The Relationship between Δ-Forced Vital Capacity (Percent Fall in Forced Vital Capacity at the PC_{20} Dose of Methacholine) and the Maximal Airway Response in Patients Who Have Mild Asthma

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ABSTRACT

Airway hypersensitivity is routinely evaluated by measuring the concentration (PC_{20}) of inhaled methacholine or histamine that causes a 20% fall in forced expiratory volume in 1 second (FEV_1). It has been suggested that a percentage fall in forced vital capacity (FVC) measured at the PC_{20} dose of inhaled agonist (ΔFVC) is a potentially useful clinical measure in patients who have asthma because it provides indirect information about gas trapping and therefore the maximal airway response. The relationships between serum eosinophil cationic protein (ECP) levels and the maximal airway response or ΔFVC are largely unknown. The aims of this study were to determine whether ΔFVC is correlated with the degree of maximal airway response and to examine the relationships between serum ECP and ΔFVC or maximal airway response in patients who have mild asthma. Fifty-eight patients with mild asthma underwent high-dose methacholine challenge testing. The PC_{20}, maximal airway response, and ΔFVC were measured on the methacholine dose–response curves. Serum ECP levels also were determined. Subjects without a maximal response plateau (n = 33) had a significantly higher level of ΔFVC (17.9 ± 4.1%) than subjects with a plateau (n = 25; 14.9 ± 4.8%). A significant correlation was found between ΔFVC and the level of maximal response plateau (r = 0.446; p = 0.026). Not only methacholine PC_{20} but also maximal airway response or ΔFVC had no relationships with serum ECP levels. Our results suggest that ΔFVC can be used as a surrogate marker of maximal airway response in patients who have mild asthma and that neither maximal airway response nor ΔFVC reflects blood eosinophil activation any more than methacholine PC_{20}. (Allergy and Asthma Proc 26: 366–372, 2005)

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Bronchial hyperresponsiveness (BHR) usually is defined as an increased sensitivity of the airways to inhaled nonsensitizing bronchoconstrictor stimuli.[1] However, there is accumulating evidence that BHR is a more complex functional abnormality that comprises more than just increased sensitivity.[2] When exposed to high concentrations of an agonist, normal subjects feature a maximal response plateau on the dose–response curve at mild degrees of airway narrowing, whereas asthmatic patients show an ex-

cessive airway narrowing, as reflected by either an elevated or absent maximal response plateau. It has been argued that excessive airway narrowing is clinically a more relevant component of BHR than airway sensitivity, because it reflects the potential severity of airway obstruction in the individual patients.[3,4] Several studies[2,5] have suggested that the mechanisms underlying maximal airway response and airway sensitivity differ, and that airway sensitivity is not an adequate measure of maximal airway response.

BHR is routinely evaluated by measuring the dose or concentration (PC_{20}) of inhaled methacholine or histamine that causes a 20% fall in forced expiratory volume in 1 second (FEV_1).[6] However, bronchoprovocation tests that use high concentrations of an agonist to measure the maximal airway response directly are not practical for routine clinical purposes because of the risks inherent in producing an excessive fall in FEV_1. It has been suggested that a percentage fall in forced vital capacity (FVC) measured at the PC_{20} dose of inhaled agonist (ΔFVC) is a potentially useful clinical measure in asthmatic patients because it provides indirect information about gas trapping and therefore the maximal airway response.[7] However, no study has been undertaken as to whether ΔFVC is actually correlated with the degree of maximal airway response.

