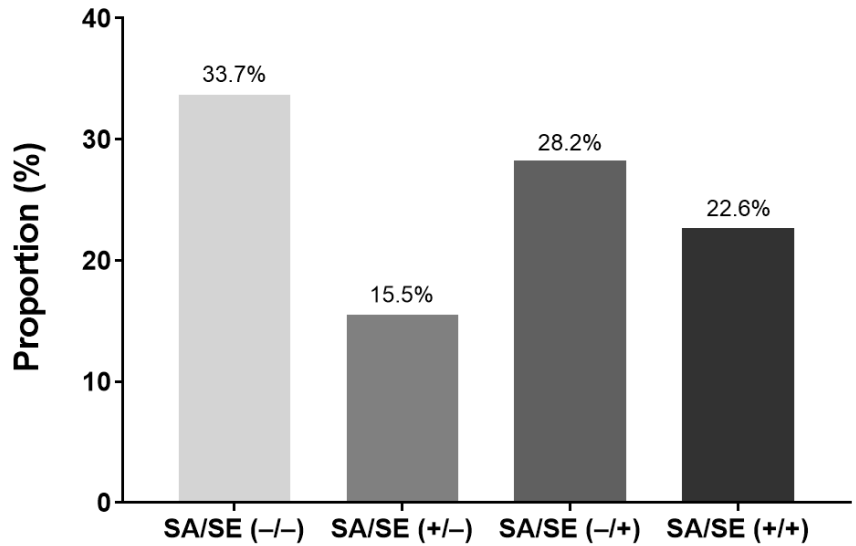
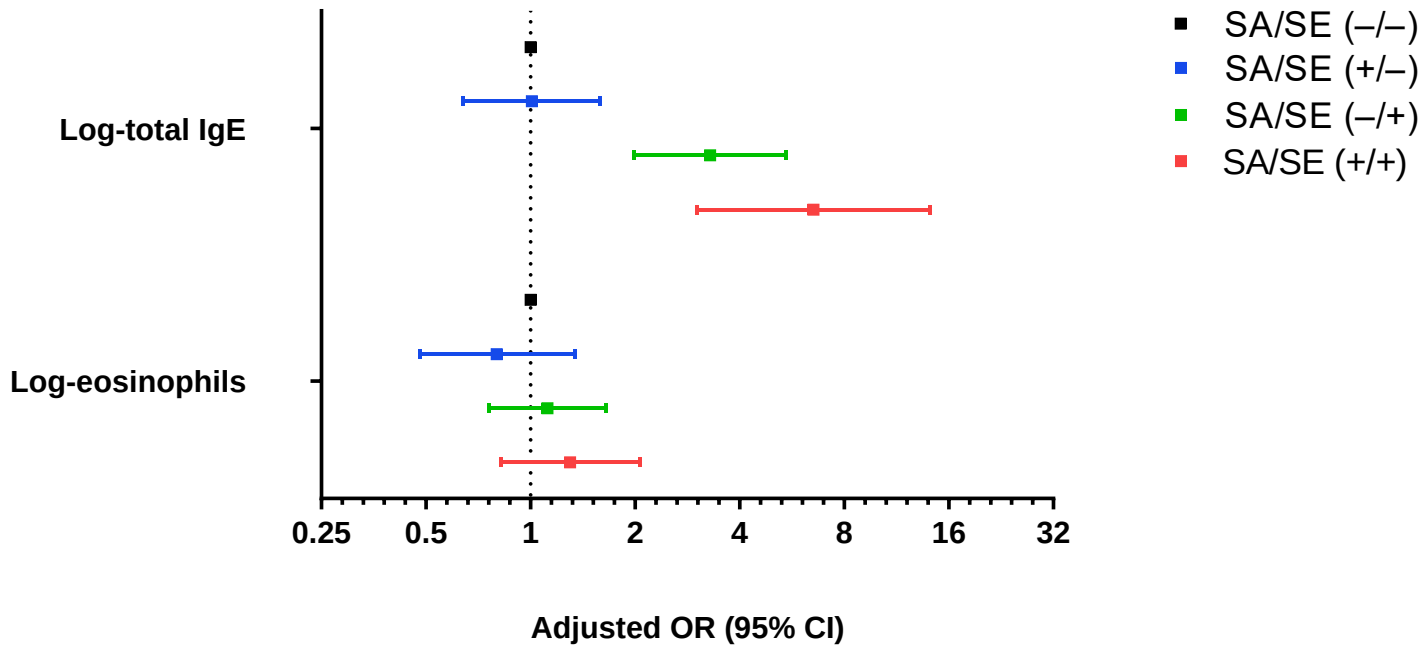


Figure 6. Multivariate logistic regression models for associated factors depending on SA nasal colonization and SE sensitization in late-onset asthmatics

