Aeroallergen sensitization correlates with PC_{20} and exhaled nitric oxide in subjects with mild-to-moderate asthma

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Background: Aeroallergen sensitization in adult asthmatic patients from a wide geographic area has not been correlated with patients' characteristics, markers of airways inflammation, and lung function.

Objective: We assessed data obtained from the Asthma Clinical Research Network trials to determine the relationship of aeroallergen sensitization to age, sex, ethnicity, and markers of inflammation and airways function.

Methods: Skin testing (14 epicutaneous) was performed on 1338 subjects with objectively diagnosed mild-to-moderate asthma from 11 Asthma Clinical Research Network studies. Skin testing used identical techniques and a quality assurance program to ensure uniformity across centers.

Results: Ninety-five percent of the subjects had at least 1 positive skin test response. Of these, 14% had positive reactions to 1 or 2 allergens and 81% had positive reactions to 3 or more allergens, and 2% of subjects reacted only to seasonal allergens, 26% only to perennial allergens, and 67% to both. Increasing IgE and exhaled nitric oxide values, decreasing PC_{20} values, and minority ethnicity significantly correlated with the number of positive skin test responses. Subjects with late-onset asthma were less likely to be sensitized; nonetheless, 89% of subjects older than 60 years had positive responses.

Conclusion: Ninety-five percent of patients with mild-to-moderate asthma might have an allergic component. Age does not significantly affect aeroallergen sensitization, but the pattern of allergic sensitization varies with ethnicity and geography. Measures used to characterize asthma, such as IgE, exhaled nitric oxide, and PC_{20} values, are correlated with aeroallergen sensitization. (J Allergy Clin Immunol 2008;121:671-7.)

Key words: *Asthma, allergy, skin testing, aeroallergens, exhaled nitric oxide, IgE, methacholine challenge*

Abbreviations used
ACRN: Asthma Clinical Research Network
eNO: Exhaled nitric oxide
FVC: Forced vital capacity

Asthma and other IgE-mediated diseases continue to increase in prevalence in the United States and throughout the world.[1] Allergens, both occupational and environmental, comprise one of the multiple triggers for asthma, along with others, including exposure to tobacco smoke, air pollution, viral infection, irritant exposure, exercise, cold dry air, sinusitis, and esophageal reflux. Skin testing, which is both sensitive and specific, has been used to objectively determine patient sensitization to allergens that can be a trigger for asthma and other allergic disorders.[2-5] Allergic sensitization to aeroallergens, as defined by skin testing, has been shown to vary by economic status, location, ethnic identity, and other factors; however, correlations between aeroallergen sensitization and asthma characteristics in a large number of patients given objective diagnoses of mild-to-moderate asthma are lacking.[6-8] A recent publication using patient recall of skin test reactivity in an observational study of those with severe asthma suggested that the vast majority of such asthmatic subjects were allergic to aeroallergens.[9] We hypothesized that the vast majority of subjects of any age with well-characterized mild-to-moderate asthma will be sensitized to aeroallergens, as defined by having at least 1 directly observed positive skin test response, and that positive skin test responses will correlate with other measures used to assess asthma.

METHODS

We assessed data from subjects who provided written informed consent and were randomized into multiple studies performed by the National Heart,

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TABLE I. Sex, age, and ethnic origin of subjects with skin test data in ACRN trials

	Category	No.	Percentage
Sex	Female	771	58
	Male	567	42
Ethnic origin	African American	279	21
	Asian and American Native	67	5
	Hispanic	111	8
	White	864	65
	Other	17	1
Age (y)		Mean = 32	Range = 12-65

Lung, and Blood Institute's Asthma Clinical Research Network (ACRN). Subjects were recruited from pulmonary and allergy clinics and through advertisements to the general public. The vast majority of subjects were recruited from non–allergy clinic populations. The studies included SOCS,[10] SLIC,[11] BARGE,[12] BAGS,[13] CIMA,[14] MICE,[15] DICE,[16] IMPACT,[17] SMOG,[18] SLIMSIT,[19] and the Predicting Response to Inhaled Corticosteroid Efficacy trial.[20] We focused our primary analysis on correlations between aeroallergen sensitization and demographics, such as ethnicity, sex, and age. We secondarily assessed other measures used in the characterization of asthma, such as methacholine reactivity, exhaled nitric oxide (eNO) level, pulmonary function, peripheral blood and sputum eosinophil numbers, and total serum IgE levels, for correlation with allergic sensitization status.

All centers performed epicutaneous skin testing by using an identical technique, as specified by the ACRN "Manual of operations." Trained certified personnel and a quality assurance program ensured expertise before research personnel participated in the ACRN studies. Allergens used in the testing panel were the house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*; the molds *Alternaria*, *Aspergillus*, and *Penicillium* species; and dog, cat, ragweed, grass mix, tree mix, weed mix, and cockroach. Appropriate negative (diluent) and positive (histamine) controls were included (Greer, Lenoir, NC). Skin testing was performed with the Multitest device (Lincoln Diagnostics, Decatur, Ill). The positive control was required to have a wheal of at least 3 mm in size with surrounding erythema to be considered a valid test. After 15 minutes of incubation, the skin test response was considered positive if the wheal exceeded that elicited by the negative control by 3 mm with documented erythema. Aeroallergen sensitization was defined as a positive response to at least 1 allergen.