The precise mechanism underlying BHR is still unclear, although it is believed that airway inflammation plays a major role.[8] Eosinophils generally are viewed as being the most important inflammatory cells.[9] In asthma, the presence of peripheral blood eosinophilia and increased serum levels of eosinophilic granular proteins, such as eosinophil cationic protein (ECP), are well recognized.[10] Investigators increasingly are suggesting that serum ECP level may reflect the intensity of airway inflammation because it is closely related to eosinophilic airway inflammation as evaluated by sputum or bronchoalveolar lavage samples.[11,12]

Several studies have examined the relationship between serum ECP levels and airway sensitivity. Some[13,14] have found strong relationships, whereas in others[15,16] these relations were weak or absent. On the other hand, the relationship between serum ECP levels and the maximal airway response or ΔFVC is largely unknown. These are relevant issues because airway eosinophilic inflammation has been suggested to be related more closely to maximal airway response than to airway sensitivity.[17,18] The aims of this study were to determine whether ΔFVC is correlated with the degree of maximal airway response and to examine the relationship between serum ECP and ΔFVC or maximal airway response in patients who have mild asthma.

METHODS

Sixty-three children with mild asthma were recruited from the allergy clinic at Seoul National University Children's Hospital. All subjects had a history of episodic wheezing and/or dyspnea and had been diagnosed as having asthma based on airway reversibility (an increase in FEV_1 $>$ 15% after bronchodilator administration) or a methacholine $PC_{20} < 16$ mg/mL. In all cases asthma was mild, stable, and controlled by bronchodilators on an as-needed basis, with or without low-dose inhaled corticosteroids. Patients with a history of near fatal asthma or major exacerbations necessitating the use of systemic corticosteroids were excluded. Skin-prick tests were performed on all children to evaluate atopic status. Atopy was defined as at least one positive reaction (wheal size $>$3 mm) to 12 common airborne allergens. All subjects provided blood samples for the determination of total eosinophil counts and serum ECP levels, and all underwent high-dose methacholine challenge tests. The patients stopped using inhaled bronchodilators or other medications 48 hours before the study and inhaled corticosteroids 7 days before the study. There was no history of upper respiratory infections for at least 4 weeks before the study.

High-dose methacholine inhalation tests were performed using a modification of the method described by Chai *et al.*[19] Spirometric measurements (FEV_1 and FVC) were made using a computerized spirometer (Microspiro-HI 298; Chest, Tokyo, Japan), in accordance with the recommendations of the American Thoracic Society.[20] The time course of the preceding inspiration was standardized, *i.e.*, rapid maximal inspiration without an end-inspiratory pause, and the FVC maneuver was continued until a pause in the forced expired volume curve was obvious by visual inspection; the minimum duration of the FVC maneuver was 6 seconds. Subjects who were unable to perform spirometric tests reproducibly or who had a low FEV_1 ($<$70% predicted)[21] were excluded. Methacholine (Sigma Diagnostics, St. Louis, MO) solutions were prepared at different concentrations (0.075, 0.15, 0.3, 0.625, 1.25, 2.5, 5, 10, 25, 50, 100, 150, and 200 mg/mL) in buffered saline solution (pH 7.4). A Rosenthal-French dosimeter (Laboratory for Applied Immunology; Baltimore, MD), triggered by a solenoid valve set to remain open for 0.6 seconds was used to generate the aerosol from a DeVilbiss 646 nebulizer (DeVilbiss Health Care, Somerset, PA), with pressurized air at 20 lb/in^2. Each subject inhaled five inspiratory capacity breaths of buffered saline solution and increasing concentrations of methacholine at 5-minute intervals. This gave an output of 0.009 $\pm$ 0.0014 mL (mean $\pm$ SD) per inhalation. FEV_1 and FVC were measured 60–90 seconds after inhalation at each concentration level, and the largest value of triplicate FEV_1 or FVC was used for analysis. The procedure was terminated when the FEV_1 level fell to $<$50% of the postsaline solution value or when a maximal response plateau had been established. This was considered to occur if three or more data points at the highest concentrations fell within a 5% response range.[2] For safety reasons, subjects were given the opportunity to discontinue challenge tests. Response, expressed as a percentage fall in FEV_1 from the postsaline solution value, was plotted against the log of the concentrations of inhaled methacholine. Dose–response curves were characterized by their position and maximal response,

the former expressed as PC_{20}, which was calculated by log-linear interpolation between two adjacent data points, and the latter defined as the level of maximal response plateau by averaging the consecutive points on the plateau. The ΔFVC relative to baseline FVC after saline inhalation also was calculated using log-linear interpolation.

