ORIGINAL ARTICLE
ALLERGY

Moulds and *Staphylococcus aureus* enterotoxins are relevant allergens to affect Type 2 inflammation and clinical outcomes in chronic rhinosinusitis patients

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ABSTRACT

Background: Sensitisation to moulds and *Staphylococcus aureus* enterotoxins (SEs) is associated with the pathophysiology of both asthma and chronic rhinosinusitis (CRS). The purpose of this study was to clarify the contribution of sensitisation to these allergens to Type 2 inflammation in the blood, nose and the lower airways, and clinical outcomes in CRS patients.

Methods: We prospectively enrolled 56 CRS patients who underwent endoscopic sinus surgery (ESS) (20 with comorbid asthma) and 28 healthy controls between October 2015 and December 2017. CRS patients were followed up for 12 months after surgery. Type 2 inflammation-related biomarkers were analysed using blood, resected tissue samples and sputum. 10 allergens including *Alternaria*, *Aspergillus* and SEs were measured. Type 2 inflammation-related biomarkers and clinical outcomes were compared in the stratification with the presence or absence of allergen sensitisation.

Results: Sensitisation rate to moulds and SEs in asthmatic patients was increased when changing the cut-off value of specific IgE titre from 0.35 UA·mL^{-1} to 0.10 UA·mL^{-1} (1.7- and 4.5-fold, respectively). Moulds and SEs affected the prevalence of asthma and eosinophilic CRS by interacting with each other. All Type 2 inflammation-related biomarkers except for eosinophils in sinus tissue were significantly higher in patients with mould or SE (mould/SE) sensitisation (⩾0.10 UA·mL^{-1}) (n=19) than in those without (n=37) and healthy subjects (all $p<0.05$). Meanwhile, mould/SE sensitisation did not affect longitudinal changes in clinical outcomes after ESS. Changes in serum mould/SE-IgE levels after ESS remained unclear.

Conclusion: Mould/SE sensitisation (⩾0.10 UA·mL^{-1}) may affect the development of Type 2 inflammation and clinical outcomes in CRS patients.

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***Alternaria*, *Aspergillus* and *S. aureus* enterotoxins are important allergens affecting Type 2 inflammation and clinical outcomes in CRS patients. Sensitisation to moulds/SEs (⩾0.10 UA·mL^{-1}) would be meaningful in the pathophysiology of CRS.** https://bit.ly/3bUG8ZT

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Introduction

Sensitisation to allergens is a significant risk factor for adult onset asthma in the general population [1, 2]. It is also common in patients with chronic rhinosinusitis (CRS), its prevalence ranging from 50 to 84% [3]. Generally, we consider patients as having sensitisation to allergens when one or more specific immunoglobulin E (IgE) titres against allergens exceed 0.35 UA·mL^{-1} [4]. However, several studies indicate the association of a threshold of ⩾0.10 UA·mL^{-1} in serum-specific IgE titres, including *Staphylococcus aureus* enterotoxins (SEs) and *Aspergillus*, with onset, severity and poor control of asthma [5–9]. However, the contribution of a lower threshold (⩾0.10 UA·mL^{-1}) of serum-specific IgE titres against allergens to Type 2 inflammation and clinical outcomes in allergic diseases remains little known.

Long-term colonisation of the nose and lower airways by moulds and *S. aureus* plays a role in their sensitisation both systemically and locally [10, 11]. The presence of IgE in the nose and lower airways is associated with local eosinophilic inflammation [12, 13]. *Alternaria* and *Aspergillus* are well-known fungal allergens associated with severe asthma [14, 15], along with *S. aureus* [6–8]. The evidence suggests that environmental microorganism-related allergens may be strong activators of Type 2 inflammation throughout airways and contribute to worse clinical outcomes in CRS patients as compared to other common allergens.

The objective of this study was to explore different cut-off levels of sensitisation to moulds and SEs in relation to Type 2 inflammation-related biomarkers in the blood, nose and lower airways, and the clinical relevance in patients with CRS. Furthermore, we assessed whether mould and SE sensitisation had any effect on the clinical and inflammatory outcomes 12 months after endoscopic sinus surgery (ESS).

Methods

This study is a *post hoc* analysis of our previous study which evaluated the pathophysiological link between upper and lower airways in CRS patients [16]. We prospectively enrolled 56 CRS patients who agreed to ESS (20 with and 36 without comorbid asthma) and 28 healthy controls between October 2015 and December 2017. The diagnosis of asthma and CRS was made according to guidelines [17, 18]. These were described in our previous report along with inclusion and exclusion criteria used in the study [16]. We excluded current smokers in this study. We confined our patient sample to those who underwent ESS in this study because tissue eosinophilia in the sinuses, particularly in nasal polyps (NPs), is associated with the presence of asthma [19, 20]. We also have suggested that upper airway tissue eosinophilia is pathophysiologically linked to Type 2 lower airway inflammation. Indeed, we have demonstrated the association of tissue eosinophilia in sinus and NPs with Type 2 lower airway inflammation in our previous study [16]. This study was approved by the Ethics Committee of our hospital (1165) and was registered on the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (Registry ID UMIN000018672). Written informed consent was obtained from all participants.

