

Skin Testing in Predicting Response to Nasal Provocation with *Alternaria*

John H. Krouse, MD, PhD; Anand G. Shah, MD; Kristy Kerswill, BSc

Objective: Examine the efficacy of epicutaneous and intradermal testing in predicting response to nasal provocation with *Alternaria* antigen. **Study Design and Setting:** Prospective study. Subjects were tested with the Multi-Test II (MT) epicutaneous testing device. Subjects with negative wheals were then tested with a 1:500 weight:volume intradermal injection of *Alternaria*. They had baseline assessment of nasal cross-sectional area (CSA) using acoustic rhinometry and underwent nasal provocation with increasing *Alternaria* concentrations. CSA was assessed at each concentration. A nasal allergen provocation score (NAP) of nasal symptoms as well as a nasal visual analogue scale (VAS) were also completed with each concentration. **Results:** Sensitivity and specificity of MT in predicting nasal response to *Alternaria* were poor at 42% and 44%, respectively. The addition of intradermal testing increased sensitivity only modestly to 58%. hierarchical linear modeling analysis demonstrated that subjects positive to *Alternaria* on skin testing did not show a significant reduction in nasal CSA on acoustic rhinometry or significant elevations in two nasal symptom scores with direct nasal provocation. **Conclusion and Significance:** Skin testing with either epicutaneous or intradermal testing may not be an accurate or sufficient technique in the assessment of *Alternaria* reactivity. These results suggest that mold allergies may involve more complex immune mechanisms than simply an immunoglobulin (Ig)E mediated type I immediate hypersensitivity response alone. An alternate model for mold sensitivity, as well as modifications in testing methods, may be required in the evaluation of mold allergy. **Key Words:** Allergy, skin testing, rhinometry, sensitivity, *Alternaria*.

Laryngoscope, 114:1389–1393, 2004

INTRODUCTION

Skin endpoint titration (SET) has been used for the testing of inhalant allergy for many years. SET was de-

veloped through the work of several individuals, dating to Hansel in the 1920s.¹ Observations by Phillips demonstrated that the safe starting dose for immunotherapy appeared to correlate well with the size of the skin wheal on intradermal testing.² SET was standardized in the 1950s by Rinkel, who noted that use of fivefold dilutions of antigens for testing was clinically effective in the preparation of potent treatment vials for the management of inhalant immunotherapy.³ Krouse and Krouse⁴ further demonstrated that immunotherapy based on SET brought about a significant reduction in both symptomatology and nasal antigenic responsivity after 6 months of immunotherapy.

Otolaryngic allergists have recently begun using blends of epicutaneous and intradermal testing methods for the evaluation of inhalant allergy.⁵ Epicutaneous testing devices such as the Multi-Test II (Lincoln Diagnostics, Decatur, IL) have been demonstrated to have excellent clinimetric properties, with a reported sensitivity of 100% and specificity of 95.5% in one study.⁶ The results noted with testing from the Multi-Test II have been estimated to correlate with an SET dilution of between #3 (1:2500) and #4 (1:12,500).⁷

A question that has concerned otolaryngic allergists involves the clinical importance of positive intradermal tests with highly concentrated antigens, such as 1:500 and 1:100 weight:volume (w:v). This discussion is based on differing interpretations of positivity and on anecdotal observations of clinical efficacy and utility. In one recent study, positive intradermal tests were shown to be less specific than prick testing in representing true inhalant allergy.⁸ Physicians who do implement immunotherapy on the basis of intradermal test results argue that prick testing is insensitive to the detection of allergy and that intradermal testing is warranted to increase the sensitivity of the testing battery.

The present research was designed to assess the sensitivity and specificity of both intradermal testing and epicutaneous testing using the Multi-Test II device in demonstrating the presence of allergy to *Alternaria* antigen. In addition, it was designed to assess the clinical significance of intradermal positivity in patients who are Multi-Test II negative for allergy to *Alternaria* but who are positive to the antigen at intradermal SET dilution #2 (1:500). Patients were assessed with progressive nasal

Presented at the Middle Section Meeting of The Triological Society, Marco Island, FL, January 8, 2004.

From the Department of Otolaryngology, Wayne State University, Detroit, MI, U.S.A.

This study was supported, in part, by a grant from Lincoln Diagnostics, Inc., Decatur, IL.

Editor's Note: This Manuscript was accepted for publication February 12, 2004.