Blood samples were withdrawn using a 21-gauge butterfly needle with an attached syringe; care was taken to avoid hemolysis. Eosinophil numbers were counted using an automated hematology analyzer (Coulter Counter, STKS; Beckman Coulter, Fullerton, CA). Serum ECP measurements were made according to the method of Venge.[22] Blood samples (4 mL) were collected in Vacutainer SST tubes (BD Biosciences, Franklin Lakes, NJ) and allowed to stand for 60 minutes at room temperature. They were then centrifuged at 1300 × *g* for 10 minutes, and sera were stored at −70°C until the ECP concentration was determined using an ECP radioimmunoassay kit (Amersham Biosciences, Piscataway, NJ). All assays were performed in duplicate, and mean values were used for statistical analysis. The detection limit of the assay was 2 μg/L, and the intra-assay coefficient of variation was 6.8%. Parents gave written informed consent for their children to participate in the study. The study protocol was approved by the Hospital Ethics Committee.

Statistical Analysis

The values of FEV_1 and FVC are expressed as percentages of predicted based on data from our local population.[21] PC_{20} values were logarithmically transformed before analysis and are expressed as geometric means with a range of 1 SD. Other values are presented as mean ± 1 SD. Values of subjects with and without a maximal response plateau were compared using the Student's *t*-test. Correlation analyses were performed using Pearson correlation coefficients. All analyses were made using Stat View II (Abacus Concept, Inc., Berkeley, CA) on a Macintosh computer (Apple Computer, Inc., Cupertino, CA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

A total of 63 subjects were enrolled in the study. Of these, five were excluded due to test interruption because of discomfort or dyspnea. Complete data were available for 58 subjects. The mean ± SD age was 11.2 ± 2.1 years with a male/female ratio of 1.9:1. Spirometric values, results of high-dose methacholine challenge tests, and blood eosinophil marker data are summarized in Table I. Seventy-eight percent of the subjects were found to be atopic and the majority (89%) of these developed a reaction to house-dust mites. Two subjects had a methacholine PC_{20} above 16 mg/mL, which was defined as the BHR cutoff. Twenty-five subjects featured a maximal response plateau on their dose–response curve to methacholine.

TABLE I

Summary of Spirometric Values, Results of High-Dose Methacholine Challenge Tests, and the Values of Blood Eosinophil Markers in All Subjects ($n = 58$)

FEV_1 (% predicted)*	94.7 ± 9.5
FVC (% predicted)*	99.3 ± 10.6
FEV_1/FVC (%)*	85.4 ± 5.1
Methacholine PC_{20} (mg/mL)#	5.00 (1.96–12.76)
Maximal response plateau (%; $n = 25$)*	41.1 ± 6.1
ΔFVC (%)*	16.6 ± 4.6
Serum ECP (μg/L)*	30.6 ± 20.1
Total eosinophil count (/μL)*	413.1 ± 224.9

