

Role of Local Immunoglobulin E Specific for *Alternaria alternata* in the Pathogenesis of Nasal Polyposis

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Objective/Hypothesis: The role of fungal pathogens in the etiology of nasal polyposis remains unclear. The aim of this study was to determine whether there was a correlation between the presence of *Alternaria*-specific immunoglobulin (Ig)E antibodies, eosinophilic inflammation, and the development of nasal polyps.

Study Design: Prospective study.

Methods: Serum and nasal tissue homogenates from 21 patients with manifestations of chronic sinusitis with nasal polyps were compared with specimens from 13 chronic sinusitis patients without polyps and 8 healthy controls. The Phadia ImmunoCAP and enzyme-linked immunosorbent assay were used to quantify levels of total IgE and *Alternaria*-specific (IgE, IgG, and IgA) antibodies. Eosinophil cationic protein (ECP) and tryptase levels were measured in tissue homogenates, whereas the inflammatory response was evaluated using tissue eosinophil counts in tissue samples.

Results: Serum analysis revealed no difference in the levels of total IgE and *Alternaria*-specific IgE, IgG, and IgA antibodies between the study groups. In contrast, the levels of *Alternaria*-specific IgE in tissue with polyps were significantly higher than in nonpolyp tissue. Increases in total tissue IgE paralleled increased levels of *Alternaria*-specific IgG and IgA antibodies in chronic sinusitis with nasal polyps as compared with control groups. A positive correlation was found between *Alternaria*-specific IgE and ECP in tissue. Increased mean levels of ECP corre-

sponded to increased eosinophil counts in the group of patients with polyps.

Conclusions: *Alternaria*-specific IgE and eosinophilic inflammation in nasal tissue correlates with the incidence of nasal polyps irrespective of specific IgE antibodies in serum. Together, the correlation between the local immune responses and the eosinophilic inflammation in nasal polyps suggests a possible role of *Alternaria* in the pathogenesis of nasal polyposis.

Key Words: *Alternaria*, nasal polyps, immunoglobulin E, human, eosinophilic inflammation.

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INTRODUCTION

Chronic rhinosinusitis (CRS) is among the most common health problems in Western countries. CRS has been classified broadly as CRS with nasal polyps (CRSwNP), CRS without nasal polyps (CRSw/oNP), and allergic fungal rhinosinusitis.¹ CRSwNP may have a complex pathogenesis and is thought to arise from multiple factors including allergy.² Eosinophilia is a major pathogenic factor that has been associated with nasal polyps. Increased release of eosinophil cationic protein (ECP) from eosinophil granules contributes to the development of nasal polyps, determining edema and an increased recruitment of inflammatory cells.³

A previous study from Mayo Clinic emphasized the role of fungal organisms in the pathogenesis of CRSwNP.⁴ According to the Mayo Clinic criteria, fungal organisms are prevalent in almost all patients suffering from CRS with or without polyposis. Immune hyperresponsiveness to colonizing fungi has been proposed as a mechanism of nasal polyposis.⁵ Among various fungi, *Alternaria alternata* is one of the most frequently encountered species that present as a normal contaminant of nasal mucus.⁶ Moreover, *Alternaria* DNA was demonstrated in all polypoid tissues from CRS patients.⁷ Several studies have demonstrated the presence of humoral immune responses to fungi, including *Alternaria*, in patients with CRS; however, the primary role of those responses in the development of CRS has been questioned.^{8,9} Locally produced

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Alternaria-specific immunoglobulin (Ig)E antibody might contribute to the pathogenesis of nasal polyposis because evidence has been emerging that this local mechanism is involved in some patients with nasal polyps,¹⁰ rhinitis,¹¹ or fungal sinusitis.¹²

The goal of the present study was to determine whether antibodies specific to *A. alternata* are associated with nasal polyps and to investigate the possible effect of these antibodies on eosinophilic inflammation. In this study, we evaluated tissue and serum from subjects with CRSwNP for *Alternaria*-specific IgE, IgG, and IgA antibodies and total IgE levels and compared the results with those from CRSw/oNP and controls without CRS (w/o CRS). Furthermore, we analyzed whether there is a relationship between *Alternaria*-specific antibodies and markers of eosinophil activation. We hypothesized that nasal mucosal allergy to *Alternaria* drives an enhanced eosinophilic inflammation and thus underlies the pathogenesis of nasal polyposis.