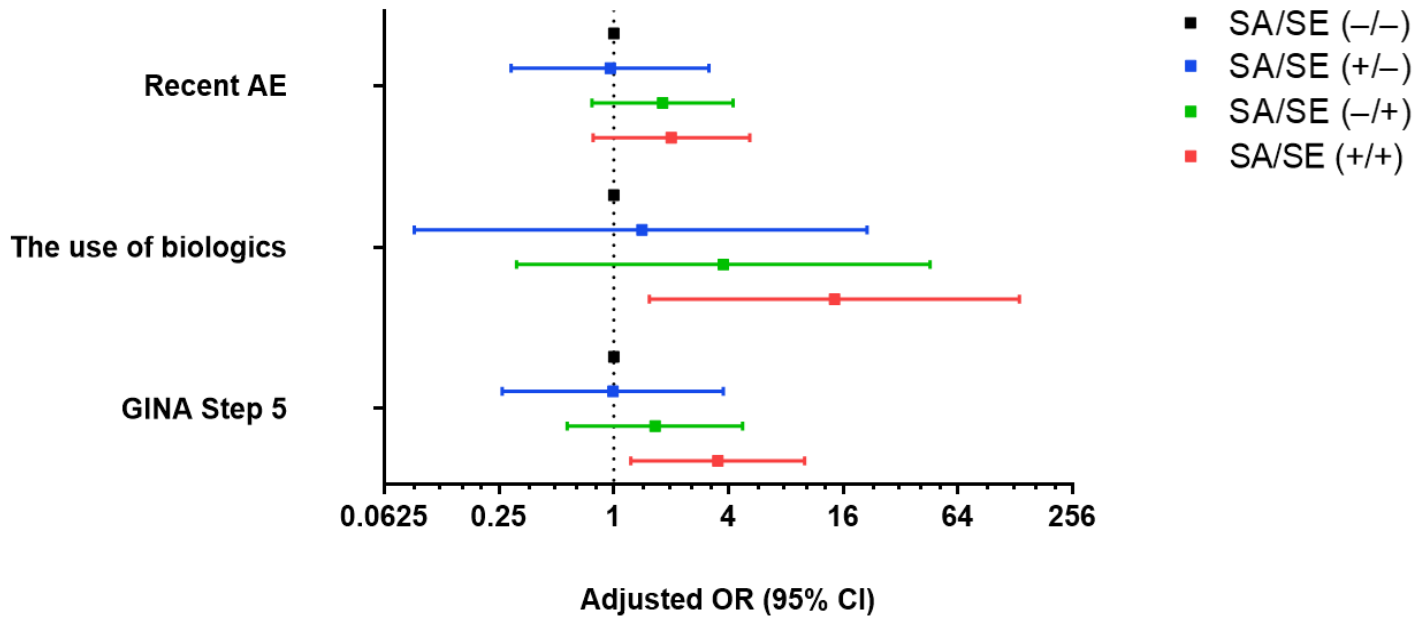
(A) Log-total IgE and Log-eosinophils



Adjusted for age, sex, smoking, and BMI

Figure 6. Multivariate logistic regression models for associated factors depending on SA nasal colonization and SE sensitization in late-onset asthmatics

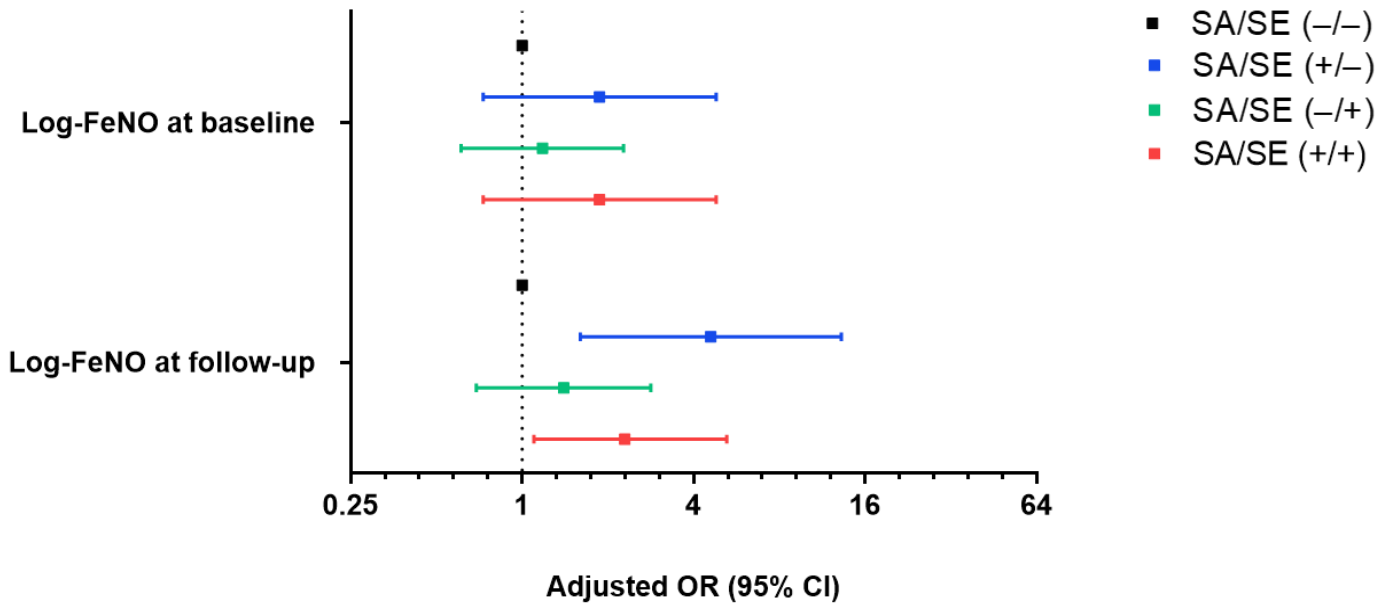
(B) Recent AE, the use of biologics, and GINA step 5



