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Original article

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Sensitisation to staphylococcal enterotoxins and asthma severity:

a longitudinal study in the EGEA cohort

Ina Sintobin*¹, Valerie Siroux*², Gabriële Holtappels¹, Christophe Pison^{3,4}, Rachel Nadif^{5,6}, Jean Bousquet J^{5,6,7,8}, Claus Bachert^{1,9}

¹Upper Airways Research Laboratory, Ghent University, Ghent, Belgium

²Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Inserm, CNRS, University Grenoble Alpes, Institute for Advanced Biosciences (IAB), U1209 Joint Research Center, Grenoble, France

³Université Grenoble Alpes, F-38000 Grenoble, France

⁴Clinique Universitaire de Pneumologie, Pôle Thorax et Vaisseaux, CHU de Grenoble; Grenoble, France; Inserm1055, Grenoble, France

⁵Inserm, U1168, VIMA: Aging and chronic diseases. Epidemiological and public health approaches, F-94807, Villejuif, France

⁶Univ Versailles St-Quentin-en-Yvelines, UMR-S 1168, F-78180, Montigny le Bretonneux, France

⁷Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Comprehensive Allergy Center, Department of Dermatology and Allergy, Berlin, Germany

⁸MACVIA-France, Fondation partenariale FMC VIA-LR, Montpellier, France.

⁹Division of ENT diseases, CLINTEC, Karolinska Institute, University of Stockholm, Sweden

* the Authors equally contributed to the manuscript

Corresponding author:

Claus Bachert

Upper Airways Research Laboratory, Ghent University

C Heymanslaan 10, 9000 Gent – Belgium

claus.bachert@ugent.be

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Abstract

Introduction: Evidence is accumulating that *Staphylococcus aureus* plays an important role as disease modifier in upper and lower airway diseases. Sensitisation to *Staphylococcus aureus* enterotoxins (SEs) was associated with an increased risk for severe asthma in previous cross-sectional studies, but evidences from longitudinal studies are lacking. We aimed to assess associations between SE-sensitisation and the subsequent risk for asthma severity and exacerbations.

Methods: This is a nested case-control study from the 20-year Epidemiological study on the Genetics and Environment of Asthma-EGEA cohort, including 225 adults (75 without asthma, 76 with mild and 75 with severe asthma) in EGEA2 (2003-2007). For 173 of these individuals, SE-sensitisation was measured on samples collected 11 years earlier (EGEA1). Cross-sectional associations were conducted for EGEA1 and EGEA2. Longitudinal analyses estimated the association between SE-sensitisation in EGEA1 and the risk of severe asthma and asthma exacerbations assessed in the follow-up. Models were adjusted on gender, age, smoking, parental asthma/allergy and skin prick test to house dust mite.

Results: SE-sensitisation varied between 39% in controls to 58% and 76% in mild and severe asthma in EGEA1. An adjusted cross-sectional association showed that SE-sensitisation was associated with an increased risk for severe, but not for mild asthma. SE-sensitisation in EGEA1 was associated with severe asthma (adjusted-OR 2.69, 95%CI [1.18-6.15]) and asthma exacerbations (adjusted-OR 4.59, 95%CI [1.40-15.07]) assessed 10 to 20 years later.

Conclusion: For the first time, this study shows that being sensitised to SEs is associated with an increased subsequent risk of severe asthma and asthma exacerbations.

Key words:

Severe asthma, asthma exacerbations, *Staphylococcus aureus* enterotoxins, IgE

Summary

Sensitisation to *Staphylococcus aureus* enterotoxins (SEs) is increased in asthmatic subjects over controls; as shown for the first time, SE-IgE serum antibodies are associated with an increased subsequent risk of severe asthma and asthma exacerbations.