Measurements

All participants underwent spirometry, fractional nitric oxide (F_{eNO}) measurement (oral expiratory flow rate 50 mL·s^{-1}), an olfactory function test as assessed by the Open Essence method (ranging from 0 to 12, and lower scores showing impaired olfaction) [21] and sputum induction, and answered the Sinonasal Outcome Test-22 (SNOT-22) (ranging from 0 to 110, and higher scores showing worse sinonasal-related quality of life (QoL)) [22] before ESS, as did healthy subjects. In one patient we were unable to measure F_{eNO} levels at enrolment due to apparatus failure. Additionally, blood and tissue sample collection and a computed tomography (CT) scan of the sinuses were performed only in CRS patients. Serum Type 2 inflammation-related biomarkers were also determined in 20 healthy subjects using stored serum samples. Inflamed sinus tissue samples and NPs were taken from 54 and 38 patients, respectively, under general anaesthesia by an otorhinolaryngology specialist (M.S.). Eosinophil counts and periostin levels were measured using blood and sputum samples. The number of eosinophils in sinus and NPs (high-power field (HPF), 400×) and the Lund–Mackay score (LMS; ranging from 0 to 24, and higher scores showing severe CRS) from CT of the sinus were determined by a pathologist (A.M.) and a radiologist (Y.O.) in a

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blinded manner, respectively. We defined eosinophilic CRS when eosinophils in sinus or NP tissue showed ⩾70 HPF [19]. We re-evaluated all measurements aside from tissue collection at 12 months after ESS in CRS patients. Patients with asthma also completed the Asthma Quality of Life Questionnaire (AQLQ) [23] (comprising four domains, score ranging from 0 to 7, with lower scores showing worse asthma-related QoL) both before and 12 months after ESS. We obtained permission to use the SNOT-22 and AQLQ for this study from Professor Jay Piccirillo, Washington University, USA, and Professor Elizabeth Juniper, McMaster University, Canada, respectively. Furthermore, an otorhinolaryngologist (M.S.) assessed the recurrence of NPs and three asthma specialists (Y.K., M.T. and A.N.) assessed new asthma onset for 12 months following ESS. Details of these measurements are described in a previous report [16].

Allergens measurement

We determined serum total IgE and specific IgE antibody against 10 allergens: house dust mite, cat, dog dander, Japanese cedar pollen, mixed Gramineae pollens (orchard grass, sweet vernal grass, Bermuda grass, Timothy grass and reeds), mixed weed pollens (ragweed, mugwort, goldenrod, dandelion and oxeye daisy), *Alternaria alternata*, *Aspergillus fumigatus* and *Staphylococcus aureus* enterotoxins A and B (SEA and SEB, respectively) (ImmunoCAP®; Phadia K.K., Tokyo, Japan). The detection limit for each specific IgE titre was 0.10 UA·mL^{-1}. Table 1 shows the proportion of each sensitised allergen in CRS patients when the cut-off value of serum-specific IgE titre was set at 0.10 UA·mL^{-1} or 0.35 UA·mL^{-1}. Sensitisation rate to moulds and SEs in asthmatic patients was increased when changing the cut-off value of specific IgE from 0.35 UA·mL^{-1} to 0.10 UA·mL^{-1} (1.7- and 4.5-fold, respectively) (table 1). However, sensitisation to other allergens such as mite and pollen was similar between two cut-off values of specific IgE titres. When a cut-off value was set at 0.10 UA·mL^{-1}, there were significant differences in sensitisation to moulds or SEs between CRS patients with and without asthma (table 1). This suggests that sensitisation to moulds or SEs (⩾0.10 UA·mL^{-1}) would be meaningful in the pathophysiology of both asthma and CRS. Thus, we considered patients as having sensitisation to moulds or SEs if they showed a serum-specific IgE titre of ⩾0.10 UA·mL^{-1}. On the other hand, we considered participants as having sensitisation to other conventional allergens if they showed one or more specific IgE titre against remaining allergens of ⩾0.35 UA·mL^{-1}.

To compare the prevalence of mould and SE sensitisation between CRS patients and healthy subjects, we also measured serum total IgE levels and specific IgE titres against *Alternaria*, *Aspergillus* and SEs in healthy subjects. Sensitisation rates to either moulds or SEs were similar between all CRS patients and healthy subjects (table 1).