Adjusted for age, sex, smoking, and BMI

Figure 6. Multivariate logistic regression models for associated factors depending on SA nasal colonization and SE sensitization in late-onset asthmatics

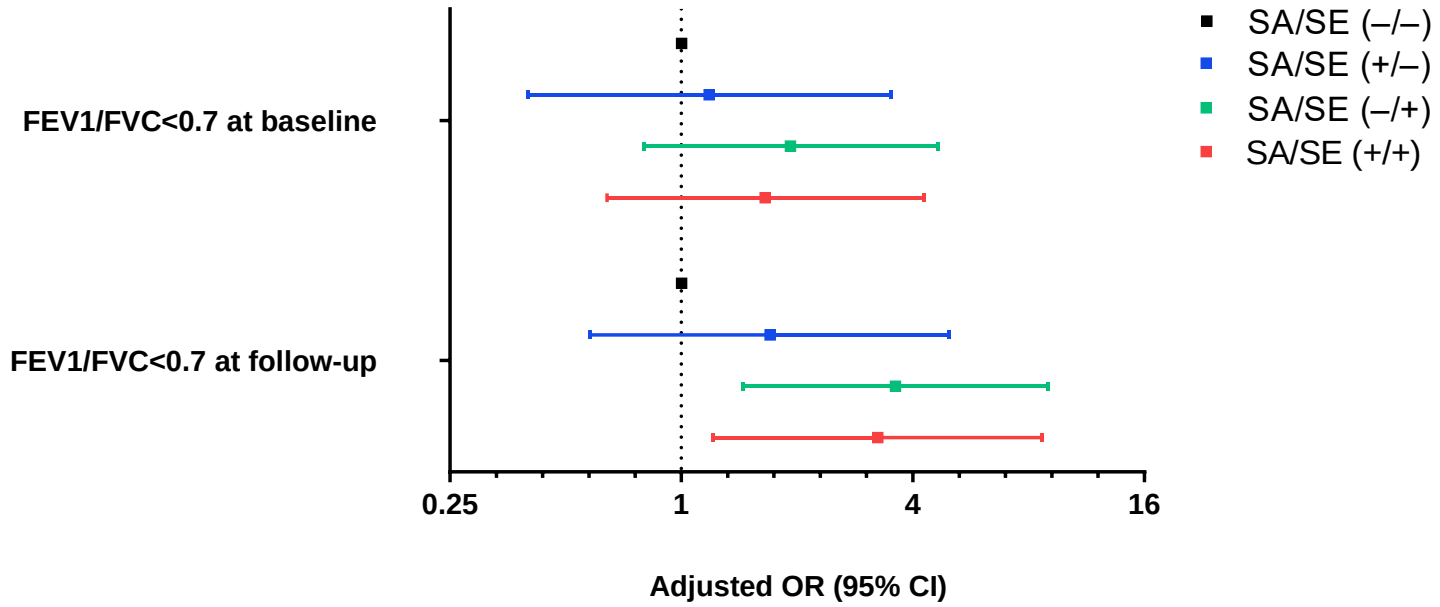
(C) Log-FeNO at baseline and Log-FeNO at follow-up



Adjusted for age, sex, smoking, and BMI

Figure 6. Multivariate logistic regression models for associated factors depending on SA nasal colonization and SE sensitization in late-onset asthmatics

(D) FEV1/FVC ratio<0.7 at baseline and FEV1/FVC ratio<0.7 at follow-up



Adjusted for age, sex, smoking, and BMI

Figure 7. The effects of Spls on decrease in airway epithelial integrity *in vitro*

