

Predicting the results of allergen bronchial challenge by simple clinical methods

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Summary

In this study, the ability to predict allergenic bronchial reactivity from non-specific bronchial reactivity, measured by free corridor-running, and the level of skin reactivity to whole rye grass pollen extract (WRGPE—*Lolium perene*) and house-dust mite extract (HDM—*Dermatophagoides pteronyssinus*) was examined in asthmatic children. Discriminant analysis of the data showed that, for the concentration of extracts used and the conditions of bronchial challenge for both allergens, the level of skin sensitivity to undiluted extract and the percentage fall in peak flow-rate on exercise best-predicted the outcome of bronchial challenge for WRGPE (100% of cases) and for HDM (89% of cases).

The results suggest that it may be possible to predict allergenic bronchial-reactivity to different allergens in children by simple clinical methods, if conditions of provocation and potency of allergen extracts can be standardized.

Introduction

Asthmatic children have an increased level of intrinsic bronchial-reactivity to physical and chemical stimuli (Mellis *et al.*, 1978; Kattan *et al.*, 1978; Silverman & Anderson, 1972). In late childhood, these patients show high levels of IgE antibodies to common environmental allergens (McNicol & Williams, 1973). Currently, the role of hyper-sensitivity to these allergens in asthma can only be defined by formal bronchial-challenge. These tests are time consuming and may be dangerous.

Studies of adult, asthmatic subjects have demonstrated that the effect of allergen bronchial-challenge is determined by both the level of IgE-allergenic sensitivity and the degree of non-allergenic bronchial hyper-reactivity (Bryant & Burns, 1976; Killian *et al.*, 1976; Cockcroft *et al.*, 1979). These observations suggest it may be possible to predict the outcome of allergen bronchial-challenge by measuring these parameters, using simple means. In this study, the value of using simple measurements to predict allergen bronchial-reactivity was examined. In it, the bronchial response of asthmatic

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children to two common allergens was studied and related to the level of IgE-allergenic sensitivity, measured by skin tests, and to bronchial hyper-reactivity, measured by exercise lability.

Materials and methods

Patients

Thirty Melbourne (Australia) children, between the ages of 8–12 years, participated in this study. They were selected on the basis of answers to a questionnaire regarding asthma, randomly distributed to 1000 urban schoolchildren. At the time the patients entered the study, they were under the care of family physicians. There were eight girls and twenty-two boys. All had a history of recurrent wheezing of at least 3 years' duration, and eleven had wheezed since early childhood. In the year prior to the trial, they had all reported at least four episodes of wheezing, but ten had had more than seven episodes of wheezing during this same period. Nine patients were using prophylactic asthma-medication. Eight were inhaling sodium cromoglycate and one beclomethasone dipropionate. The patient using beclomethasone dipropionate, and three of those using sodium cromoglycate were also using inhalational nebulized bronchodilators on a regular basis. All the other patients had used intermittent oral, or aerosol bronchodilators in the year prior to this study. All were asymptomatic and stable at the time they were admitted to hospital for these investigations. Asthma medication ceased 12 hr before the study commenced.

Allergen solutions

Standard, commercial allergen-solutions were provided by Commonwealth Serum Laboratories (CSL, Parkville, Australia) as 10 000 protein nitrogen units (PNU)/ml for skin test. Solutions used for bronchial provocation were made up in 1.0 ml volumes by diluting the whole rye grass pollen extract (WRGPE—*Lolium perene*) to 5000 PNU/ml and the house-dust mite extract (HDM—*Dermatophagoides pteronyssinus*) to 200 PNU/ml with propylene glycol respirator diluent. Uniform batches of allergen were used throughout this study and dilutions of solutions were made up daily.

Skin tests

Routine. All patients had skin prick tests to the following twelve common allergens: rye grass; cat; dog; capeweed; mite; house dust; *Alternaria*; *Cladosporium*; *Aspergillus*; milk; egg; and wheat. The skin tests were performed on the right and left forearms and the results compared with histamine and a control solution. Measurement of the mean weal diameter, elicited at 15 min, was recorded, but where a reaction occurred to control solution, this was deducted from the weal induced by allergen. A weal diameter greater than 2 mm was regarded as positive for these allergen skin-tests.

