The Belgian IgE study: *Staphylococcus aureus* toxins in adult severe asthma

Florence Schleich, MD, PhD[a], Eléonore Maury, PhD[b], Claus Bachert, MD, PhD[c,d], Mieke Jansen, PhD[b], Sandra Gurdain, MD[b], and Jan Van Schoor, MD, PhD[b], on behalf of the Belgian IgE study investigators*

Clinical Implications

Our study suggests that *Staphylococcus aureus* toxins should be tested in patients with severe asthma, especially in those considered nonatopic to common perennial aeroallergens, because this may allow the identification of an *S aureus*-monosensitized subgroup with type 2 inflammation.

Staphylococcus aureus (*StA*) is a gram-positive bacterium known to colonize the human upper airways and skin and to release enterotoxins and toxic shock syndrome toxin (TSST).[1] These toxins can act as (super)antigens, eliciting a specific IgE (sIgE) response, inducing polyclonal T-cell activation including proallergenic T_H2 cells, increasing type 2 inflammation.[2,3] The Belgian IgE study revealed that sensitizations to *StA* toxins were highly prevalent in patients with severe asthma; however, these perennial aeroallergens (PAAs) are not routinely tested in clinical practice.[4] Here, we specifically explore the results obtained in relation to staphylococcal enterotoxins (SE) A, B, and C, and staphylococcal TSST in patients with severe asthma, and the potential link between sensitization toward *StA* toxins and disease characteristics.

The cross-sectional Belgian IgE study was composed of 175 adults with severe asthma from 16 centers across Belgium, whose blood samples were drawn during a single outpatient consultation. Eligible patients were adults with respiratory physician-diagnosed severe asthma (American Thoracic Society/European Respiratory Society definition)[5] established 6 months or more before enrollment, 8 weeks or more after the most recent exacerbation, and receiving maintenance treatment corresponding to Step 5 by the Global Initiative for Asthma.[6] Exclusion criteria were treatment with omalizumab in the year before enrollment, or with maintenance systemic glucocorticosteroids.[5] Dupilumab was not on the market during the study. We collected data from the medical files, except for the quantification of serum total IgE (IgE_{tot}) and sIgE directed against 43 PAAs, performed as described elsewhere.[4] The cutoff value for sensitization was defined as an sIgE level of 0.10 kU/L or greater.[7-9] The study was approved by the Ethics Committee of the University Hospital Sart-Tilman of Liège (Study No 2019-310) and by those of the participating sites; all patients gave written informed consent.[4]

Almost half (49.1%) had one or more sensitization to *StA* toxins. Serum IgE_{tot} progressively increased with the number of *StA* toxin sensitizations ($P < .05$ vs *StA*-negative group) (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). The original population[4] was subdivided into four subgroups (Table I) according to a recent report.[7] Sensitizations (1) to both *StA* toxins and other PAAs was found in 40.6% of patients, and (2) to PAAs other than *StA* toxins in 35.4%; (3) 15.4% of patients were nonsensitized (full panel negative); and (4) 8.6% of *StA*-monosensitized patients (ie, sensitized to one or more *StA* toxins but not to any other PAAs) had a mean of 1.3 ± 0.2 *StA* sensitizations (Table I). The *StA*-monosensitized group had higher serum IgE_{tot} compared with nonsensitized patients ($P < .05$, group 3 vs group 1) (Table I). Of the 32 patients who tested positive for only one *StA* toxin (n = 11 in group 3 and n = 21 in group 4) (Table I), 21 (65.6%) tested positive for TSST, five (15.6%) for SEC, three (9.4%) for SEA, and three (9.4%) for SEB (Figure 1), demonstrating that none of the four test results was redundant.

The distribution of serum sIgE concentrations of the four *StA* toxins showed higher levels of sIgE against TSST compared with the three individual SEs, and these differences were statistically significant in the overall cohort ($P < .01$) (Figure 1). Sensitization to TSST was also the most prevalent among the four tested toxins (Figure 1).

