

Prevalence of hypersensitivity to specific fungal allergens as determined by intradermal dilutional testing

ROBERT H. STROUD, MD, KAREN H. CALHOUN, MD, STEVEN T. WRIGHT, BS, and KYLE L. KENNEDY, MD, Galveston and Amarillo, Texas

OBJECTIVE: To determine fungal allergen reactivity prevalence by intradermal dilutional testing in patients with and without chronic rhinitis or rhinosinusitis symptoms.

STUDY DESIGN: Prospective comparison of fungal allergen reactivity prevalence in symptomatic and asymptomatic patients.

SETTING: University medical center.

METHODS: Group I (chronic rhinitis and/or rhinosinusitis symptoms) and Group II (asymptomatic) patients underwent intradermal dilutional testing with usual and fungal allergens.

RESULTS: Fungal reactivity occurred in 65% (13/20) of Group I, and 13% (4/30) of Group II ($P < 0.0002$ by χ^2 testing). Group I was more reactive to non-fungal allergens (85% vs. 33%, $p < 0.0004$), and to all allergens considered together (95% vs. 40%, $p < 0.0001$).

CONCLUSIONS: Patients with chronic rhinitis and rhinosinusitis symptoms were more reactive to fungal and nonfungal allergens. Fungal allergens were as likely as nonfungal to elicit reactivity.

SIGNIFICANCE: These findings suggest a role for fungal hypersensitivity in chronic rhinitis and chronic rhinosinusitis. (Otolaryngol Head Neck Surg 2001;125:491-4.)

Current opinions about the role of fungi in chronic rhinitis or rhinosinusitis range from minimal to virtually universal. Numerous studies have estimated the prevalence of allergic fungal sinusitis (AFS) in patients undergoing sinus procedures for chronic rhinosinusitis (CRS) to be between 2% and 10%.¹⁻⁵ In contrast, another group

reports that 93% of patients with CRS meet the diagnostic criteria for AFS.⁶ Since Type I reactivity to fungal allergens would, in most schema, be a prerequisite for fungal causality in rhinitis or rhinosinusitis, the overall prevalence of fungal hypersensitivity becomes important in considering rhinosinusitis etiology. This study was undertaken to define the prevalence of Type I hypersensitivity by serial intradermal testing (IDT) to a panel of fungal and nonfungal allergens. This was investigated in patients with symptoms of chronic rhinitis or rhinosinusitis, as well as in subjects without any such symptoms.

MATERIALS AND METHODS

The Institutional Review Board at the University of Texas Medical Branch at Galveston (UTMB) approved the protocol for recruitment and testing of subjects within the study. Informed consent was obtained from all patients prior to testing. Exclusion criteria were age less than 18 years, prior diagnosis of AFS, prior sinus surgery with the finding of thick tenacious mucin typical of AFS even in the absence of other histologic criteria for AFS, use of medications contraindicated in IDT (beta-blockers, tricyclic antidepressants, antihistamines, etc.) or current acute bacterial sinusitis or upper respiratory infection (URI).

Group I (subjects with chronic rhinitis or rhinosinusitis symptoms) consisted of patients referred from the Otolaryngology clinic for IDT as a part of the work-up for chronic sinonasal complaints. The major inclusion criterion was a history of rhinitis or rhinosinusitis symptoms. Computed tomography or other objective evidence of chronic sinusitis was not part of the evaluation to assess candidacy for inclusion. These patients underwent IDT with an individualized panel of allergens consisting of no less than 6 fungal allergens. Nonfungal allergens were chosen based on history supplied by the patient. IDT was performed in the standard format with the placement of saline solution, glycerine, and histamine controls. Reactivity was recorded as a 2 mm or greater increase in wheal size read at 10 minutes postinjection. Testing was begun at the number 6 dilution and continued to number 2 dilution or until an endpoint was determined.

Group II (subjects without chronic rhinitis or rhinosinusitis symptoms) was made up of subjects recruited from employees and students of UTMB. All subjects completed a brief survey regarding rhinitis or rhinosi-

From the Department of Otolaryngology (Drs Stroud and Calhoun, and Mr Wright), The University of Texas Medical Branch, Galveston, and the Center for Diseases of the Nose and Sinuses (Dr Kennedy), Amarillo.

