Original articles

Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma

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Background: Nasal polyps often are associated with asthma. The phenotype of these patients is unknown.
Objective: To identify the mucosal factors associated with asthma comorbidity, we analyzed the inflammatory patterns of nasal polyps.
Methods: Nasal polyps from 70 Belgian patients, 34% with asthma, were analyzed for type of inflammation, T-cell cytokines, and IgE antibodies to *Staphylococcus aureus* enterotoxins. The same investigations were repeated in 93 Chinese patients with polyps, a group with a low asthma comorbidity rate (8%).
Results: In Belgian patients with polyps, 54% of samples showed eosinophilic inflammation. A classification tree evaluation identified IL-5 as the main positive determinant. Enterotoxin IgE in tissue (37%) was associated with significantly increased total IgE and eosinophil cationic protein concentrations. Expression of enterotoxin IgE, total IgE at greater than 1,442 kU/L, and eosinophil cationic protein at greater than 17,109 μg/L in samples with a total IgE concentration of greater than 246 kU/L significantly predicted asthma (odds ratio, 5.8-13). Only 7.5% of the samples from Chinese patients with polyps showed eosinophilic inflammation. IL-5 was confirmed as a positive determinant of eosinophilic inflammation, and enterotoxin IgE in tissue (17% of patients) was associated with significantly increased total IgE and eosinophil cationic protein concentrations. The expression of IL-5 or total IgE at greater than 790 kU/L in samples with an IL-5 concentration of greater than 194 pg/mL significantly predicted comorbid asthma (odds ratio, 17.2-96).
Conclusion: Mucosal inflammation in nasal polyps orchestrated by T_H2 cytokines and amplified by *S aureus* enterotoxins is characterized by an increased eosinophilic inflammation and formation of IgE antibodies. This phenotype is associated with comorbid asthma in white and Asian patients with nasal polyps. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: *Chronic rhinosinusitis, nasal polyps, asthma, T-cell cytokines,* Staphylococcus aureus *enterotoxins, IgE*

Chronic rhinosinusitis affects up to 15% of US and European populations and causes considerable impairment of performance and loss of quality of life, as well as high socioeconomic costs.[1,2] It is frequently linked to asthma comorbidity; however, the links are poorly understood.

Chronic rhinosinusitis can be differentiated based on the T-effector cell pattern,[2,3] resulting in eosinophilic or neutrophilic inflammation. In white subjects chronic rhinosinusitis with nasal polyps is considered to be orchestrated by T_H2 cells, with IL-5 as major cytokine, resulting in increased eosinophil survival and an eosinophilic type of inflammation, which might be associated with IgE formation.[1,4] However, the predominant T-effector cell in Asian patients with polyps is the T_H17 cell, with IL-17 as the key cytokine resulting in a predominance of neutrophils.[2] Both types of polyps shared a lack of regulatory T cells and low concentrations of TGF-β1 in comparison with control tissue.[5] Asian and white patients also showed a marked difference in the prevalence of comorbid asthma, with this disease being rare in Chinese and Thai patients.[5] The phenotypes that are associated with asthma comorbidity are unknown thus far.

Enterotoxins derived from nasal *Staphylococcus aureus* might amplify the T_H2 polarization and inhibit regulatory T-cell function and related cytokine production.[6-8] These superantigens are able to activate plasma cells and induce IgG4 and IgE synthesis, resulting in an overexpression of IgE specificities and high total IgE concentrations in the polyp tissue, which has been addressed as polyclonal IgE.[9] IgE antibodies against superantigens detected in tissue homogenates from mucosal tissue serve as a marker of effect of those enterotoxins on the local inflammation.

To assess the inflammatory phenotype linking nasal polyps with asthma, we studied the effect of eosinophilic and neutrophilic types of inflammation and of polyclonal IgE in nasal polyps

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Abbreviations used
ECP: Eosinophilic cationic protein
MPO: Myeloperoxidase
OR: Odds ratio
SE: *Staphylococcus aureus* enterotoxin

of white and Asian patients because differences between the populations might help to elucidate the inflammatory pattern associated with comorbidity.[3] We analyzed the inflammatory patterns in terms of predominance of granulocytes, contribution of key T-cell cytokines, and effect of *Staphylococcus aureus* enterotoxins (SEs).

METHODS

Patients

Nasal tissue and sera were obtained from 70 adult patients (mean age, 47.7 years; age range, 11-73 years; 51 male and 19 female patients) who underwent surgical intervention for the treatment of nasal polyps at the Ear, Nose, and Throat Department, University Hospital Ghent, Ghent, Belgium. Samples were obtained during routine endonasal sinus surgery in consecutive patients scheduled for surgical intervention unrelated to the study. The diagnosis of sinus disease was based on history, clinical examination, nasal endoscopy, and computed tomographic scanning of the sinuses. All patients fulfilled the criteria of bilateral nasal polyps according to the EP^{3}OS guidelines.[1]

The atopic status of all patients was evaluated by screening for IgE antibodies to the most frequent inhalant allergens (birch, timothy, mugwort, cat, dog, horse, mold [*Cladosporium herbarum*], and house dust mite *Dermatophagoides pteronyssinus*]; Phadia, Uppsala, Sweden). The diagnosis of asthma was confirmed by a chest physician according to Global Initiative for Asthma 2006 guidelines based on symptoms and pulmonary function tests.[10] None of the subjects used oral or nasal corticosteroids or antibiotic treatment 4 weeks before the operation.

As an independent cohort, 93 adult Chinese patients with nasal polyps according to the abovementioned criteria (mean age, 37.6 years; age range, 16-63 years; 56 male and 37 female patients) who underwent surgical intervention for the treatment of nasal polyps at the Ear, Nose, and Throat Departments in Zhongshan or Chengdu, China, were investigated in the same way.

The study was carried out after approval by the Ethics Committees of Ghent University Hospital, Belgium; West China Hospital, Sichuan University, Chengdu; and Zhongshan Hospital, China, and written informed consent was obtained from each patient before inclusion in the study.