Abbreviations

CRS	Chronic rhinosinusitis
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SEs	<i>Staphylococcus aureus</i> enterotoxins
SE-IgE	IgE to <i>S. aureus</i> enterotoxins
EGEA	Epidemiological study of Genetics and Environment of Asthma
SPT	Skin prick test
GINA	Global Initiative for Asthma
ICS	Inhaled corticosteroids
HDM	House dust mite

Introduction

Asthma is one of the most common chronic diseases in the world; it is estimated that around 300 million people currently have asthma¹. Trials demonstrated that guideline-defined asthma control can be attained and maintained for the majority of patients. However, asthma control is not achieved in many patients, including some patients with severe asthma despite optimal anti-inflammatory treatment². Among patients with severe adult-onset asthma, comorbid conditions, including chronic rhinosinusitis (CRS) and specifically CRS with nasal polyposis, are frequent³⁻⁵.

Staphylococcus aureus (*S. aureus*), a common human pathogen, is frequently part of the normal microflora found in the nose and on the skin⁶. *S. aureus* is responsible for an array of diseases and evidence is accumulating that it plays an important role as disease modifier in upper and lower airway diseases⁷. Enterotoxins from *Staphylococcus aureus* (*S. aureus* enterotoxins, SEs) may induce polyclonal IgE formation, associated with allergic multi-morbidity in adolescents⁸. The mechanisms behind the association of *S. aureus* and IgE (in tissue and in serum) have been summarized in a recent review⁹. Shortly, SEs can act as superantigens and are able to activate T-cells by binding to the variable β -chain of the T-cell receptors, which allows the polyclonal activation of a substantial number of T-cells. Furthermore, SEs may activate B-cells, eosinophils, epithelial cells and others, resulting in a cytokine storm locally in the tissue and the generation of a strong inflammatory response⁹.

There is an increasing body of epidemiological literature on the association between SE-sensitisation and asthma-related outcomes. Birth cohorts suggested an association between SE-sensitisation and an increased risk for persistent wheezing and asthma^{10,11}. Moreover, *S. aureus* nasal colonization was associated with increased risk of asthma prevalence, symptoms and exacerbations in children and young adults¹². SE-sensitisation was associated with an increased risk of asthma severity, hospitalisations, oral corticosteroid use and low lung function¹³. Furthermore, specific IgE to SEs in serum (which is considered as a marker for contact with *S. aureus* and its enterotoxins) is associated with asthma Europe-wide¹⁴, and is specifically associated with late-onset asthma in the elderly, particularly severe eosinophilic asthma with chronic rhinosinusitis comorbidity¹⁵. Nevertheless, the association between SE-sensitisation and an increased risk for asthma, especially severe asthma, was suggested only by cross-sectional studies¹³⁻¹⁵ and evidences from longitudinal studies are lacking.

We aimed to analyse whether sensitisation to SEs is associated with severe asthma and asthma exacerbations later in life, by taking advantage of the Epidemiological study of the Genetics and Environment of Asthma (EGEA), a cohort based on an initial group of asthma cases, first-degree relatives of cases, and controls followed-up over 20 years¹⁶⁻¹⁸.

Materials and methods

Study design

This is a nested case-control study from the EGEA cohort, a 20-year follow-up study on asthma cases, their family members and controls.

Population

The EGEA1 cohort (1991-1995) is composed of a group of 2,047 participants, including 348 cases with asthma enrolled in chest clinics, 1,244 first-degree relatives and 415 controls^{16, 17}. Participants were recruited through a self-completed questionnaire and underwent a complete examination, including detailed questionnaires, total serum IgE, lung function tests (spirometry and methacholine challenge test), and skin prick tests (SPT) to 11 aero-allergens (cat, *Dermatophagoides pteronyssinus*, *Blattella germanica*, olive, birch, *Parietaria judaica*, timothy grass, ragweed pollen, *Aspergillus*, *Cladosporium herbarum*, *Alternaria tenuis*). Positive SPT result was defined by a mean wheal diameter of 3 mm or more¹⁸⁻²⁰. About 11 years later, 1,601 participants (77.1% of the original cohort + 58 new family members) took part to the first follow-up (EGEA2) including a detailed questionnaire and a complete examination, as performed in EGEA1²¹. The third survey (EGEA3, 2011-2013) consisted of a self-completed questionnaire that was sent to the participants and achieved a high response rate (79.2%)²². Ethical approval was obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris). Written informed consent was signed by all participants.