(A) Immunofluoresence staining for Zo-1 and E-cadherin

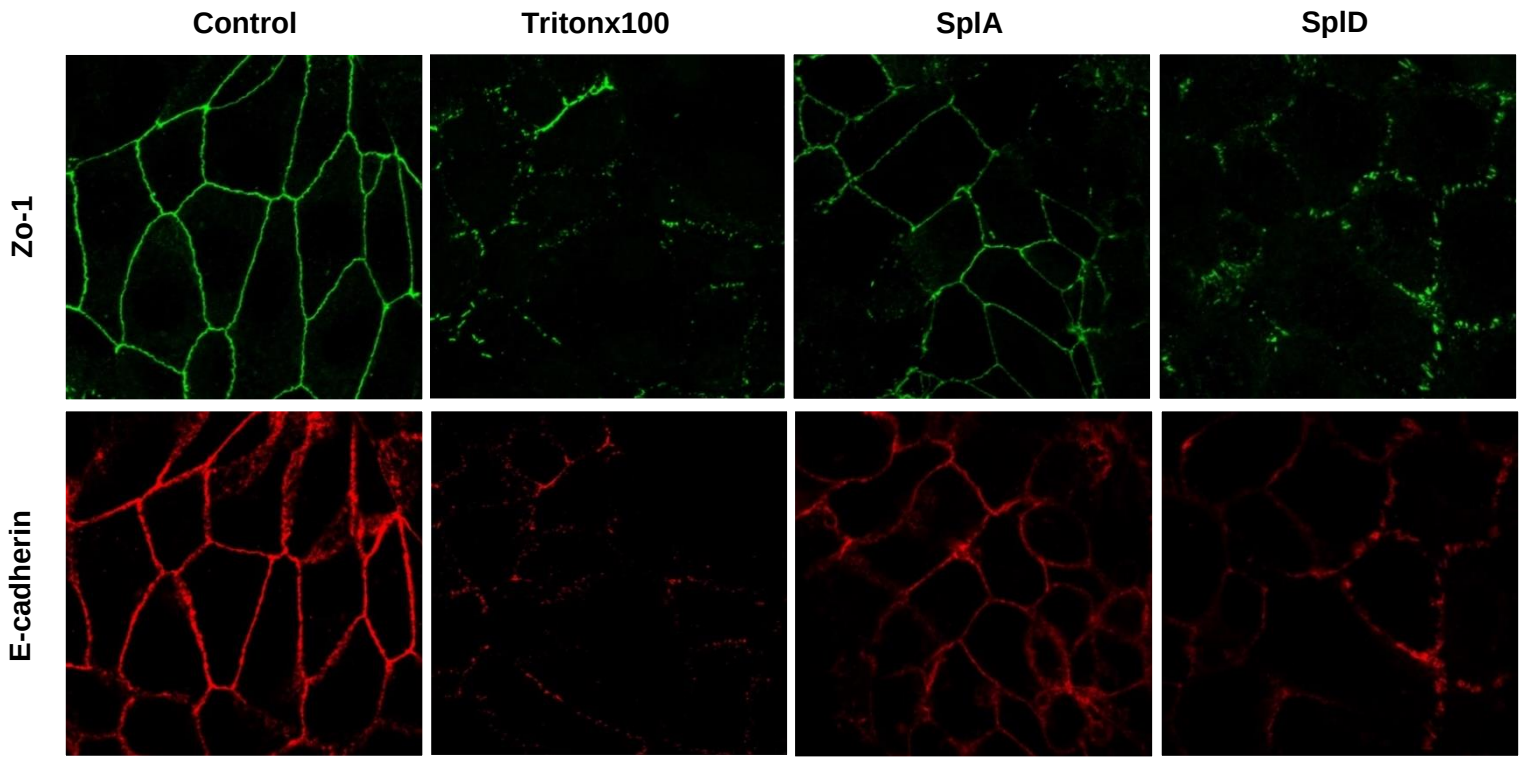


Figure 7. The effects of Spls on decrease in airway epithelial integrity *in vitro*

(B) Western blots

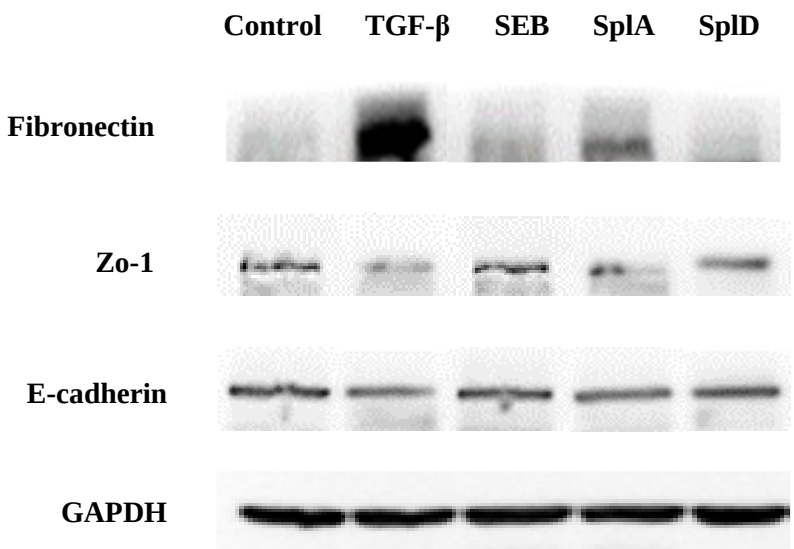


Figure 7. The effects of Spls on decrease in airway epithelial integrity *in vitro*