Skin test titration. Extracts of HDM and WRGPE were diluted with phenol saline for prick testing to the following concentrations: 10 000, 1000 and 100 PNU/ml.

These skin tests were done on the right and left forearms and the results recorded as above. For each of these two allergens, a semi-log dose-response curve of the mean weal diameter, elicited by each concentration of allergen, was constructed. The mean weal diameter equivalent to 5000 PNU/ml WRGPE and 200 PNU/ml HDM (i.e., the doses of allergen used in the bronchial provocation-test) were calculated for both allergens from this dose-response curve.

Lung-function studies

Detailed lung-function studies were performed prior to exercise and allergen challenges. The FEV₁ and FVC were measured, using a Godart spiograph. An air-conditioned displacement body plethysmograph was used to measure thoracic gas-volume, total lung-capacity, residual volume and maximum expiratory-flow volume curves. Four patients showed some disturbance in one or more of these tests at the time of this study.

Bronchial provocation-tests

Bronchial provocation-tests (BPT) were performed between 9 and 10 a.m. Allergen extracts were inhaled through one limb of a T-piece placed in the mouth. Solutions were inhaled after nebulization by a Wright nebulizer, powered by an air-compressor which generated an air flow of 8.0 l/min. Expired air, containing allergen, was removed from the room by a closed-circuit, connected to the third limb of the mouth T-piece. The children were instructed to breathe at normal tidal breathing, and each volume of allergen was inhaled for five, ten, fifteen, twenty and forty consecutive inspirations, until the allergen under investigation was completely inhaled or a reaction occurred. Only one concentration of each allergen was used in this study. These concentrations had been previously investigated, and the response elicited, demonstrated to be safe and antigenically specific. After each set of consecutive inspirations, the peak expiratory flow-rate (PEFR) was checked with the Wright peak flow-meter and again after a 10-min delay. If the PEFR was stable, the next series of inspirations was performed until the whole volume of extract under investigation was inhaled. After completion of the challenge, or if a positive reaction occurred, the PEFR was checked at 10-min intervals for 30 min, then hourly for 10 hrs. The highest of three consecutive PEFR manoeuvres was taken as the relevant reading after each set of inspirations.

Asthmatic reactions developing within 30 min of challenge were designated immediate asthmatic reactions (IAR) and if later than this, they were late asthmatic reactions (LAR). Where an asthmatic reaction caused distress, inhalation of nebulized 2.0% Oreiprenaline respirator solution was given. Any fall of the PEFR below the pre-challenge base-line level was regarded as a positive reaction, but in all cases this exceeded 20% of the basal PEFR on the day of challenge, as well as the PEFR at the corresponding time of the control day for LAR.

For the purposes of this study, all asthmatic reactions, regardless of whether they were isolated immediate, or isolated late reactions or a combination of both these, were regarded as positive.

Measurement of exercise-induced bronchial lability

This was assessed by 6 min of free corridor-running. The tests were undertaken between 9 and 10 a.m. and all children achieved a pulse rate greater than 160 per min after 2 min of running; this rate of running was maintained for a total of 6 min. Peak expiratory flow-rate was measured, using the Wright peak flow-meter, before the commencement of exercise, at 2-min intervals during the exercise and at 1, 3, 5, 7 and 10 min after cessation of exercise. The exercise lability is reported as the maximum fall in PEFR related to the initial PEFR thus:

$$\% \text{ Fall in PEFR} = \frac{(\text{Initial PEFR} - \text{the lowest PEFR for exercise})}{\text{Initial PEFR}} \times 100$$

In this study all children completed the 6 min of exercise tolerance-test, except for one child whose asthmatic response to exercise was so vigorous that at 3 min the test was ceased and bronchial obstruction relieved.

Conduct of trial

The following procedure was adopted:

- Day 1. Bronchial challenge with phenol saline propylene glycol control solution and hourly basal PEFR readings commenced. Detailed lung function studies undertaken.
- Day 2. Six minutes' free corridor-running exercise tolerance-test. Hourly PEFR readings continued.
- Day 3. Bronchial challenge with 1.0 ml of HDM (200 PNU) or WRGPE (5000 PNU).