Cytokine, mediator, and IgE measurements in tissue homogenates

The nasal polyp tissue was processed according to methods already published.[11] Snap-frozen tissues were added to 1 mL of 0.9% NaCl solution per every 0.1 g of tissue. The tissue was then homogenized with a mechanical homogenizer (B. Braun-Melsungen, Melsungen, Germany) at 1,000 rpm for 5 minutes on ice. After homogenization, the suspensions were centrifuged at 3,000 rpm for 10 minutes at 4°C, after which the supernatants were separated and stored at −80°C until analysis for cytokines and mediators. Supernatants were assayed for eotaxin, soluble IL-2 receptor α, IL-5, IFN-γ, IL-10, IL-1β, IL-6, IL-17A, TGF-β1, and myeloperoxidase (MPO) by using commercially available ELISA kits (Quantikine ELISA; R&D Systems, Minneapolis, Minn; MPO was from Oxis International, Portland, Ore). Concentrations of eosinophilic cationic protein (ECP), total and specific IgE to allergens, and SE were measured by using the UniCAP system (Phadia, Uppsala, Sweden). An ECP/MPO ratio was calculated (cutoff value for "eosinophilic" inflammation = 1). Specific IgE was determined for a mixture of SEA, SEC, and toxic shock syndrome toxin 1, which proved to be both more sensitive than the single enterotoxins and highly specific (cutoff = 0.1 kU/L).[11] No nonspecific IgE reactivity to the SE mixture was found for non–antibody-active IgE (E myeloma) at concentrations of up to 1,000 kU/L. Samples positive for IgE antibodies to the mixture tested negative to a control CAP test without enterotoxins bound.

Statistical analysis

Variables in the dataset were analyzed as continuous or binary when appropriate. Issues of values below the detection limit made grouping observations as below the detection limit and not below the detection limit necessary. As an exploratory analysis, classification trees were built[12] and supported by random forests.[13] These 2 statistical approaches were used to analyze several variables simultaneously to select the best variables that distinguish between 2 groups of interest (eg, presence vs absence of comorbid asthma). In addition, they organize the use of these most distinguishing variables in sequence to best separate groups of interest. Variables used in classification tree analysis can be analyzed as categorical variables (eg, presence or absence of IL-5 in polyps, see detection limits in Table E1 in this article's Online Repository at www.jacionline.org) or as continuous variables (eg, IL-5 in polyps expressed as picograms per milliliter). In the latter case the analysis selects a variable and its cutoff value that best distinguishes between the 2 groups. Random forests were constructed to support the classification trees, allowing investigation of consistency in results. The importance of the variables in the dataset can be measured by using importance scores[14]; here we used the mean decrease in Gini index. The ranking of an index indicates its importance; the 3 most important predictors for an ECP/MPO ratio of greater than 1 and asthma comorbidity are shown in Table E2 in this article's Online Repository at www.jacionline.org.

For further explanation, please refer to the Methods section in this article's Online Repository at www.jacionline.org.

When comparing the medians of concentrations between 2 groups, nonparametric statistical analysis was performed with the Mann-Whitney *U* 2-tailed test for unpaired comparisons. Each time, the significance level was specified as an α value of .05.

All analyses for the classification trees and the random forests were performed in R (R Development Core Team, http://www.r-project.org/). Odds ratios (ORs) were calculated with the package epitools in R by using a small sample adjustment on the normal approximation of the OR and corresponding confidence limits; ORs might therefore not be equidistant to the lower and upper bounds of the CI.

RESULTS

Demographic characteristics of the patients

Patient groups from Belgium and China did not differ in terms of age, sex distribution, allergic disease comorbidity, smoking habits, severity of disease judged by computed tomographic scan, and nasal endoscopy (see Table E3 in this article's Online Repository at www.jacionline.org). However, as predicted, they significantly differed in the number of patients with asthma comorbidity, with significantly fewer in the Chinese population (34% vs 9%, $P < .01$).

The patient's age, sex, atopic status, or smoking habits did not predict the T-cell phenotype or the type of inflammation (eosinophilic vs neutrophilic) in any of the populations.

Inflammatory patterns of nasal polyps

When a cytokine is measurable in nasal polyp tissue homogenates, we report the sample as positive for this cytokine (for detection thresholds, see Table E1). Cytokine patterns in nasal polyps of Belgian and Chinese patients showed great differences, with 83% of the polyp samples being IL-5 positive in the Belgian population but only 16% in the Chinese population. Forty-three