Then, we explored the potential involvement of sensitization to *StA* toxins in the pathophysiology of asthma. First, using univariate correlation analysis, we further found that sIgE against each *StA* toxin was highly significantly correlated with IgE_{tot} (coefficient of correlation r, 0.53-0.56; $P < .0001$) (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). Multiple linear regression analysis using the presence of the four *StA* toxin sIgE levels as independent variables further indicated that TSST sIgE was a main predictor of serum IgE_{tot} (coefficient of determination $R^2 = 0.20$; $P < .0001$). The sIgEs to the other *StA* toxins were also considered significant contributors to the level of IgE_{tot} ($R^2 = 0.37$ with SEB [$P < .0001$], SEC [$P < .0001$], and SEA [$P < .05$]).

Although sensitizations to *StA* toxins are significant determinants of IgE_{tot} levels, the importance of sensitizations to other PAAs cannot be disregarded. Indeed, the prevalence of overall sensitizations to PAAs was higher in patients with concomitant sensitizations to *StA* toxins (Table I). Moreover, multiple linear regression analysis using the presence of all PAA sIgEs as independent variables showed that sIgEs to TSST ($P < .0001$), SEA ($P < .0001$), SEB ($P < .001$), *Dermatophagoides pteronyssinus* ($P < .0001$), *Aspergillus fumigatus* ($P < .0001$), cat dander ($P < .001$), and moth ($P < .0001$) combined strongly predict serum IgE_{tot} levels ($R^2 = 0.85$), and the concomitant presence of sIgE to TSST ($P < .0001$) and *A fumigatus* ($P < .0001$) is already a strong predicting factor ($R^2 = 0.72$).

In addition, TSST sIgE was positively correlated with the FeNO level (Spearman's correlation coefficient r = 0.17; $P < .05$; not shown).

We further investigated whether sensitization to *StA* toxins could be associated with the development of nasal polyps. In the total population, 32 patients (18.3%) had a formally proven diagnosis of nasal polyps (ie, established by nasal endoscopy). The prevalence of sensitization to TSST was markedly higher in patients with proven nasal polyps (56.3%) compared with patients without them (36.4%). The sensitization to TSST was significantly associated with the diagnosis of nasal polyps ($P < .05$ by