MATERIALS AND METHODS

Patients

Nasal tissue and serum specimens were obtained from 21 CRSwNP, 13 CRSw/oNP (diseased-control), and 8 healthy, non-diseased control patients. All control subjects had intranasal abnormalities, and none had a history of CRS or allergic disease (asthma, rhinitis). CRSwNP and CRSw/oNP patients underwent endoscopic sinus surgery; controls underwent appropriate nasal or septal surgery based on their presenting symptoms. Patients undergoing endoscopic sinus surgery met the criteria for CRS as defined by the guidelines established by the American Academy of Otolaryngology–Head and Neck Surgery Task Force for Defining Adult Chronic Rhinosinusitis.⁴ All subjects underwent endoscopy and a sinus computed tomography scan confirming CRS. Nasal tissue specimens were collected from the nasal polyps when present (CRSwNP group), osteomeatal complex region (CRSw/oNP diseased-control group), or inferior turbinates (w/o CRS control group) at the time of surgery. In addition, serum samples were collected from all study subjects, and eosinophil counts were measured. The Institutional Review Board of the NYU School of Medicine approved this study, and informed consent was obtained for all subjects included in the study.

Histology and Microscopic Analysis

Surgical tissue specimens were embedded in paraffin, sectioned into 5- μ m slices, mounted into glass slides, and stained with hematoxylin-eosin and Gomori's methenamine silver for routine pathologic examination. Eosinophils were counted in surgical tissue specimens, and the results were expressed as the mean number of cells per field. All freshly obtained tissue specimens were immediately washed in saline to completely remove any nasal secretion and weighed, and phosphate-buffered saline was added to tissue. The tissue was then homogenized with a mechanical homogenizer at 22,000 rpm on ice. After homogenization, the suspensions were centrifuged at 3,000 rpm for 10 minutes at 4°C, and supernatants were collected. Serum was obtained from the whole blood samples after centrifugation and frozen until analyzed.

Measurement of Mediators

ECP in tissue homogenates (diluted 1:2) were quantified using the Immulite 2000 Chemiluminescent Enzyme Immunoassay (IBT Reference Laboratory, Lenexa, KS). Tryptase levels in

tissue homogenates were quantified by using a Mast Cell Degranulation Assay Kit (Chemicon International, Temecula, CA).

Measurement of Total and Specific Antibodies

All supernatants and sera were assayed for total and specific IgE antibodies to common airborne fungi, including *Alternaria alternata*, *Aspergillus fumigatus*, *Candida albicans*, *Cladosporium herbarum*, and *Penicillium notatum* by the Phadia ImmunoCAP system (Portage, MI). In addition, the atopic status of 12 patients with allergic rhinitis or bronchial asthma was evaluated by assaying sera for a broad panel of specific IgE antibodies or by skin prick testing with a panel of allergens or by both. Specific IgE antibodies in serum included multi-allergen panel, mold profile, weed profile, tree profile, grass profile, dust/mite profile, and dog and cat dander, and the panel of allergens used in skin testing included molds (*Alternaria alternata*, *Cladosporium herbarum*, and *Penicillium notatum*), pollens (tree mix, grass mix, and ragweed), dust mites, cat, and dog. Allergen-specific IgE levels greater than 0.70 kU/L were considered a positive result. Analytical measurements for total and specific IgE antibodies were performed either by the Johns Hopkins Dermatology, Allergy and Clinical Immunology Reference Laboratory (Baltimore, MD) or the ARUP laboratories (Salt Lake City, UT). A positive skin test response was defined by a wheal of 3 mm or greater in size than that produced by the saline control. Histamine was used as a positive control. All tissue homogenates and sera were assayed for *Alternaria*-specific IgA and IgG by using an enzyme-linked immunosorbent assay (AlerCHECK, Inc., Portland, ME). Optical density was measured at 450 nm with a microplate reader (Bio-Tek Instrument, Winooski, VT).

Statistics

Data are expressed as median and interquartile range. The Mann-Whitney *U* test was used to compare levels of eosinophils, ECP, tryptase, total IgE, and specific IgA and IgG antibody. Differences were significant at $P \leq .05$. Fisher's exact test was used for analysis of eosinophils and allergen-specific IgE. Pearson's correlation was used to examine the relationship between the levels of *Alternaria*-specific IgE and ECP.