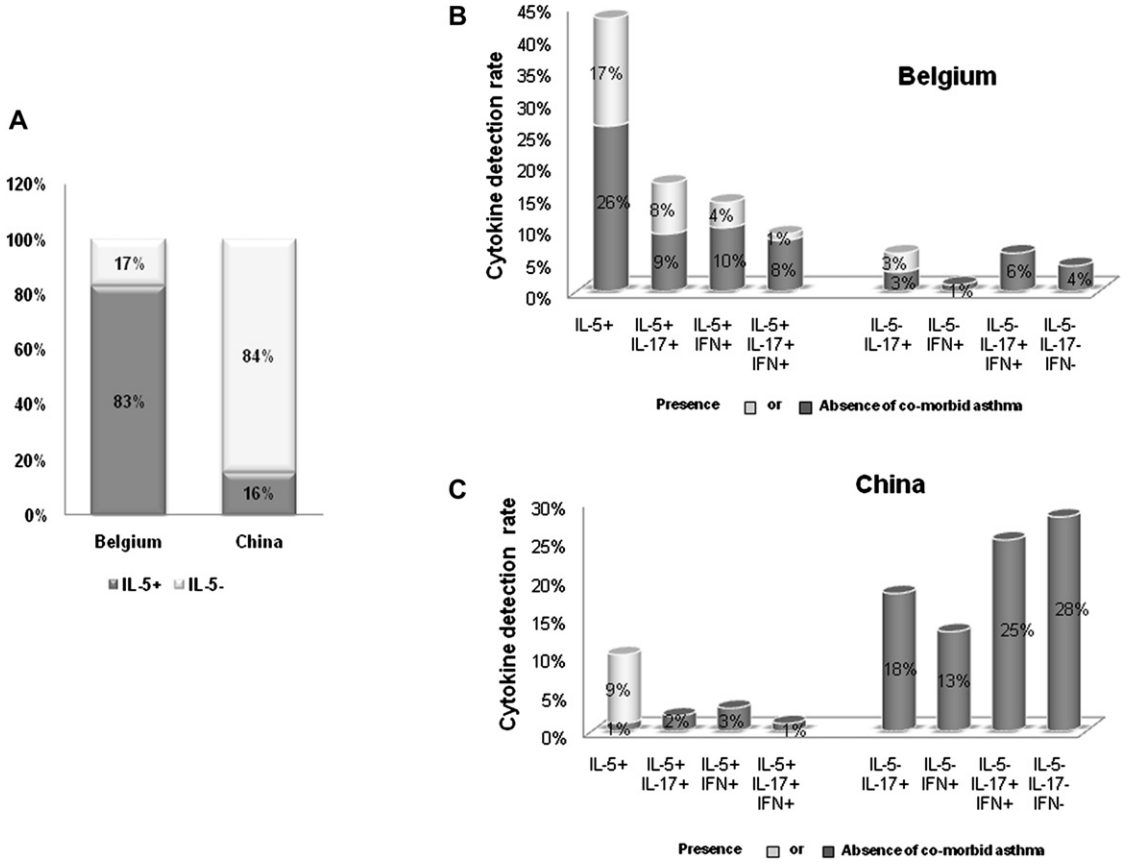

FIG 1. Cytokine expression in nasal polyp tissue homogenates: differentiation of 70 Belgian and 93 Chinese nasal polyp samples. **A**, Eighty-three percent of Belgian but only 16% of Chinese polyp samples were IL-5 positive. **B** and **C**, The distribution of cytokine patterns and asthma comorbidity is provided for Belgian (Fig 1, *B*) and Chinese (Fig 1, *C*) patients with polyps. The presence *(gray)* or absence *(black)* of comorbid asthma is indicated for each group.

percent of Belgian samples expressed IL-5 but no IFN-γ or IL-17 protein; 28% of all Chinese samples expressed none of the proteins IL-5, IFN-γ, or IL-17. The expression pattern of T-cell cytokines in all samples is demonstrated in Fig 1.

Correlations between inflammatory patterns

The effect of the expression of key T_H cytokines on other variables measured in the tissue was evaluated. In the Belgian and Chinese polyps the presence of IL-5 protein in tissue was associated with significantly increased concentrations of ECP, IgE, and SE IgE but significantly decreased concentrations of IL-1β and IL-17 (see Table E4 in this article's Online Repository at www.jacionline.org). The presence of IL-17 protein in tissue was associated with significantly increased concentrations of IL-1β, IL-6, and MPO but decreased concentrations of IL-5 protein (reaching significance in Chinese patients only). Finally, measurable IFN-γ protein was associated with significantly increased concentrations of IL-1β protein but significantly decreased concentrations of IL-5 protein in both populations.

In the Belgian population we detected SE IgE in 25 of 58 tissue samples in the IL-5–positive group but only 1 patient in the IL-5–negative group (1/12). The presence of SE IgE was associated with significantly increased total IgE and ECP concentrations in tissue ($P < .01$ for both parameters).

As in the Belgian patients with polyps, the presence of SE IgE (16/93) was also associated with significantly increased total IgE and ECP tissue concentrations in the Chinese population ($P < .01$ for both parameters, see Table E4).

In both the Belgian and Chinese populations, total IgE concentrations were significantly higher in tissue compared with that seen in sera. This was especially true for the SE IgE–positive samples in both groups and for total IgE and SE IgE concentrations (see Table E5 in this article's Online Repository at www.jacionline.org). These observations support the suggestion of a local IgE production.

Differentiation of disease by T_H cytokine pattern

We designed a classification tree and searched for parameters that would be predictive for the typical eosinophilic inflammation seen in patients with polyp disease based on the ratio of the eosinophil-derived ECP to the neutrophil-derived MPO protein concentration in tissue homogenates. The mean ECP/MPO ratio was 3.5 ± 6.0 (SD) for the Belgian population and 0.3 ± 0.5 (SD) for the Chinese population.

The categorical approach identified IL-5 protein as the main positive determinant in Belgian patients and IL-17 as the main negative marker (Fig 2, *A*). Two high-risk groups were observed with ORs of 14 (95% CI, 3.3-131.4) and 8.8 (95% CI, 1.9-112.4;

