

Do diagnostic procedures other than inhalation challenge predict immediate bronchial responses to inhaled allergen?

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Summary

To investigate the relationships between allergen inhalation challenge and other diagnostic procedures, inhalation challenge with house dust (HD) allergen, intradermal skin tests with HD allergen, inhalation challenge with methacholine and circulating HD allergen-specific IgE levels were examined in 104 patients with bronchial asthma. Using the single exposure method, allergen inhalation challenge was performed. Forty-three patients had positive bronchial responses to allergen and 61 patients had negative bronchial responses. With serially diluted HD allergen (10^{-3} to 10^{-6} , w/v), skin-test sensitivity was expressed as the highest dilution required to produce a weal of more than 9×9 mm. With the continuous exposure method, bronchial responsiveness to methacholine was evaluated as the number of units of inhaled methacholine (PD₃₅-Grs) from the start to the point at which Grs had decreased by 35% from its baseline value. The level of circulating HD allergen-specific IgE was measured with the Phadebas® RAST system and the results were assessed as a RAST score. Using discriminant analysis, in which the independent variables were skin-test sensitivity, PD₃₅-Grs and the RAST score, only in 30% of all patients was bronchial responsiveness to inhaled HD allergen predictable. Therefore, we suggest that inhalation challenge with allergen is an essential test for determining the role of a specific allergen in airways at present.

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Introduction

Inhalation challenge with allergen has been used as a diagnostic procedure for determining the role of specific allergens in bronchial asthma and estimating the efficacy of immunotherapy and other therapeutic agents. Since inhalation challenge with allergen has several demerits, namely complexity of technique, induction of bronchoconstriction in the late phase, and deterioration in clinical asthma and non-specific bronchial hyper-responsiveness, some investigators have tried to evaluate results of allergen inhalation challenge by simpler and safer diagnostic procedures. Consequently, using multiple regression analysis, it has been demonstrated that the degree of airway responsiveness to inhaled allergen in patients with positive bronchial responses can be predicted from skin-test sensitivity to allergen and bronchial responsiveness to

inhaled histamine [1,2] or from the level of allergen-specific IgE in serum, airway responsiveness to inhaled methacholine and baseline airway caliber [3]. In addition, using the skin-prick test, Ollier *et al.* reported a fine study [4] on prediction of a positive response to inhalation provocation testing.

As previously reported, we have developed an apparatus [5] for inhalation challenges with methacholine [6] and house dust (HD) allergen [7]. Using the apparatus, throughout both inhalation challenges, bronchial responses can be continuously and graphically evaluated as a change in total respiratory resistance (Rrs) measured with the oscillation technique [8]. Since these two methods entail very little discomfort for the patients, many outpatients have undergone bronchial challenges with methacholine and allergen in our clinic.

The present study was done to examine whether the occurrence of immediate bronchoconstriction to inhaled HD allergen is predictable from the combination of results of other diagnostic procedures, such as inhalation

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challenge with methacholine, intradermal skin test with HD allergen, circulating HD allergen-specific IgE assay, and baseline pulmonary function testing.

Subjects and methods

Subjects

One-hundred and four patients with bronchial asthma participated in the present study. All subjects had a history of episodic dyspnea with wheezing and a documented increase in FEV₁ of more than 20%, either spontaneously or after medication. In all patients, a skin weal greater than 6 × 6 mm was produced in response to HD allergen (10⁻³ w/v). Before each inhalation challenge test, beta-adrenergic stimulating agents and phosphodiesterase inhibitory agents were withheld for at least 12 hr and anti-allergic drugs, including sodium cromoglycate, tranilast and ketotifen, were withheld for at least 72 hr. None of the subjects received antihistamine and glucocorticosteroid agents.

Inhalation challenge

On 2 different days within 2 weeks, all patients underwent first a non-specific bronchial challenge with methacholine and then a specific bronchial challenge with HD allergen.

Inhalation challenge with methacholine was performed using the method described by Takishima *et al.* [6]. Throughout the test, Rrs was measured continuously and graphically with the oscillation technique. An aerosol of saline followed by two-fold increasing concentrations of methacholine (0.049–25 mg/ml) was inhaled for 1 min continuously. A 2-min inhalation of aerosolized 2% metaproterenol was begun immediately after Rrs reached twice the baseline value. All patients were released from the test after their Rrs had returned to the baseline value. The results were expressed as the number of units (PD₃₅-Grs) from the start of methacholine exposure to the point at which Grs decreased by 35% from its baseline value, because all had positive bronchial responses to methacholine. One unit of inhaled methacholine represents a 1-min inhalation of 1 mg/ml of methacholine.

