

Predictors of early- and late-phase reactions to bronchial allergen challenge

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The influence of inhaled steroids and predictive factors on the response to bronchial allergen challenge (BCA) was evaluated in 80 asthmatics allergic to *Dermatophagoides pteronyssinus* (Der p). All underwent BCA with Der p and measurement of early (EAR) and late asthmatic reaction (LAR). The cumulative dose of allergen producing 20% fall in FEV₁ in the EAR (PD₂₀) was calculated. Bronchial histamine provocation, conjunctival provocation test (CPT), and skin prick test with Der p extract were performed. Specific IgE to Der p in serum (RAST), blood eosinophil (EOS) count, serum eosinophil cationic protein, and eosinophil protein X were measured. Thirty patients (38%) were treated with inhaled steroids. All patients had at least a 20% fall in FEV₁ in EAR. Some 42% of nonsteroid- and 33% of steroid-treated patients had LAR with fall in peak flow of at least 20%. For patients not treated with steroid, 35% of variation in PD₂₀ was explained by RAST and histamine reactivity, and 53% of variation of observed PD₂₀ could be predicted. The baseline FEV₁, EOS, and EAR explained 28% of variation in LAR, and 28% of variation in observed LAR could be predicted. For patients treated with steroids, 38% of variation in PD₂₀ was explained by EOS and histamine reactivity, and only 18% of variation of observed PD₂₀ could be predicted. For patients treated with steroids, it was impossible to predict LAR. We conclude that to achieve a quantitative estimation of allergen-specific EAR and LAR, BCA cannot be replaced by the tests used in this study. Treatment with inhaled steroids modifies the response to BCA, making quantitative prediction of EAR less accurate and prediction of the magnitude of LAR impossible.

Key words: asthma; bronchial allergen challenge; inhaled steroids; quantitative prediction.

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Bronchial inhalation challenge with allergen (BCA) is used to assess the specific bronchial response to an allergen in bronchial asthma. The indications for BCA are as follows:

- 1) to reach a specific diagnosis when other tests are inadequate
- 2) to monitor changes in specific bronchial reactivity resulting from pharmacologic intervention or specific treatment, including allergen avoidance and immunotherapy (1, 2).

Allergen challenge is a time-consuming procedure, and 50–80% of patients have a late asthmatic reaction (LAR) (3, 4), entailing the risk of deterioration of the disease and increase in nonspecific bronchial reactivity for a period. Therefore, alternative ways have been sought to predict the response to inhalation challenge by other clinical and

laboratory variables. The variables used for prediction have been the skin prick test, specific IgE, nonspecific bronchial reactivity, blood eosinophil count, and baseline lung function; to some degree, it has been possible to predict whether or not a patient will exhibit an early asthmatic reaction (EAR) (5–10) or an LAR (4, 11, 12). When BCA is indicated for measurement of changes in specific bronchial reactivity, a quantitative result is required. A quantitative expression of the result of BCA is also a prerequisite for the standardization of the test (2, 13).

Many patients are treated with inhaled steroids, which have been shown to reduce the EAR and LAR, probably by dampening bronchial inflammation (11, 14–18). The influence of steroids on the predictability of the response to a BCA is not known.

This study aimed to evaluate the quantitative prediction of EAR and LAR in a group of patients with positive EAR to the same allergen. Furthermore, we wanted to study the possible prediction of EAR and LAR in a group of patients treated with inhaled steroids, as this has not been reported previously.

Material and methods

Patients

Eighty patients were recruited for the study: 30 female and 50 male patients with a mean age of 28 years (range, 10–64 years). The patients included in the study had the following characteristics:

- 1) typical history of bronchial asthma (19)
- 2) positive bronchial challenge test with histamine
- 3) positive skin prick test (SPT) with house-dust mite (*Dermatophagoides pteronyssinus* (Der p) extract
- 4) positive reaction to bronchial challenge with Der p extract
- 5) positive RAST (Phadebas[®] RAST, Pharmacia, Sweden; ≥ 0.35 PRU/ml) to Der p.

No patients with positive SPT to molds or storage mites participated, and patients with animal-dander allergy had no regular contact with animals. The patients had mild to moderate asthma, and none received systemic corticosteroids. Thirty of the patients were treated with inhaled steroids; 13 patients with a dose of budesonide 400 µg daily, and 17 patients with a dose of budesonide 800 µg daily. Informed consent to participate in the study was given by all patients, and the protocol was approved by the ethical committee of Aarhus County (Denmark).