TABLE 1 The proportion of sensitised allergens

	⩾0.35 UA·mL^{-1}						⩾0.10 UA·mL^{-1}					
	CRS patients	Asthma	Without asthma	Healthy subjects	p-value[#]	p-value[¶]	CRS patients	Asthma (n=20)	Without asthma	Healthy subjects	p-value[#]	p-value[¶]
Subjects n	56	20	36	20			56	20	36	20		
House dust mite	19 (34)	10 (50)	9 (25)			0.08	27 (48)	13 (65)	14 (39)			0.09
Dog dander	3 (5)	1 (5)	2 (6)			>0.99	8 (14)	4 (20)	4 (11)			0.44
Cat	2 (4)	0 (0)	2 (6)			0.53	7 (13)	0 (0)	7 (19)			0.04
Japanese cedar	29 (52)	12 (60)	17 (47)			0.41	32 (57)	13 (65)	19 (53)			0.41
Mixed Gramineae	7 (13)	4 (20)	3 (8)			0.23	10 (18)	5 (25)	5 (14)			0.47
Mixed weed	9 (16)	5 (25)	4 (11)			0.26	14 (25)	6 (30)	8 (22)			0.53
Alternaria	5 (9)	4 (20)	1 (3)	1 (5)	>0.99	0.0497	12 (21)	8 (40)	4 (11)	1 (5)	0.16	0.02
Aspergillus	5 (9)	4 (20)	1 (3)	1 (5)	>0.99	0.0497	8 (14)	6 (30)	2 (6)	2 (10)	>0.99	0.02
Moulds	7 (13)	6 (30)	1 (3)	2 (10)	>0.99	0.006	14 (25)	10 (50)	4 (11)	2 (10)	0.21	0.003
SEA	4 (7)	1 (5)	3 (8)	0 (0)	0.57	>0.99	9 (16)	5 (25)	4 (11)	6 (30)	0.20	0.26
SEB	5 (9)	1 (5)	4 (11)	0 (0)	0.32	0.64	13 (23)	7 (35)	6 (17)	3 (15)	0.54	0.19
SEs (A and/or B)	6 (11)	2 (10)	4 (11)	0 (0)	0.33	>0.99	15 (27)	9 (45)	6 (17)	8 (40)	0.27	0.03

Categorical data are presented as n (%). CRS: chronic rhinosinusitis; SEA: *Staphylococcus aureus* enterotoxin A; SEB: *Staphylococcus aureus* enterotoxin B; SEs: *Staphylococcus aureus* enterotoxins. [#]: Compared between all CRS patients and healthy subjects; [¶]: Compared between with asthma and without asthma. We could not measure serum-specific IgE titres in eight healthy subjects because of shortage of sample amount.

Statistics

Data were analysed using JMP 14 Start Statics (SAS Institute Inc., Cary, NC, USA) and presented as a median (25th percentile, 75th percentile). We evaluated the interactive effect between moulds and SEs sensitisation for clinical outcomes in CRS patients using ANCOVA. A p-value <0.05 was taken to show that, moulds and SEs affected the development of clinical outcomes by interacting with each other. We also evaluated the interactive effect of smoking or other conventional allergens with mould or SE sensitisation for clinical outcomes. We stratified patients according to the presence or absence of sensitisation to allergens (allergen^{+} and allergen^{-} groups). We adopted the Kruskal–Wallis test followed by Steel–Dwass analysis or Chi-squared test when comparing indices among patients with and without allergen sensitisation and healthy subjects. We also applied the Wilcoxon rank-sum test or Fisher exact test when comparing between allergen^{+} and allergen^{-} groups. We analysed changes in indices with ESS using the Wilcoxon single rank test. A p-value $\leqslant$0.05 was considered significant when α error was set at 5%.

Results

Characteristics of participants are presented in table 2. The proportion of females was lower in participants with mould or SE sensitisation than in healthy subjects. A history of smoking was less frequent in healthy subjects than CRS patients; however, it was unrelated to sensitisation to either moulds or SEs ($\geqslant$0.10 UA·mL^{-1}) in CRS patients. There were no obvious differences in age and body mass index among the three groups. When confined to patients with CRS, the prevalence of asthma and sensitisation to other allergens were more frequent in patients with mould or SE sensitisation than in those without (table 2). However, other general indices such as previous history of ESS and disease duration were similar between the two groups (table 2).