RESULTS

The clinical characteristics of the CRSwNP, CRSw/oNP, and w/o CRS patients are presented in Table I. There were no significant differences in age, sex, previous sinus surgeries, serum total IgE, and mold-specific IgE levels between the study groups ($P > .05$). Elevated allergen-specific IgE antibody levels in serum or a positive skin prick test for at least one allergen was present in all CRSwNP and CRSw/oNP patients with allergic rhinitis and all CRSwNP patients with asthma. Sensitization (IgE antibody positivity) to *Alternaria* was demonstrated in three of seven (43%) CRSwNP patients with allergic rhinitis. Three subjects from the CRSwNP group and two from the CRSw/oNP group had allergic mucin without the presence of fungal organisms.

Table II presents the levels of eosinophils, total IgE, and *Alternaria*-specific (IgE, IgG, and IgA) antibodies present in the tissue and serum specimens. Tissue eosinophil density was 220-fold higher in the CRSwNP group as compared with the CRSw/oNP group ($P < .05$). In contrast, serum eosinophil counts were not significantly different ($P > .05$), although there was an increased trend in the CRSwNP group. ECP levels in polyp tissue from CRSwNP subjects were fourfold higher than in tissue from

TABLE I.
Clinical Characteristics of Patients With and Without Chronic Rhinosinusitis.

	CRSwNP (n = 21)	CRSw/oNP (n = 13)	w/o CRS (n = 8)
Median age (yr)*	45.4 (27–64)	35.3 (22–61)	34.5 (21–49)
Sex (M/F)	11/10	8/5	4/4
Sinus surgery (n)*	0.56 (0–4)	0	0
Allergic rhinitis*	7 (33%)	2 (15%)	0 (0%)
Bronchial asthma*	4 (19%)	1 (8%)	0 (0%)
Total IgE (kU/L)†	90.6 (41.4–218)	84 (26.6–201)	111.9 (86.8–158.5)
Specific IgE to molds*‡	4 (19%)	2 (15%)	0 (0%)

*Data are number or mean (range or percent).

†Data are medians (25%–75% percentile) of total IgE in serum.

‡Specific serum IgE to common airborne fungi (*Alternaria alternata*, *Aspergillus fumigatus*, *Candida albicans*, *Cladosporium*, and *Penicillium notatum*).

CRS = chronic rhinosinusitis; NP = nasal polyps; Ig = immunoglobulin.

CRSw/oNP subjects ($P < .001$). This level of change was supported by a 30-fold increase over w/o CRS controls ($P < .001$). On the other hand, there was no significant difference in the tryptase levels between the groups ($P > .05$).

To determine the role of local and systemic humoral immune responses to *Alternaria* in the pathogenesis of nasal polyposis, levels of total IgE and *Alternaria*-specific (IgE, IgG, and IgA) antibodies were compared among the CRSwNP, CRSw/oNP, and w/o CRS controls. Tissue analysis showed increased total IgE levels in nasal polyps as compared with those in nonpolyp tissue (vs. CRSw/oNP: 4-fold increase, $P < .05$; vs. w/o CRS: 7-fold increase, $P < .05$). Local IgE antibodies specific to *Alternaria* were demonstrated in 10 of 21 CRSwNP patients. The levels of *Alternaria*-specific IgE in nasal polyp tissue were significantly higher than those in nonpolyp tissue from CRSw/oNP (2 of 13 patients) and w/o CRS controls (0 of 8 patients) (vs. CRSw/oNP and vs. w/o CRS: $P < .05$).

Importantly, 6 of 10 patients with nasal polyps were positive for specific IgE, but they did not have detectable *Alternaria*-specific IgE in their serum. The level of tissue-derived specific IgE from the CRSwNP subjects was over threefold higher than in the CRSw/oNP group ($P = .017$). *Alternaria*-specific IgE antibodies were detected in fewer serum specimens than in tissue specimens (CRSwNP group: 4 of 21 patients; CRSw/oNP group: 1 of 13 patients) without difference among the study groups ($P > .05$).

IgG and IgA antibodies specific to *Alternaria* were present in both the tissue and in the serum. In the CRSwNP patients, the levels of *Alternaria*-specific IgG and IgA antigen in tissue were significantly higher (4–6-fold) as compared with the CRSw/oNP ($P < .001$) or w/o CRS ($P < .001$) groups. There was no difference in patient serum levels ($P > .05$).