Supported by a Research Grant from the American Academy of Otolaryngic Allergy Foundation.

Presented at the Annual Meeting of the American Academy of Otolaryngic Allergy, September 22, 2000, Washington, DC.

Reprint requests: Karen H. Calhoun, MD, Professor and Vice Chair, Department of Otolaryngology, The University of Texas Medical Branch, 301 University Boulevard, Route 0521, Galveston, TX 77555; e-mail, kcalhoun@utmb.edu.

Copyright © 2001 by the American Academy of Otolaryngology—Head and Neck Surgery Foundation, Inc.

0194-5998/2001/\$35.00 + 0 23/1/119969

doi:10.1067/mhn.2001.119969

Table 1. Allergen panel used for testing asymptomatic subjects (group II)

Fungal allergens	
<i>Penicillium</i>	<i>Mucor</i>
<i>Alternaria</i>	<i>Cladosporium</i>
<i>Aspergillus</i>	<i>Helminthosporium</i>
<i>Fusarium</i>	<i>Stemphyllium</i>
<i>Pullularia</i>	<i>Epicoccum</i>
<i>Curvularia</i>	
Nonfungal allergens	
Short ragweed	Mountain cedar
Rough marshelder	Bermuda grass
Dust mite	Cat hair

nusitis symptoms and their use of prescription or over-the-counter medications for the relief of such symptoms. Subjects were excluded if responses indicated a likelihood of chronic rhinitis or chronic rhinosinusitis. All subjects then underwent a head and neck examination looking for signs of sinonasal disease performed by one examiner who completed a standardized examination sheet. Patients who showed evidence of chronic rhinitis or rhinosinusitis (copious rhinorrhea, nasal crusting, markedly erythematous or polypoid mucosa, frank pus, or polyps) were eliminated from the study. All subjects underwent IDT as described above to a standardized panel of 11 fungal allergens and 6 nonfungal allergens as listed in Table 1. In order to expedite testing and minimize the number of injections, dilutions 6, 4, and 2 were used with dilutions 5, 3, and 1 placed only as necessary to determine an endpoint. Testing was uneventful in both groups and no untoward events were experienced.

All data were entered in a database recording positive responses as reactive and negative as nonreactive. The prevalence of reactivity to fungal allergens, nonfungal allergens, and to each allergen was calculated as a percentage of subjects testing positive. These proportions were compared using the χ^2 test. In addition, the likelihood of reactivity to fungal versus nonfungal allergens within each group was compared.

RESULTS

Group I consisted of 20 patients (3 male, 17 female) with chronic rhinitis or rhinosinusitis symptoms. The average age was 45 ± 13 years. Median age was 41 years. Group II consisted of 30 asymptomatic subjects (14 males, 16 females). The average age of group II was 27 ± 5 years. Median age was 25 years. The difference in average age between the 2 groups was statistically significant ($P < 0.0001$).

In group I, 13 (65%) of the 20 patients reacted to at least 1 of the fungal allergens tested and 10 reacted to 3 or more fungal allergens. One patient reacted to all 10

fungal allergens in the test panel. (He had undergone 2 endoscopic sinus procedures with minimal polyposis but no allergic mucin and did not meet the diagnostic criteria for AFS.) Seventeen of the 20 (85%) patients reacted to at least 1 of the nonfungal allergens. Two of the 3 patients who did not react to the nonfungal allergens reacted to 1 fungal allergen. One patient did not react to any of the fungal or nonfungal allergens tested. Nineteen (95%) of 20 reacted to at least 1 fungal or nonfungal allergen. The difference in the prevalence of reactivity to fungal as compared with nonfungal allergens was not significant ($P = 0.144$).

