
Multiallergen nasal challenges in nonallergic rhinitis

Dania A. Wierzbicki, MD*; Amee R. Majmundar, MD†; Diane E. Schull, RN, PhD‡; and David A. Khan, MD*

Background: It has been proposed that some patients with nonallergic rhinitis may have “localized allergy” of the nasal mucosa. Nasal challenges with aeroallergens can help determine whether a patient is clinically allergic via an IgE-mediated pathway.

Objective: To determine the prevalence of localized allergy in patients with negative skin prick test results via nasal challenges with an array of allergens.

Methods: Twenty individuals with perennial rhinitis and negative epicutaneous test results to common perennial aeroallergens underwent nasal challenges to glycerin, *Alternaria*, cockroach, timothy grass, cat hair, and *Dermatophagoides pteronyssinus*. Total symptom scores, peak nasal inspiratory flow rates, and nasal eosinophil counts were determined.

Results: Of 20 patients with nonallergic rhinitis, 4 were hyperresponsive to glycerin and were not subsequently challenged. Eleven patients had negative nasal challenges. Five patients developed positive challenges (total symptom score ≥ 5) to 7 allergens. These 5 patients returned for nasal provocation testing to their offending allergens, and these repeated challenges were negative. Three control subjects with allergic rhinitis developed positive challenges after nasal allergen challenge.

Conclusions: Although some individuals with nonallergic rhinitis can have positive nasal allergen challenges, these results were not reproducible in the present patient population.

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INTRODUCTION

Chronic rhinitis is a common disorder affecting up to 40% of the general population.¹ Allergic rhinitis is a symptomatic disorder of the nose defined by IgE-mediated inflammation of the nasal mucosa after allergen exposure. In contrast, the diagnosis of nonallergic rhinitis is given to patients with perennial nasal congestion, rhinorrhea, itching, or sneezing with no identifiable origin.²

There is an increasing body of literature suggesting that many patients with nonallergic rhinitis may actually have “localized allergy.” Powe et al^{3,4} proposed the term *entopy* to define the phenomenon of localized nasal allergy in patients with “nonatopic” rhinitis. They demonstrated, using full-thickness inferior turbinate specimens, the presence of mast cells, IgE-positive cells, and grass specific IgE in patients with idiopathic rhinitis.^{3,4}

In 1975, Huggins and Brostoff⁵ described 14 individuals with positive nasal challenges to *Dermatophagoides pteronyssinus* and specific IgE in nasal secretions despite negative skin prick test results and negative serum radioallergosorbent test results. More recently, Carney et al⁶ performed nasal

challenges with dust mite, cat, dog, and grass pollen on individuals with idiopathic rhinitis or perennial allergic rhinitis and controls. Of the 23 patients with idiopathic rhinitis, 13 (57%) tested positive to 1 of the allergens based on anterior rhinomanometry. Of those 13 patients, 11 (85%) reacted to dust mite.⁶

We investigated the prevalence of localized allergy in individuals with negative skin test results via nasal challenges with a broader array of allergens. The primary end point was the widely used total symptom score (TSS) after nasal challenge.⁷ This system has been shown to correlate with mediator release in the nose and has been used by a variety of investigators.^{8–11} Secondary end points evaluated included change in nasal eosinophil counts and peak nasal inspiratory flow (PNIF) rate.

METHODS

Study Patients

Individuals 18 years or older were recruited from the local community, including patients from the University of Texas Southwestern Medical Center outpatient allergy clinic, the county hospital outpatient allergy clinic, and medical center personnel. All the patients were classified as having symptomatic perennial nonallergic rhinitis based on history, physical examination findings, and negative epicutaneous test results with a panel of common aeroallergens (Hollister-Stier Laboratories LLC, Spokane, Washington). Exclusion criteria included nasal polyposis, septal deformity, infection-related rhinitis in the previous 2 weeks, uncontrolled asthma, uncontrolled heart or lung disease, nasal surgery in the previous 6

Affiliations: * Division of Allergy and Immunology, University of Texas Southwestern Medical Center, Dallas, Texas; † Advanced Allergy & Asthma Care SC, Orland Park, Illinois; ‡ Parkland Health and Hospital System, Dallas, Texas.

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weeks, chronic sinusitis, pregnancy or lactation, dermographism, and current immunotherapy. Medications that could interfere with nasal challenges were withheld before nasal challenges. First-generation antihistamines were withheld for 3 days, second-generation antihistamines for 7 days, nasal and oral corticosteroids for 1 week, nasal cromolyn/nedocromil for 4 weeks, oral decongestants for 1 day, topical decongestants for 1 week, ipratropium nasal spray for 1 week, ocular antihistamines for 1 week, injected corticosteroids for 3 months, and tricyclic antidepressants for 6 months. The study was approved by the University of Texas Southwestern Medical Center institutional review board, and all the patients provided written informed consent.