To determine whether there was a correlation between *Alternaria*-specific IgE antibody and eosinophilic

TABLE II.
Laboratory Characteristics of CRSwNP, Diseased-Control (CRSw/oNP), and w/o CRS Control Subjects.*

	CRSwNP (n = 21)	CRSw/oNP (n = 13)	P†	w/o CRS	P‡ (n = 8)
Tissue					
Eosinophils (no./HPF)	33 (10–100.5)	0.15 (0–10)	<.05	NA	NA
ECP (ng/mL)	397 (234–640)	90.9 (78.5–151)	<.001	29.7 (17.6–121)	<.001
Total IgE (kU/L)	69 (26–167.8)	17.7 (6.7–27.6)	<.05	9.9 (7–12.4)	<.001
Specific IgE to <i>Alternaria</i>	0.71 (<0.7–1.1, 48%)	<0.7 (<0.7–<0.7, 15%)	<.05	<0.7 (<0.7–<0.7, 0%)	<.05
Specific IgG (OD)	2.3 (0.9–3.1)	0.6 (0.4–0.9)	<.001	0.7 (0.5–1.1)	<.001
Specific IgA (OD)	1 (0.3–1.3)	0.15 (0.1–0.2)	<.001	0.17 (0–0.31)	<.001
Tryptase (mmol/L)	0.39 (0.2–0.55)	0.48 (0.3–0.58)	NS	0.32 (0.23–0.32)	NS
Serum					
Eosinophils (no.)	4×10^8 (1.5– 6.5×10^8)	1×10^8 (1– 3.5×10^8)	NS	NA	NA
Specific IgE to <i>Alternaria</i>	0.14 (<0.7–<0.7, 19%)	0.13 (<0.7–<0.7, 8%)	NS	<0.7 (<0.7–<0.7, 0%)	NS
Specific IgG (OD)	0.45 (0.29–0.6)	0.37 (0.23–0.45)	NS	0.17 (0.16–0.24)	NS
Specific IgA (OD)	0.72 (0.48–1.19)	0.7 (0.48–0.96)	NS	0.54 (0.4–0.63)	NS

Tissue and serum samples were assayed for total IgE and *Alternaria*-specific IgE antibodies by Pharmacia ImmunoCAP system. Detection limits of the assays are 0.70 kU/L.

*Data are medians (25%–75% percentile, proportion of subjects with detectable antibodies).

†CRSwNP vs. CRSw/oNP.

‡CRSwNP vs. w/o CRS controls.

CRS = chronic rhinosinusitis; NP = nasal polyps; HPF, high-powered field; NA = not applicable; ECP = eosinophil cationic protein; Ig = immunoglobulin; OD = optical density; NS = not significant.

inflammation in nasal polyps, we compared ECP and eosinophil numbers in nasal polyp samples with the presence of specific IgE antibodies to *Alternaria* (n = 10) versus the absence of IgE antibodies (n = 11). Surprisingly, the mean levels of ECP (Fig. 1A) as well as eosinophil numbers (Fig. 1B) were significantly higher in nasal polyps positive for IgE antibodies to *Alternaria* than in *Alternaria*-specific IgE negative samples (ECP, $P < .05$; eosinophil numbers, $P < 0.05$). Similarly, the total IgE levels were significantly higher ($P < .01$) in nasal polyps with *Alternaria*-specific IgE, than those in nasal polyps without *Alternaria*-specific IgE (Fig. 1C). There was no correlation between the levels of *Alternaria*-specific IgG or IgA antibodies and ECP and the presence or absence of nasal polyps ($P > .05$).

Furthermore, we aimed to determine the relationship between *Alternaria*-specific antibodies and ECP in a group of nasal polyps positive subjects who tested positive for *Alternaria*-specific IgE antibodies (Fig. 2). Levels of specific IgE to *Alternaria* showed striking correlations with ECP levels in this group ($r = 0.83$; $P = .0027$).

DISCUSSION

In the current study, we provide empirical evidence for the presence of a local humoral immune response to *Alternaria* in nasal polyps. The tissue analysis showed

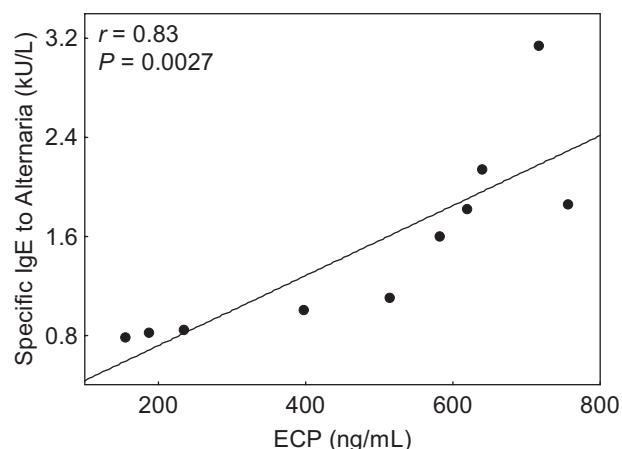


Fig. 2. Correlation of levels of *Alternaria*-specific immunoglobulin (Ig)E with eosinophil cationic protein (ECP) in nasal polyps (n = 10).

