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Predictive accuracy of late asthmatic reaction to *Dermatophagoides pteronyssinus*

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Sixteen patients with asthma were challenged with solutions of Dermatophagoides pteronyssinus extracts. Fifteen patients demonstrated an early asthmatic reaction, and 10 patients also demonstrated a late asthmatic reaction, as demonstrated by a 15% decrease of FEV₁ within 1 hour or between 3 and 8 hours after challenge, respectively. Airway responsiveness to inhaled methacholine, expressed as the dose causing a fall of FEV₁ of 15% (Pd15), and the serum IgE level (RAST), expressed as percent of the total radioactivity added, were also measured. Multiple regression analysis of data demonstrated that both early asthmatic reaction, expressed as the allergen Pd15, and late asthmatic reaction, expressed as the FEV₁ maximum fall from control values, can be predicted with similar accuracy (explained variance of 63% and 68%, respectively) by a model that incorporates simultaneously methacholine Pd15, percent of total radioactivity added by RAST, and baseline values of FEV₁. We conclude that the late asthmatic reaction is more likely to occur and is more severe in patients with more severe underlying disease. (J ALLERGY CLIN IMMUNOL 78:908-13, 1986.)

The occurrence of late asthmatic reactions to inhaled allergens was first described in 1873 by Blackley,¹ but it is only in recent years that its relevance in clinical asthma has been emphasized.²⁻⁸ In spite of this increasing interest, the pathogenesis of the late asthmatic reaction remains unclear. In a few recent studies,⁹⁻¹⁴ the correlates of late asthmatic reaction have been investigated. Generally, it has been found that patients with dual asthmatic reaction tended to have higher concentrations of IgE in serum⁹⁻¹¹ than patients with early reactions only but similar prick tests and

Abbreviations used

TRA%:	Percent of total radioactivity added
AU:	Arbitrary units
MEF50; MEF25:	Maximum expiratory flows at 50% and 25% of forced vital capacity
Pd15:	Provocative dose that causes a 15% fall of FEV ₁

bronchial response to histamine.¹²⁻¹⁴ To date, no evidence has been provided that late asthmatic reactions can be predicted from the knowledge of nonspecific reactivity or allergic hypersensitivity or any particular clinical feature.

The purpose of this study was to investigate if the occurrence and the magnitude of late asthmatic re-

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Received for publication Nov. 1, 1985.

Accepted for publication April 12, 1986.

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TABLE I. Synopsis of individual results

Patients No.	IgE RAST TRA%	Baseline			Methacholine Pd15 FEV ₁ (mg)	Allergen Pd15 FEV ₁ (AU)	Early	Late
		FEV ₁	MEF50	MEF25			max FEV ₁ fall	max FEV ₁ fall
		% Of reference value					% Fall from control	
1	32	85	61	52	0.1	16.7	31	76
2	32	89	53	40	0.03	20	30	61
3	29	80	63	41	0.03	15	25	58
4	24	75	59	64	0.04	5.8	25	47
5	18.2	79	57	48	0.06	27.5	36	45
6	34.8	93	105	89	0.04	12	20	36
7	21	86	38	35	0.05	80	26	26
8	18	79	46	35	0.16	37.5	46	24
9	10.5	97	86	43	0.14	250	22	23
10	18	86	60	56	0.1	31.2	45	21
11	19	103	117	142	0.05	50	38	14
12	34.6	86	65	56	1	85	23	14
13	15	137	150	129	0.05	137	41	8
14	2.9	84	55	40	0.17	62.5	35	8
15	28	90	89	81	10	125	24	5
16	3	96	85	80	0.3	>500	2	8

Patients are numbered in the order of the magnitude of the late FEV₁ maximum fall.

action can be predicted by a model that simultaneously takes into account nonspecific bronchial reactivity, IgE levels, and baseline airway caliber.

METHODS

Patient selection

We have studied 16 consecutive attack-free patients with asthma (10 male and six female, aged 13 to 45 years) sensitized to *Dermatophagoides pteronyssinus*, as proved by the positivity of both prick tests (glycerol-preserved allergen from Lofarma, Milan, Italy) and RAST (Pharmacia, Uppsala, Sweden). Informed consent was obtained from each patient before entering into the study.