Patients recruited into the studies were required to have evidence of asthma, as defined by either a positive methacholine challenge result (<8 mg/mL), defined as PC_{20}, an improvement of FEV_1, or both of 12% or greater with 2 to 8 puffs of albuterol. For safety, skin tests were not performed if the FEV_1 was less than 60% of predicted value or if the patient had a recent history of asthma instability. Demographics, peak flow measurement, spirometry, methacholine testing, eNO quantization, sputum induction, and screening laboratory tests were performed on entrance into and periodically during the studies and on termination. Spirometry, methacholine testing, eNO measurement, sputum test for eosinophils, and genotyping were performed as outlined in the ACRN manual of operations and as described in previous publications.[10-20]

The total number of positive skin test responses was summarized into the categories of 0, 1 to 2, and 3 or more. The associations between this measure and the demographic information and measures of asthma severity were then evaluated by using the Cochran-Mantel-Haenszel nonparametric test of correlation adjusted for the ACRN trial. The adjustment for the ACRN trial accounted for the different types of run-in periods included in each trial. For significant associations, the mean, median, or geometric mean of each measure at each level of allergic sensitization was reported. Partial Kendall τ correlation coefficients were also calculated between allergic status and the demographic and asthma severity measures adjusted for the ACRN trial.

The categorization of allergic status into no allergic sensitization, seasonal only, perennial only, and at least one of each was evaluated by using the Cochran-Mantel-Haenszel nonparametric test of mean differences to determine whether associations existed between this variable and the demographic and asthma severity measures adjusted for ACRN trial. For significant associations, the mean, median, or geometric mean of each measure at each level of the seasonal/perennial categorization was reported.

RESULTS

One thousand three hundred thirty-eight subjects were included in this analysis, and Table I summarizes their baseline characteristics. Additional demographics are available in Table II. Patients ranged in age from 12 to 65 years, with 58% being female and 42% being male. Ethnicity distribution included 65% white, 21% African American, 8% Hispanic, and 6% "other." Skin test results to specific antigens and to positive and negative controls are noted in Table III.

Ninety-five percent of subjects had at least 1 positive skin test response and thus, for the purposes of this study, are considered to have aeroallergen sensitization. Fourteen percent of subjects had 1 to 2 positive test responses, and 81% had a positive response to 3 or more allergens. The average number of positive test responses was 5. Of those who reacted, 2% did so only to seasonal allergens, 26% only to perennial antigens, and 67% to both categories. The percentage of positive test responses decreased slightly, although not significantly, with increased decades of life (Fig 1), and those 60 to 70 years of age had a higher mean and median number of positive skin test responses than younger subjects (Fig 2), and 89% had at least 1 positive skin test response. Subjects with onset of asthma before 30 years of age were more likely to be sensitive to aeroallergens (96%) than those whose onset of asthma was later (88%, $P \leq .001$).

Skin tests also varied by ethnicity (Fig 3), and all nonwhite subjects, defined as all nonwhite minorities, had a higher frequency of 3 or more skin tests than white subjects ($P = .024$, Table IV). Ninety-six percent of male subjects and 94% of female subjects were aeroallergen sensitive. Although both FEV_1 (male subjects, 81.7%; female subjects, 87.1%; $P = .001$) and the FEV_1/forced vital capacity (FVC) ratio (male subjects, 69.9%; female subjects, 74.5%; $P = .001$) varied by sex, sex did not influence aeroallergen sensitization ($P = .276$), nor did FEV_1 percentage or FEV_1/FVC ratio for either sex vary by aeroallergen sensitivity. We also assessed skin tests by ACRN site geographic location (Table V) to determine regional distribution of positive skin test responses. Of note are the high prevalence of positive skin test responses to cat and dust mite and a relatively uniform distribution of positive test responses across the ACRN sites. Notable exceptions to this uniform distribution were the observed and expected low prevalence of dust mite and cockroach sensitization in Denver and the low prevalence of positive reactions to ragweed in San Francisco but also the unexpected low prevalence of positive reactions to tree mix and dog in San Francisco. In all regions of the country, sensitivity to *Aspergillus* and *Cladosporium* species was lower than that noted to essentially all other allergens tested.

When measures used to characterize asthma were compared with aeroallergen sensitization (Table IV) both eNO and IgE levels increased significantly with an increased number of positive test responses ($P = .008$ and $P < .001$, respectively), whereas PC_{20} decreased as the number of positive skin test responses increased ($P < .001$). The average values for all 3 (ie, eNO, IgE, and PC_{20}) were greatest in those subjects with either positive skin test responses to perennial allergens alone or to both seasonal and perennial allergens compared with those who responded only to seasonal allergens (Table VI). We did not find