Among the subjects in group II, 4 (13%) of the 30 reacted to fungal allergens. All of those who reacted did so to at least 2 of the 11 fungal allergens tested. One patient reacted to 5 of the 11 fungal allergens. Ten (33%) of the 30 subjects reacted to at least 1 of the 6 nonfungal allergens. Eight of the 10 reacted to multiple nonfungal allergens. Two patients reacted to multiple fungal allergens yet did not react to any nonfungal allergens. Twelve (40%) of the 30 reacted to at least 1 fungal or nonfungal allergen. The difference in the prevalence of reactivity to fungal as compared with nonfungal allergens approached but did not reach significance ($P < 0.07$).

Subjects in group I exhibited a significantly greater prevalence of reactivity to fungal allergens than those in group II ($P < 0.0002$). Those in group I also showed a higher rate of hypersensitivity to nonfungal allergens ($P < 0.0004$). The results of testing with each individual allergen are summarized in Table 2.

DISCUSSION

Patients with self-designated chronic rhinitis or rhinosinusitis symptoms (CR/RSSx) had a high (95%) sensitivity to test allergens, with dust mite most common. Previous studies show up to 80% of chronic rhinosinusitis (CRS) patients with allergies, particularly dust mite.^{7,8} Our positive test prevalence was higher, but our group was highly selected, with both history and symptoms of inhalant allergy, and poor response to pharmacotherapy and avoidance. Our humid coastal climate causes greater exposure to environmental molds than other North American climates.⁹

We found a moderate sensitivity rate (40%) among subjects without CR/RSSx. These may have preclinical disease, more symptom tolerance, or less awareness. Kerlchhof et al¹⁰ found that 43% of subjects with inhalant allergen specific IgE were asymptomatic. There was a significant difference between our 2 groups' reactivity to all nonfungal allergens except cat and mountain cedar.

Our symptomatic patients were older (45 ± 13 years) than control subjects (27 ± 5 years). Total allergen

Table 2. Rates of reactivity to individual allergens tested in both groups

Allergen	Group I	Group II	P value
	Reactive/tested (percentage)	Reactive/tested (percentage)	
<i>Alternaria</i>	7/18 (39%)	2/30 (6.7%)	0.0056
<i>Epicoecum</i>	4/17 (24%)	0/30 (0%)	0.0054
<i>Penicillium</i>	2/13 (15%)	1/30 (3%)	0.15
<i>Pullularia</i>	6/16 (38%)	0/30 (0%)	0.0003
<i>Helminthosporium</i>	6/16 (38%)	1/30 (3%)	0.0021
<i>Cladosporium</i>	1/14 (7%)	2/30 (6.7%)	0.95
<i>Stemphyllium</i>	5/14 (36%)	2/30 (6.7%)	0.014
<i>Mucor</i>	5/18 (28%)	1/30 (3%)	0.013
<i>Fusarium</i>	11/19 (58%)	1/30 (3%)	< 0.0001
<i>Aspergillus</i>	7/19 (37%)	1/30 (3%)	0.0020
<i>Curvularia</i>	6/16 (38%)	1/30 (3%)	0.0021
Short ragweed	7/15 (47%)	2/30 (6.7%)	0.0015
Rough marshelder	6/13 (46%)	5/30 (17%)	0.042
Cat hair	2/8 (25%)	4/30 (13%)	0.42
Dust mite	11/19 (58%)	8/30 (27%)	0.028
Mountain cedar	4/13 (31%)	3/30 (10%)	0.09
Bermuda grass	4/16 (25%)	3/30 (10%)	0.18

exposure increases with age. Since Type I hypersensitivity develops after antigen exposure, serial testing can show development of sensitivity to previously nonreactive allergens.¹¹ An older control group might have had greater Type I hypersensitivity, and thus a smaller difference between 2 groups.

There was a gender difference between our groups (I, 85% female; II, 53%). Sears et al¹² found a moderate hypersensitivity difference between genders in 13-year-old children, insufficient to explain the differences between our groups.

Nearly two thirds (65%) of our symptomatic patients reacted to at least 1 fungal allergen versus 13% of controls. This suggests that fungal sensitivity is not ubiquitous, but does accompany CR/RSSx, and may play a role in their development.