For the current study, 225 participants were selected at EGEA2, including all subjects with moderate and severe asthma (n=74), 76 subjects with mild asthma (randomly selected) and 75 control participants without asthma ever, no atopic dermatitis and no nasal polyps (defined by a negative answer to the following questions “Have you ever had atopic dermatitis?” and “Have you ever had a surgery for nasal polyps?”). Both the mild asthma group and the control group were matched on age and sex with the moderate-severe asthma group (at the group level). Specific IgE to SEs were measured in EGEA2 serum samples (see below) for these 225 selected participants; in EGEA1, serum samples for 173 of these participants were available for SE-sensitisation measurement. Of the 225 selected participants at EGEA2, 203 participants completed the questionnaire at EGEA3.

Asthma, asthma severity and asthma exacerbation

The definition of ever asthma was based on a positive answer to either « Have you ever had attacks of breathlessness at rest with wheezing?» or “Have you ever had asthma attacks?” or being recruited as an asthma case.²³

Asthma severity (mild vs. moderate/severe) was defined at EGEA1 and EGEA2 with the available data in the study, following the principle of the GINA2002 guidelines, by combining the report of at least one asthma attack in the past 12 months, a clinical score and the type of asthma treatment used in the past 12 months (none, treatment not including inhaled corticosteroids (ICS), treatment including ICS). The clinical score was defined on the basis of asthma attack frequency (from 0 to 3), level of symptoms between asthma attacks (from 0 to 3), and hospitalization for asthma over the past 12 months (yes/no); the clinical score thus ranged from 0 to 7. Subjects were identified with “*moderate-to-severe asthma*” if they reported at least one asthma attack in the past 12 months and either 1) use of ICS treatment in the past 12 months (whatever the asthma score), or 2) an asthma score ≥ 3 (whatever the asthma treatment) or 3) an asthma score = 2 and use of asthma treatment other than ICS. The other subjects were classified as « past or mild » asthma. This asthma severity classification has previously been used^{24, 25}.

The presence of at least one asthma exacerbation over the past 12 months was defined at EGEA2 and EGEA3 by the report of a hospitalisation for asthma attack or the use of oral glucocorticosteroids to help breathing anytime in the past 12 months.

Laboratory measurements

Total serum IgE and SE-specific IgE (SE-mix including SE-A, SE-C and toxic shock syndrome toxin (TSST)) were measured with the ImmunoCAP 100 system (ThermoFisher, Uppsala, Sweden). The lower limit of SE-mix detection was set as 0.10 kU/L as in former studies^{14, 26}. The presence of SE-specific IgE in the serum is considered as a marker for exposure and an immune reaction to *S. aureus* and SEs.

Statistical analysis

Cross-sectional analyses were conducted for EGEA1 and EGEA2. To assess the risk of severe asthma associated with SE-sensitisation, multinomial logistic models were applied when considering the 3-class asthma severity outcome (controls without asthma was the reference category); in analyses restricted to participants with asthma, binomial logistic models were conducted (moderate-severe asthma vs. mild asthma). Models were adjusted for gender, age (year), smoking habit (never, ex or current smoking), parental asthma or allergy (≥ 1 parent with asthma or allergy vs. none) and skin prick test for house dust mite (negative/positive).

Longitudinal analyses were conducted to assess the association between sensitisation to *S. aureus* enterotoxins in EGEA1 and the subsequent risk of 1) severe asthma at EGEA2 and 2) asthma exacerbations reported either at EGEA2 or EGEA3.

A sensitivity analysis considering the levels of SE specific IgE was conducted (<0.1kU/L; 0.1 – 0.35 kU/L; >0.35 KU/L).