Allergen extracts and SPT

The extract used was partially purified, standardized extract of Der p whole-mite culture (ALK Laboratories, Hørsholm, Denmark). A glycerol solution of allergen (Soluprick SQ[®]) was used for SPT. A drop of solution was placed on the volar side of the forearm, and the skin gently perforated down into the epidermis with a skin-puncture needle with a 1-mm stylus. The tests were made in duplicate with an allergen concentration of 10 histamine equivalent prick (HEP) (20). Histamine dihydrochloride (10 mg/ml) was used as positive control, and diluent as negative control. After 15 min, the contours of the wheals were outlined with a ballpoint pen and transferred to patients' records with transparent cellulose tape. The result was expressed as a skin index (SI), calculated by

adding the largest diameters of the duplicate wheals and the diameters perpendicular to these, and then dividing this sum by the sum of the corresponding diameters of the duplicate histamine wheals.

Blood samples and analyses

Blood samples were drawn immediately before bronchial allergen challenge. Eosinophils in whole blood were counted after eosin staining. Serum concentrations of eosinophil cationic protein (ECP) and eosinophil protein X (EPX) were measured by radioimmunoassay, as previously described (21). Total serum IgE antibody levels were determined by the Pharmacia PRIST method, as recommended by the manufacturer. Results were expressed as kU/l. Serum-specific IgE antibody levels to Der p were determined by Phadebas RAST, and the results were expressed in Phadebas RAST units per ml (PRU/ml).

Bronchial challenge tests

For bronchial challenge tests, we used a standard Wright nebulizer driven with compressed air at a pressure of 0.5–0.7 bar. The nebulizers were calibrated to give an output of 0.13–0.15 ml/min. A flow regulator ensured a constant pressure to the nebulizer. The aerosol was inhaled via a mouth-piece at tidal breathing, and the nose was clipped to ensure oral breathing. Forced expiratory volume in 1 s (FEV₁) was measured with a dry bellow spirometer (Vitalograph 20600, Buckingham, UK), and peak expiratory flow (PEF) with a Mini-Wright peak flow meter. All measurements of lung function performed during the study were repeated three times, and the highest value was used for calculations.

Oral bronchodilators were withheld 24 h before challenge; inhaled β_2 -agonists and steroids for 12 h. None of the patients had a respiratory tract infection within 4 weeks before the challenges.

Bronchial challenge with allergen

An aqueous solution of Der p extract (AquaGen SQ[®]) was used for the bronchial challenge test. SPT titration with Der p was performed, and the allergen concentration giving a 2–3-mm wheal used as the starting concentration. FEV₁ and PEF were measured before starting the challenge. Lung function measured 5 min after eight inhalations of diluent was used as baseline for the BCA, which was started with two inhalations of the initial concentration. Throughout the challenge, FEV₁ was measured 5 and 10 min after inhalation. The

challenge was continued with doubling doses (two, four, eight inhalations) and 10-fold increments in allergen concentration until a fall $\geq 20\%$ in FEV_1 was obtained. PD_{20} was determined by linear interpolation on the log dose-response curve (22). Lung function was followed in the laboratory for at least 60 min after the last dose of allergen and usually normalized. Patients were not given bronchodilators after challenge, and they measured PEF every hour for the next 10–12 h, taking only inhaled β_2 -agonists when necessary. A significant LAR was defined as a fall in PEF of $\geq 20\%$ of pre-challenge value, measured 2–10 h after challenge, with a typical gradual deterioration of lung function after spontaneous reversal of the EAR.

Bronchial challenge with histamine

The bronchial challenge test with histamine dihydrochloride was performed as a modification of the method described by Cockcroft et al. (23). The same type of Wright nebulizer as for BCA was used. The patients inhaled for 2 min at tidal breathing. Challenge was started with inhalation of isotonic saline. FEV_1 measured 90 s after this inhalation was used as baseline. Then histamine dihydrochloride was inhaled in doubling concentrations, starting with 0.03 mg/ml and rising to maximum concentration of 32 mg/ml. FEV_1 was measured 30 and 90 s after each inhalation, and the challenge was continued with intervals of 5 min between inhalations until a drop in $FEV_1 \geq 20\%$ was obtained. PC_{20} was calculated by linear interpolation on the log dose-response curve.