Interactive effect between moulds and SEs for clinical outcomes in CRS patients

We evaluated whether moulds and SEs have an interactive effect for the development of clinical outcome in CRS patients. Moulds and SEs significantly increased serum periostin levels and sputum eosinophil counts

TABLE 2 Participant characteristics

	All participants, except where noted	Moulds^{+}	Moulds^{-}	SEs^{+}	SEs^{-}	Healthy subjects	p-value$^{\#}$	p-value¶
Subjects n	84	14	42	15	41	28		
General indices								
Age years	60 (50, 67)	62 (44, 66)	61 (51, 67)	63 (50, 66)	60 (51, 68)	59 (42, 67)	0.61	0.68
Female sex	35 (42)	3 (21)§	16 (46)	2 (13)§	17 (41)	16 (57)	0.06	0.01
Body mass index kg·m^{-2}	23.3 (20.6, 25.3)	24.0 (20.1, 26.3)	23.5 (21.5, 25.1)	22.7 (19.9, 26.0)	23.9 (21.9, 25.5)	21.6 (20.6, 24.3)	0.59	0.43
Smoking history, never	45 (54)	7 (50)§	16 (38)^{f}	5 (33)§	18 (44)^{f}	22 (79)	0.003	0.003
Pack-years$^{\#\#}$	15.5 (5.3, 30)	10 (1.6, 26.3)	20 (7.3, 30)	17.5 (8.1, 30.4)	16.3 (5, 30)	14 (4.9, 32)	0.50	0.97
Disease indices								
Past history of ESS¶¶	11 (20)	3 (21)	8 (19)	4(27)	7 (17)			
Presence of asthma¶¶	20 (36)	**10 (71)**$^{+}$	10 (24)	**9 (60)**$^{+}$	11 (27)			
Duration of sinusitis years¶¶	3 (1, 10)	2.5 (1, 8)	3.5(1, 10)	2 (1, 7.5)	3.5 (1, 10)			
Duration of asthma years^{++}	5 (1, 12)	11 (4, 13)	3 (1, 6)	2.5 (1, 6)	10 (3, 14)			
ICS dose µg·day^{-1}$^{++}$	450 (340, 640)	520 (380, 680)	450 (240, 640)	500 (360, 840)	400 (320, 640)			
GINA2015 Treatment step n (2/3, 4)$^{++}$	10, 10	4, 6	6, 4	4, 5	6, 5			
Conventional allergen sensitisation¶¶								

Data are presented as median (25th percentile, 75th percentile) or n (%), unless otherwise stated. ESS: endoscopic sinus surgery; ICS: inhaled corticosteroid; mould: *Alternaria* and *Aspergillus*; SEs, *Staphylococcus* enterotoxins A and B; conventional allergens: house dust mite, dog dander, cat, Japanese cedar, mixed Gramineae and mixed weed; H: healthy subjects. $^{\#}$: Compared among moulds^{+}, moulds^{-} and H using Kruskal–Wallis test or Chi-squared test; ¶: compared among SEs^{+}, SEs^{-} and H using Kruskal–Wallis test or Chi-squared test; $^{+}$: p<0.05 for moulds or SEs^{+} *versus* moulds or SEs^{-}; §: p<0.05 for moulds or SEs^{+} *versus* H; ^{f}: p<0.05 for moulds or SEs^{-} *versus* H, analysed by Steel–Dwass analysis, Wilcoxon rank-sum test or Fischer's exact test. $^{\#\#}$: n=46 (mould$^{+/-}$: 7/26, SEs$^{+/-}$: 10/23, H: 6); ¶¶: n=56; $^{++}$: n=20 (mould$^{+/-}$: 10/10, SEs$^{+/-}$: 9/11).

and affected the prevalence of asthma and eosinophilic CRS by interacting with each other (supplementary table E1). This result suggests that moulds and SEs induce Type 2 inflammation systemically and locally by interacting with each other. Indeed, 10 patients (53%) were sensitised to both moulds and SEs. Therefore, we categorised these allergens into the same group (moulds/SEs) to evaluate their relevance to clinical outcomes in CRS patients. However, other conventional allergens did not have an interactive effect with mould/SE allergens on prevalence of asthma and eosinophilic CRS (data not shown).

The impact of sensitisation to moulds/SEs (⩾0.10 UA·mL^{-1}) on clinical outcomes

We evaluated the impact of sensitisation to moulds/SEs (⩾0.10 UA·mL^{-1}) on clinical outcome (table 3). All Type 2 inflammation-related biomarkers apart from eosinophil counts in sinus tissue were significantly higher in the moulds/SEs^{+} group than in the moulds/SEs^{-} group (table 3). These values were not significant except for serum total IgE levels upon setting a cut-off value at 0.35 UA·mL^{-1} (supplementary table E1). Type 2 lower airways inflammation in the moulds/SEs^{+} group was also more predominant than in healthy subjects, while a significant difference between the moulds/SEs^{-} group and healthy subjects was only seen in sputum periostin levels (table 3). Conventional allergens had an interactive effect with mould/SE allergens by increasing serum periostin levels and sputum eosinophil counts, whereas smoking did not have any interactive effect with mould/SE sensitisation for the development of clinical outcomes in CRS (data not shown).

Sensitisation to moulds/SEs (⩾0.10 UA·mL^{-1}) did not affect olfaction, radiological CRS severity, or sinonasal- and asthma-related QoL in CRS patients (table 3).