Inhalation challenge with HD allergen was carried out as previously reported [7]. Briefly, an aerosol of 1:5 w/v HD allergen was inhaled for 1 min, after it had been confirmed that a 1-min inhalation of physiologic saline did not change baseline Rrs. Patients began to inhale aerosolized 2% metaproterenol for 3 min if their Rrs reached twice the baseline value within 10 min of the start of allergen exposure. Otherwise, we observed Rrs for 10 min after the start of allergen exposure, and then aerosolized 2% metaproterenol was also inhaled for 3 min. All patients were released from the test after their

Rrs had returned to the baseline value. We considered the response to be positive when Grs decreased by more than 35% from its baseline value within 10 min of the start of allergen exposure. In positive cases, we expressed the results as the time (PT₃₅-Grs) from the start of allergen exposure to the point at which Grs had decreased by 35% from its baseline value.

All 104 patients were asymptomatic and baseline Rrs differed by less than ±15% on these 2 days.

Intradermal skin tests with HD allergen

Before inhalation challenge with HD allergen, serially diluted HD allergen (10⁻³ to 10⁻⁶ w/v) was injected into the forearm and the weal diameters were measured 10 min after the injection. Skin test sensitivity was expressed as the highest dilution required to produce a weal of more than 9 × 9 mm. We considered the response to be negative when a 10⁻³ w/v dilution induced a weal of less than 9 × 9 mm.

Circulating HD allergen-specific IgE assay

Venous blood was taken prior to intradermal skin tests with HD allergen. HD allergen-specific IgE was measured by means of the Phadebas® RAST (Pharmacia Diagnostics, Uppsala, Sweden). Briefly, the serum was mixed with a paperdisc-coupled HD allergen. After washing, radioactively labelled antibodies against IgE were added. The radioactivity was then measured in a gamma counter. The counter rates were compared directly with the counter rates obtained with a reference series run in parallel with the samples. The results were expressed as a RAST score from 0 to 4: 0, negative; 1, doubtful; and 2, 3 and 4, positive with progressively increasing amounts of specific IgE.

Allergen

HD allergen was made by using the original method of Hollister-Stier Labs, Spokane, WA. The lyophilized allergen was dissolved in 10 ml of physiologic saline, producing a 1:5 w/v HD allergen solution. In each patient, the same lot of allergen was used for the single exposure method and intradermal skin tests.

Statistical analysis

Logarithmic transformation of skin-test sensitivity and PD₃₅-Grs was used for all analyses. The unpaired Student's *t*-test was used to determine the significance of differences in age, age at onset of disease, skin-test sensitivity, PD₃₅-Grs, RAST score and baseline Rrs

Table 1. Comparison of various parameters between patients with positive and negative bronchial responses to inhaled house dust (HD) allergen

	Patients with positive bronchial responses	Patients with negative bronchial responses
No of patients	43	61
M/F	27/16	38/23
Age (yr)	29.3 ± 14.2	32.4 ± 12.6
Age at onset of disease	18.0 ± 15.7	23.9 ± 15.3
Baseline Rrs	4.7 ± 1.5	4.3 ± 1.4
-log (STS)	3.42 ± 1.07*	2.36 ± 1.00
RAST score	1.95 ± 1.23*	0.90 ± 1.15
log(PD ₃₅ -Grs)	0.24 ± 0.55†	0.63 ± 0.62
PT ₃₅ -Grs	216 ± 86	> 600

* $P < 0.001$; † $P < 0.005$.

STS, skin-test sensitivity to HD allergen; RAST, circulating HD allergen-specific IgE; PD₃₅-Grs, bronchial responsiveness to inhaled methacholine; PT₃₅-Grs, bronchial responsiveness to inhaled allergen.

between patients with positive and negative bronchial responses to HD allergen.

For analysis of data from the 43 patients with positive bronchial responses to allergen, linear multiple regression analysis was carried out. The dependent variable was PT₃₅-Grs and the independent variables were skin-test sensitivity, PD₃₅-Grs, RAST score and baseline Rrs. The backward elimination method was used for selecting independent variables. The level of significance of multiple correlation coefficients was estimated by analysis of variance (ANOVA).

To discriminate between patients with positive and negative bronchial responses, the linear discriminant function was used. The independent variables were skin-test sensitivity, PD₃₅-Grs, RAST score and baseline Rrs. When discriminant scores were greater than 0, the patients were evaluated as predicted-positive cases and when the scores were less than 0, they were evaluated as predicted-negative cases.