**Mean ± SD.*
#Geometric mean (range of 1 SD).

A comparison of ΔFVC in subjects with and without a maximal response plateau is shown in Fig. 1 *a*. The ΔFVC was significantly higher in subjects without a plateau (17.9 ± 4.1%) than in those with a plateau (14.9 ± 4.8%; $p = 0.017$). The relationship between ΔFVC and the maximal response plateau level among subjects with a plateau is shown in Fig. 1 *b*. There was a significant correlation between ΔFVC and the maximal response plateau level ($r = 0.446$; $p = 0.026$). Regression plots of ΔFVC against methacholine PC_{20} in all subjects are shown in Fig. 2. No significant correlation was found between ΔFVC and methacholine PC_{20} ($r = -0.182$; $p = 0.172$). The relationship between maximal airway response and methacholine PC_{20} also was examined. Methacholine PC_{20} did not differ significantly between subjects without a maximal response plateau (geometric mean, 4.47 mg/mL [range of 1 SD, 1.51–13.18]) and those with a plateau (mean, 5.89 [range, 2.95–11.75]; $p = 0.242$). No significant relationship was found between methacholine PC_{20} and the maximal response plateau level among subjects with a plateau ($r = -0.220$; $p = 0.291$) (data not shown).

Serum ECP levels of subjects with and without a maximal response plateau are shown in Fig. 3 *a*. The serum ECP level of subjects with a plateau was 28.0 ± 12.3 μg/L, which was not significantly different from serum ECP levels (32.5 ± 24.5 μg/L) in subjects without a plateau ($p = 0.365$). The relationship between serum ECP concentration and the maximal response plateau level among subjects with a plateau is shown in Fig. 3 *b*. There was no significant correlation between serum ECP concentration and the maximal response plateau level ($r = 0.250$; $p = 0.228$). Regression plots of serum ECP levels against ΔFVC in all subjects are shown in Fig. 4. No significant correlation was found between serum ECP levels and ΔFVC ($r = 0.186$; $p = 0.163$). The relationship between serum ECP levels and methacholine PC_{20} also was examined, but no significant correlation was found ($r = -0.185$; $p = 0.166$; data not shown).