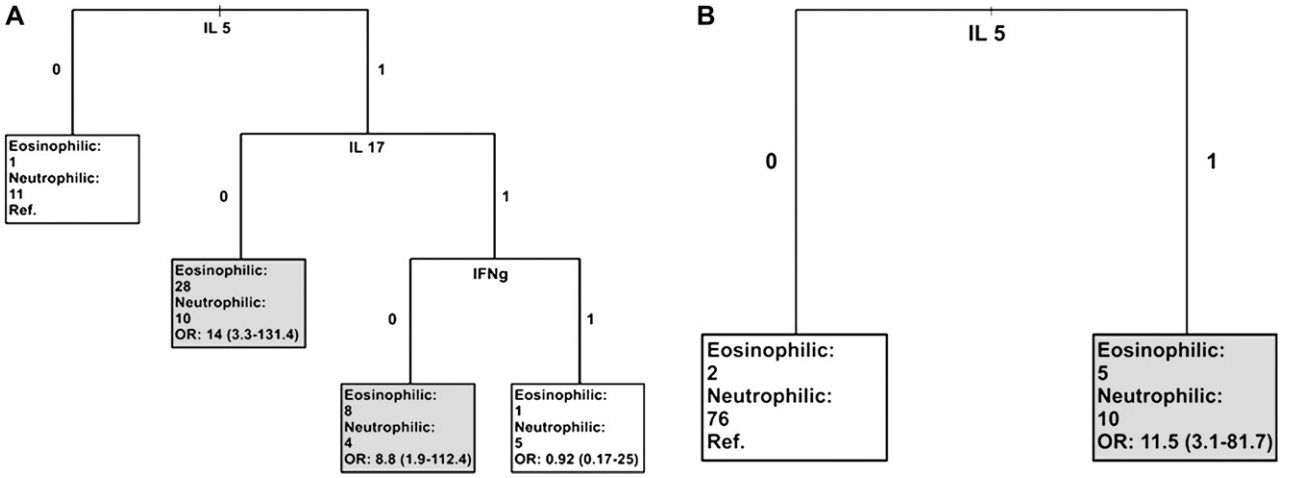

FIG 2. Classification tree for the presence of eosinophilic mucosal inflammation expressed as ECP/MPO ratio (cutoff value of the ratio = 1). Eosinophilic inflammation was defined as an ECP/MPO ratio of greater than 1 and neutrophilic inflammation as a ratio of 1 or less: Belgian (n = 68; **A**) and Chinese patients (n = 93; **B**) with polyps for categorical determinants. The presence or absence of the respective cytokine in polyp tissue is referred to as "1" and "0." The OR is given with 95% CIs in comparison with the IL-5 protein–negative group as reference.

significantly different to 1), respectively, both expressing IL-5 protein with either a lack of IL-17 protein, presence of IFN-γ protein, or both.

Although the expression pattern of T-effector cell cytokines in Chinese samples was remarkably different from that seen in Belgian patients with polyps, IL-5 protein again was identified as the main positive determinant for an eosinophilic type of inflammation (ECP/MPO ratio > 1; Fig 2, *B*). For the ECP/MPO ratio, sensitivity and specificity of the predictors found for the Belgian group and tested in the Chinese patients were 0.71 and 0.9, respectively, confirming the findings of the Belgian patients.

The random forest analysis confirmed the classification tree data for both Belgian and Chinese groups (see Table E2). In the Belgian data IL-5, IL-17, and IFN-γ are the 3 parameters with the largest mean decrease in Gini index (in this order). This supports the presence of IL-5 in the root of the tree and IL-17 and IFN-γ as the subsequent splits in the classification tree (Fig 2, *A*). For the Chinese patients, the same cytokines were identified as the top 3 parameters. These results again support IL-5 as the root in the corresponding classification tree (Fig 2, *B*). The reason that only IL-5 appears in the classification tree for the Chinese samples is that of the 93 subjects included in the study, only 7 had a ratio of greater than 1. Only 1 variable in the tree is needed to distinguish between patients having a ratio equal to 0 or 1.

Amplification of T_H2-based inflammation by SE IgE and asthma comorbidity

A classification tree was developed for the parameter of comorbid asthma; the categorical approach identified SE IgE as positive and IFN-γ as negative determinants in Belgian patients (Fig 3, *A*). Two groups at high risk were observed, with ORs of 5.8 (95% CI, 1.8-29.6) and 5.1 (95% CI, 1.4-38), respectively: SE IgE–positive patients with polyps and SE IgE–negative/IFN-γ–negative/IL-17–positive subjects. The continuous approach found total IgE values of greater than 1,442 kU/L and ECP values of greater than 17,109 μg/L (in samples with IgE values between 246 and 1,442 kU/L) as the main positive predictors (ORs of 13 [95% CI, 2.21-305] and 8 [95% CI, 1.3-256] respectively; significantly higher than 1). It also identified a second IgE-low, ECP-low, IL-6–high risk group (OR of 3.7 [95% CI, 0.6-90.6] vs reference; Fig 3, *B*). The prevalence of asthma comorbidity in the Belgian nasal polyp group was 34% overall. However, the prevalence of comorbid asthma was significantly increased among those subjects who had SE IgE positivity in nasal polyp tissue (57%) versus those with SE IgE negativity (20%, $P < .01$; the OR adjusted for small sample size is 4.8 vs reference [95% CI, 1.9-15.9]). Atopy and asthma were associated with significantly increased tissue IgE concentrations; however, only asthma was associated with significantly increased ECP and SE IgE concentrations in nasal polyp tissue.

In the Chinese patient group the classification tree analysis for the parameter of comorbid asthma identified IL-5 protein in the categorical approach with an OR of 52.3 (95% CI, 9.9-794; Fig 4). The continuous approach confirmed an IL-5 protein concentration of 194 pg/mL or greater as the single positive determinant (OR, 96 [95% CI, 16.6-1,990]) and furthermore that a total IgE concentration of greater than 790 kU/L in combination with an IL-5 protein concentration of 194 pg/mL or greater significantly increased the risk of comorbid asthma (OR of 17.2 [95% CI, 9.9-794]). All 8 patients with asthma expressed IL-5 protein in the mucosal tissue; no IL-5 protein−negative patient had asthma. Five of 8 mucosal samples from patients with asthma were SE IgE positive. For comorbid asthma, sensitivity and specificity of the categorical predictors found for the Belgian group and generalized to the Chinese group were 0.6 and 0.7, and for continuous data, they were 0.8 and 0.7, respectively.

In the Belgian data the random forests analysis revealed as the top 3 parameters predictive of asthma comorbidity SE IgE, IFN-γ, and IL-17 for the categorical approach and IgE, ECP, and IFN-γ for the continuous approach (see Table E2). For the categorical approach (Fig 3, *A*), this confirms the presence of SE IgE as the root of the classification tree and of IFN-γ and IL-17 as the subsequent splits. For the continuous approach (Fig 3, *B*), the