Nearly 60% of our symptomatic group reacted to *Fusarium*. In this group, 36% to 39% reacted to these fungi: *Alternaria*, *Pullularia*, *Helminthosporium*, *Curvularia*, *Aspergillus*, and *Stemphyllium*. In contrast, the most common control group fungal reactivities were *Alternaria*, *Cladosporium*, and *Stemphyllium*, each 6.7%. Only 1 patient (3%) reacted to *Fusarium*. This marked difference in sensitivities suggests a possible relationship between Type I reactivity to *Fusarium* and CR/RSSx.

Allergic fungal sinusitis (AFS) is a subset of chronic rhinosinusitis (CRS) thought to be caused by Type I fungal hypersensitivity. AFS diagnostic criteria are: (1) Type I fungal hypersensitivity, (2) nasal polyposis, (3) characteristic radiographic findings, (4) eosinophilic mucus without fungal tissue invasion, and (5) positive fungal stain on the surgical specimen.² Clinically, AFS is charac-

terized by nasal polyposis, "double density" on CT scan, and thick, brownish (peanut-butter) sinus contents. The most common organisms are dematiaceous fungi (*Bipolaris*, *Dreschlera*, *Alternaria*, *Curvularia*, *Exserohilum*, *Rhizopus*, *Fusarium*, and *Chrysosporium*).⁵ (Antigen suppliers often refer to *Bipolaris*, *Dreschlera*, and *Exserohilum* as *Helminthosporium*.)

Bent and Kuhn² suggest that AFS results from sinus fungal colonization, causing an intense, prolonged immune response. If this is true, early identification and aggressive treatment of fungal hypersensitivity may diminish the occurrence or severity of AFS. Recent successes with postoperative immunotherapy support a causal role for systemic hypersensitivity to fungal allergens.¹³⁻¹⁵

Some patients partially fulfill AFS diagnostic criteria. Allphin et al.¹ reported 11 patients with typical allergic mucin without identifiable fungi in sinus contents. Ferguson¹⁶ describes patients with eosinophilic mucin, without fungal elements on histopathology. The role of fungal Type I hypersensitivity in these patients is not yet known. Allphin et al provided no allergy testing data. Such data were available for 23 of Ferguson's 29 patients. Seventeen (73%) showed reactivity (allergy test method not specified). Seven (54%) of the 13 with IgE data had elevated levels, and 13 (81%) of 16 had eosinophilia. Ferguson terms these findings "eosinophilic mucin rhinosinusitis." She postulates a disease distinct from AFS, resulting from systemic immunologic malfunction rather than fungal hypersensitivity. Fungal immunotherapy would probably not influence the course of such a disease. Others suggest

that these patients have pre-AFS that would progress to classic AFS if not treated.¹

Ponikau et al⁶ recently suggested a wider role for fungal hypersensitivity in CRS. They cultured fungi from nasal washings of 96% of CRS patients and 100% of controls. Only 42% of their CRS patients had Type I hypersensitivity to fungal allergens by skin testing vs 21% of their controls. They believe that fungi are causal agents in most CRS and postulate that local (nasal mucosal) IgE production causes local eosinophil mobilization in response to fungal colonization. Patients with elevated fungal-specific serum IgE have systemic recognition of the disease, but the circulating IgE is not causal. Patients with elevated total serum IgE and fungi cultured from nasal washings are thought to have allergic rhinitis without the mucosal changes seen in AFS. They purport that allergic rhinitis (AR) and AFS can be co-morbid conditions, with elevated total serum IgE, eosinophilic mucus, and marked polyposis, but that elevated IgE or Type I hypersensitivity need not be present for the diagnosis of AFS. Based on these assumptions, they recommend a changing terminology from AFS to eosinophilic fungal rhinosinusitis. Using their criteria of (1) CRS confirmed by CT, (2) allergic (eosinophilic) mucin, and (3) fungal organisms in the mucin (by histology or culture), they found a 93% incidence of AFS in patients with CRS.

The concept of mucosal allergy was addressed by Shatkin et al¹⁷ in a meta-analysis of 9 studies investigating the correlation between intranasal and systemic IgE. They found 19% of 180 patients to have strictly nasal mucosal allergy (positive RAST on polyp homogenate, nasal secretion, or inferior turbinate, with negative serum RAST). This suggests a patient subset with nasal hypersensitivity without systemic evidence of reactivity.