Of the thirty children, eighteen were challenged with HDM and twelve with WRGPE.

Results

The results of the fall in PEFR on exercise, BPTs to WRGPE and HDM and the skin-weal diameters, elicited by prick test to two concentrations of each of these two allergens, is shown in Tables 1 and 2. The two sets of prick test results, to the two allergens shown, were to those concentrations of allergens used for routine skin tests (10 000 PNU/ml WRGPE and 10 000 PNU/ml HDM) and in the BPTs (5000 PNU/ml WRGPE and 200 PNU/ml HDM).

Bronchial provocation test

Of the twelve children challenged with WRGPE, eight were BPT-positive; all had an

Table 1. Inter-relation of exercise bronchial-capability, WRGPE skin-sensitivity and WRGPE bronchial-reactivity.

Patient	WRGPE* (skin-weal diam.) (mm)	WRGPE† (skin-weal diam.) (mm)	BPT	PEFR fall (%)
L.P.	12	10	+	55
A.N.	0	0	—	42
J.T.	4	3.5	+	63
S.C.	8.5	7.5	+	51
C.M.	11.5	9.5	+	21
A.T.	10	10	+	44
P.L.	4	3.5	—	38
J.H.	11.5	10	+	47
L.N.	9.0	6.0	+	45
I.H.	11.5	9.5	+	35
J.E.	4.0	2.5	—	40
S.N.	6.0	5.0	—	2

* 10 000 PNU/ml

† 5000 PNU/ml

Table 2. Inter-relation of exercise bronchial-lability, HDM skin-sensitivity and HDM bronchial-reactivity.

Patient	HDM* (skin-weal diam.) (mm)	HDM† (skin-weal diam.) (mm)	BPT	PEFR fall (%)
A.P.	8.0	3.5	+	74
M.U.	7.5	1.8	+	29
S.N.	8.5	3.0	+	60
D.E.	10	3.0	+	38
G.B.	0	0	—	8
G.M.	7	3.5	+	59
P.W.	0	0	—	6
E.M.	9	4	—	7
B.T.	9	1.5	—	2
D.D.	8.5	3.5	—	30
M.G.	8.0	4.0	—	2
B.R.	0	0	—	2
A.S.	7.0	0.5	—	15
K.M.	0	0	—	2
M.W.	0	0	—	2
J.O.	8.0	3.0	+	18
M.R.	12	3.5	+	73
P.B.	7	4.0	+	65

* 10 000 PNU/ml.

† 200 PNU/ml.

IAR, and four of these were followed by an LAR. For HDM, eight positive responses followed challenge; two had an IAR, followed by an LAR, but in the remaining six cases, an isolated LAR occurred without any preceding immediate response. All these reactions were regarded as positive. Ten patients challenged with HDM did not show bronchial reactivity to it.

Relation between bronchial reactivity to allergens and skin sensitivity

WRGPE (see Table 1). For WRGPE BPTs, all the positive patients showed skin reactivity to the 5000 PNU concentration of allergen used in the BPT. Three of the four patients who were negative to WRGPE on BPT showed skin-test sensitivity to WRGPE. Similar discrepancies between the results of skin sensitivity and bronchial reactivity were noted when 10 000 PNU/ml WRGPE skin-test was used. The Mann-Whitney U test showed that the BPT-positive patients had significantly larger skin-weal diameters than the BPT-negative group for both 10 000 ($P < 0.05$) and 5000 PNU/ml skin tests ($P < 0.05$). If the analysis was confined to rye grass skin-sensitive patients the weal-size diameter was still greater in the BPT-positive than BPT-negative patients ($P < 0.05$) for both concentrations of WRGPE.

HDM (see Table 2). The eight patients, BPT-positive to 200 PNU/ml HDM, showed skin reactivity to this concentration of allergen. Of the ten BPT-negative patients, five showed skin sensitivity to this concentration of allergen. No difference

was seen in this relation of skin sensitivity to BPT when 10 000 PNU/ml HDM was used for skin tests. The Mann-Whitney U test showed the BPT-positive patients' skin-weal diameters were not significantly greater than the BPT-negative patients' skin tests with either 200 or 10 000 PNU/ml HDM. If this analysis was confined to the skin-test positive patients alone, again there was no statistical difference in skin sensitivity between the HDM BPT-positive and -negative groups.