Send Correspondence to Dr. John H. Krouse, Department of Otolaryngology, Wayne State University, 540 E. Canfield, 5E-UHC, Detroit, MI 48201, U.S.A. E-mail: jkrouse@med.wayne.edu

antigen provocation as a “gold standard” of response, correlating the direct effects of increasing concentration of antigen on the end organ with skin responsiveness. Support for the use of nasal provocation as an appropriate reference standard was confirmed by Olive-Perez.⁹ It was the hypothesis of this proposed research that nasal challenge testing with *Alternaria* antigen would correlate well with epicutaneous testing using the Multi-Test II and that additional testing with 1:500 intradermal injections would add little clinical utility to the diagnosis of *Alternaria* allergy.

METHODS

Sample

The sample consisted of 44 adults with a history of allergic rhinitis, collected consecutively by means of a public advertisement. Three groups were defined on the basis of each individual's response to skin testing: 1) 17 subjects who were positive to *Alternaria* by Multi-Test II with a wheal that was at least 3 mm larger than a negative control; 2) 8 subjects who were negative to testing with Multi-Test II but positive to *Alternaria* at a SET dilution of #2 with a 7 mm intradermal wheal; and 3) 19 subjects who were negative to testing for *Alternaria* by both Multi-Test II and with SET dilution #2 intradermal tests.

Inclusion Criteria

History of allergic rhinitis.
Adults, ages 18 to 70.
Ability to consent to participation in the study.

Exclusion Criteria

Use of an oral antihistamine within 1 week.
Pregnancy at the time of the study.
Concurrent use of leukotriene receptor antagonists.
Concurrent use of 5-OH-lipoxygenase inhibitors.
Use of nasal or systemic corticosteroids within 1 week.
History of endoscopic, intranasal, or external sinus surgery.

Setting

The study was conducted through the offices and clinics of University Otolaryngology, P.C. and the Wayne State University Department of Otolaryngology–Head and Neck Surgery. All subjects were volunteers accrued through open advertisement in the university community.

Procedure

Individuals with a history suggestive of allergic rhinitis were identified as prospective candidates for this study. They were required to be symptomatic with allergic rhinitis for a minimum of 2 years before the study. Those individuals who satisfied the diagnostic and inclusion/exclusion criteria were invited to participate. The nature of the study was then explained to each individual, who was then asked to enroll in the study. Consent was completed, and each subject was asked to read and sign the consent form. The study design and consent procedures were reviewed and approved by the Human Investigation Committee of Wayne State University. Subjects who agreed to participate were included in the study.

Subjects were tested for inhalant allergy using two different methods of skin testing. They were first tested for 24 inhalant antigens, including *Alternaria*, using epicutaneous testing with

the Multi-Test II along with appropriate controls.¹⁰ Subjects who were positive to *Alternaria* antigen with a wheal size at least 3 mm greater than a negative control were included in group 1, the *Alternaria*-positive group. Subjects who were negative to *Alternaria* were then tested with a #2 dilution (1:500 w:v) intradermally. If positive to the #2 dilution (7 mm intradermal wheal and a negative glycerin control), these patients were included in group 2, the *Alternaria*-#2 group. Subjects negative to *Alternaria* with both Multi-Test II and intradermal testing at the #2 dilution were included in group 3, the *Alternaria*-negative group.

Each subject then proceeded to the nasal provocation portion of the study. The *Alternaria* antigen used in this study was provided at a concentration of 1:20 w:v, prepared as a suspension with 50% glycerin added as a preservative (Antigen Laboratories, Liberty, MO). Challenge solutions consisted of progressive dilutions of *Alternaria* extract in 0.9% saline and 0.4% phenol, and included SET #3 (1:2,500), SET #2 (1:500), and SET #1 (1:100). The challenge volume was delivered under pressure into each nostril with a 2-second spray from a standard DeVilbiss spray bottle.

Each subject first completed a nasal allergen provocation scale (NAP) and a visual analogue scale (VAS), reflecting his/her global allergic symptomatology before antigen challenge. Each subject also completed a baseline nasal cross-sectional area (CSA) measurement by acoustic rhinometry (AR) (Eccovision, Hood Laboratories, Pembroke, MA). The subject then received a control challenge consisting of an aqueous suspension of 0.9% saline, 50% glycerin, and 0.4% phenol to demonstrate lack of response to the diluent medium. At the conclusion of a 5-minute period, the subject again completed the NAP, VAS and AR. The subject waited 10 minutes for any effects to abate and was then challenged with the #3 (1:2,500) dilution of *Alternaria* antigen. After a 5-minute period, the subject again completed the NAP, VAS, and AR. If there was a 15% reduction in nasal CSA, the procedure was terminated. If a lesser change was noted, after a 10-minute hiatus, a challenge was given with the #2 (1:500) dilution of *Alternaria*. The same progression was followed with antigen challenge and outcome assessment until the patient was positive at one dilution or negative at all dilutions. At the conclusion of the study, all subjects who completed the nasal challenge received a payment of \$50 for their participation.