Conjunctival provocation test (CPT)

A drop was applied in the lower conjunctival sac, alternating between the two eyes. Aqueous diluent served as control, and Der p extract was administered in concentrations beginning with 100 SQ-U and increased in half-log steps with intervals of 10 min to a maximum of 320 000 SQ-U. A positive reaction was recorded when both itching and congestion of the conjunctival mucosa occurred (24).

Statistics

Data analysis was performed with the Statistical Package for the Social Sciences (SPSS) (25). Comparison of means was performed with one-way analysis of variance, and multiple linear regression analysis was used to analyze explanatory variables for early and late allergic reactions. The forward selection procedure with a probability of 0.05 to enter the equation was used in multiple regression. The test for nonnormal distribution was performed

with the Lilliefors test, and normal distributions were obtained for the following variables after logarithmic transformation: PD_{20} , PC_{20} , IgE, EOS, ECP, and EPX.

Results

Comparisons of means were performed according to treatment with inhaled steroids and the occurrence of LAR. Thirty patients were treated with inhaled steroids and 50 were not. All patients were hyperreactive with a mean PC_{20} 0.50 mg/ml (range 0.02–8.86 mg/ml) and had a mean RAST value to Der p of 17.8 PRU/ml (range 0.6–33 PRU/ml). Many patients had moderate to pronounced eosinophilia, 32 of 80 having an eosinophil count above $0.4 \times 10^9/l$, whereas levels of ECP generally were low, only 18 of 80 having more than 15 $\mu g/l$.

Patients without inhaled steroids tended to have higher baseline FEV_1 than patients with steroids (85% [range 48–129%] vs 79% [range 51–108%]) and higher PC_{20} values (0.60 vs 0.39 mg/ml histamine), but the difference was not significant, whereas RAST values were significantly higher (19.9 vs 14.4 PRU/ml). Other variables including EAR, LAR, EOS, and ECP were very similar in the two groups and did not differ significantly (Table 1).

Measurement of PEF (26) with regard to LAR was available in 78/80 patients. Forty-five patients had LAR $\geq 20\%$; 33 did not. LAR was found

Table 1. Comparison of clinical and immunologic parameters between patients treated with (+) and without (–) inhaled steroids

Parameter	Inhaled steroids			Significance level
	Mean	+ (n=30)	– (n=50)	
log PD_{20} allergen (SQ-U)	1.851	1.839	1.859	0.997
PC_{20} histamine (mg/ml)	0.51	0.39	0.60	0.173
Baseline FEV_1 (% predicted)	82.6	79.1	84.7	0.142
RAST (PRU/ml)	17.8	14.4	19.9	0.008
IgE (kU/l)	131.9	111.6	145.9	0.262
EOS ($10^9/l$)	0.32	0.31	0.32	0.803
Early reaction (% fall in FEV_1)	31.1	31.5	30.5	0.721
LAR (% fall in FEV_1)	22.8	22.0	23.2	0.772
sECP ($\mu g/l$)	9.4	10.7	8.7	0.227
sEPX ($\mu g/ml$)	25.5	24.5	26.4	0.777

in 33% of steroid-treated patients, and 42% of nonsteroid-treated patients. Patients with LAR tended to have lower baseline FEV₁ (80% vs 87%) and significantly larger EAR than patients without LAR (34% vs 27%), whereas other variables were very similar without significant differences (Table 2).

Analysis of explanatory variables for early and late asthmatic reaction was performed separately in steroid- and nonsteroid-treated patients. The correlations between possible predictors for PD₂₀ and LAR were examined in a Pearson correlation matrix (Tables 3 and 4). Steroid-treated and nonsteroid-treated patients exhibited different patterns of correlation between possible predictors. Common to both groups was the correlation between PC₂₀, histamine and PD₂₀ and FEV₁ percent predicted. Furthermore, ECP and EPX were closely correlated ($r=0.78-0.88$). In nonsteroid-treated patients (Table 3), there was a significant correlation between PD₂₀ and RAST to Der p ($r=-0.52$) and EAR ($r=-0.48$). This suggests that patients with high RAST values have low PD₂₀ values and are very sensitive to allergen challenge. Furthermore, patients with low PD₂₀ values obtained the highest percent fall in FEV₁ during EAR. This association was also revealed in the positive correlation between RAST and EAR ($r=0.33$). The percent fall in PEF

Table 2. Comparison of clinical and immunologic parameters between patients with (+) and without (-) LAR