TABLE I. Patient characteristics according to presence of sensitizations

Characteristics	*StA* toxin negative and other PAA negative (group 1) (n = 27; 15.4%)	*StA* toxin negative and other PAA positive (group 2) (n = 62; 35.4%)	*StA* toxin positive and other PAA negative (group 3) (n = 15; 8.6%)	*StA* toxin positive and other PAA positive (group 4) (n = 71; 40.6%)
Historical data				
Sex, female, n (%)	19 (70.4)	40 (64.5)	6 (40.0)	32 (45.1)
Menopausal, n (%)	13 (68.4)	29 (72.5)	4 (66.7)	21 (65.6)
Age, y (mean ± SEM)	55.6 ± 2.9	55.9 ± 1.8	60.9 ± 2.2	58.1 ± 1.6
Demography, n (%)				
Wallonia	15 (55.6)	34 (54.8)	8 (53.3)	31 (43.7)
Flanders	12 (44.4)	20 (32.3)	6 (40.0)	31 (43.7)
Brussels	0	8 (12.9)	1 (6.7)	9 (12.7)
Urban area	4 (14.8)	37 (59.7)	9 (60.0)	39 (54.9)
Body mass index, kg/m^2 (mean ± SEM)	28.3 ± 0.9	27.7 ± 0.7	29.4 ± 1.6	27.0 ± 0.6
Obesity, n (%)	11 (40.7)	21 (33.9)	5 (33.3)	21 (29.6)
Smoking status, n (%)				
Never smoker	11 (40.7)	41 (66.1)	8 (53.3)	28 (39.4)
Ex-smoker	13 (48.1)	17 (27.4)	6 (40.0)	31 (43.7)
Current smoker	3 (11.1)	4 (6.5)	1 (6.7)	12 (16.9)
Age at asthma onset, y (mean ± SEM)	35.0 ± 4.2	25.5 ± 2.2	40.8 ± 5.5	31.1 ± 2.5
<18, n (%)	5 (20.0)	23 (37.7)	3 (20.0)	20 (30.8)
≥18 y, n (%)	20 (80.0)	38 (62.3)	12 (80.0)	45 (69.2)
Age at asthma diagnosis, y (mean ± SEM)	36.2 ± 4.0	30.4 ± 2.4	44.6 ± 4.8	34.3 ± 2.6
Allergic asthma, n (%)	8 (29.6)	43 (69.4)	4 (26.7)	54 (76.0)
Allergic rhinitis, n (%)	8 (29.6)	30 (48.4)	3 (20.0)	44 (62.0)
Allergic dermatitis, n (%)	1 (3.7)	9 (14.5)	1 (6.7)	13 (18.3)
Nasal polyposis, n (%)	4 (14.8)	9 (14.5)	3 (20.0)	16 (22.5)
Loss of smell, n (%)	1 (25.0)	4 (44.4)	2 (66.7)	7 (43.8)
Nasal obstruction, n (%)	4 (100)	7 (77.8)	2 (66.7)	7 (43.8)
Treatment, n (%)	3 (75.0)	6 (66.7)	2 (66.7)	13 (81.3)
Surgery, n (%)	2 (50.0)	6 (66.7)	1 (33.3)	12 (75.0)
Aspirin-exacerbated respiratory disease, n (%)	0	4 (6.5)	0	6 (8.5)
Drug allergy, n (%)	5 (18.5)	12 (19.4)	4 (26.7)	11 (15.5)
Food allergy, n (%)	3 (11.1)	7 (11.3)	1 (6.7)	11 (15.5)
Gastroesophageal reflux disease, n (%)	15 (55.5)	25 (40.3)	8 (53.3)	29 (40.8)
Eosinophils, cells/μL (mean ± SEM)	287.2 ± 36.7	490.8 ± 57.7	522.8 ± 84.6	530.8 ± 49.5
FeNO, ppb (mean ± SEM)	40.2 ± 11.1	37.7 ± 5.4	55.1 ± 10.6	43.3 ± 6.1
Global Initiative for Asthma control level, n (%)				
Controlled	12 (44.4)	32 (51.6)	5 (33.3)	47 (66.2)
Partly controlled	12 (44.4)	27 (43.5)	7 (46.7)	19 (26.8)
Uncontrolled	3 (11.1)	3 (4.8)	3 (20.0)	6 (8.5)
Exacerbations in previous 12 mo, n (%)				
0	19 (70.4)	48 (77.4)	10 (66.7)	51 (71.8)
1	5 (18.5)	8 (12.9)	1 (6.7)	9 (12.7)
2	2 (7.4)	2 (3.2)	2 (13.3)	5 (7.0)
≥3	1 (3.7)	4 (6.5)	2 (13.3)	6 (8.5)

(continued)

TABLE I. *(Continued)*

Characteristics	*StA* toxin negative and other PAA negative (group 1) (n = 27; 15.4%)	*StA* toxin negative and other PAA positive (group 2) (n = 62; 35.4%)	*StA* toxin positive and other PAA negative (group 3) (n = 15; 8.6%)	*StA* toxin positive and other PAA positive (group 4) (n = 71; 40.6%)
Asthma maintenance therapy, n (%)				
Inhaled corticosteroid	27 (100)	62 (100)	15 (100)	71 (100)
Inhaled long-acting β_2-agonist	27 (100)	62 (100)	15 (100)	71 (100)
Inhaled long-acting muscarinic antagonist	7 (25.9)	19 (30.6)	7 (46.7)	20 (28.2)
Anti-IL-5/anti-IL-5R monoclonal antibody	12 (44.4)	39 (62.9)	11 (73.3)	50 (70.4)
Prospective data				
StA toxin sensitizations, n (mean ± SEM)	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 0.2	2.5 ± 0.1
sIgE against one *StA* toxin, n	—	—	11	21
sIgE against two *StA* toxins, n	—	—	3	16
sIgE against three *StA* toxins, n	—	—	1	10
sIgE against 4 *StA* toxins, n	—	—	0	24
Other PAA sensitizations, n (mean ± SEM)	0.0 ± 0.0	6.8 ± 0.6	0.0 ± 0.0	11.5 ± 0.9*
Serum total IgE, kU/L	34.0 ± 6.7†	185.3 ± 33.0	139.3 ± 26	586.6 ± 94.8‡
Prevalence per individual *StA* toxin (%)				
SEA			13.3	54.3§
SEB			14.3	61.4§
SEC			26.7	56.3§
TSST			80.0	81.7§