(C) Western blots – Quantification graph for Fibronectin

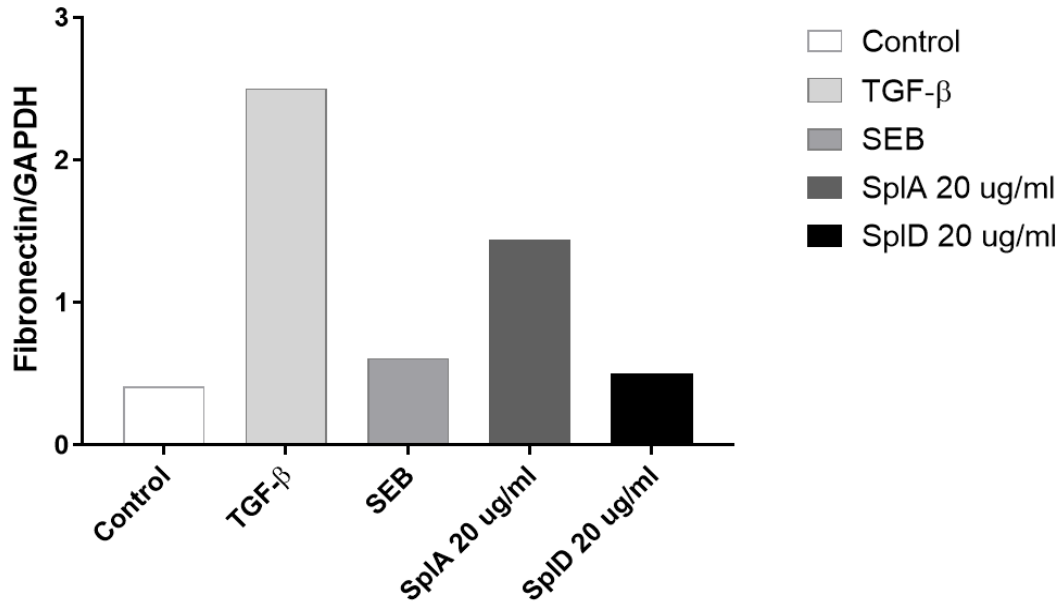


Figure 7. The effects of Spls on decrease in airway epithelial integrity *in vitro*

(D) Western blots - Quantification graph for Zo-1

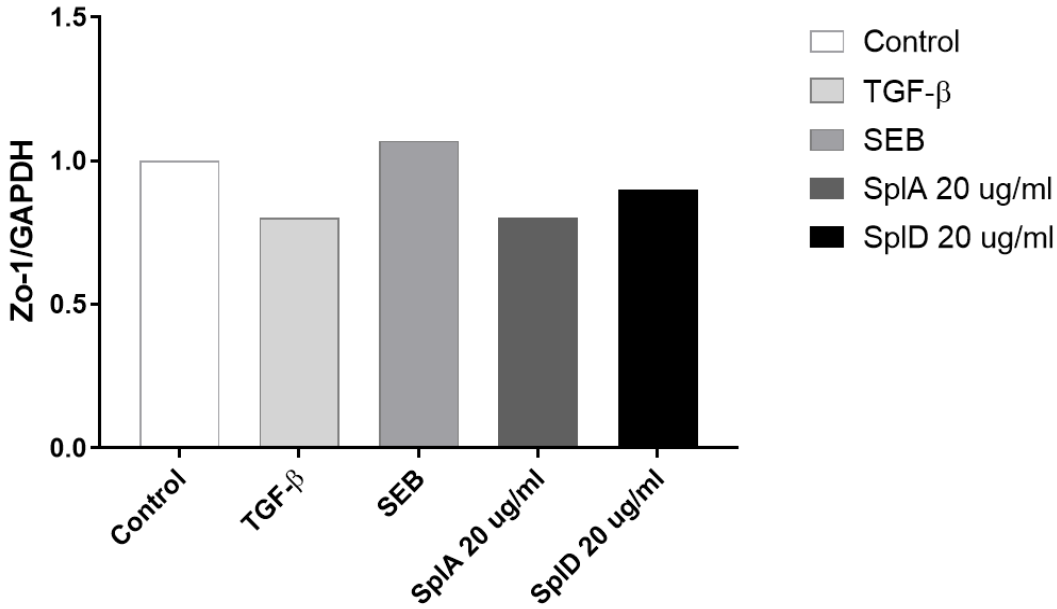


Figure 7. The effects of Spls on decrease in airway epithelial integrity *in vitro*