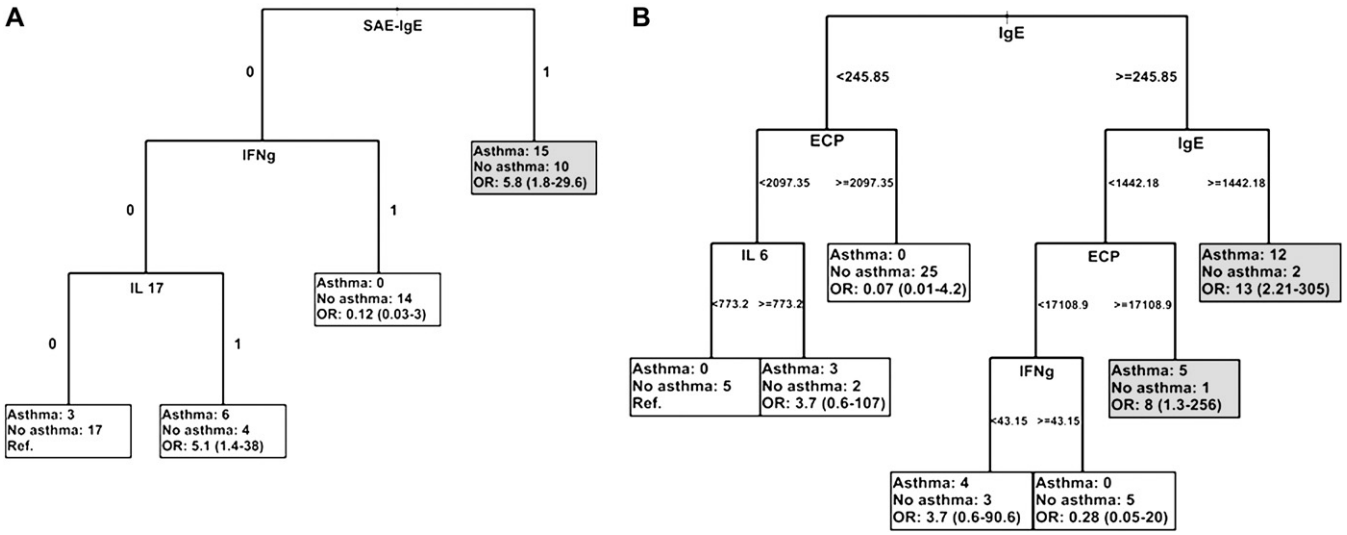

FIG 3. Classification tree for comorbid asthma in the Belgian population: categorical (n = 69; **A**) and continuous classifying determinants (n = 66; **B**; 3 samples without detectable IgE). The presence or absence of the respective parameter is referred to as "1" and "0." ORs with 95% CIs in comparison with the SE IgE–negative, IFN-γ–negative, IL-17–negative group in Fig 3, *A*, and the IgE-low, ECP-low, IL-6–low group in Fig 3, *B*, are shown as a reference.

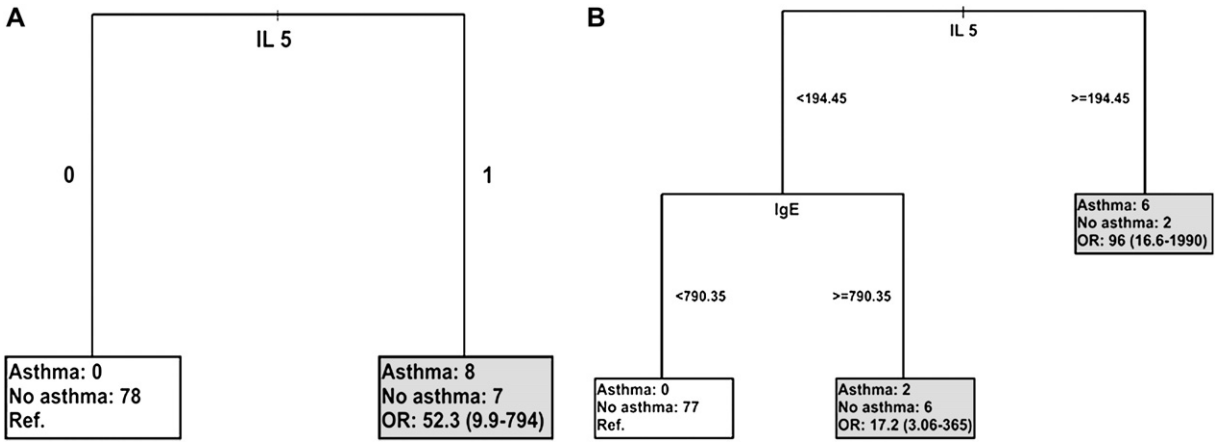

FIG 4. Classification tree for comorbid asthma in the Chinese population: categorical (n = 93; **A**) and continuous classifying determinants (n = 93; **B**). The presence or absence of the respective parameter is referred to as "1" and "0." ORs with 95% CIs in comparison with the IL-5–negative group in Fig 4, *A*, and the IL-5–low, IgE-low group in Fig 4, *B*, are shown as a reference.

situation is more complex because the left and right child nodes of the root have further splits. However, it is clear from the classification tree that IgE is the most important variable because it is the root of the tree and that ECP is the second most important variable because it is the next split in the left and right child node of the tree. Therefore the classification tree and random forest analysis do not contradict each other.

Similarly, for the Chinese data, the order of variable importance (from high to low) supported by random forest analysis was IL-5, SE IgE, and IFN-γ for the categorical and IL-5, IgE, and ECP for the continuous approach (see Table E2). For the categorical approach (Fig 4, *A*), this supports the presence of IL-5 as the root of the classification tree. After IL-5, SE IgE and IFN-γ are the most important predictors for asthma according to the random forest analysis. Although they do not appear in the classification tree (Fig 4, *A*), we observe the same order of importance in prediction capability in the classification tree of the Belgian data (Fig 3, *A*). For the continuous approach to the Chinese data (Fig 4, *B*), the presence of IL-5 as the root of the classification tree and of IgE as the second split is confirmed in the random forest analysis. Although ECP is not present in the classification tree (Fig 4, *B*), we observe again the same order of importance in terms of prediction in the classification tree for the Belgian data (Fig 3, *B*).

DISCUSSION

Our study confirms the heterogeneity of mucosal inflammation in patients with chronic rhinosinusitis and identifies a T_H2-biased inflammation and its amplification by SEs, for which we used tissue IgE antibodies to classical enterotoxins as marker

determinants for comorbid asthma in Belgian patients with nasal polyp disease. Sensitivity and specificity values of the predictors found for the Belgian group and generalized to the Chinese patients confirmed the role of those determinants. Thus we here identify a specific phenotype within patients with nasal polyps that is characterized by asthma comorbidity.