higher levels and a higher prevalence of *Alternaria*-specific IgE in CRSwNP as compared with CRSw/oNP. Importantly, 6 of 10 patients with positive specific IgE with nasal polyps had an absence of *Alternaria*-specific IgE in their serum, supporting the identification of local production. There was also a correlation between the level

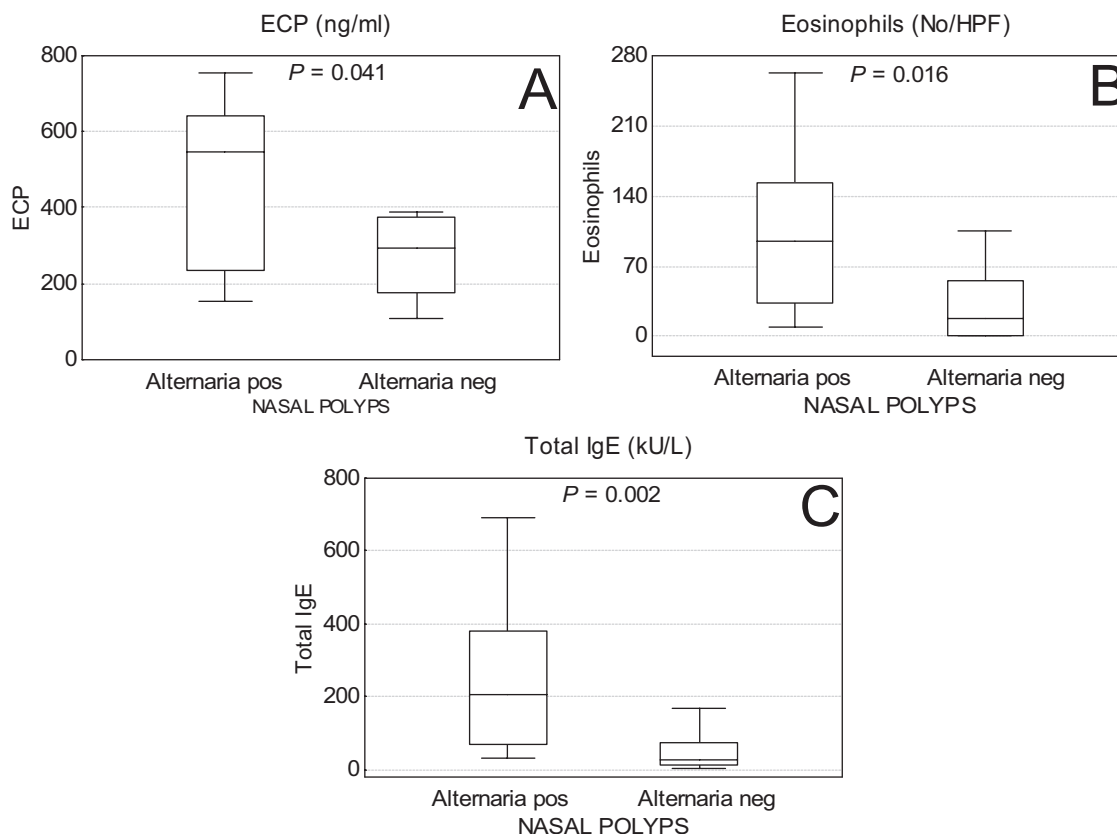


Fig. 1. Comparison of eosinophil cationic protein (ECP) (Fig. 1A), eosinophil numbers (Fig. 1B), and total immunoglobulin (Ig)E (Fig. 1C) in nasal polyp samples with the presence of specific IgE antibodies to *Alternaria* (Alternaria pos; n = 10) versus samples without (Alternaria neg; n = 11). The box and whisker plot represent the median, the lower to upper quartile, and the minimum to maximum value. HPF = high-powered field.

of *Alternaria*-specific IgE and the eosinophil marker ECP in nasal polyps. This suggests that a localized IgE-dependent eosinophilic degranulation in nasal polyps may play a role in the pathogenesis of nasal polyposis.