Outcome Measures

Outcome measures for the study included 1) AR of the minimal CSA of the nasal cavity; 2) NAP for subjective assessment of nasal symptoms; and 3) VAS for subjective assessment of nasal symptoms.

AR was conducted using an Eccovision acoustic rhinometer (Hood Laboratories, Pembroke, MA). Minimal CSA of each nasal cavity was assessed before challenge. The side of the nose with the greater CSA was chosen for subsequent nasal provocation challenge and that CSA was used as the baseline measurement. A positive response to antigen challenge was defined as a reduction in CSA by 15% or greater from baseline in that nasal cavity. A positive response at one dilution resulted in termination of the test and was judged positive for all more concentrated dilutions.

The NAP scale was completed before challenge and after each nasal challenge. The scale consisted of three questions. Subjects were asked to rate the severity of their nasal secretions, nasal irritation, and distant symptoms (watery eyes, cough). Each question was scored from 0 to 2, with 6 the highest possible score. The total score therefore ranged from 0 to 6.

The VAS was completed before challenge and after each nasal challenge. The scale consisted of a 10 cm line drawn on paper with marks at 1 cm intervals. Subjects were asked to globally assess their symptoms, including congestion, stuffiness,

itching, sneezing, and rhinorrhea and placed a single mark along the VAS to demonstrate their perceived symptomatology.

RESULTS

On the basis of skin testing, 44 consecutive adult volunteers with symptoms of allergic rhinitis were classified into one of three groups:

1. Positive to Multi-Test II ($n = 17$).
2. Negative to Multi-Test II but positive to intradermal testing ($n = 8$).
3. Negative to both Multi-Test II and intradermal testing ($n = 19$).

A breakdown of subjects as a function of Multi-Test II positivity and nasal provocation response is presented in Table I. These same results including intradermal positivity are presented in Table II.

Analysis of nasal provocation results among those subjects positive and negative to *Alternaria* antigen by Multi-Test II demonstrates a sensitivity of 42% (8/19) and a specificity of 44% (11/25). In subjects who were negative to epicutaneous testing, intradermal tests with 1:500 *Alternaria* antigen were performed. Eight of these 27 subjects had a 7 mm wheal to *Alternaria* after 10 minutes, demonstrating a positive response. The addition of intradermal positivity to the results of the Multi-Test II increased the sensitivity to 58% (11/19), without changing the specificity.

To compare the numbers of patients reaching the 15% change in nasal CSA across the patient groups, a chi-square analysis using Fisher's exact test was performed. To model the change in nasal CSA, NAP, and VAS across the four provocation assessments, hierarchical linear modeling (HLM) was used. Separate analyses were performed for each of the outcome measures. HLM was used because not all subjects had complete data for all four possible concentration values. To compare the two patient groupings in the HLM, dummy coding was used.

Chi-square analysis revealed no significant difference in the percent of subjects reaching the 15% reduction in nasal CSA between those subjects positive to skin testing with Multi-Test II, those subjects positive with intradermal testing, and those subjects negative to testing with both methods ($\chi^2 = 0.218$, $df = 2$, $P = .897$). In addition, with HLM analysis, subjects who were positive to skin testing by either epicutaneous or intradermal testing did not demonstrate a significant reduction in nasal CSA as the concentration of nasal antigen increased when com-

TABLE I.
Multi-Test II Response and Nasal Provocation.

	Reached 15%		
	Negative	Positive	Total
Multi-Test II			
Negative	16	11	27
Positive	9	8	17
Total	25	19	44

TABLE II.
Intradermal (ID) Response and Nasal Provocation.

	Reached 15%		
	Negative	Positive	Total
Multi-Test II and ID			
Negative	11	8	19
Positive	14	11	25
Total	25	19	44

pared with those subjects who were negative to skin testing ($t = -714$, $df = 41$, $P = .479$).

Analysis of the NAP data using HLM did not show a significant difference in subjective nasal symptoms between those subjects testing positive to *Alternaria* antigen by either epicutaneous or intradermal testing and those testing negative ($t = 0.165$, $df = 41$, $P = .870$). Subjects who were positive to skin testing did not demonstrate more severe nasal symptoms on the NAP scale with increasing concentrations of nasally delivered *Alternaria* antigen than those subjects who were negative to skin testing ($t = 1.875$, $df = 41$, $P = .068$).