Parameter	Late asthmatic reaction			Significance level
	Mean	+(n=45)	-(n=33)	
PD ₂₀ allergen (SQ-U)	1.842	2.192	1.621	0.416
PC ₂₀ histamine (mg/ml)	0.52	0.55	0.50	0.785
Baseline FEV ₁ % predicted	83.0	87.3	80.2	0.057
RAST (PRU/ml)	17.7	17.5	17.8	0.986
IgE (KU/l)	130.0	142.5	122.5	0.528
EOS (10 ⁹ /l)	0.31	0.34	0.30	0.439
Early reaction (% fall in FEV ₁)	31.2	27.4	34.1	0.017
Skin index at 10 HEP	1.20	1.20	1.20	0.990
Conjunctival test (threshold conc. SQ-U)	60.4	57.0	62.9	0.894
s-ECP (μg/l)	9.3	7.7	10.7	0.057
s-EPX (μg/ml)	25.2	23.3	26.6	0.537
Inhaled steroids (%)	37.5	33.3	42.0	0.425

Boldface indicates statistical significance.

Table 3. Pearson correlation matrix for 50 nonsteroid-treated patients. Values are Pearson correlation coefficient with significance below (two-sided test)

	2	3	4	5	6	7	8	9
1. PC ₂₀	0.43	-0.23	0.05	0.04	-0.28	0.57	-0.15	-0.09
	<0.01	0.11	0.72	0.78	-0.05	<0.01	0.31	0.55
2. PD ₂₀		-0.20	-0.05	0.03	-0.52	0.18	-0.48	-0.03
		0.16	0.74	0.85	<0.01	0.23	<0.01	0.83
3. EOS			0.09	0.22	0.21	-0.21	0.04	-0.25
			0.53	0.13	0.14	0.15	0.78	0.09
4. ECP				0.78	0.03	-0.17	-0.06	0.18
				<0.01	0.83	0.24	0.66	0.20
5. EPX					-0.01	-0.24	-0.04	0.15
					0.97	0.11	0.80	0.30
6. RAST						-0.10	0.33	0.06
						0.51	0.02	0.70
7. FEV ₁							-0.13	-0.32
							0.36	0.03
8. EAR								0.32
								0.03
9. LAR								

Boldface indicates statistical significance.

Table 4. Pearson correlation matrix for 30 steroid-treated patients. Values are Pearson correlation coefficient with significance below (two-sided test)

	2	3	4	5	6	7	8	9
1. PC ₂₀	0.43	0.18	0.15	0.09	0.20	0.50	0.18	-0.08
	0.02	0.35	0.43	0.62	0.30	0.01	0.35	0.69
2. PD ₂₀		-0.17	-0.07	0.02	-0.04	0.32	-0.30	-0.23
		0.37	0.72	0.91	0.82	0.10	0.11	0.22
3. EOS			0.09	0.04	-0.14	0.12	0.11	-0.28
			0.65	0.85	0.48	0.55	0.56	0.14
4. ECP				0.88	-0.04	-0.13	0.21	0.19
				<0.01	0.83	0.52	0.28	0.32
5. EPX					-0.13	-0.29	0.15	0.01
					0.51	0.13	0.43	0.94
6. RAST						0.07	0.77	0.74
							-0.01	-0.10
7. FEV ₁							0.93	0.62
8. EAR								0.13
								0.50
9. LAR								

Boldface indicates statistical significance.

in LAR was negatively correlated to baseline lung function ($r=-0.32$) and positively to the fall in FEV₁ in EAR ($r=0.32$).

For nonsteroid-treated patients, 35% of the variation in PD₂₀ could be explained by RAST and PC₂₀ (Table 5), whereas baseline FEV₁, IgE, EOS, ECP, EPX, SI, and CPT did not explain any significant amount of variation in PD₂₀. The correlation between predicted and observed PD₂₀ was 0.73 ($P<0.01$); thus, 53% of the variation of observed PD₂₀ could be predicted.