(E) Western blots - Quantification graph for E-cadherin

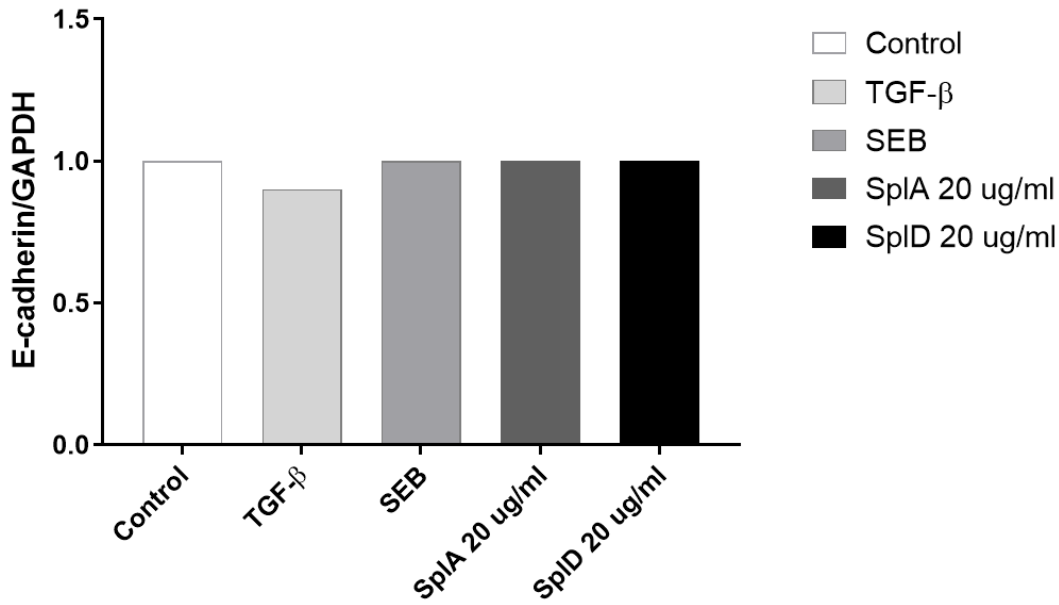


Figure 8. The effects of Spls on airway epithelial cells inflammation *in vitro*

(A) mRNA expression of CST1 in BEAS-2B cells

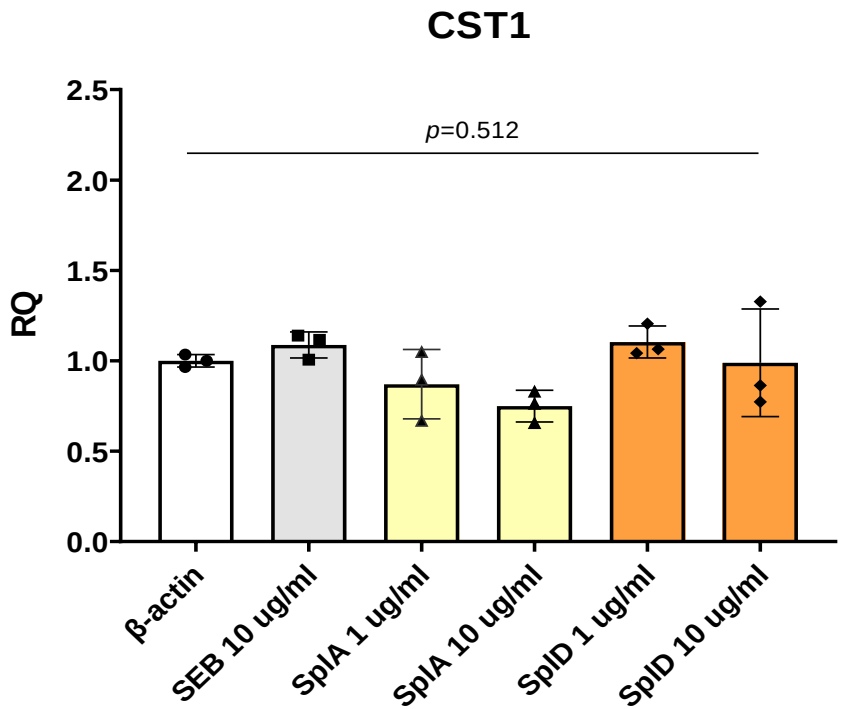


Figure 8. The effects of Spls on airway epithelial cells inflammation *in vitro*
(B) mRNA expression of CST1 in A549 cells

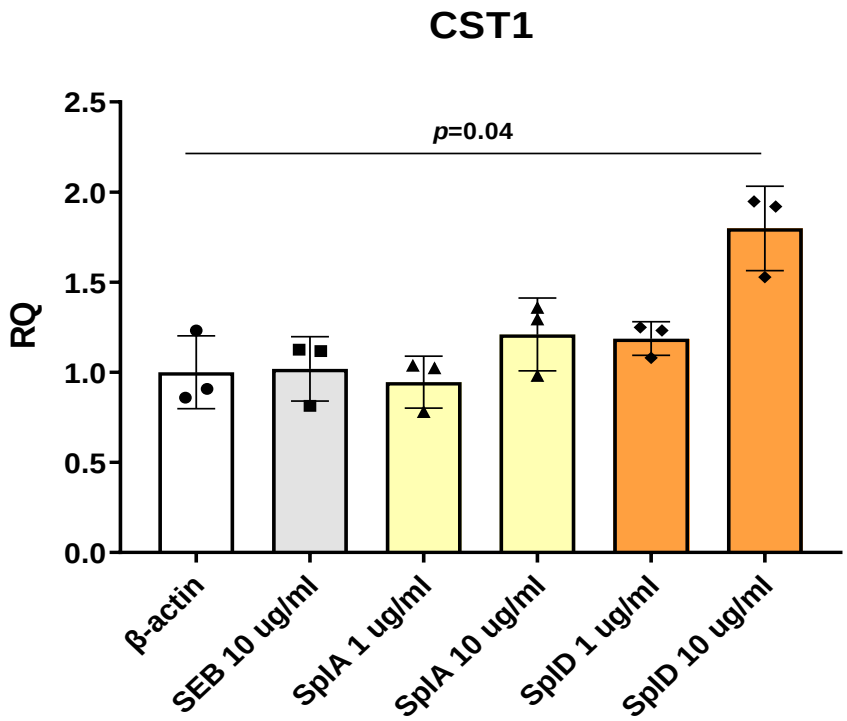


Figure 8. The effects of Spls on airway epithelial cells inflammation *in vitro*
(C) mRNA expression of CCL-11, IL-33, and CST1 in HNEpC