Results

Description of the study population

A description of the population in EGEA1 (n=173) and EGEA2 (n=225) is provided in Table 1. By design, the number of participants in each group (control, past or mild asthma or moderate-severe asthma) was well balanced and there was no significant difference in mean age or gender between the 3 groups. No difference was observed for smoking between the 3 groups. In EGEA1, one third of the moderate-severe asthma participants had a positive skin prick test to house dust mites, compared to two thirds of the participants with mild or past asthma and 12.1% of the control participants. By definition, participants with moderate-severe asthma used significantly more inhaled corticosteroids compared to the participants with mild-past asthma and control participants (p<0.001 at both points in time). Among the participants with asthma, 43 (42.2%) and 31 (20.9%) had an asthma exacerbation in the past 12 months in EGEA1 and EGEA2, respectively. Both in EGEA1 and EGEA2, the number of participants with high total IgE (>100 kU/L) was higher in asthmatics compared to controls. In the time course between EGEA1 and EGEA2 (n=120 asthmatics), 47 participants had persistent mild asthma, 22 changed from mild to moderate-severe asthma, 17 changed from moderate-severe to mild asthma, and 34 had persistent moderate-severe asthma.

Cross sectional association between SE-IgE and asthma phenotype accounting for disease severity

In EGEA1, SE-IgE positivity varied between 39.1% in controls to 57.6% in mild asthma and 75.6% in severe asthma (p=0.006) (Table 2). SE-IgE was significantly associated with both moderate-severe asthma and mild-past asthma as compared to the control group (OR [95% CI] were 4.81 [2.09-11.07] and 2.12 [1.04-4.29], respectively) (Table 2). After adjustment for several confounders, SE-sensitisation was associated with a significant increased risk for moderate-severe asthma (OR 7.97, 95% CI [2.78-22.84]) but was not associated with past-mild asthma (OR 0.98, 95% CI [0.38-2.55]) (Table 2). In this multiple regression model, SPT to house dust mite was not associated with an increased OR for moderate-severe asthma (OR 2.25, 95%CI [0.75-6.78]) but increased the risk for past-mild asthma (OR 12.04, 95%CI [4.49-32.32]).

The same analysis in EGEA2 showed a similar pattern of associations, although with lower magnitudes. A statistically significant higher SE-sensitisation rate was observed in moderate-severe asthmatics (62.2%), compared to past-mild asthmatics (43.4%) and controls (33.3%) (overall p = 0.002) (Table 2). SE-IgE positivity was significantly associated with moderate-severe asthma, but not

with past-mild asthma, as compared to the control group (OR 3.29,[95% CI][1.68-6.43] and 1.54, [0.79-2.97], respectively). The multiple logistic regression analysis showed that sensitisation to SE was associated with an increased risk of moderate-severe asthma (OR 2.09, 95%CI [0.98-4.45]), but not associated with past-mild asthma(OR 0.91, 95%CI [0.42-1.99]) (Table 2). In this multiple regression model, positive SPT to house dust mite was positively associated in EGEA2 with both, moderate-severe asthma (OR 10.53, 95%CI [3.56-31.15]) and past-mild asthma (OR 19.43, 95%CI [6.57-57.49]).

The sensitivity analysis considering the levels of SE-IgE did not show any trend for a dose-response relationship, both at EGEA1 and EGEA2 (table 3).

Cross sectional association between SE-IgE and severe asthma among subjects with asthma

When only considering participants with asthma, and comparing participants with moderate/severe asthma to participants with past-mild asthma, SE-sensitisation was associated with a significant increased OR of moderate-severe asthma in EGEA1 (OR 7.30, 95%CI [2.22-24.01]) and EGEA2 (OR 2.18, 95%CI [1.08-4.44]) (Table 2). Among the co-factors included in the model, a positive SPT to house dust mite was associated with a decreased OR for moderate-severe asthma in EGEA1 (OR 0.20, 95%CI [0.07-0.59]), however not in EGEA2. None of the co-factors were associated with moderate-severe asthma in EGEA2. A non-significant trend for a positive association between sensitisation to SEs and asthma exacerbations was observed in EGEA1(adjusted-OR 1.79; 95%CI [0.68-4.66]) and EGEA2 (1.66, 95%CI [0.69-3.96]).