All of the BPT-positive patients showed skin sensitivity to the allergen to which they demonstrated bronchial reactivity.

Exercise lability and skin reactivity in predicting bronchial reactivity to allergen.

WRGPE. The discriminant function equation,

$$0.07 \times \% \text{ fall PEFR} + 0.48 \text{ skin test diam. (10 000 PNU)} - 6.47,$$

was derived from these studies and correctly classified 100% of cases, with regard to bronchial reactivity to the rye extract. This classification was based on the results of skin test to 10 000 PNU/ml and the percentage fall in PEFR on exercise.

HDM. Stepwise discriminant-analysis of this data correctly classified 89% of the cases into BPT-positive or -negative. This classification was based on the results of 10 000 PNU/ml skin test and the percentage fall in PEFR on exercise. The discriminant function equation on which this classification was based was:

$$0.06 \times \% \text{ fall PEFR} + 0.06 \text{ skin test diam. (10 000 PNU)} - 2.021.$$

Discussion

The findings in this study are similar to investigations in adults, where measurement of the level of skin sensitivity and degree of histamine bronchial-reactivity have been shown to predict the outcome of allergen bronchial-challenge (Bryant & Burns, 1976; Bryant, Burns & Lazarus, 1975). In our children, stepwise discriminant-analysis showed that for both WRGPE and HDM, the level of skin sensitivity to undiluted extract together with fall in PEFR on exercise most accurately predicted the results of bronchial challenge. However, these predictions were based on the data used to generate the discriminant equations, and cannot be universally applied because of the variation in potency of allergen extracts and lack of standardization of bronchial challenge-tests. The validity of these derived equations need to be evaluated in prospective studies to test them under carefully standardized conditions.

One study of children which examined the inter-relation of skin sensitivity, bronchial hyper-reactivity and allergenic bronchial-reactivity (Van Lookeren Campagne, Knol & de Vries, 1969) showed skin sensitivity to a house dust extract was not significantly associated with bronchial reactivity to this allergen. However, when bronchial hyper-reactivity, measured by response to histamine challenge and the levels of skin sensitivity to this allergen were taken into account, only the patients with increased bronchial-reactivity to histamine and a positive skin test showed a positive BPT with this allergen. Our results for HDM were similar and statistical analysis confirmed the value of using parameters of both bronchial reactivity and allergen skin-sensitivity to predict the outcome of challenge with both this allergen and grass pollen.

It was hoped that measuring the skin reactivity to the concentration of allergen, used in the bronchial provocation-system, would increase the accuracy of predicting

the outcome of BPT but this was not demonstrated. Indeed statistical analysis demonstrated that the size of the weal diameter to the higher allergen-concentrations used routinely in the clinic, more accurately predicted the outcome of bronchial provocation. Furthermore, comparison of the skin-weal diameter elicited by prick tests to the concentrations of allergens used in BPTs showed that the biological activity of WRGPE, in terms of skin reactivity were significantly greater than HDM.

Other studies, which have examined the importance of intrinsic bronchial-reactivity in determining the outcome of allergen bronchial-challenge, have used inhalations of histamine. In children, there is good correlation between hyper-reactivity to histamine and bronchial lability measured by a fall in PEFR on tread-mill exercise (Mellis *et al.*, 1978). In this study, bronchial lability was assessed by free corridor-running because it is simple to perform, is widely available and there is some evidence that it is more asthmagenic than tread-mill running (Shapiro *et al.*, 1979).

The findings of this study have some clinical applications. It has shown that the demonstration of a positive skin-test is not very useful in predicting the outcome of allergen BPT. Assigning a level of skin sensitivity to an allergen by measuring the skin-weal diameter to an undiluted extract of moderate, biological activity improves this predictability. However, for the allergens used in this study a knowledge of both the skin sensitivity and level of bronchial reactivity to exercise, most accurately predict the outcome of allergen bronchial-challenge in childhood.

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