Epicutaneous Testing

Epicutaneous testing for 16 patients recruited from the university's outpatient allergy clinic and the county hospital outpatient allergy clinic included a panel of 38 aeroallergens endemic to the area along with histamine (184 mg/mL) and saline control (Hollister-Stier Laboratories LLC). Epicutaneous tests were performed using a bifurcated needle at the university clinic and using the Multi-Test device (Lincoln Diagnostics Inc, Decatur, Illinois) at the community clinic. The 4 remaining patients (recruited from the community) underwent skin testing to a southern grass mix, tree mix, weed mix, mold mix, cat, and cockroach using a bifurcated needle (Greer Laboratories Inc, Lenoir, North Carolina). After 15 minutes, results were considered negative if the wheal diameter was less than 2 mm. A positive histamine control was defined as a wheal diameter greater than 8 mm using 1M histamine.

Total Symptom Score

Patients rated their symptoms according to the TSS.^{7,8} Symptoms were scored on a scale from 0 to 12. A positive challenge was defined as a TSS of 5 or greater. Specifically, this system scores the number of sneezes (0–2 = 0 points, 3–4 = 1 point, and ≥ 5 = 3 points), rhinorrhea (anterior = 1 point, posterior = 1 point, both = 2 points, and marked = 3 points), nasal blockage (breathing with difficulty = 1 point, 1 nostril blocked = 2 points, and both nostrils blocked = 3 points), nasal itch (present = 1 point), palate or ear itching (present = 1 point), and conjunctivitis (present = 1 point).

Nasal Provocation Testing

Patients waited 30 minutes before testing to allow nasal mucosa to acclimate. Baseline TSSs, PNIF rates, and nasal smears for eosinophil counts were obtained. One hundred

microliters of control solution, 50% glycerin, was placed on the inferior turbinate of the least congested side via a pipette. Nasal challenges were performed in a single-masked manner. Fifteen minutes later, TSSs, nasal smears for eosinophil counts, and 3 PNIF measurements were obtained.¹² If provocation with control solution did not elicit a positive TSS, then the patient was challenged with 100 μ L of *Alternaria tenuis*, 1:20 wt/vol (Fig 1). Again, TSSs, nasal cytologic samples, and PNIF measurements were obtained after 15 minutes. If the provocation elicited a TSS greater than or equal to 5, the remaining challenges were postponed for another day. If the TSS was negative, subsequent challenges with cockroach mix (100 μ L of 1:5,000 wt/vol), timothy grass (100 μ L of 2,000 bioequivalent allergy units [BAU]/mL),¹³ cat hair (100 μ L of 1,000 BAU/mL),¹⁴ and *D pteronyssinus* (100 μ L of 1,000 arbitrary units/mL)¹⁵ were performed (Greer Laboratories Inc). Positive nasal challenges were repeated 3 to 5 months after the patient's original challenge. The repeated challenge protocol was similar to that of the first challenge except that after the initial glycerin challenge, patients were again challenged to only a single allergen. Patients were not informed of which allergens provoked their symptoms.

PNIF Measurement

Baseline PNIF rates were obtained using a nasal inspiratory flow meter with a cushioned face mask (In-Check; Clement Clarke International, Harlow, England). The In-Check has an accuracy of $\pm 10\%$ or 10 L/min (whichever is greater).¹⁶ Patients were instructed to inspire as forcefully as possible through the nose with their mouth closed while pressing the face mask firmly against their face. The best of 3 efforts was used to determine PNIF.¹⁷ A significant decline in PNIF was defined as a decrease greater than 20% from the baseline value.

Nasal Eosinophil Counts

Nasal cytologic samples were obtained by having the patient blow into wax paper. A Hansel stain was used to identify eosinophils. For each specimen, high-power fields (Olympus BH2 microscope with a $\times 10$ ocular and $\times 40$ objective lens; Olympus America Inc, Center Valley, Pennsylvania) with a maximum number of eosinophils were identified and recorded as eosinophils per high-power field.^{18,19} There were generally too few eosinophils on each smear to count contiguous high-power fields.

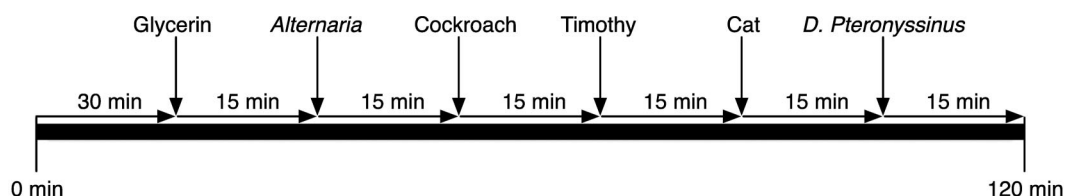


Figure 1. Nasal challenge protocol. Before each challenge, total symptom scores, nasal smears, and peak nasal inspiratory flow rates were obtained.