Beta₂-stimulants, sodium cromoglycate, and theophylline, if any of these were taken, were stopped 12 hours before the study. None of the patients was receiving steroids or antihistamines.

In a prestudy selection day, a standard spirometry was performed before and 15 minutes after inhalation of control saline solution. In order to assess spontaneous intraday variations, spirometric measurement was repeated hourly for 10 hours. The apparatus consisted of a heated Fleisch No. 3 pneumotachograph (COSMED, Rome, Italy) coupled to a differential pressure transducer. Flow was measured at the mouth, and volumes were obtained by electric integration of the flow signal. FVC, FEV₁, MEF50, and MEF25 were calculated by an interfaced microcomputer. Measured values were compared with reference values for age, sex, and height.¹⁵ To be included into the study, patients were required to have an FEV₁ >75% of the reference value without circadian variations exceeding 10%.

Bronchial challenges

On two consecutive days, patients had a nonspecific bronchial challenge with methacholine (day 1) and a specific bronchial challenge with *D. pteronyssinus* (day 2). Solutions were aerosolized through an ampule-dosimeter device that, according to the manufacturing firm (Mefar, Brescia, Italy), delivers aerosols with an aerodynamic mass median diameter of particles ranging between 1.53 and 1.61 μ m. The dosimeter, which consisted of a breath-activated solenoid valve and a source of compressed air <1.5 kg/cm, was set to deliver, during 1 second, 5 μ l of solution for each breath. Patients inhaled through the mouth during quiet tidal volume breathing, triggering the delivering device with inspiratory flow; 5 seconds were allowed between breaths.

The same ampule, batch of methacholine, and lot of allergen were used for all patients. Methacholine challenges were started from a dose of 0.02 mg, obtained by allowing four breaths of a 1 mg/ml solution. Thereafter, doses were twofold incremented by changing the methacholine concentration and/or the number of breaths. Challenges were stopped when the FEV₁, measured immediately after nebulization, was decreased by >20% of control, otherwise at the maximum dose of 20 mg. *D. pteronyssinus* solutions were extemporaneously obtained by adding redistilled water to single-use vials containing dried allergen (Lofarma) predosed in arbitrary units by RAST-inhibition technique¹⁶ by use of an in-house (Lofarma) standard sera pool and a 5000 AU of *D. pteronyssinus* reference extract (160 μ g/ml of protein). Specific bronchial challenges were started with a dose of 2 AU by allowing 50 breaths of an 8 AU/ml solution. Doses were twofold incremented by changing allergen con-

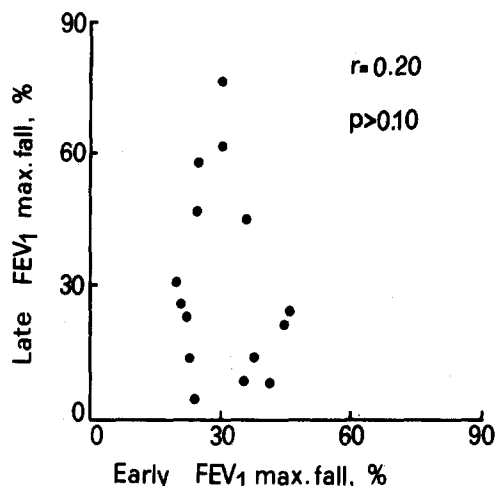


FIG. 1. Relationship between early (within 1 hour) and late (3 to 8 hours) FEV₁ maximum falls as percent of control. Early asthmatic reactions of similar magnitude were followed by late asthmatic reactions of largely different magnitude.

centration until the FEV₁ measured 15 minutes after nebulization was decreased by >20% of control, otherwise up to the maximum dose of 500 AU. The bronchial response after *D. pteronyssinus* challenge was monitored hourly for 8 hours.