For steroid-treated patients, PC₂₀, histamine and EOS explained 38% of PD₂₀, whereas baseline FEV₁, IgE, RAST, ECP, EPX, SI, and CPT did not

Table 5. Multiple linear regression R^2 is proportion of variation in dependent variable explained by successive steps in regression equation

Dependent variable: PD ₂₀ allergen in nonsteroid-treated patients				
Explanatory variable	B	SE of B	R ²	Sig. of B
RAST (PRU/ml)	-0.039	0.011	0.252	0.001
Log PC ₂₀ histamine (units of 0.01 mg/ml)	0.420	0.314	0.346	0.015
Constant	1.117			

explain any significant amount of variation in PD₂₀ (Table 6). The correlation between predicted and observed PD₂₀ was 0.42 ($P=0.02$); thus, only 18% of the variation in PD₂₀ could be predicted.

Significant explanatory variables for LAR as a continuous variable were found only in nonsteroid-treated patients. Three variables (baseline FEV₁, EOS, and EAR) explained 28% of the variation in LAR, whereas IgE, RAST, ECP, EPX, SI, and CPT did not explain any significant amount of variation in LAR (Table 7). In Table 7, the correlation between log EOS and LAR was negative, as EOS were counted as 10⁹/l, and thus in almost all cases were less than 1. The correlation between predicted and observed LAR was 0.54 ($P<0.01$), and 28% of the variation in observed LAR could be predicted. Steroid-treatment patients had no significant explanatory variables, and it was not possible to predict LAR to any degree.

Discussion

This study included asthmatic patients allergic to house-dust mite. They were challenged with a partially purified standardized extract, and all had positive EAR. This allowed us to compare and study separately two large subgroups of patients; patients treated with inhaled steroid and nonsteroid-treated patients. In both groups, more than one-third of the patients had positive LAR, including patients receiving inhaled steroids for a long period (14–17). The study was not randomized for steroid treatment. It is probable that patients treated with inhaled steroids had more severe asthma, and the two groups of patients were not directly comparable without the inclusion of inhaled steroid treatment.

Earlier studies have established the central role of methacholine or histamine reactivity (4–8, 10, 27) and the degree of sensitization expressed by RAST or SPT in the prediction of the EAR (4–10), and this was confirmed in this analysis. Histamine reactivity was a significant explanatory variable for PD₂₀ in both nonsteroid- and steroid-treated patients. The degree of sensitization was a

Table 6. Multiple linear regression R^2 is proportion of variation in dependent variable explained by successive steps in regression equation

Dependent variable: PD ₂₀ allergen in steroid-treated patients				
Explanatory variable	B	SE of B	R ²	Sig. of B
Log PC ₂₀ histamine (units of 0.01 mg/ml)	0.770	0.204	0.273	0.008
Log EOS (10 ⁹ /l)	-0.683	0.334	0.376	0.048
Constant	0.172			

significant explanatory variable in only nonsteroid-treated patients. Only RAST, not SPT, was significant in this study. In previous studies, RAST and SPT reaction have been used, and they have been shown to be associated. The differences between studies were probably due to differences in patient selection and to the fact that some other studies (7, 9) used serially diluted extracts for SPT. This increases the range of responses in SPT and probably the explanatory power. Sensitization expressed as CPT reaction was not a significant explanatory variable. Previous studies have found no association between the outcome of allergen challenge in lung, nose, and eyes (27, 28).

The blood eosinophil count was a significant explanatory variable for PD₂₀ in steroid-treated and for LAR in nonsteroid-treated patients, as demonstrated in previous studies for EAR (10) and LAR (10, 11). Eosinophil granule proteins in serum, ECP and EPX, have been shown to be important for the outcome of LAR (11), but they were not significant explanatory variables in this study.

To summarize, we have identified significant explanatory variables for both EAR and LAR in nonsteroid-treated patients. The power of prediction, however, was not strong enough to allow replacement of BCA with other tests in situations where a quantitative expression of specific bronchial reactivity was needed. We have demonstrated that explanatory variables for PD₂₀ are distinctly different in steroid- and nonsteroid-treated patients

Table 7. Multiple linear regression R^2 is proportion of variation in dependent variable explained by successive steps in regression equation

Dependent variable: LAR in nonsteroid-treated patients				
Explanatory variable	B	SE of B	R ²	Sig. of B
Baseline FEV ₁ (% predicted)	-0.420	0.169	0.099	0.017
Log EOS (10 ⁹ /l)	-24.023	9.809	0.200	0.019
EAR (% fall in FEV ₁)	0.437	0.209	0.275	0.043
Constant	34.226			

and that prediction of PD₂₀ in steroid-treated patients is poor and clinically insignificant. Furthermore, prediction of LAR was not possible in steroid-treated patients.

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