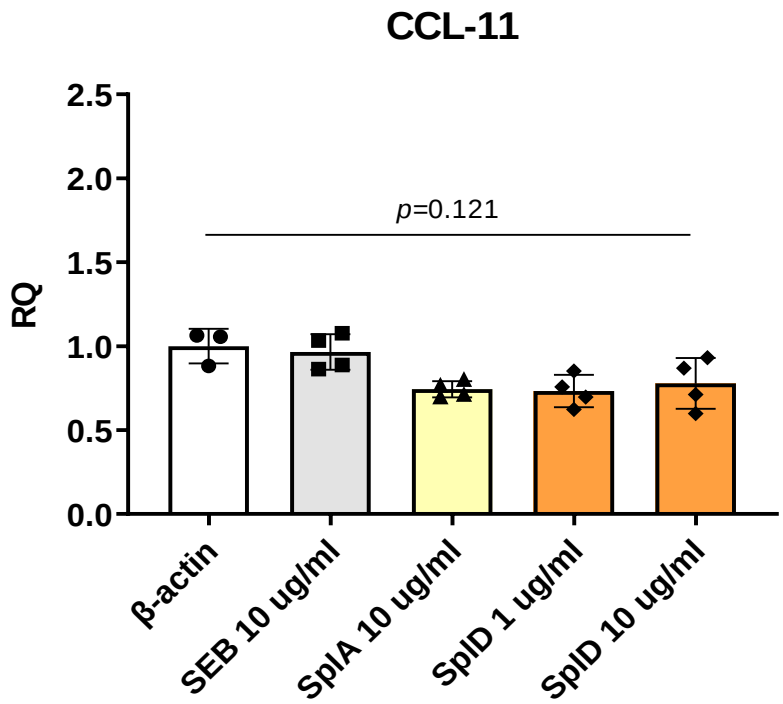


Figure 8. The effects of Spls on airway epithelial cells inflammation *in vitro*

(D) mRNA expression of CCL-11, IL-33, and CST1 in HNEpC

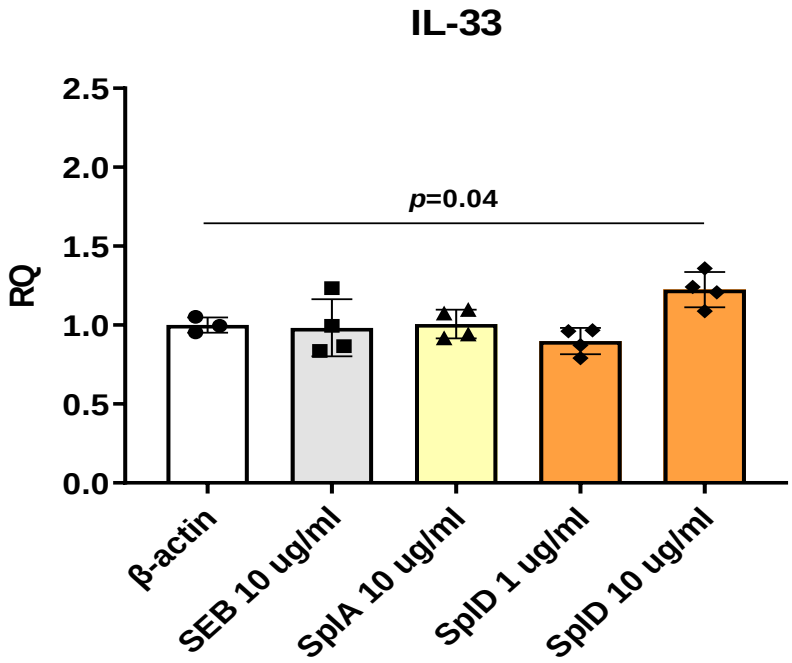
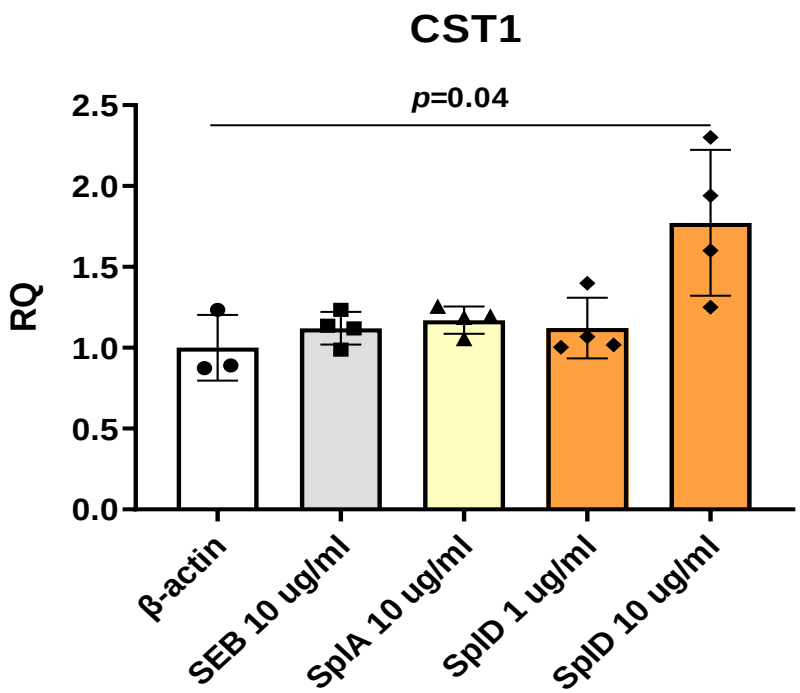