Statistical Analyses

The data were analyzed using a statistical software program (SPSS for Windows 10.0; SPSS Inc, Chicago, Illinois). The Pearson correlation coefficient tested bivariate correlations for PNIF values and nasal eosinophil numbers. $P < .05$ was regarded as statistically significant.

RESULTS

Total Symptom Scores

Twenty patients with perennial rhinitis symptoms but negative skin test results underwent nasal allergen challenges. Six were men and 14 were women. The median age was 47 years (range, 24–62 years). For comparison, 3 positive control subjects with allergic rhinitis were also studied. Two controls were sensitive to dust mites and 1 to cat. Of the patients with idiopathic rhinitis, 4 developed positive nasal challenges after the glycerin control. These individuals with hyperreactive nasal mucosa did not undergo further nasal allergen challenges. Eleven patients had negative symptom scores after sequential nasal challenges with *Alternaria*, cockroach, timothy grass, cat hair, and dust mite.

Five of the 20 patients (25%) with nonallergic rhinitis developed positive nasal challenges after 1 or more allergen nasal challenges (Table 1). All 5 participants returned for repeated nasal challenges to the allergens that provoked their positive symptom score approximately 3 months after their initial challenge. Patients were masked as to which allergens initially provoked their symptoms. All the patients, on rechallenge, had negative nasal challenges (Table 1).

Compared with patients with nonallergic rhinitis, patients with allergic rhinitis had significantly higher TSSs on antigen challenge ($P = .04$). The 2 positive control subjects sensitized to Der p 1 had positive TSSs of 9 and 10 after nasal challenge with dust mite. The control subject sensitized to Fel d 1 had a positive nasal challenge score of 11 after nasal challenge with cat.

PNIF Measurements

Two of the 4 patients hyperresponsive to glycerin by TSS also had a significant decline in their PNIF rate (23% and 29%). Because patients' baseline PNIF values ranged from

70 to 210 L/min, we believed that it was necessary to compare the relative percentage change from baseline ($[(\text{post} - \text{pre})/\text{pre} \times 100]$) between patients as well.²⁰ The percentage change in PNIF for the 4 nasal hyperresponders significantly differed from the change in PNIF of the other 16 nonallergic patients after glycerin challenge ($P = .02$). However, 1 patient with a negative TSS after glycerin control also had a greater than 20% decline in PNIF. Of the 7 positive nasal challenges in 5 patients after allergen challenge, only patient 18 had a significant decrease in PNIF of 30%. This patient's PNIF again declined 20% on repeated nasal allergen challenge despite a negative symptom score with that challenge. The percentage change in PNIF for patients with a positive challenge did not significantly differ from the percentage change for the other patients challenged after each respective allergen. Likewise, there was no significant correlation between symptom scores and change in PNIF values after allergen challenges.

Nasal Eosinophil Counts

Nasal eosinophils were present in very low numbers, obviating the need for statistical analysis. There was no correlation between TSSs and eosinophil counts on nasal smears after allergen challenge in patients with negative challenges and those with positive challenges.

DISCUSSION

The purpose of this study was to evaluate the intranasal response in patients with nonallergic rhinitis to a variety of aeroallergens because recent publications suggest that local allergy confined to the nasal mucosa may contribute to rhinitis symptoms. These results reveal that although some patients with nonallergic rhinitis may develop positive symptom scores after nasal allergen challenge, these results were not reproducible.

Of the 20 patients with perennial nonallergic rhinitis, 4 were hyperresponsive to glycerin and were not subsequently challenged. Sixteen patients underwent sequential nasal challenges with *Alternaria*, cockroach mix, timothy grass, cat hair, and *D pteronyssinus*. We detected a positive TSS after 7 nasal challenges in 5 patients. These positive symptom

Table 1. Positive Nasal Allergen Challenges

Patient No.	Allergen	Initial challenge		Repeated challenge	
		TSS	% Δ PNIF from previous challenge	TSS	% Δ PNIF from previous challenge
1	Cockroach	5	10	4	31
	Dust mite	8	–15	3	–9
3	Cat	6	–6	3	–8
12	Dust mite	5	–16	3	0
18	Timothy grass	5	–30	2	0
19	Cat	6	27	3	–3
	Dust mite	7	–11	4	6

Abbreviations: TSS, total symptom score (a positive challenge is a score ≥ 5); % Δ PNIF, percentage change in peak nasal inspiratory flow after allergen challenge compared with previous PNIF.

scores did not correlate with changes in PNIF values or nasal eosinophil numbers; however, the sample size was not adequately powered to address these secondary outcomes. These 5 patients returned for repeated single-masked nasal challenges with the allergen that initially provoked their positive score. None of the patients developed a positive symptom score on repeated challenges.