Results

Table 1 shows a comparison of various parameters between patients with positive and negative bronchial responses to inhaled HD allergen. There was no statistical difference in age and age at onset of disease between them. The baseline Rrs (mean ± s.d.) on inhalation challenge with HD allergen of positive cases and negative cases were 4.7 ± 1.5 and 4.3 ± 1.4 cm H₂O/l per sec, respectively. Thus, we did not find any statistical difference in baseline

Rrs at the time of the test. However, there were statistically significant differences in skin-test sensitivity ($P < 0.001$), PD₃₅-Grs ($P < 0.005$) and RAST score ($P < 0.001$) between them.

Table 2 shows the results of the multiple linear regression analysis of data from 43 patients with positive bronchial responses to HD allergen. Skin-test sensitivity, PD₃₅-Grs RAST score and baseline Rrs were used as independent variables in the first step; skin-test sensitivity PD₃₅-Grs and RAST score in the next step; and skin-test sensitivity and PD₃₅-Grs in the last step. When skin-test sensitivity and PD₃₅-Grs were used as independent variables, the F value was maximum, although the multiple correlation coefficient was the smallest. In addition, under this condition, both Mallows' Cp and Akaike's information criteria were the smallest. Thus, when skin-test sensitivity and PD₃₅-Grs were used as independent variables, PT₃₅-Grs was predicted most exactly ($r = 0.391$, $P < 0.05$) and the linear multiple regression equation was as follows: PT₃₅-Grs = 324.9 + 34.1 × log(skin-test sensitivity) + 50.6 × log(PD₃₅-Grs).

A histogram of discriminant scores of all 104 subjects is shown in Fig. 1, when skin-test sensitivity, PD₃₅-Grs and RAST score were used as independent variables. The combination of these three parameters had the highest rate of accuracy of prediction and the linear discriminant function equation, in which all independent variables significantly ($P < 0.005$) contributed to the discrimination, was as follows: $z = -0.91 \times \log(\text{skin-test sensitivity}) - 1.42 \times \log(\text{PD}_{35}\text{-Grs}) + 0.6 \times \text{RAST score} - 2.89$. Thirty-one out of 45 patients who were

Table 2. Results of linear multiple regression analysis of 43 patients with positive bronchial responses to inhaled allergen

Independent variables	Partial regression coefficients	Partial correlation coefficients	Multiple correlation coefficients	F
STS	32.4	0.345	0.403	1.84
PD ₃₅ -Grs	57.4	0.298		
RAST score	-6.6	-0.091		
Rrs base	4.0	0.059		
STS	32.2	0.343	0.399	2.46
PD ₃₅ -Grs	52.9	0.301		
RAST score	-6.2	-0.085		
STS	43.1	0.370	0.391	3.62
PD ₃₅ -Grs	50.6	0.292		

STS, skin-test sensitivity to house dust (HD) allergen; PD₃₅-Grs, bronchial responsiveness to inhaled methacholine; RAST, circulating HD allergen-specific IgE.

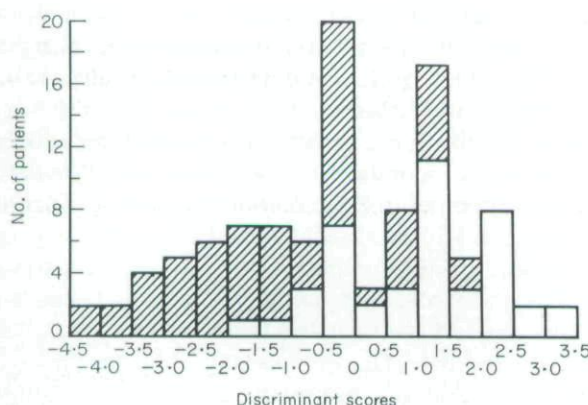


Fig 1. Histogram of discriminant scores. Discriminant score (Z) is calculated as follows: $Z = -0.91 \times \log(\text{skin-test sensitivity}) - 1.42 \times \log(\text{PD}_{35}\text{-Grs}) + 0.61 \times \text{RAST score} - 2.89$. ($Z > 0$, predicted-positive cases; $Z < 0$, predicted-negative cases; □, observed-positive cases; ▨, observed-negative cases).

predicted to be positive cases had actually had positive bronchial responses to inhaled allergen, and 12 out of 59 patients who were predicted to be negative cases had actually had positive bronchial responses. Thus, the true positive rate was about 69% and the false-negative rate was about 20%. However, as shown in Fig. 1, in only 30% of all subjects whose discriminant scores were greater than +2.0 or less than -2.0, could we confidently discriminate between subjects with positive and negative bronchial responses to inhaled allergen.