Figure 7. The effects of Spls on airway epithelial cells inflammation *in vitro*
(E) mRNA expression of CCL-11, IL-33, and CST1 in HNEpC



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국문초록

후기 발병 천식 환자에서 포도상 구균과 초항원이 천식 중증도 및 폐기능에 미치는 영향

원 하 경

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서론: 포도상 구균은 주로 사람의 피부와 상기도에서 발견되는 빈번한 상재균이다. 최근 연구에 따르면, 포도상 구균은 천식 및 천식의 중증도와 관련이 있는 것으로 알려져 있다. 그러나 후기 발병 천식에서 SE-IgE의 장기적인 영향과 비강 내 포도상 구균 상재와 장독소 감작에 따른 임상적 연관성에 대한 연구는 거의 없다. 더욱이, 실험적으로 세린 단백질 분해 효소 유사 단백질이 기도 상피에 대한 염증과 상피 무결성에 대한 영향은 아직 연구되지 않았다. 따라서 우리는 장독소와 세린 단백질 분해 효소 유사 단백질을 포함한 포도상 구균이 후기 발병 천식에서 천식 중증도에 기여한다는 가설을 검증하고자 하였다.

방법: 첫번째로, 총 223명의 노인 천식 환자와 89명의 대조군을 분석하였고, 환자의 인구 통계, 만성 비부비동염 병력, 천식 기간, 급성 악화 빈도, 폐기능에 대해 등록 시 평가한 후 2년 동안 전향적으로 추적했다. 혈청 총 IgE 및 장독소 특이 IgE 수준을 기준선에서 측정했다. 두번째로, 전향적 관찰 연구로서 총 181명의 후기 발병 천식 환자에 대해, 등록 시 천식 유병 기간, 급성 악화, 호기산화질소, 초기 폐기능을 포함한 인구 통계학적 요인을 포함하였고, 등록 후 18-24개월 후에 측정한 호기산화질소 및 폐기능을 후향적으로 수집하였다. 비강 내 포도상구균 상재 및 장독소 특이 IgE를 얻기 위해 비강 면봉 및 혈청 샘플을 얻었다. 대상자는 비강내 포도상 구균 상재 및 장독소 감작 상태에 따라 4개의 그룹으로 구분하였다. 세번째로, 세린 단백질 분해 효소 유사 단백질을 기도 상피 세포에 노출시켰을 때, 기도 상피 세포의 변화를 형광염색, 웨스턴 블롯과 메신저 리보핵산 발현으로 확인하였다.

결과: 연구 등록 시, 기류 폐쇄의 비율은 29.1%였다. 기류 폐쇄가 동반된 환자는 남성이 많았고, 흡연 이력, 만성 비부비동염 동반 및 더 높은 수준의 장독소 특이 IgE가 기류 폐쇄가 없는 환자보다 높았다. 다변량 로지스틱 회귀 분석은 기류 폐쇄가 기준선에서 현재 흡연 및 장독소 특이 IgE 감작과 유의하게 연관되어 있음을 보여주었다. 2년 추적 조사 후 초기 장독소 특이 IgE 감작은 지속적으로 고정 기류 폐쇄와 관련이 있었다. 한편, 연간 급성 악화 횟수는 장독소 특이 IgE 수치와 유의한 상

관관계가 있었다. 두번째로, 후기 발병 천식에서 등록 시 비강내 포도상 구균 상재의 유병률은 약 38.1% 였다. 비강 내 포도상 구균 상재 및 장독소 감작에 따라 분류된 4개 그룹 중 두가지 모두 양성인 그룹은 대조군에 비해 남성, 현재 흡연력, 높은 총 IgE, 치료 중증도 및 기류 폐쇄와 관련이 있었다. 다변량 로지스틱 회귀 분석에서 두가지 모두 양성인 그룹은 다른 그룹에 비해 높은 총 IgE, 더 높은 수준의 치료와 18-24개월 측정 시 증가한 호기산화질소 및 고정 기류 폐쇄와 일관된 연관성을 보였다. 세번째로, 세린 단백질 분해 효소 유사 단백질은 BEAS-2B 세포에서 상피 무결성 감소 유발을 형광염색과 웨스턴 블롯에서 확인하였고, A549 세포와 인간 비강 상피 세포에서 Cystatin SN의 증가를 유발하였다.

결론: 장독소 특이 IgE 감작은 노인 천식 환자에서 2년 추적 관찰 후 천식 악화 횟수 및 고정 기류 폐쇄와 유의한 관련이 있었고, 후기 발병 천식에서 비강 내 포도상 구균 상재 및 장독소 감작은 천식 중증도 및 고정 기류 폐쇄와 관련이 있었다. 또한, 세린 단백질 분해 효소 유사 단백질은 실험적으로 상피 무결성을 감소시키고, 기도 염증을 유발하였다. 따라서, 향후 포도상 구균이 천식 중증도와 병태 생리에 미치는 영향에 대해 추가 연구가 필요하겠다.

주요어: 천식, 후기 발병, 포도상 구균, 장독소, 중증도

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