Analysis considering change in asthma severity between EGEA1 and EGEA2 shows that the proportion of subjects with SE-sensitisation (at EGEA1 and EGEA2) was the highest among those with persistent severe asthma and persistent severe asthma was the only phenotype showing statistically significant association when compared to the persistent mild asthma group (Table 4).

Longitudinal association between SE-IgE at EGEA1 and severe asthma and asthma exacerbation at EGEA2 or EGEA3

Longitudinal adjusted logistic regression analysis showed that SE-sensitisation in EGEA1 was significantly associated with moderate-severe asthma in EGEA2 (adjusted-OR 2.69, 95%CI [1.18-6.15]), and a non-significant trend was observed for past-mild asthma (Figure 1). In this multiple regression model, positive SPT to HDM in EGEA1 was significantly associated with mild-past asthma, as well as with moderate-severe asthma in EGEA2 (adjusted-OR 7.80, 95%CI [3.01-20.71] and 3.73, 95% CI [1.41-9.83] respectively). Being sensitised to SEs in EGEA1 was also related to a significant increased risk for subsequent asthma exacerbations in EGEA2 or EGEA3 (adjusted-OR 4.59, 95%CI

[1.40-15.07]) (Figure 1). Conversely, a positive SPT to house dust mite did not contribute to the risk for having an asthma exacerbation in the future (adjusted-OR 0.46, 95%CI [0.15-1.45]).

Discussion

This study showed that there is a significantly higher SE-sensitisation rate in severe asthmatics compared to mild asthmatics and controls. Moreover, sensitisation to SEs was associated with a strong and significantly increased risk for moderate to severe asthma, but not for past or mild asthma. When considering only participants with asthma, there was a significantly increased risk for having moderate-severe asthma when sensitised to SEs. For the first time, we were able to show longitudinal relationships between SE-sensitisation and asthma, and particularly moderate/severe asthma. The current study showed that being sensitised to SEs was a significant risk factor to have moderate-severe asthma about a decade later, whereas it did not contribute to the risk of having mild or past asthma. Moreover, being sensitised to SEs was a risk factor for having asthma exacerbations years later, whereas a positive SPT to house dust mite does not contribute to that risk.

Discussion of results

We previously described that serum SE-IgE was associated with asthma in a large European cohort¹⁴. Previous reports showed a relationship between SE-sensitisation and the prevalence of asthma and/or asthma severity in different age groups. Semic-Jusufagic showed an increased sensitisation rate to SEs in children with eczema and wheeze, with an increasing proportion of subjects sensitised to SE-mix with increasing symptom severity¹⁰. We further showed that asthmatics at age 20 were more often sensitised to SEs compared to controls²⁷ and also in an elderly Asian population, a significant association between SE-IgE and late onset severe asthma was shown¹⁵. Other groups also confirmed that increased SE-IgE levels were associated with lower respiratory function, which may be a marker for asthma severity²⁸. The results of the current study are in line with these findings, but extend on these findings by showing that SE-sensitisation was specifically associated with severe asthma. This association is reinforced in the analysis considering change in asthma status over time, indicating stronger associations between SE-sensitisation and persistent severe asthma than between SE-sensitisation and severe asthma reported at a single time point, which may result from a more accurate asthma severity definition.

Although a previous epidemiologic study in more than 3000 subjects showed a significant association of undefined asthma with slightly higher levels (0.3 kU/L) of SE-IgE¹⁴, in this study we do not show

any trend for a dose-response relationship. This might be related to the fact that SE-IgE is often associated with polyclonality (induced by *S. aureus*), and thus concentrations stay on the low side.