These studies highlight the uncertain role of fungal hypersensitivity in chronic sinonasal disease. Although fungi are ubiquitous within nasal cavities, only some develop systemic Type I allergic responses or clinical symptoms. If localized nasal mucosal allergy occurs without systemic allergen-specific IgE, systemic immunotherapy may not alter the course of this disease. It is not known whether classic AFS is a distinct variant of chronic sinonasal disease, or is part of a continuum ranging from mild rhinosinusitis through classic AFS. These are some of the questions to be addressed in further research as we seek optimal treatment for patients with these problems.

CONCLUSION

Type I fungal hypersensitivity is common among our patients with refractory chronic rhinitis or rhinosinusitis complaints. It is far less common among asymptomatic subjects. The exact role of this hypersensitivity in the development of chronic sinonasal disease remains to be elucidated. Long-term follow-up of patients with fungal hypersensitivity and minimal symptoms may provide further insight into fungal allergy and its role in the natural history of CRS and AFS.

REFERENCES

1. Allphin AL, Strauss M, Abdul-Karim FW. Allergic fungal sinusitis: problems in diagnosis and treatment. *Laryngoscope* 1991;101:815-20.
2. Bent JP, Kuhn FA. Diagnosis of allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1994;111:580-8.
3. Cody DT, Neel B, Ferreiro JA, et al. Allergic fungal sinusitis: the Mayo Clinic experience. *Laryngoscope* 1994;104:1074-9.
4. Corey JP, Delsupehe KG, Ferguson BJ. Allergic fungal sinusitis: allergic, infectious, or both? *Otolaryngol Head Neck Surg* 1995;113:110-9.
5. Houser SM, Corey JP. Allergic fungal sinusitis: pathophysiology, epidemiology and diagnosis. *Otolaryngol Clin N Am* 2000;33:399-408.
6. Ponikau JU, Sherris DA, Kern EB, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999;74:877-84.
7. King HC, Mabry RL, Mabry CS. Allergy in ENT practice. New York: Thieme; 1998. p. 367-78.
8. Emanuel IA, Shah SB. Chronic rhinosinusitis: allergy and sinus computed tomography relationships. *Otolaryngol Head Neck Surg* 2000;123:687-91.
9. Ferguson BJ, Barnes L, Bernstein JM, et al. Geographic variation in allergic fungal rhinosinusitis. *Otolaryngol Clin N Am* 2000;33:441-9.
10. Kerlchhof M, Schouten JP, DeMondy JG. The association of sensitization to inhaled allergens with allergy symptoms: the influence of bronchial hyperresponsiveness and blood eosinophil count. *Clin Exp Allergy* 2000;30:1387-94.
11. Linneberg A, Nielsen NH, Madsen F, et al. Smoking and the development of allergic sensitization to aeroallergens in adults: a prospective population-based study. *The Copenhagen Allergy Study*. *Allergy* 2001;56:328-32.
12. Sears MR, Burrows B, Flannery EM, et al. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. *Clin Exp Allergy* 1993;23:941-8.
13. Folker RJ, Marple BF, Mabry RL, et al. Treatment of allergic fungal sinusitis: a comparison trial of postoperative immunotherapy with specific fungal antigens. *Laryngoscope* 1998;108:1623-7.
14. Mabry RL, Marple BF, Folker RJ, et al. Immunotherapy for allergic fungal sinusitis: three years' experience. *Otolaryngol Head Neck Surg* 1998;119:648-51.
15. Mabry RL, Marple BF, Mabry CS. Outcomes after discontinuing immunotherapy for allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 2000;122:104-6.
16. Ferguson BJ. Eosinophilic mucin rhinosinusitis: a distinct clinicopathological entity. *Laryngoscope* 2000;110:799-813.
17. Shatkin JS, Delsupehe KG, Thisted RA, et al. Mucosal allergy in the absence of systemic allergy in nasal polyposis and rhinitis: a meta-analysis. *Otolaryngol Head Neck Surg* 1994;111:553-6.