The clinical methods used here follow the current guidelines for chronic rhinosinusitis and asthma diagnosis.[1,10] The measurement of cytokines in mucosal samples has been demonstrated to differentiate disease subtypes,[4] and the appropriateness of the SE mixture has been established.[11] The ECP/MPO ratio was used to describe the eosinophilic or neutrophilic types of inflammation because both mediators reflect the activation and not the mere presence of eosinophilic or neutrophilic granulocytes; the possible dissociation between the presences of eosinophils and their activation status in nasal polyps has been demonstrated recently.[3]

For the first time, we demonstrate that the 3 major T-effector cell cytokines described until recently (IFN-γ, IL-5, and IL-17) can coexist in the upper airway mucosa; their presence and pattern affect and differentiate mucosal inflammation and suggest at least 2 distinct molecular mechanisms. Whereas T_H1 and T_H2 cells and their functions are well characterized, IL-17–producing T_H17 cells were only recently introduced as a separate lineage, orchestrating neutrophilic inflammation and enlarging the T-cell immunology picture.[15,16] We recently described T_H17 as the predominant T-effector cells in a group of Asian patients with polyps, resulting in the predominance of neutrophil granulocytes.[3,5] Here we show that IL-17 also is expressed in individual white patients with nasal polyps, whereas IL-5 protein is found in a minority of Asian patients with polyps only. Although the expression pattern of T-effector cell cytokines in Chinese and Belgian polyp samples were remarkably different, with only 16% of the patients expressing IL-5 protein in the Chinese population versus 83% in the Belgian population, IL-5 protein was identified as the main positive determinant for eosinophilic inflammation in both groups.

Our results suggest that upper airway disease can be amplified by *S aureus*–derived superantigens, leading to the formation of specific IgE antibodies to SEs in the tissue in approximately 37% of the Belgian patients; the prevalence was lower in Chinese patients with polyps (17%). The presence of SEs in both polyp groups was associated with a significant increase in the eosinophilic inflammation marker ECP and a massive increase in the synthesis of polyclonal IgE antibodies within mucosal tissues; consequently, IgE antibodies to SEs might serve as a marker of the effect of superantigens on mucosal inflammation. The significantly higher concentrations of total IgE and SE IgE in tissues versus sera in both the Belgian and the Chinese patients support the suggestion of a local immune response.[9]

However, this amplification of the inflammatory pattern only can be seen in the IL-5 protein–positive subgroup in both populations. Of great interest, we here also show that the presence of SE IgE in the polyp mucosal tissue bears a significant risk of asthma for the white population (OR of 4.8, categorical data analysis). Asthma comorbidity was 57% in SE IgE–positive versus 20% in SE IgE–negative patients with polyps (all IL-5–positive polyps, $P < .01$) and 75% versus 17% in the only IL-5–positive polyp type ($P < .01$). This link was confirmed by means of continuous data analysis, with excessive IgE (>1,442 kU/L) and ECP (>17,109 pg/mL) concentrations in tissue as determinants (ORs of 13 and 8 vs reference), with both parameters being significantly increased as a consequence of the effect of SEs. In contrast, as demonstrated in Fig 3, the presence of IFN-γ in the absence of SE IgE (categorical data) and low concentrations of ECP and IgE (continuous data) showed a decreased OR (0.1 and 0.1, respectively).

As reported before, asthma comorbidity in the Chinese population studied here was low. The risk constellation for comorbid asthma in the Chinese population, although showing a clearly different inflammatory and clinical phenotype than the white population, confirmed the eosinophilic inflammation as background (IL-5 protein) and the amplification of the IgE synthesis (total IgE >790 kU/L) as the second determinant (OR, 17.2-96). All 8 Chinese patients with asthma expressed IL-5 protein, and 5 of 8 mucosal samples from patients with asthma were SE IgE positive. The presence of SE IgE was again associated with significantly increased total IgE and ECP concentrations, whereas in IL-5–negative samples, the presence of SE IgE was associated with an amplification of the IL-17–associated IL-1β protein only.

Although comprising 163 patients with nasal polyps, one limitation of our study is the limited number of patients with respect to the comorbidity question. In particular, the number of Chinese patients with IL-5 expression in the nasal polyp homogenates was small, leading to uncertainty in estimating the cutoff (branching) values, as evidenced by their wide 95% CIs. Further studies with cluster analysis approaches should certainly aim to include considerably higher numbers of patients and will have to make use of collaborative networks. However, combining the information of the 2 datasets (ie, Belgian and Chinese patients with nasal polyps) and combining 2 statistical approaches (ie, classification trees and the Random Forests), one can conclude that there is an extensive overlap between these 2 datasets, which does support the findings.

Products of *S aureus* have been demonstrated to heavily activate T and B lymphocytes, as well as other inflammatory cells,[6,8] and to bias the T-cell pattern toward T_H2 and against regulatory T cells.[7,8] We have recently shown that SEB and *Staphylococcus* protein A contributes to T-cell activation and mast cell degranulation in upper airway disease, respectively.[8] Superantigens have the potential to induce IgE synthesis, resulting in an overexpression of various IgE specificities and high total IgE concentrations in the polyp tissue.[9] We speculate that the polyclonal IgE formed in the polyp tissue might further contribute to chronic inflammation by IgE-related or independent mast cell degranulation and that anti-IgE treatment might be especially effective in patients with nasal polyp disease with polyclonal IgE formation[17]; however, this still needs to be demonstrated.

In conclusion, we have shown that SEs significantly modify the severity of upper airway inflammation in a subgroup of patients with upper airway disease, specifically those with IL-5 protein–expressing nasal polyps. This inflammatory phenotype also carries a considerable risk of comorbid asthma.

Clinical implications: Nasal polyps can be differentiated on the basis of tissue IL-5 protein and IgE antibodies to staphylococcal enterotoxins because these parameters are related to type of inflammation and asthma comorbidity.