Several studies have demonstrated a localized allergic response to environmental allergens or microbial products within nasal polyp tissue, which may be more important to the etiology of polyp formation than a systemic allergic response.^{10,11,13} Of our group, 10 of 21 patients had demonstrable IgE antibody in nasal polyp tissue homogenates, which supports the hypothesis that immediate (type 1) hypersensitivity may be involved in polyp pathogenesis. Only 4 of these 10 had a corresponding specific IgE antibody that was detectable in serum. Because IgE antibody is routinely not measured in polyp tissue, this suggests that relying solely on serum IgE measurements may cause the clinician to miss fungal allergy as a possible underlying cause. Because 11 of 21 of CRSwNP patients did not demonstrate either a local or systemic IgE response to *Alternaria*, allergy may be only one of the causes of polyp formation.

The present study revealed an association between the presence of *Alternaria*-specific IgE antibodies and increased ECP and eosinophil levels in patients with nasal polyps. The higher concentration of ECP in nasal polyp tissue suggests the involvement of more highly activated eosinophils in the disease. These findings suggest that local IgE-mediated hypersensitivity to *Alternaria* allergens may drive local eosinophilic inflammation in nasal polyps. One possible mechanism is that specific IgE bound to cell-surface receptors on eosinophils may bind *Alternaria* proteins, which results in eosinophil degranulation and release of ECP.

The appearance of tissue-localized specific IgE antibody in nasal polyps could result from an increased exposure to *Alternaria* or an increased genetic predisposition to mount a specific IgE response to its allergenic proteins. In the present study, the levels of the group 1 *Alternaria* antigen, Alt a 1, in tissue homogenates and sinonasal mucus were not different between the groups (data not shown). Therefore, greater genetic susceptibility to mounting immune responses to *Alternaria* may underlie the pathogenesis of nasal polyposis.

The present study detected *Alternaria*-specific IgG and IgA antibodies irrespective of the presence of specific IgE antibodies in the nasal polyp tissue or the concomitant serum. These findings suggest that increases in fungal antigen-specific antibodies of different isotypes reflect the individual's ability to mount a nonallergic humoral immune response. Previously, several studies demonstrated receptors for IgA and IgG antibodies on the surface of eosinophils, suggesting a role for these antibodies in eosinophil degranulation within nasal polyps.^{14,15} The results of the present study do not support the role of *Alternaria*-specific IgG and IgA antibodies in eosinophil degranulation because the levels of these antibody isotypes showed a poor correlation with ECP levels within nasal polyps.

Another outcome of the present study was an increased level of total IgE in nasal polyps, especially in a group of nasal polyps containing *Alternaria*-specific IgE

antibodies. However, *Alternaria*-specific IgE constituted only a small fraction of the IgE antibodies that were detected. Therefore, the increased total IgE level may better reflect a predisposition to produce IgE antibody to a broad spectrum of aeroallergens. However, it is clear from early studies that total serum IgE may not be diagnostic for atopy because there is a wide overlap in the total serum IgE levels among atopic and nonatopic populations. This is why total serum IgE levels are clinically judged against 95% confidence intervals established with serum from age-adjusted, healthy, nonatopic individuals.¹⁶ Our study data question the diagnostic value of polyp tissue total IgE levels because no difference in the levels of total IgE were observed between the study groups.

We acknowledge a number of limitations related to our study. First, the numbers of tissue analyses performed were limited by the volume of specimens that were available. Second, because of limited specimens, we were only able to screen for specific antibody to *Alternaria*, which is believed by the allergy community to be a primary fungal aeroallergen. No doubt, there may have been IgE antibodies specific for other fungi or common aeroallergens that we were unable to assess. Previous studies demonstrated multiclonal IgE production in a group of nasal polyps that was associated with the presence of staphylococcal superantigen.¹⁷ Recently, *Alternaria* was proposed as both a classical allergen and a superantigen.¹⁸ A similar mechanism of multiclonal IgE production resulting from *Alternaria*'s action as a superantigen may also apply in the present study.

CONCLUSION

This study represents the first quantification of *Alternaria*-specific IgE antibody within nasal polyp tissue and its correlation with markers of eosinophil activation. Tissue-localized IgE-mediated hypersensitivity to *Alternaria* antigen may serve as an important pathogenic mechanism that drives localized eosinophilic inflammation in CRSwNP and, thus, may be of relevance in the pathogenesis of nasal polyposis. Further studies will be required to more specifically dissect the localized specific immunologic mechanisms and to correlate their presence with the markers of eosinophil activation in the nasal polyps. This will involve screening for IgE antibody to a broader spectrum of aeroallergen specificities and evaluating a larger number of subjects and tissue specimens.

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