Similarly, analysis of the VAS data using HLM did not show a significant difference in subjective nasal symptoms between those subjects testing positive to *Alternaria* antigen by either epicutaneous or intradermal testing and those testing negative ($t = 0.478$, $df = 41$, $P = .634$). Subjects who were positive to skin testing, however, did demonstrate more severe nasal symptoms with increasing concentrations of nasally delivered *Alternaria* antigen than those subjects who were negative to skin testing ($t = 2.060$, $df = 41$, $P = .045$).

DISCUSSION

The results of this study suggest that mold allergy may not be accurately assessed through either epicutaneous or intradermal testing. In the present study, epicutaneous testing using the Multi-Test II did not appear to represent a sufficient method for the detection of allergy to *Alternaria*. The sensitivity of this epicutaneous technique was 42% in the present sample when evaluated against nasal provocation with *Alternaria* antigen. Subjects who clearly responded to direct stimulation of the end organ with *Alternaria* antigen were often not detected with this testing method. The Multi-Test II also demonstrated a low specificity of 44% in the present sample. Subjects who did demonstrate skin test positivity often failed to objectively demonstrate end organ responsiveness to nasal challenge, although positive subjects did have more nasal symptoms as the concentration of nasally delivered antigen increased. These properties suggest that use of an epicutaneous technique may have limitations in testing for *Alternaria* allergy.

Furthermore, the current results suggest that intradermal testing may offer little additional benefit in the diagnosis of *Alternaria* allergy. The sensitivity with the combined prick/intradermal approach for *Alternaria* was 58%. Although the addition of intradermal testing mod-

estly improved sensitivity, the overall poor percentage again challenges the role of skin testing in the detection of mold allergy. Although *Alternaria* skin tests did produce wheals in select patients, these results were not reliably demonstrated at the end organ with nasal CSA.

Many in vivo testing techniques, including SET, have been clinically accepted for the diagnosis of inhalant allergy.¹⁻⁴ A review of the literature, however, shows that few studies have examined direct response to molds at the end organ. A report by Lynch and coworkers¹¹ showed that bronchial challenge and skin testing results with *Alternaria* did not correlate significantly, whereas correlations between testing results and end organ challenge with timothy grass antigen were excellent. In addition, in vitro immunoglobulin (Ig)E levels by radioallergosorbent test (RAST) also failed to correlate with end organ bronchial challenge in the Lynch study using *Alternaria* antigen. Similarly, a study by Kewson and colleagues¹² showed that the sensitivity and specificity of the Multi-Test II device for *Alternaria*, when judged against inhaled bronchial challenge of nebulized *Alternaria* antigen, were only approximately 50%. These two reports, which focused on direct responses at the end organ, are consistent with the present findings.

One possible explanation for the present results involves the hypothesis that patient sensitivity to mold antigens may not be mediated through a simple, IgE mediated type I immediate hypersensitivity response alone. Because both epicutaneous and intradermal testing involve examination of the skin's response after 10 to 20 minutes, these approaches primarily assess histamine release mediated by a type I IgE response. The poor sensitivity and specificity noted in the current study suggest that additional immune mechanisms may be involved in the pathogenesis of mold allergy. Other mechanisms, including a T-cell mediated, type IV delayed hypersensitivity response may also contribute to the expression of symptoms in this population. Clinical observations frequently demonstrate persistence of whealing with mold antigens over several days, although the significance of these observations is currently speculative.

There may also be variability in the methods of preparation of mold antigens for testing among various antigen suppliers. This variability may also reflect inherently different responses when mold antigen is used percutaneously and when it is applied directly to mucosal surfaces. Aas et al.¹³ used prick testing to compare extracts of five common molds obtained from different manufacturers. Considerable differences were found between the extracts with respect to the pattern of positive and negative tests noted in mold-allergic individuals and nonallergic controls. Aas and colleagues therefore questioned the reliability and validity of skin tests in the accurate representation of mold allergies.