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METHODS

Statistical analysis

Two statistical approaches were used to analyze several variables simultaneously to select the best variables that distinguish between 2 groups of interest (eg, presence vs absence of comorbid asthma). In addition, they organize the use of these most distinguishing variables in sequence in an algorithm to best separate the 2 groups of interest. Variables used in classification tree analysis can be analyzed as categorical variables (eg, presence or absence of IL-5 in polyps) or as continuous variables (eg, IL-5 in polyps expressed as picograms per milliliter). In the latter case the analysis selects a variable and its cutoff value that best distinguishes between the 2 groups.

Classification tree analysis is a data-mining tool used to predict membership of cases or objects in the classes of a categorical dependent variable (eg, asthma) from their measurements on 1 or more predictor variables. They are typically used to construct a hierarchical system for sorting potential predictor variables. In these analyses the ECP/MPO ratio (cutoff value = 1) and asthma were used as response variables. After building the trees, they were pruned to avoid overfitting. Overfitting occurs when a tree characterizes too much detail and noise of the training data on which it is built. Therefore when overfitting is not properly addressed (eg, by pruning the tree), generalizability of obtained trees to new data will be poor. Pruning was based on using a cost-complexity parameter of 2.5. A balance was sought while relaxing this assumption to maintain sufficient structure in the trees or while enforcing additional pruning of children's nodes that showed no variance in the predicted classes. For each tree, we defined different groups of subjects according to the terminal nodes of the tree. One of these groups was chosen as the reference group for that particular tree. ORs were calculated for all other terminal nodes of the tree in comparison with the reference group together with their 95% CIs. These ORs were adjusted for small sample sizes. It is generally known that a small perturbation in the input variables or new samples can lead to very different classification trees. Therefore to support the classification trees, random forests[E1] for both the Belgian and Chinese data were constructed. As the name indicates, random forests constitute a collection of classification trees, allowing investigation of the consistency in results. In this type of analysis, every tree is constructed on a bootstrap sample of the data and is unpruned so that a random forest does not suffer from overfitting.

Moreover, the importance of the variables in the dataset can be measured based on certain important scores. One such evaluation criterion is the mean decrease in Gini index. It measures what is called the node purity of a split for every node in a tree. For binary classification problems (ie, settings in which there are 2 outcomes, such as 0 and 1), the Gini index is presented as 2p0p1, where p1 is the proportion of subjects having outcome 1 at that node and p0 = 1 − p1. When this Gini index for the 2 descendent nodes is less than that of the parent node, a split of the node is made on the variable. Adding up the Gini index decreases for a particular variable over all trees in the forest gives an idea about the importance of the variable. The higher the sum of decreases, the more important the variable.[E2] The Gini index can be used to produce a ranking of the variables in terms of importance for prediction of the outcome (eg, asthma). The ranking of the indices should be interpreted rather than the value itself. Therefore the 3 most important predictors for an ECP/MPO ratio of greater than 1 and asthma comorbidity are shown in Table E2.

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TABLE E1. List of detection limits of the assays used for this study

	Detection limit (ELISA)	Detection limit (after sample/handling dilution)
IL-5	0.6 pg/mL	7.0 pg/mL
IL-17	1.3 pg/mL	14.1 pg/mL
IL-1β	0.9 pg/mL	9.4 pg/mL
IL-6	1.8 pg/mL	20.1 pg/mL
IFN-γ	7.8 pg/mL	42.9 pg/mL
MPO	2.5 ng/mL	13.8 ng/mL
IgE	0.35 kU/L	1.9 kU/L
SAE IgE	0.35 kU/L	1.9 kU/L
ECP	2.0 μg/L	11.0 μg/L

TABLE E2. Random forest analysis for the Belgian and Chinese patient groups for an ECP/MPO ratio of greater than 1 and asthma comorbidity, specifying the importance of the variables in terms of prediction

Three most important predictors (Belgian patients)	Mean decrease in Gini index	Three most important predictors (Chinese patients)	Mean decrease in Gini index
ECP/MPO ratio >1, categorical values			
IL-5	5.0	IL-5	2.6
IL-17	3.2	IFN-γ	0.5
IFN-γ	1.8	IL-17	0.4
Asthma comorbidity: categorical values			
SE IgE	4.0	IL-5	5.6
IFN-γ	2.0	SE IgE	1.1
IL-17	1.3	IFN-γ	0.9
Asthma comorbidity: continuous values			
IgE	7.6	IL-5	4.1
ECP	5.8	IgE	2.9
IFN-γ	2.8	ECP	1.4

TABLE E3. Demographic characteristics of Belgian and Chinese patients with chronic rhinosinusitis with nasal polyps

	Chinese patients with nasal polyps	Belgian patients with nasal polyps	Nonparametric group comparisons
No. of patients	93	70	
Age (y [range])	37.6 (18-63)	47.7 (11-73)	NS
Female/male sex	37/56	19/51	NS
Asthma	8/93 (8.6%)	24/70 (34.3%)	$P < .01$
Continuous inhalant steroid use in asthmatic subjects	8/8 (100%)	19/24 (79.2%)	NS
FEV_1 (% predicted) in asthmatic subjects	69.9 ± 14.5	67.2 ± 12.8	NS
Phadiatop positive	39/93 (41.9%)	28/70 (40%)	NS
Computed tomographic score (Lund and Mackay)	11.6 (7-23)	12 (10-21)	NS
Polyp score (Davos)	5 (4-6)	4 (4-6)	NS
Total symptom score* (maximum 15)	10 (6-12)	9 (7-11)	NS
Nasal congestion	3 (2-3)	3 (2-3)	NS
Rhinorrhea	3 (1-3)	1 (0-2)	NS
Sneezing	1 (0-3)	0 (0-2)	NS
Loss of smell	2 (2-3)	3 (2-3)	NS
Headache	2 (0-3)	2 (1-2)	NS
Smoker	16/93 (17.2%)	5/70 (7.1%)	NS

Chinese patients significantly differed in the number of patients with asthma comorbidity, with significantly fewer compared with the Belgian population with polyps ($P < .01$). Data are expressed as medians and interquartile ranges. The level of significance was obtained by using the Fisher exact test for categorical outcomes and the Mann-Whitney test for quantitative outcomes and set at an α value of .05.