TABLE 3 The impact of sensitisation to moulds/*Staphylococcus aureus* enterotoxins (SEs) (⩾0.10 UA·mL^{-1}) on clinical outcomes

	All participants, except where noted	Moulds/SEs^{+}	Moulds/SEs^{-}	Healthy subjects	p-value$^{\#}$	p-value¶ moulds/SEs^{+} *versus* $^{-}$	p-value¶ moulds/SEs^{+} *versus* H	p-value¶ moulds/SEs^{-} *versus* H
Subjects n	84	19	37	28				
Systemic biomarkers								
Blood eosinophil count per μL^{++}	246 (143, 526)	449 (191, 737)	198 (117, 361)			0.01		
Serum total IgE IU·mL$^{-++,\dagger+}$	137 (26, 431)	513 (389, 1020)	69 (24, 172)	44 (11, 357)	0.004	<0.0001	0.0002	0.99
Serum periostin ng·mL$^{-1++,+}$	86 (74, 108)	112 (93, 159)	80 (73, 97)	84 (74, 101)	0.009	0.008	0.01	0.95
Upper airway markers								
Sinus eosinophils per HPF^{+}	66 (20, 168)	100 (56, 178)	60 (12, 156)			0.17		
Nasal polyps, presence§	38 (68)	15 (79)	23 (62)			0.24		
Nasal polyps, eosinophils per HPF$^{\S f}$	85 (6, 145)	125 (65, 262)	33 (1, 97)			0.007		
Eosinophilic CRS, presence^{++}	33 (59)	15 (79)	18 (49)			0.04		
Lund–Mackay score^{++}	12 (7, 16)	14 (9,17)	11 (7, 16)			0.41		
SNOT-22 score	15 (3, 35)	35 (23, 53)	22 (12, 41)	2 (0, 4)	<0.0001	0.21	<0.0001	<0.0001
Open Essence score	7 (3, 9)	4 (0, 8)	5 (1, 8)	9 (7, 10)	<0.0001	0.98	0.002	<0.0001
Lower airway markers								
AQLQ points$^{\#\#}$	5.8 (5.5, 6.7)	5.9 (5.2, 6.7)	5.6 (5.5, 6.7)			0.91		
Sputum eosinophils %¶¶	0 (0, 3.2)	5.5 (1.8, 57.5)	0 (0, 2.8)	0 (0, 0.4)	0.0005	0.01	0.0006	0.29
Sputum periostin ng·mL$^{-1\P\P}$	7.1 (1.5, 16.3)	23.0 (11.7, 42.9)	9.0 (2.0, 14.9)	1.6 (0.5, 3.4)	<0.0001	0.004	<0.0001	0.001
F_{eNO} ppb	25.8 (17.7, 38.7)	41.7 (27.9, 73.8)	26.2 (18.4, 38.0)	20.6 (16.1, 26.2)	0.0003	0.04	0.0001	0.13

Data are presented as median (25th percentile, 75th percentile) or n (%), unless otherwise stated. Moulds: *Alternaria* and *Aspergillus*; SEs, *Staphylococcus* enterotoxins A and B; H: healthy subjects; HPF: high-power field; eosinophilic CRS: defined when eosinophils in sinus or NP tissue show ⩾70 HPF; SNOT-22: Sinonasal Outcome Test-22; AQLQ: Asthma Quality of Life Questionnaire; F_{eNO}: fractional nitric oxide (could not measure F_{eNO} in one patient because of apparatus failure). $^{\#}$: Analysed by Kruskal–Wallis test; ¶: Analysed by Steel–Dwass analysis, Wilcoxon rank-sum test or Fischer's exact test; $^{+}$: n=56; §: n=54 (moulds/SEs$^{+/-}$: 18/36); ^{f}: n=38 (moulds/SEs$^{+/-}$: 15/23); $^{\#\#}$: n=20 (moulds/SEs$^{+/-}$: 13/7); ¶¶: n=65 (moulds/SEs$^{+/-}$: 15/30, H: 20); $^{++}$: n=76 (CRS/H: 56/20).

TABLE 4 Longitudinal changes in clinical outcomes in chronic rhinosinusitis (CRS) patients when stratified according to the presence or absence of sensitisation to moulds/*Staphylococcus aureus* enterotoxins (SEs) (⩾0.10 UA/mL)