Pd15 of either methacholine or allergen was calculated by interpolating in the dose-response curves and transformed to logarithmic form for statistical analysis.

RAST results were expressed as TRA%. The Rast negative control value was always <2%.

Statistical analysis

Single and multivariate regression analysis was carried out. The dependent variables were the allergen Pd15 or the FEV₁ maximum fall (as percent of control values) 3 to 8 hours after challenge, and the independent variables were the methacholine Pd15, RAST TRA%, and baseline FEV₁, or maximal expiratory flows as percent of reference values. Predicted values for allergen Pd15 or late FEV₁ maximum fall were obtained by solving multiple regression equations of the following general form:

$$Y = a + b_1 \cdot X_1 + \dots + b_n \cdot X_n$$

where each regression coefficient ($b_1 \dots b_n$) denotes the regression coefficient of Y on each independent variable ($X_1 \dots X_n$) that would be expected if the other independent variables had been held constant. Simple and multiple coefficients of correlation and their squares (coefficient of determination, r^2) were calculated to assess the statistical significance and the proportion of the variance of the dependent variables explained by stepwise addition of correlated independent variables.¹⁷ The correlation between the dependent variables and each independent variable of multiple regression equations was assessed by calculating the partial coefficients of correlation.

TABLE II. Determinants of early and late asthmatic reaction

Independent variables	Dependent variables	
	Early Pd15 FEV ₁ , AU (r^2)	Late % Max FEV ₁ fall (r^2)
Methacholine + IgE + baseline FEV ₁	0.63	0.68
Methacholine + IgE	0.55	0.56
Methacholine + baseline FEV ₁	0.41	0.47
IgE + baseline FEV ₁	0.31	0.36
Methacholine	0.25	0.26
IgE	0.25	0.25
Baseline FEV ₁	0.12	0.18

Methacholine: Log PD15 FEV₁, mg; IgE: RAST, TRA%; baseline FEV₁: percent of reference values.

RESULTS

Individual data are presented in Table I. Fifteen of 16 patients exhibited a positive early asthmatic reaction to *D. pteronyssinus* as indicated by an FEV₁ fall exceeding 15% of control within 1 hour from the end of challenge. Ten patients also demonstrated a positive late asthmatic reaction, as indicated by an FEV₁ fall exceeding 15% of control, in one occasion at least, between 3 and 8 hours after the end of challenge. One patient did not respond to the maximum dose of allergen and, therefore, was excluded from the subsequent statistical analysis.

No correlation was found between the early and late maximum fall of FEV₁ (Fig. 1). Patients with early reactions of similar magnitude (20% to 46% of FEV₁ maximum fall) exhibited very different late responses (5% to 76% of FEV₁ maximum fall).

Fig. 2 illustrates the predictive accuracy of allergen Pd15 (left) and late maximum fall of FEV₁ (right) achieved by the following relations, respectively:

$$\begin{aligned} \text{Log all.Pd15(AU)} = & 3.92 + 0.38 \times \\ & \log \text{meth.Pd15(mg)} - 0.05 \times \\ & \text{IgE (TRA\%)} + 0.02 \times \text{baseline FEV}_{1}(\%) \end{aligned}$$

$$\begin{aligned} \text{Late FEV}_{1} \text{ fall (\%)} = & 36.5 - 8.15 \times \\ & \log \text{meth.Pd15(mg)} + 1.11 \times \\ & \text{IgE(TRA\%)} - 0.54 \times \text{baseline FEV}_{1}(\%) \end{aligned}$$

The deviations of measured from predicted values were randomly scattered around the line of identity in both cases, suggesting that a large part of the residual unexplained variance was likely caused by the random experimental error. All the partial coefficients of correlation between the above dependent variables and any of the independent variables considered in this