PAA, perennial aeroallergen; *SE*, staphylococcal enterotoxin; *sIgE*, specific IgE; *StA*, *Staphylococcus aureus*.
Continuous data are represented as means ± SEMs. Comparisons among four groups were carried out using one-way ANOVA followed by Tukey's test or Kruskal-Wallis test followed by Dunn's test, as appropriate (all vs group 3). Mann-Whitney test was used to compare two groups (groups 2 and 4).
*$P < .001$ (vs group 2).
†$P < .05$ vs *StA*-monosensitized (group 3).
‡$P < .01$ vs *StA*-monosensitized (group 3).
§Groups not compared.

Fisher exact test; odds ratio = 2.201; 95% CI, 1.005-4.642). The prevalences of sensitization to the other PAAs including SEA, B, and C, on the other hand, were not different.

These results show that patients with *StA*-MS display a type 2 inflammatory profile and may represent a subgroup incorrectly considered to be nonsensitized. Testing for *StA* toxins may therefore be indicated in this group. Sensitization to TSST is most frequent, but none of the four tests is redundant. To the best of our knowledge, this study is the first to explore the potentially different contribution of individual *StA* toxins, the TSST in particular, to nasal polyps. Further investigation into the role of sensitization to *StA* toxins in asthma and nasal polyps is warranted to understand their pathophysiology and develop targeted interventions for these conditions.

Data availability

Additional information is available from the corresponding author (eleonore.maury@novartis.com) on request (including the prevalence of sensitization to *S aureus* toxins in patient groups 3 and 4, in patients with two or three positive test results to *S aureus* toxins, or in patients with nasal polyposis).

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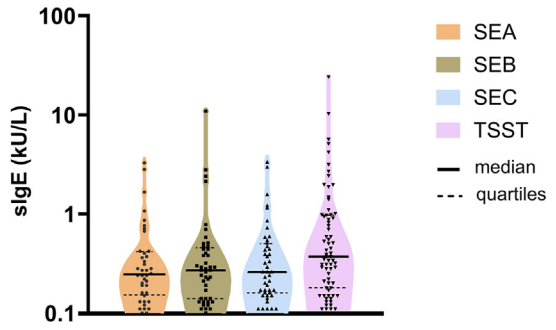

N (% of total cohort)	40 (23.5)	45 (26.8)	44 (25.4)	70 (40.5)
25% Percentile	0.153	0.140	0.160	0.180
Median	0.245	0.270	0.260	0.370
75% Percentile	0.415	0.455	0.500	0.973
Mean, total cohort	0.112	0.178	0.125	0.515
Standard Error of Mean	0.029	0.070	0.030	0.161
p value, vs. TSST	0.0006	0.0077	0.0031	-

FIGURE 1. Violin plot of distribution of specific IgE levels for the four staphylococcal toxins. Only patients with levels of 0.1 kU/L or greater are shown in the plot. Significant difference was determined in the entire cohort using Kruskal-Wallis test and *post hoc* Dunn's multiple comparisons test ($P < .01$, sIgE to three individual staphylococcal enterotoxin [SEs] vs toxic shock syndrome toxin [TSST]).