Allergic asthma is the most common asthma phenotype in the general population of patients with asthma²⁹. Non-allergic asthma is less common, typically of late onset, related to nasal polyposis, and tends to be more severe³⁰. Bachert et al. already showed that the association between SE-IgE and severe asthma is not purely a reflection of atopy¹³, as subjects may have negative skin prick test results and lack specific serum IgE antibodies to inhalant allergens. In this EGEA study population, 10 of 21 (EGEA1) and 11 of 29 asthmatics (EGEA2) were SE mono-sensitized, and showed no further sensitisation. These data are in line, although slightly higher, with what was reported earlier¹³ in a severe cross-sectional asthma cohort, demonstrating that SE-IgE positivity (but not HDM or grass pollen IgE) was associated with asthma severity (oral steroid use, hospitalisations and lung function) also. We here confirm these data, again separating the associations with SE-IgE from other inhalant allergen IgEs, using an independent cohort, and expand our observation with regard to prediction. Recently, proteins of *S. aureus* including SEs have been demonstrated within the sinus mucosa in nasal polyp disease³¹ and been identified as triggers for IL-5 release³². *S. aureus* proteins have recently also been found in house dust samples³³ and may be inhaled, as *S. aureus* also belongs to the microbiome of house dust mites³⁴. HDM may act as a carrier for antigens from bacteria and thus may cause sensitisation to both, house dust mite and bacterial antigens³⁵. The current study also shows a high sensitisation rate to house dust mites in the asthmatic population; however, logistic regression models reveal a significant relationship between SE-IgE positivity and severe asthma independent from the sensitisation to HDM. Moreover, SE-IgE positivity is associated with an increased risk for asthma exacerbations in the future, whereas a positive SPT to HDM does not contribute to that risk.

Strengths and weaknesses

The main strength of this study lies in the longitudinal nature of the data allowing to address first the robustness of the cross-sectional association by showing similar patterns of association in a population investigated 12-years apart, and secondly the role of SE-sensitisation on the subsequent risk for asthma severity and asthma exacerbations about 12-years later. Therefore, these results provide new evidence to the causality of the observed association. A further strength relates to the detailed phenotypic characterization of the EGEA population, allowing a comprehensive asthma severity definition, adjustment for potential confounders in the association and the comparison of the magnitude of the association observed for HDM sensitisation and SE-sensitisation. Nevertheless, the association reported could be partly biased by residual confounding, either due to missing

potential confounders in the regression model (i.e. asthma comorbidities), or due to limited accuracy in the assessment of some independent variables (i.e. the smoking variable does not take into account for the amount and duration of smoking).

One of the weaknesses regards the sample size of the study that lead to wide 95%CI of the estimated ORs and did not allow investigating the role of SE-IgE in changes in asthma severity over time. Previous studies have shown that staphylococcal enterotoxins are strongly associated with chronic rhinosinusitis with nasal polyps, particularly in the subpopulation of patients with asthma³⁶. In the current study, we only have information on “ever had surgery for nasal polyps” and only 5 asthmatics at EGEA1 and 14 asthmatics in EGEA2 answered yes to that question. As no nasal endoscopy was performed during clinical examination, we could not further analyze this comorbidity in our study. The analyses on asthma exacerbation rely on the report at EGEA2 or EGEA3 of oral steroids use or hospitalization for asthma in the past 12 months. The number of asthma exacerbation from EGEA1 would have been more relevant, but this information was not available in the study. Finally, although our results rely on longitudinal data, the direction of associations, and thus causality, could be a matter of debate. ICS use is part of the definition of asthma severity and there is some concerns about long term ICS use in terms of risk for infections, including upper and lower respiratory tract infection^{37 38} and mycobacterial infections³⁹. While *S aureus* is suspected to secrete enterotoxins that induce corticosteroid resistance⁴⁰, and as there is no strong support in the literature that ICS act as an immunomodulator increasing the risk for *S. Aureus*, we cannot totally avoid this possible effect.