	Moulds/SEs^{+} (n=15)			Moulds/SEs^{-} (n=33)			p-value¶
	Before	12 months after	p-value$^{\#}$	Before	12 months after	p-value$^{\#}$	
Systemic biomarkers							
Blood eosinophil count per µL	449 (262, 653)	341 (203, 487)	0.08	178 (85, 310)	177 (80, 317)	0.42	0.10
Serum periostin ng·mL^{-1}	112 (93, 159)	102 (64, 125)	0.36	80 (72, 100)	86 (69, 111)	0.80	0.16
Sensitisation to moulds	11 (73)	10 (67)		0 (0)	0 (0)		
Sensitisation to SEs	11 (73)	7 (47)		0 (0)	6 (18)		
Upper airway markers							
Nasal polyps recurrence, presence, n (%)$^{+}$		4 (27)			3 (9)		0.18
Lund–Mackay scores	14 (10, 15)	9 (6,13)	0.004	11 (7, 16)	5 (2, 11)	<0.0001	0.81
SNOT-22 scores	31 (22, 51)	24 (5, 32)	0.03	20 (12, 33)	10 (5, 20)	0.0003	0.80
Open Essence scores	4 (0, 8)	6 (0, 9)	0.06	5 (0, 8)	6 (4, 8)	0.08	0.67
Lower airway markers							
New asthma onset, presence§		1 (25)			6 (24)		>0.99
AQLQ points^{f}	5.9 (4.9, 6.7)	6.4 (6.0, 6.7)	0.02	5.6 (5.5, 6.57)	6.2 (6.1, 7)	0.03	0.82
Sputum eosinophils %$^{\#\#}$	3.3 (0.9, 27.2)	3.4 (0.5, 12.1)	0.76	0 (0, 2.0)	1.0 (0, 4.5)	0.09	0.33
Sputum periostin ng·mL$^{-1\#\#}$	27.2 (14, 47.8)	8.1 (1.7, 20.8)	0.02	9.8 (2.3, 15.6)	3.3 (1.8, 9.0)	0.004	0.24
F_{eNO} ppb¶¶	50.4 (33.6, 73.8)	32.7 (24.6, 54.6)	0.56	26.4 (17.6, 38.0)	26.3 (15.4, 46.0)	0.86	0.34

Data are presented as median (25th percentile, 75th percentile) or n (%), unless otherwise stated. Moulds: *Alternaria* and *Aspergillus*; SEs: *Staphylococcus* enterotoxins A and B; SNOT-22: Sinonasal Outcome Test-22; AQLQ: Asthma Quality of Life Questionnaire; F_{eNO}: fractional nitric oxide. $^{\#}$: Analysed by Wilcoxon single rank test; ¶: Analysed by Wilcoxon rank-sum test or Fischer's exact test; $^{+}$: n=34 (moulds/SEs$^{+/-}$: 12/22; §: n=30 (moulds/SEs$^{+/-}$: 5/25); ^{f}: n=18 (moulds/SEs $^{+/-}$: 10/8; $^{\#\#}$: n=39 (moulds/SEs $^{+/-}$: 13/26); ¶¶: n=47 (moulds/SEs $^{+/-}$: 14/33).

The impact of sensitisation to moulds/SEs (⩾0.10 UA·mL^{-1}) on the longitudinal clinical outcomes

We also evaluated the association of sensitisation to moulds/SEs (⩾0.10 UA·mL^{-1}) with changes in biomarkers, radiological CRS severity, olfaction and QoL by ESS intervention (table 4). We followed-up 48 CRS patients for 12 months after ESS (15 with mould/SE (⩾0.10 UA·mL^{-1}) sensitisation and 33 without). Among 48 patients, 15 had mould or SE sensitisation at enrolment (n=11 for both, respectively). Although serum mould-IgE levels tested negative in only one patient without asthma, none were sensitised 1 year after ESS. On the other hand, serum SE-IgE levels tested negative in four patients (two with asthma and two without asthma), whereas six (two with asthma and four without asthma) were newly sensitised after surgical intervention. Twenty patients had mould or SE sensitisation 1 year after ESS. We could not find any significant difference between patients whose serum mould/SE-IgE levels were changed by ESS.

ESS could improve radiological CRS severity, olfaction and sinonasal- and asthma-related QoL in CRS patients (figure 1, table 4). Moreover, levels of sputum periostin also declined significantly with ESS intervention (figure 1, table 4). When patients were divided into those with presence or absence of sensitisation to moulds/SEs (⩾0.10 UA·mL^{-1}), however, changes in biomarkers, radiological CRS severity and QoL with ESS intervention were similar between the two groups (table 4). The proportion experiencing recurrence of NPs or new asthma onset over the 12 months following ESS was also not related to mould/SE sensitisation (⩾0.10 UA·mL^{-1}) at enrolment.

Discussion

To the best of our knowledge, this is the first study to demonstrate that levels of moulds/SEs-IgE of ⩾0.10 UA·mL^{-1} rather than ⩾0.35 UA·mL^{-1} may be more useful as biomarkers that reflect systemic and local Type 2 inflammation and asthma prevalence in CRS patients. Moulds and SEs contributed to the development of clinical outcomes in CRS patients by interacting with each other. However, neither mould nor SE sensitisation (⩾0.10 UA·mL^{-1}) affected clinical outcomes and Type 2 inflammation-related biomarkers in ESS.