Several studies have now shown positive nasal allergen provocation test results in nonallergic individuals, especially with dust mite. Rondon et al²¹ recently demonstrated that 27 of 50 patients (54%) with persistent nonallergic rhinitis showed a positive nasal allergen provocation test with Der p 1, with 6 of those 27 patients (22%) having nasal specific IgE to *D pteronyssinus*. Indeed, 3 of the 7 positive nasal allergen challenges were to dust mite. Repeated dust mite challenges in these individuals were negative. This could be due to the fact that they had already been challenged with *Alternaria*, cockroach, timothy grass, and cat hair before dust mite at their initial visit; however, during repeated challenge they were administered dust mite alone.

There are a variety of other reasons that could explain the disparate results. First, the studies by Rondon et al²¹ and Huggins and Brostoff⁵ used incremental challenges of dust mite, whereas we used a single dose. Because the present study sought to identify the frequency of positive nasal challenges to several allergens, we designed the protocol to use multiple rather than single allergen challenges. It is possible that incremental challenges may have a nonspecific irritant effect that could eventually elicit a TSS greater than 5. In the present study, patients 1 and 19 seemed to have an escalation in TSS with subsequent challenges on initial testing. However, on repeated single testing, negative challenges were observed.

Another problem with comparing the present study with others that showed positive nasal challenges in nonallergic patients is the fact that the doses, method of administration, and source of allergen were all different. Huggins and Brostoff⁵ used *D pteronyssinus* dosed in protein nitrogen units from Dome Laboratories (England) delivered via aerosol. Carney et al⁶ used freshly reconstituted freeze-dried allergen solutions of *D pteronyssinus*/*Dermatophagoides farinae*, cats/dogs, and grass pollen mix in unspecified concentrations from an unspecified source via aerosol spray. Rondon et al²¹ used freshly reconstituted freeze-dried allergen solutions of *D pteronyssinus* dosed based on Der p 1 concentration from ALK-Abello Inc (Round Rock, Texas) via an aerosol spray. We used multiple allergens (with standardized extracts when available) in various concentrations available from Greer Laboratories Inc and administered them via pipette. Although freshly reconstituted freeze-dried allergen solutions would likely retain the most potency, the doses and extracts we used seemed to be sufficiently potent because they were capable of eliciting markedly positive reactions in controls with documented allergic sensitivity. We selected the pipette delivery method rather than the aerosol method to minimize dosing errors and provide a more precise dose.

Another possibility for disparate results is the fact that no studies have used the same definition for a positive challenge. These factors confound the comparison of nasal challenges in nonallergic rhinitis. For example, Carney et al⁶ used an increase of greater than 50% baseline nasal resistance when using anterior active rhinomanometry but noted that most researchers require a 100% change in resistance for the definition of a positive nasal challenge. We chose PNIF because it is simple, cost-effective, and quick and because it strongly correlates with anterior rhinoscopy findings.²² It has also been shown to be at least as sensitive as active anterior rhinomanometry and acoustic rhinometry and to correlate with subjective symptoms using patient questionnaires in some studies.^{23–28} Osterballe²⁹ originally defined the response to nasal challenge as positive when a 20% or greater decrease in PNIF was present, and this value has been used in subsequent publications.^{8,30}

There are other limitations to this study. Circadian variation of nasal congestion could be a confounding variable because we did not test all the patients during a predetermined time of day. Imaging to exclude sinusitis was not routinely performed, and this could have affected the results. A priming effect could have conceivably affected the results of patient 18 because his initial positive challenge to timothy grass occurred during the grass pollinating season, and his subsequent negative challenge was performed outside the season. There was no known exposure change to dust mite or cat allergen in the other relevant patients. Because we did not perform escalating dose challenges, it is conceivable that the threshold dose required to provoke a positive challenge had increased by the time each patient underwent their second nasal challenge, thereby creating false-negative challenges. It is possible that the threshold dose may have diminished in some patients during that same 3- to 5-month period between nasal challenges. Thus, some of the repeated nasal challenges should have remained positive if they were truly a result of localized nasal IgE production.

In conclusion, this study does not support the concept of localized allergy in the nose. The positive nasal challenges in patients with perennial nonallergic rhinitis were not reproducible on repeated allergen challenge. The positive challenges did not correlate with PNIF values or nasal eosinophil numbers. Thus, further investigation with larger numbers of patients is warranted.

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Requests for reprints should be addressed to:

David A. Khan, MD

Division of Allergy and Immunology

University of Texas Southwestern Medical Center

5323 Harry Hines Blvd

Dallas, TX 75390-8859

E-mail: dave.khan@utsouthwestern.edu