Conclusion

This study confirms previous findings of a higher SE-sensitisation rate in severe asthmatics compared to mild asthmatics or controls. Being sensitised to SEs is significantly associated with an increased risk for having moderate-severe asthma, whereas having a positive SPT to HDM was associated with a decreased risk for having severe asthma. Moreover, for the first time, we showed with longitudinal data that being sensitised to SEs is a significant risk factor for having asthma exacerbation years later whereas being sensitised to house dust mite does not contribute to that risk.

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Figure legend

Figure 1. Longitudinal logistic regression analysis between *S. aureus* enterotoxin-IgE in EGEA1 and asthma severity in EGEA2 and asthma exacerbations in EGEA 2 or EGEA 3 among subjects with asthma

Regression models were adjusted for gender, age, smoking (3 classes: never/ex/current), parental asthma or allergy (yes/no), positive skin prick test to HDM (yes/no)

Table 1. Population description in EGEA1 and EGEA2

	EGEA1 (n=173)				EGEA2 (n=225)			
	Controls (n=69)	Past-mild asthma (n=59)	Moderate- severe asthma (n=45)	P	Controls (n=75)	Past-mild asthma (n=76)	Moderate- severe asthma (n=74)	P
Age, years (mean±SD)	34.3±14.8	29.6±17.3	36.3±16.8	0.09	44.8±15.3	43.0±18.3	42.9±16.8	0.74
Gender, male (%)	39.1	42.4	37.8	0.88	38.7	39.5	40.5	0.97
Smoking status:				0.22				0.30
never smoker (%)	52.5	56.4	53.9		54.1	43.4	60.8	
ex-smoker (%)	20.3	23.1	35.9		25.7	34.2	21.6	
current smoker (%)	27.1	20.5	10.3		20.3	22.4	17.6	
≥1 positive SPT* (%)	36.4	84.5	70.0	<0.001	27.0	74.7	85.9	<0.001
Positive SPT to HDM (%)	12.1	63.8	32.5	<0.001	8.1	53.3	46.5	<0.001
Total IgE> 100 kU/L (%)	31.9	76.3	69.8	<0.001	22.7	58.7	70.3	<0.001
ICS use in the past 12m (%)	0	32.2	82.2	<0.001	2.7	45.3	82.2	<0.001
Asthma exacerbations in the past 12 months (%)	0	29.8	57.8	<0.001	0	12.2	29.7	0.009

* 11 allergens tested (cat, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, *Alternaria tenuis*, timothy grass, olive, birch, *Parietaria judaica*, ragweed, *Aspergillus*, and *Blatta germanica*)

Table 2. Cross-sectional association between *S. aureus* enterotoxin-IgE and asthma severity

	EGEA1			EGEA2		
	Controls	Past-mild asthma	Moderate-severe asthma	Controls	Past-mild asthma	Moderate-severe asthma
SE-IgE positivity, %	39.1	57.6	75.6	33.3	43.4	62.2
In All						
Unadjusted analysis						
OR [95% CI]	1	2.12[1.04-4.29]	4.81[2.09-11.07]	1	1.54[0.79-2.97]	3.29[1.68-6.43]
P value		0.04	<0.001		0.20	<0.001
Adjusted analysis*						
OR [95% CI]	1	0.98 [0.38-2.55]	7.97 [2.78-22.84]	1	0.91 [0.42-1.99]	2.09 [0.98-4.45]
P value		0.97	<0.001		0.82	0.06
In asthmatics only						
Unadjusted analysis						
OR [95% CI]	NA	1	2.27[0.97-5.35]	NA	1	2.14[1.11-4.11]
P value			0.06			0.02
Adjusted analysis*						
OR [95% CI]	NA	1	7.30 [2.22-24.01]	NA	1	2.18 [1.08-4.44]
P value			0.001			0.03

* models adjusted for gender, age, smoking (3 classes: never/ex/current), parental asthma/allergy (yes/no), positive SPT to HDM (yes/no)