Discussion

When results of diagnostic procedures can distinguish between subjects with positive and negative responses to inhaled allergen, we should accept that allergen inhalation challenge can be replaced by those procedures. Therefore, using discriminant analysis, results of other diagnostic procedures were estimated in the present study. As shown in Fig. 1, it was in only 30% of all subjects whose discriminant scores are greater than +2.0 or less than -2.0 that we could exactly discriminate between subjects with positive and negative bronchial responses to inhaled HD allergen, although the true positive rate was about 69% and the false-negative rate was about 20%. In addition, in the present study we selected an atopic and asymptomatic patient population. It is possible that the false-negative rate may be underestimated, because as many as 30% of patients with negative skin tests to HD allergen may exhibit a positive bronchial response to inhaled allergen [9]. Therefore, at present we conclude that diagnostic procedures other than inhalation challenge with allergen cannot predict the presence or absence of immediate bronchoconstriction to inhaled HD allergen completely.

Tiffeneau [10] and Zuidema [11] hypothesized in the past that bronchial responsiveness to allergen might be predictable from the results of airway responsiveness to histamine and skin sensitivity to allergen. In the last decade, however, our knowledge of the immediate allergic reaction has progressed. Thus, the existence of not only the heterogeneity in mast cells [12-15] but also low

affinity Fc receptors [16–19] on the cells, such as macrophages, eosinophils, and platelets, has been reported. Therefore, we may not simply deduce local allergic reaction along the respiratory tract from skin sensitivity to allergen. In addition, the existence of chemical mediators [20–24] other than histamine has been reported in immediate IgE-mediated responses. Therefore, bronchial responsiveness to methacholine or histamine may not simply reflect bronchoconstriction in the immediate allergic reaction.

In this study, we used the single exposure method [7] as inhalation challenge with HD allergen and the continuous exposure method [6] as inhalation challenge with methacholine, since these two methods have some advantage over the conventional methods: (i) bronchial responsiveness is evaluated within 15 min; (ii) subjects experience less discomfort; and (iii) deterioration in lung function can be monitored continuously throughout the tests. As a result, there is a major difference between inhalation challenge procedures used in the present study and those by other investigators. However, Tamura *et al.* [7] have already reported that PT_{35} -Grs obtained with the single exposure method is significantly correlated with PD_{20} -FEV₁ obtained with the dosimeter method [25] and Ishii *et al.* [26] have also observed that PD_{35} -Grs obtained with the continuous exposure method is significantly correlated with 35% decrease in specific airway conductance obtained with the conventional method. Therefore, we infer that our conclusion is not due to the differences in inhalation challenge procedures.

Cockcroft *et al.* [1,2] have previously reported that the degree of bronchial responsiveness to inhaled allergen can be predicted from the combination of cutaneous sensitivity to allergen and non-allergic bronchial responsiveness to histamine in patients with positive bronchial responses to inhaled allergen. As shown in Table 2, PT_{35} -Grs in 43 positive cases was predicted most exactly from the combination of PD_{35} -Grs and skin-test sensitivity, when all of the parameters (skin-test sensitivity, PD_{35} -Grs, RAST score and baseline Rrs) were used as independent variables and the backward elimination method was used for selecting independent variables. In addition, the linear multiple regression equation obtained in the present study was similar to that reported by them, although the *r* value in the present study was rather low compared with that in their report, and differences in subject population and the method used for assessing skin reaction to allergen might be partly responsible for this. Thus, at least in patients with positive bronchial responses to inhaled allergen, our results support the finding that the degree of bronchial responsiveness to inhaled allergen may be dependent not only on allergic reaction in airways but also on non-specific bronchial hyper-responsiveness.

Baseline pulmonary function testing is reported to be useful for predicting immediate and late asthmatic reaction to *Dermatophagoides pteronyssinus* extracts [3]. However, in the present study, there was no statistical difference in baseline Rrs between patients with positive and negative bronchial responses. In addition, baseline Rrs was not useful in predicting PT_{35} -Grs or in discriminating between subjects with positive and negative responses. One of the reasons for the discrepancy may be the difference in subjects. However, non-specific bronchoconstriction due to a forced expiratory maneuver [27] or deep inspiration [28–30] is excluded from our data, since in the single exposure method, patients perform tidal breathing through the mouthpiece throughout the test. Therefore, we may conclude that the results of a baseline pulmonary function test are not helpful in predicting bronchial responses to inhaled allergen, at least when the responses are evaluated with the single exposure method.

Using discriminant analysis, we have shown that the occurrence of immediate bronchoconstriction to inhaled HD allergen can be predicted confidently by means of a combination of diagnostic procedures other than inhalation challenge in only 30% of all subjects. This finding suggests that at present inhalation challenge with allergen is a valuable test for determining the role of allergen in airways, although relationships between a positive response to inhaled allergen and natural symptoms remain obscure. Further study should be made to establish a less noxious technique for evaluating immediate allergic reaction in airways.

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