Although the clinicometric properties of both epicutaneous and intradermal testing appear poor in the evaluation of molds such as *Alternaria*, these properties do not appear characteristic of other antigens. For example, a study by Krouse et al.¹⁴ supported the use of skin testing as an accurate methodology for the diagnosis of timothy grass sensitivity. Krouse et al. reported a sensitivity and

specificity of 87% and 86% with Multi-Test II alone when evaluated against direct nasal provocation at the end organ; the addition of intradermal testing raised sensitivity slightly to 93%.¹⁴ In a like manner, Lynch et al.¹¹ supported the use of skin testing as accurate in the diagnosis of timothy grass allergy. These findings suggest that skin testing for pollens is more reliable and valid than with antigens such as molds and more accurately reflects end organ responsiveness. Pollen allergy appears to be more directly a function of type I hypersensitivity and histamine release. Additional research is necessary to further characterize differential immune responses to skin testing when using different antigens.

The present findings suggest that assessment methods other than epicutaneous and intradermal testing for immediate response may be indicated for the comprehensive evaluation of patients with suspected mold allergies. Of note, subjects in the present study who were positive to epicutaneous testing with *Alternaria* antigen did have increasing nasal symptoms with increasing concentrations of nasally delivered antigen. Unfortunately, this relationship was not confirmed objectively by AR in this study. A more important role for direct end organ challenge in the assessment of mold allergy may be indicated. In addition, clinical observations suggest the importance of delayed responses to skin testing in the assessment of mold allergy and the provision of immunotherapy. The use of 48-hour delayed responses in mold-allergic patients may improve the overall sensitivity and specificity of assessment. Further research is necessary to evaluate the use of alternate testing methodologies and their scientific accuracy and clinical utility.

CONCLUSION

Epicutaneous testing as currently used demonstrates poor sensitivity and specificity in its ability to detect mold allergy. Although intradermal testing improves sensitivity, its representation of end organ responsiveness remains poor. These findings suggest that mold allergies may involve more complex immune mechanisms than an IgE mediated type I immediate hypersensitivity pattern alone. Further research is necessary to elicit the mechanism of mold allergy and to determine more accurate and effective methods in its diagnosis.

BIBLIOGRAPHY

1. Keenan JP. History of skin testing and evolution of skin endpoint titration. In: Mabry RL, ed. *Skin Endpoint Titration*. New York: Thieme, 1992:12-16.
2. Phillips EW. Relief of hayfever by intradermal injections of pollen extract. *JAMA* 1926;86:182-184.
3. Rinkel HJ. The management of clinical allergy. II. Etiologic factors and skin titration. *Arch Otolaryngol* 1963;77:42-75.
4. Krouse JH, Krouse HJ. Efficacy of immunotherapy based on skin end-point titration. *Otolaryngol Head Neck Surg* 2000;123:183-187.
5. Krouse JH, Mabry RL. Skin testing for inhalant allergy 2003: current strategies. *Otolaryngol Head Neck Surg* 2003;129: S33-S49.
6. Nelson HS, Lahr J, Buchmeier A, McCormick D. Evaluation of devices for skin prick testing. *J Allergy Clin Immunol* 1998;101:153-156.
7. Murphree JT, Kniker WT. Correlation of immediate skin test responses to antigens introduced by multi-test and intra-

- cutaneous routes. *Ann Allergy* 1979;43:279–285.
8. Gungor A, Houser SM, Aquino BF, et al. Comparing skin endpoint titration to other forms of allergy testing. *ENT J* 2004;83:54–60.
 9. Olive-Perez A. The nasal provocation test in the diagnosis of allergic rhinitis. Comparison with other diagnostic tests. *Rhinology* 1988;26:175–181.
 10. Hurst DS, Gordon BR, Krouse JH. The importance of glycerin-containing controls in the interpretation of allergy skin tests. *Otolaryngol Head Neck Surg* 2002;127:177–181.
 11. Lynch NR, Dunand P, Newcomb RW, et al. Influence of IgG antibody and glycopeptide allergens on the correlation between the radioallergosorbent test (RAST) and skin testing or bronchial challenge with *Alternaria*. *Clin Exp Immunol* 1975;22:35–46.
 12. Kewson DT, Kerswill KA, Janisse J, Krouse JH. The sensitivity and specificity of 1:500 dilution in predicting bronchial response to antigen challenge. Paper presented at the Annual Meeting of the American Academy of Otolaryngic Allergy, Orlando, FL, September 19, 2003.
 13. Aas K, Leegaard J, Aukrust L, Grimmer O. Immediate type hypersensitivity to common moulds. Comparison of different diagnostic materials. *Allergy* 1980;35:443–451.
 14. Krouse JH, Sadrazodi K, Kerswill KA. Sensitivity and specificity of prick and intradermal testing in predicting response to nasal provocation with timothy grass antigen. *Otolaryngol Head Neck Surg* In press.