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[a]Department of Respiratory Medicine, University Hospital Sart-Tilman, Liège, Belgium
[b]Medical Department, Novartis Pharma, Vilvoorde, Belgium
[c]Department of Otorhinolaryngology, Head and Neck Surgery, University of Muenster, Muenster, Germany
[d]Upper Airways Research Laboratory, Department of Head and Skin, Faculty of Medicine, Ghent University Hospital, Ghent, Belgium
[*]The Belgian IgE study investigators are Gwenaëlle Brui (CHWAPI Notre-Dame, Tournai), Alain Delobbe (private practice), Solange de Lovinfosse (Hôpital de Jolimont, La Louvière), Itte Dobbeleir (St Elisabeth Hospital, Herentals), Lieven Dupont (University Hospital Gasthuisberg, Leuven), Shane Hanon (University Hospital VUB, Brussel), Sofie Maddens (AZ Groeninge, Kortrijk), Olivier Michel (University Hospital ULB Brugmann, Brussel), Vicky Nowé (St Vincentius Hospital, Antwerpen), Rudi Peché (University Hospital Vésale, Charleroi), Linda Remels, (AZ St Elisabeth, Zottegem), Anna Sadowska (ZOL, Maaseik), Florence Schleich (University Hospital Sart-Tilman, Liège), Hélène Simonis (CHR La Citadelle, Liège), François Spirlet (University Hospital UCL Dinant-Godinne, Campus Dinant), Joël Thimpont (CH EpiCURA, Baudour), Bram Vandenberge (AZ St Jan, Campus Henri Serruys, Oostende[†]), and Luc Vanmaele (AZ Zeno, Knokke[†]).
[†]These centers were unable to recruit owing to the COVID-19 pandemic.

The study was designed by the sponsor, Novartis Pharma Belgium, in collaboration with the authors. The institutional review board at each participating center approved the protocol. Data were collected in accordance with Good Clinical Practice guidelines by the study investigators and were analyzed by the sponsor. All the authors vouch for the accuracy and completeness of the data and analyses, as well as for the fidelity of this report to the study protocol, which are available from the funder.

Conflicts of interest: F. Schleich reports grants and research support from GSK, Chiesi, and AstraZeneca; and honoraria or consultation fees from GSK, Chiesi, Novartis, AstraZeneca, Teva, and Amgen. C. Bachert is an advisory board member and speaker for Novartis, GSK, AstraZeneca, Sanofi, ALK, and Mylan. EM, MJ, SG, JVS are employees of the sponsor at the time of the study.

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Corresponding author: Eléonore Maury, PhD, Medical Department, Novartis Pharma NV/SA, Medialaan 40 bus 1, Vilvoorde 1800, Belgium. E-mail: eleonore.maury@novartis.com.

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TABLE E1. Correlations of sIgEs to four *Staphylococcus aureus* toxins and serum total IgE

	Serum total IgE level			
Correlation	**SEA-sIgE**	**SEB-sIgE**	**SEC-sIgE**	**Toxic shock syndrome toxin-sIgE**
Spearman's correlation coefficient r	0.53	0.56	0.55	0.56
95% CI	0.4044-0.6304	0.4442-0.6599	0.4349-0.6506	0.4386-0.6532
Statistical significance	<.0001	<.0001	<.0001	<.0001

SE, staphylococcal enterotoxin; *sIgE*, specific IgE.
Correlation analyses were performed using Spearman's test in the entire population (n = 175 patients).

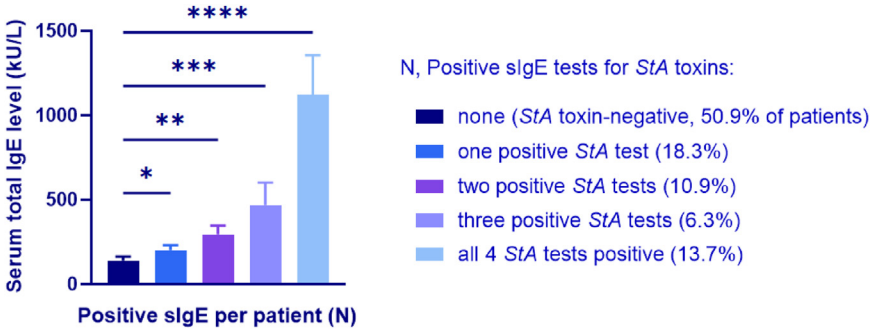

FIGURE E1. Serum total IgE level according to number of sensitizations to *Staphylococcus aureus* (*StA*) toxins. Data are represented as means ± SEMs. *$P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$ (vs negative), using Kruskal-Wallis test and *post hoc* Dunn's test. Among 18.3% of patients positive for specific IgE (sIgE) to one *StA* toxin, 65.6% were positive for toxic shock syndrome toxin, 15.6% for staphylococcal enterotoxin (SE)C, and 9.4% each for SEA and B.