Table 3. Association between *S. aureus* enterotoxin-IgE and asthma at EGEA1 and EGEA2, accounting for levels of SE-specific IgE

	EGEA1			EGEA2		
	Controls	Mild asthma	Moderate-severe asthma	Controls	Mild asthma	Moderate-severe asthma
N	69	59	45	123	62	40
SE-IgE<0.1, %	60.8	42.4	24.4	66.7	59.2	37.8
SE-IgE [0.1-0.35[, %	15.9	28.8	31.1	20.0	26.3	36.5
SE-IgE>0.35, %	23.19	28.8	44.4	13.3	14.5	25.7
Age and sex adjusted OR [95%CI]*						
SE-IgE<0.1	1	1	1	1	1	1
SE-IgE [0.1-0.35[1	2.31 [0.91-5.48]	6.02 [2.06-17.58]	1	1.43 [0.65-3.17]	3.18 [1.44-7.04]
SE-IgE>0.35	1	1.50 [0.62-3.68]	6.45 [2.35-17.67]	1	1.20 [0.46-3.14]	3.42 [1.38-8.51]

* given that some sub-groups included few subjects, co-factors in the models were restricted to age and sex

Table 4. Association between *S. aureus* enterotoxin-IgE and change in asthma severity between EGEA1 and EGEA2

	Mild_EGEA1 Severe_EGEA2	Mild_EGEA1 Severe_EGEA2	Severe_EGEA1 Mild_EGEA2	Severe_EGEA1 Severe_EGEA2
SE-IgE at EGEA1, n	38	21	16	29
SE-IgE positive at EGEA1, %	57.9	57.1	68.8	79.3
Age and sex adjusted OR [95%CI]*	1	0.69 [0.1-2.28]	2.55 [0.66-9.93]	5.64 [1.44-22.10]
SE-IgE at EGEA2, n	47	22	17	34
SE-IgE positive at EGEA2, %	46.8	63.6	47.1	64.7
Age and sex adjusted OR [95%CI]*	1	1.69 [0.57-4.97]	1.43 [0.43-4.75]	2.79 [1.05-7.43]

* given that some sub-groups included few subjects, co-factors in the models were restricted to age and sex

ASTHMA SEVERITY AT EGEA2-----

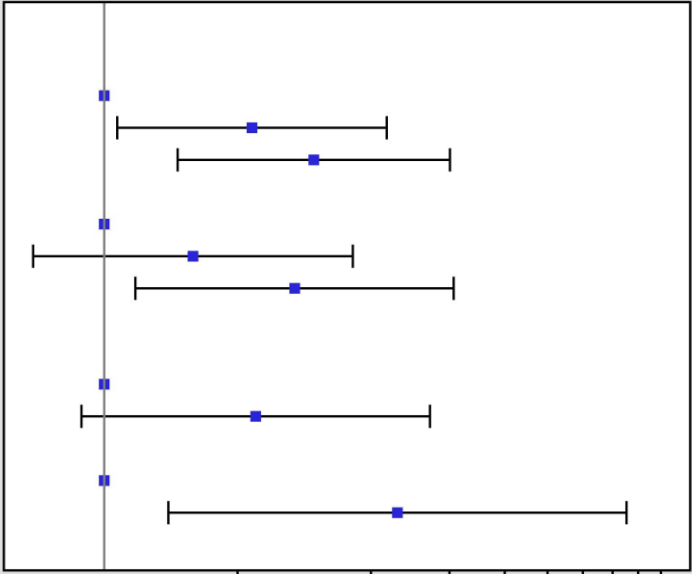
Unadjusted model.....
Control
Mild
Moderate-severe

Adjusted model.....
Control
Mild
Moderate-severe

ASTHMA EXACERBATION AT EGEA2-----

Unadjusted model.....
No
Yes

Adjusted model.....
No
Yes



OR [95% CI] associated with positive SE-IgE at EGEA1