*The total symptom scores is the sum of the scores for nasal congestion, rhinorrhea, headache, postnasal drip, loss of smell, and sneezing.

TABLE E4. Concentrations of cytokines and inflammatory markers based on the presence or absence of IL-5, IL-17, IFN-γ, and SE IgE antibodies in nasal polyp tissue homogenates from Belgian (A) and Chinese (B) patients

A

	IL-5 positive (n = 58)	***P* value**	**IL-5 negative (n = 9)**
IgE (kU/L)	410.6 (177.8 to 1573.5)	$P < .01$	61.2 (6.7 to 111.8)
SE-IgE (patients)	25/58 (43%)	$P = .03$	1 (11%)
ECP (μg/L)	7375.6 (4984.3 to 15114.3)	$P < .01$	2037.5 (1168.7 to 2891.7)
IL-1β (pg/mL)	44.2 (12.0 to 142.9)	$P < .01$	191.0 (87.2 to 384.7)
IL-17 (pg/mL)	14.3 (14.0 to 41.1)	$P < .01$	111.1 (64.7 to 170.9)
	IL17 positive (n = 26)	***P* value**	**IL17 negative (n = 41)**
MPO (ng/mL)	8195.1 (3066.9 to 15476.1)	$P < .01$	2937.9 (1445.1 to 7056.4)
IL-1β (pg/mL)	158.6 (68.5 to 397.1)	$P < .01$	26.5 (9.4 to 61.4)
IL-6 (pg/mL)	857.7 (470.0 to 3503.9)	$P = .02$	498.6 (248.6 to 903.1)
	IFN positive (n = 21)	***P* value**	**IFN negative (n = 46)**
MPO (ng/mL)	8061.9 (2936.6 to 15677.0)	$P = .01$	3225.9 (1648.9 to 7420.8)
TGFβ1 (pg/mL)	11736.3 (8032.5 to 19629.8)	$P < .01$	5550.3 (4226.7 to 9586.0)
IL-1β (pg/mL)	100.9 (27.6 to 450.3)	$P < .05$	50.4 (12.0 to 112.0)
IL-5 (pg/mL)	149.2 (20.7 to 266.1)	$P < .01$	422.4 (186.9 to 799.5)
	SE-IgE positive (n = 25)	***P* value**	**SE-IgE negative (n = 42)**
IgE (kU/L)	1274.8 (632.5 to 2681.1)	$P < .01$	173.3 (95.7 to 244.7)
ECP (μg/L)	12192.2 (8084.8 to 20163.2)	$P < .01$	4743.3 (2588.0 to 7304.3)

B

	IL-5 positive (n = 15)	***P* value**	**IL-5 negative (n = 78)**
IgE (kU/L)	1140.8 (325.7 to 1918.9)	$P < .01$	80.6 (38.4 to 154.0)
SE-IgE (patients)	8 (53%)	$P < .01$	8 (10%)
ECP (μg/L)	5099.7 (2544.5 to 11843.8)	$P < .01$	1023.3 (488.2 to 2173.8)
IL-1β (pg/mL)	30.7 (27.7 to 115.3)	$P < .01$	143.3 (61.5 to 350.5)
IL-17 (pg/mL)	7.3 (7.3 to 7.3)	$P = .03$	32.5 (7.3 to 132.6)
	IL17 positive (n = 45)	***P* value**	**IL17 negative (n = 48)**
MPO (ng/mL)	9495.0 (5783.3 to 17226.5)	$P = .01$	20554.8 (8369.8 to 69586.2)
IL-1β (pg/mL)	231.8 (141.5 to 516.3)	$P < .01$	49.1 (27.7 to 109.4)
IL-6 (pg/mL)	646.6 (328.4 to 1706.2)	$P < .01$	208.4 (52.8 to 1231.2)
IL-5 (pg/mL)	9.8 (7.0 to 10.0)	$P < .01$	59.3 (7.0 to 61.4)
	IFN positive (n = 39)	***P* value**	**IFN negative (n = 54)**
IL-1β (pg/mL)	210.2 (97.3 to 560.3)	$P < .01$	81.6 (28.5 to 190.7)
IL-5 (pg/mL)	7.0 (6.9 to 10.0)	$P < .01$	52.1 (9.8 to 61.1)
	SE positive (n = 16)	***P* value**	**SE negative (n = 77)**
IgE (kU/L)	406.3 (88.8500 to 969.0)	$P < .01$	86.4 (44.0 to 171.2)
ECP (μg/L)	2579.5 (1463.0 to 5099.7)	$P < .01$	1015.6 (496.4 to 2066.7)

TABLE E5. Concentrations of IgE and SE IgE for Belgian and Chinese patients in tissue and serum

All samples (Belgian patients)	**Serum (n = 70)**	***P* value**	**Tissue (n = 70)**
IgE (kU/L)	123.5 (46-211)	<.01	241.6 (117.8-113.7)
SE IgE–positive samples (Belgian patients)	**Serum (n = 22)**	***P* value**	**Tissue (n=22)**
IgE (kU/L)	313 (172.4-789.5)	<.01	1,052 (554.6-2,860.6)
SE IgE	1.57 (0.8-5.2)	<.01	10.1 (5.9-20.4)
All samples (Chinese patients)	**Serum (n = 87)**	***P* value**	**Tissue (n = 87)**
IgE (kU/L)	78 (42.2-176)	<.01	156.2 (70.5-705.4)
SE IgE–positive samples (Chinese patients)	**Serum (n = 13)**	***P* value**	**Tissue (n=13)**
IgE (kU/L)	222.5 (61.9-467.4)	<.04	523.2 (259.2-1,132.8)
SE IgE	0.5 (0.1-1.0)	<.01	13.5 (5.4-87.6)

Data are expressed as medians and interquartile ranges. Statistical evaluation: Mann-Whitney *U* 2-tailed test; significance level specified as an α value of .05.