Specific IgE titres are measured using fluorescence enzyme immunoassay. A cut-off value for this assay was set at 0.35 UA·mL^{-1} following the discovery of a strong correlation with the radioallergosorbent test in 1990 [4]. The detection limits of specific IgE titres have improved over the last two decades from 0.35 UA·mL^{-1} to 0.10 UA·mL^{-1}. We have demonstrated the importance of a lower cut-off value of moulds/SEs-IgE (⩾0.10 UA·mL^{-1}) on the development of Type 2-predominant inflammation in nose and

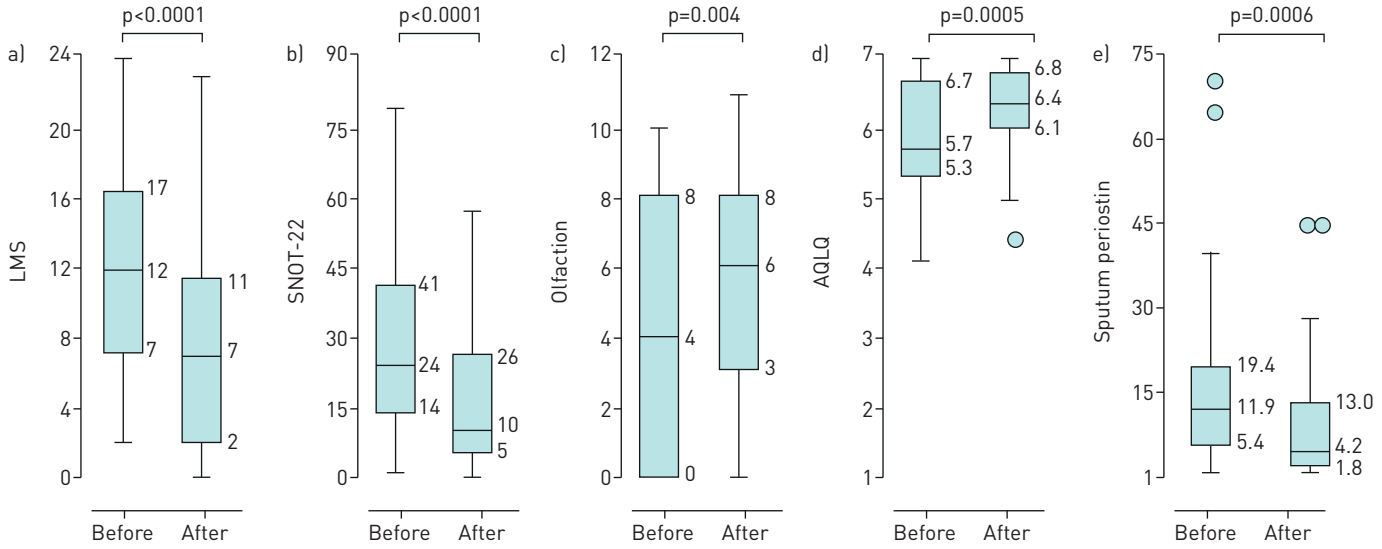

FIGURE 1 The efficacy of endoscopic sinus surgery in chronic rhinosinusitis (CRS) patients. Box and whisker plots show the change in a) the Lund–Mackay score (LMS), b) the Sinonasal Outcome Test-22 (SNOT-22) score, c) olfactory score, d) the Asthma Quality of Life Questionnaire (AQLQ) score and e) levels of sputum periostin with endoscopic sinus surgery. The horizontal line in the box interior shows the median values of indices. The length of the box represents the distance between the 25th and 75th percentiles. The circles represent outliers if data were above upper whiskers or below lower whiskers. before: before surgery; 12 m after: 12 months after surgery.

lower airways. The sensitisation rate to moulds and SEs in asthmatic patients rose from 10–30% to 45–50% when the cut-off value was set at 0.10 UA·mL^{-1} compared with 0.35 UA·mL^{-1} (table 1). However, the change in cut-off value from 0.35 UA·mL^{-1} to 0.10 UA·mL^{-1} hardly affected the frequency of patients who have sensitisation to other allergens such as house dust mite, cat and pollen (table 1). Moulds and SEs might induce Type 2-predominant airway inflammation to a lesser degree than more conventional allergens. Recent studies have reported a significant association of SE sensitisation determined by a threshold of 0.10 UA·mL^{-1} with the prevalence [2] and severity of asthma [7]. Thus, a lower cut-off value of 0.10 UA·mL^{-1} in moulds and SEs would be meaningful in the pathophysiology of allergic diseases such as asthma and CRS. Moulds and SEs may be common allergens in patients with asthma as well as more conventional allergens.