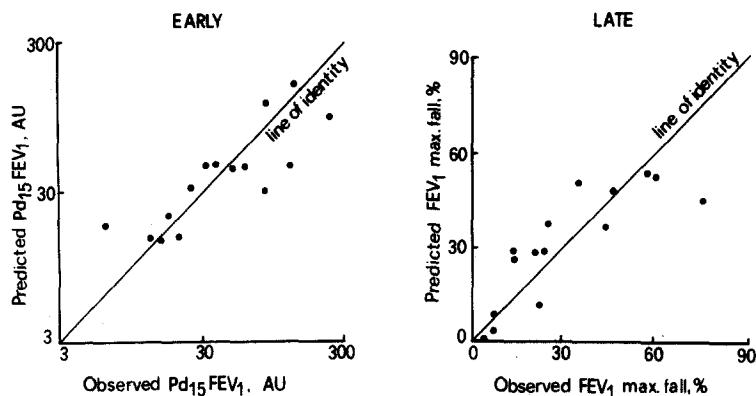


FIG. 2. Predictive accuracy of allergen Pd15 (*left*) and late FEV₁ maximum fall (*right*) obtained by multiple regression equations (see text) in which methacholine Pd15, IgE level, and baseline FEV₁ were simultaneously included. The coefficients of determination (r^2) are presented in Table II.

study were significant ($p < 0.05$), whereas no statistical significance was found between any of these independent variables.

Table II presents the degree of dependence (r^2) of both allergen Pd15 and late maximum fall of FEV₁ from control values on each individual independent variable considered in this study or on their possible combinations. Similar results were obtained when baseline values of MEF50 or MEF25 were used instead of FEV₁.

Fig. 3 illustrates the relationship between the late maximum fall of FEV₁ and the maximum dose of allergen inhaled for achieving Pd15. A highly significant negative correlation was found ($r = -0.764$; $p < 0.001$).

DISCUSSION

The results of the present study provide evidence that the occurrence and severity of late asthmatic reaction after inhalation of *D. pteronyssinus* can be predicted from the combination of nonspecific bronchial reactivity with specific IgE levels and baseline airway caliber. We have also demonstrated that late asthmatic reactions occur more frequently and severely in patients in whom small amounts of allergen are sufficient to elicit an early asthmatic reaction.

Late asthmatic reactions have been reported^{3, 14} to occur in 47% to 67% of adult patients who exhibit an early asthmatic reaction after the inhalation of house-dust allergen. In a previous study¹⁸ on patients characterized by strongly positive skin reactions and/or high specific IgE levels in serum, we have found that dual asthmatic reaction to *D. pteronyssinus* occurred in most (77%) adult patients without steroid therapy. We, therefore, postulated that the wide variability of the rate of occurrence of late asthmatic reaction ob-

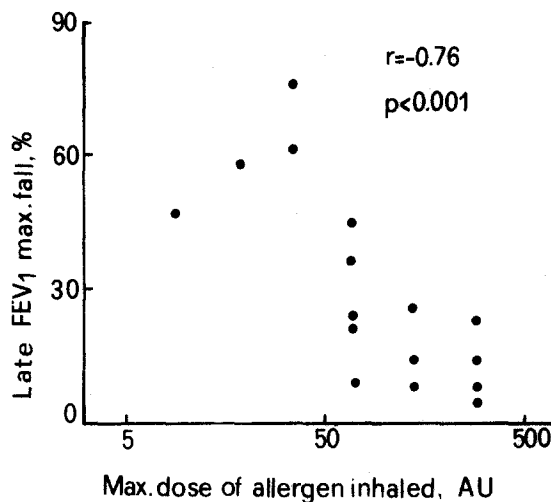


FIG. 3. Relationship between the maximum dose of allergen administered to obtain early asthmatic reactions (FEV₁ fall $>20\%$ of control) and the late maximum fall of FEV₁.

served in different studies might be accounted for by different criteria used for patients' selection or, in other words, by different severity of disease.