Alternaria and *Aspergillus* are classified as moulds, whereas *S. aureus* are bacteria. Both moulds and *S. aureus* colonise in particular the NPs of CRS patients [24–26]. We found that moulds and SEs have an interactive effect on the development of clinical outcomes and Type 2 inflammation in CRS patients; however, different mechanisms are associated with the development of Type 2-predominant inflammation. Although enterotoxins produced by *S. aureus* are well-known inducers of Type 2-related cytokines and eosinophilic inflammation in both nose and lower airways by acting as superantigens [27, 28], *S. aureus* can directly induce interleukin-33 and thymic stromal lymphoprotein production *via* airway epithelium through binding to Toll-like receptor 2 [29]. *Alternaria alternata* and *Aspergillus fumigatus* have very similar epitopes [30]. This suggests that their sensitisation may elicit a similar immune response. Protease produced by *Alternaria* and *Aspergillus* can evoke release of Type 2-related cytokines [31], airway hyperresponsiveness [32] and activation of eosinophils [33]. Furthermore, the humoral immune response to moulds is also thought to be associated with increased production of Type 2-related cytokines [34]. A lesser degree of sensitisation to moulds and SEs may be related to induction of the Type 2 inflammatory cascade in the nose and lower airways, as stated above.

Airways are anatomically continuous from the nose to the lower airways. The sino/nasobronchial reflex is thought to be associated with the development of both upper and lower airway inflammation. Inhalation of allergens through the nose and lower airways can induce Type 2 inflammation of the lower airways and nose, respectively [35, 36]. Nasal colonisation by *S. aureus* is also related to the development of eosinophilic NPs (*i.e.* eosinophil cationic protein production) [25] and asthma prevalence [37]. This evidence supports the presence of an interaction between upper and lower airway inflammation by neuronal reflex. However, all Type 2 inflammation-related biomarkers of lower airways except for sputum periostin did not decline after ESS. Furthermore, the presence of moulds/SEs did not improve clinical outcomes or change levels of Type 2 inflammation-related biomarkers 1 year after ESS. Although we did not evaluate levels of moulds/SEs-IgE in NPs, it remains unclear whether ESS produces changes in levels

of serum moulds/SEs-IgE. These results indicate that systemic allergen sensitisation induces Type 2 inflammation in the nose and lower airways separately. Neuronal reflex through local allergen sensitisation may not be the major cascade in the development of Type 2 airway inflammation throughout the airways. Indeed, treatment of CRS does not always improve asthma outcomes [3]. Furthermore, most Type 2 inflammation-related biomarkers declined in number after ESS in the moulds/SEs^{+} group. Sample size may not be sufficient to clarify the impact of moulds/SEs on longitudinal changes in clinical outcomes and Type 2 inflammation-related biomarkers in CRS patients who underwent ESS. Further larger cohort studies are necessary to elucidate how mould/SE sensitisation affects clinical symptoms and inflammation of the nose and lower airways after ESS.

There are some limitations to the present study. First, we did not evaluate local levels of IgE against moulds/SEs in either sputum or sinus samples due to the lack of storage samples. Therefore, the association between lower threshold ($\geqslant$0.10 UA·mL^{-1}) of serum IgE titres and local sensitisation to allergens remains to be elucidated. However, the present study provides clinicians with data for the clinical importance of sensitisation to moulds/SEs ($\geqslant$0.10 UA·mL^{-1}) as indicators of the presence of Type 2 inflammation and comorbid asthma in CRS patients. Both otorhinolaryngology and respirology specialists could potentially identify comorbid asthma or eosinophilic CRS by evaluating specific IgE against moulds/SEs. Second, our cohort was enriched with the more severe CRS patients who required ESS. Therefore, the present findings might not be applicable to patients with mild/moderate CRS or those responsive to treatment, who are underrepresented in this study. Therefore, we need to compare the impact of mould/SEs sensitisation ($\geqslant$0.10 UA·mL^{-1}) on Type 2 inflammation-related biomarkers and clinical outcomes between severe and non-severe CRS patients in future studies. Finally, follow-up of patients ceased 12 months after ESS. Therefore, the association of sensitisation to moulds/SEs ($\geqslant$0.10 UA·mL^{-1}) with long-term prognosis for asthma and CRS after ESS remains unclear. Further longitudinal studies are necessary to clarify the clinical implications of sensitisation to moulds/SEs determined by $\geqslant$0.10 UA·mL^{-1} in terms of the pathophysiology of asthma and sinusitis.

In conclusion, moulds/SEs are important allergens in the development of comorbid asthma and eosinophilic CRS and in inducing Type 2 inflammation in CRS patients. A lower threshold value of these allergens ($\geqslant$0.10 UA·mL^{-1}) would be meaningful to evaluate Type 2 inflammation and clinical outcomes in CRS patients. Further studies are warranted to clarify the clinical implications of mould/SE sensitisation as determined by a threshold of $\geqslant$0.10 UA·mL^{-1} in the pathophysiology of asthma and CRS.

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