Several authors have, indeed, speculated that the occurrence of the late asthmatic reaction may depend on the severity of the disease, as reflected by the severity of symptoms and the early onset of disease in childhood.^{5, 14} Such relationships, however, were not found by Booijs-Noord et al.³ Others⁹⁻¹⁴ attempted to determine if those factors that are known as determinants of early asthmatic reaction, i.e., the nonspecific bronchial hyperreactivity and the atopic status, are also determinants of late asthmatic reaction. However, neither skin reactivity^{13, 14, 19} nor bronchial reactivity¹²⁻¹⁴ were found to be significantly different

in patients with or without late asthmatic reaction, but serum concentrations of specific IgE tended to be higher in dual responders.⁹⁻¹² In addition, MacIntyre and Boyd¹⁴ and Price et al.²⁰ observed that late asthmatic reactions are more likely to occur in patients who develop an early asthmatic reaction with small doses of allergen.

Our present study, unlike studies previously published,⁹⁻¹⁴ was designed to assess the predictive accuracy not only of the occurrence but also of the magnitude of bronchial reactions to allergen. Thus, it is crucial that the technique of bronchial challenge provides quantitatively reproducible results. By use of similar equipment and the same procedure as in this study, we have previously determined that both early and late asthmatic reactions to inhaled house-dust allergen are satisfactorily reproducible with similar coefficients of variation of about 14%.¹⁸ Therefore, our method of bronchial challenge with allergen extracts appears to be suitable for quantitative studies.

We have found that the maximum fall of FEV₁ of late asthmatic reaction can be predicted by a multivariate model that simultaneously takes into account bronchial reactivity, specific IgE, and baseline airway caliber. These findings confirm and extend those findings previously published⁹⁻¹¹ that demonstrated that IgE concentrations tended to be higher in patients with dual reaction compared to patients with isolated early reaction. Furthermore, our findings demonstrate that nonspecific bronchial hyperreactivity and baseline airway caliber are also determinants of late asthmatic reaction. Such relationships have not been previously published.¹²⁻¹⁴ In these studies, however, the effect of each variable was separately considered and, therefore, could have been masked by the influence of any other variable that was not removed experimentally or statistically. This interpretation is confirmed by the observation that in our study all the nonsignificant or barely significant simple coefficient of correlation were changed to definitely significant partial coefficient of correlation when other variables were held constant.

From data in Table II it appears that the relative importance of the above three variables was similar in predicting both early and late responses, methacholine sensitivity being the most important and baseline airway caliber the least important. Since the Pd15 of early reaction and the maximum fall of FEV₁ of late reaction are predictable by similar models, it is not surprising that late asthmatic reactions may occur more frequently and also more severely in patients who exhibited an early reaction after inhalation of a small amount of allergen. Nevertheless, it is not clear why patients in whom the magnitude (fall of FEV₁)

of early asthmatic reactions was constrained to be similar may exhibit quite different late responses. The pathogenesis of the late asthmatic reaction has not yet been completely elucidated. Recently, the time course of mediator release in dual asthma has been described both in animals and humans. In rats, Oertel and Kaliner²¹ have demonstrated that delayed skin inflammation can be induced by mast cell granules purified in vitro, and a neutrophilic polymorphonuclear response is greatest 8 hours after administration. In humans, Nagy et al.²² and Durham et al.²³ have demonstrated that in patients with dual asthmatic reactions two peaks of histamine and neutrophil chemotactic agent occur, whereas single peaks occur in patients with early asthmatic reaction only. De Monchy et al.²⁴ found that in patients with dual reaction to inhaled *D. pteronyssinus* the number of eosinophils in bronchoalveolar lavage fluid was significantly increased only after the late reaction. These findings suggest that the late asthmatic reaction could be the result of mediator release by inflammatory cells that are activated during the exposure to allergen. We can speculate that patients with more severe disease, as reflected by higher bronchial reactivity and/or higher IgE level, are more susceptible of developing dual reactions because of a persistency of higher levels of mediators or infiltration by inflammatory cells.¹⁹⁻²⁶

In conclusion, we have demonstrated that late asthmatic reactions, like early asthmatic reactions, can be predicted on the basis of the baseline conditions, i.e., bronchial hyperreactivity, baseline airway caliber, and IgE levels, and that they are more severe in patients with more severe underlying disease.

We gratefully thank Mr. Domenico Pozzolo for the valuable technical assistance.

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