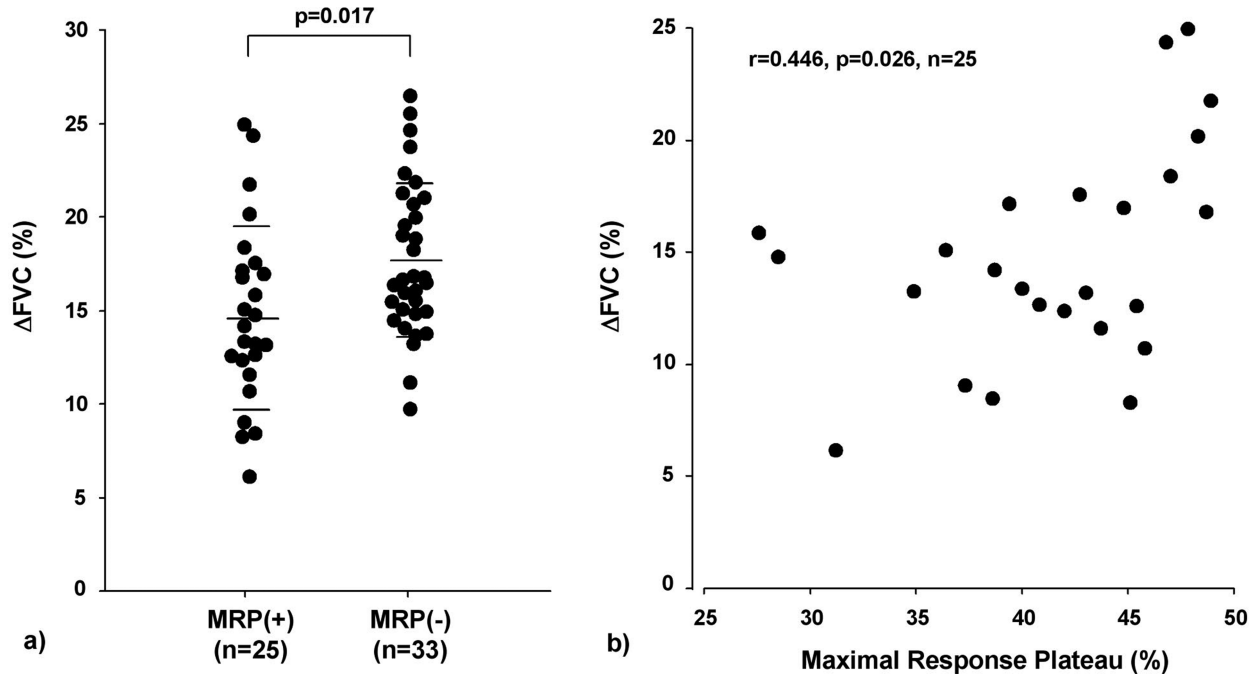

Figure 1. (a) A comparison of ΔFVC in subjects with and without a maximal response plateau (MRP). Horizontal bars represent mean ± SD. (b) The relationship between ΔFVC and the maximal response plateau level among the subjects with a plateau.

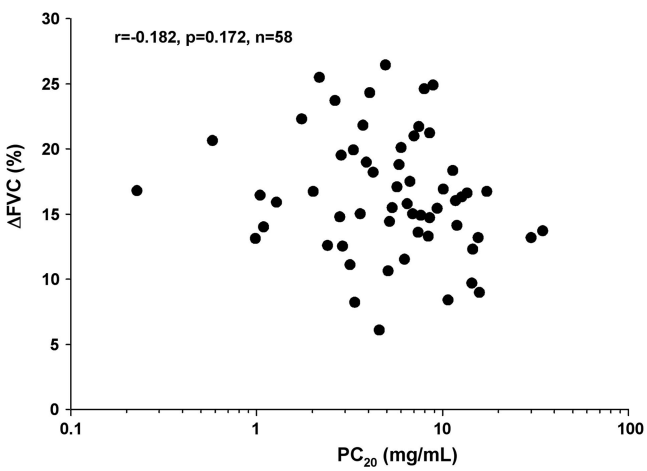

Figure 2. Regression plots of ΔFVC against methacholine PC_{20} in all subjects.

DISCUSSION

This study shows that ΔFVC is significantly correlated with the maximal airway response in patients who have mild asthma. This substantiates the hypothesis that ΔFVC may reflect gas trapping because of excessive bronchoconstriction. Neither maximal airway response nor ΔFVC had a relationship with serum ECP levels in patients with mild asthma.

Excessive bronchoconstriction is presumably the most important pathophysiological abnormality in asthma because it puts patients who have asthma at risk for serious illness.[3,4] Excessive bronchoconstriction is reflected by an absent or elevated maximal response plateau on the methacholine dose–response curve.[2] However, its measurement is neither safe nor easy to perform, because of the problems inherent in provoking an excessive fall in FEV_1. Therefore, a new means of quantifying excessive bronchoconstriction is desirable. Gibbons *et al.*[7] proposed a novel indirect method for the detection of excessive bronchoconstriction in patients with mild, newly diagnosed asthma. They retrospectively measured ΔFVC, which reflects the gas trapped at that point of dose–response curve caused by excessive bronchoconstriction. Indeed, it has long been appreciated that residual volume increases[23] and vital capacity falls[24] significantly in patients with asthma during induced bronchoconstriction. Assuming that total lung capacity remains constant,[25] the gas trapping so induced should be easily measurable as a dose-dependent fall in vital capacity. Unlike the PC_{20}, ΔFVC was found significantly related to the average number of oral corticosteroid prescriptions per month, which suggests that it may be a more useful index of disease severity in asthma than PC_{20}. After this report, several studies[26,27] have indicated that ΔFVC has potential as a clinical marker in identifying patients with asthma at risk for serious disease. However, the relationship between ΔFVC and the degree of excessive bronchoconstriction has not been investigated to date.

In this study, mild asthma patients were selected because a maximal response plateau usually is not measurable in patients who have moderate to severe asthma, reflecting the potential for excessive airway narrowing. Even in patients with mild asthma, the plateau was not detectable in more than one-half (56.9%) of the subjects, making the evaluation of the correlation complicated. Given this reservation, asth-

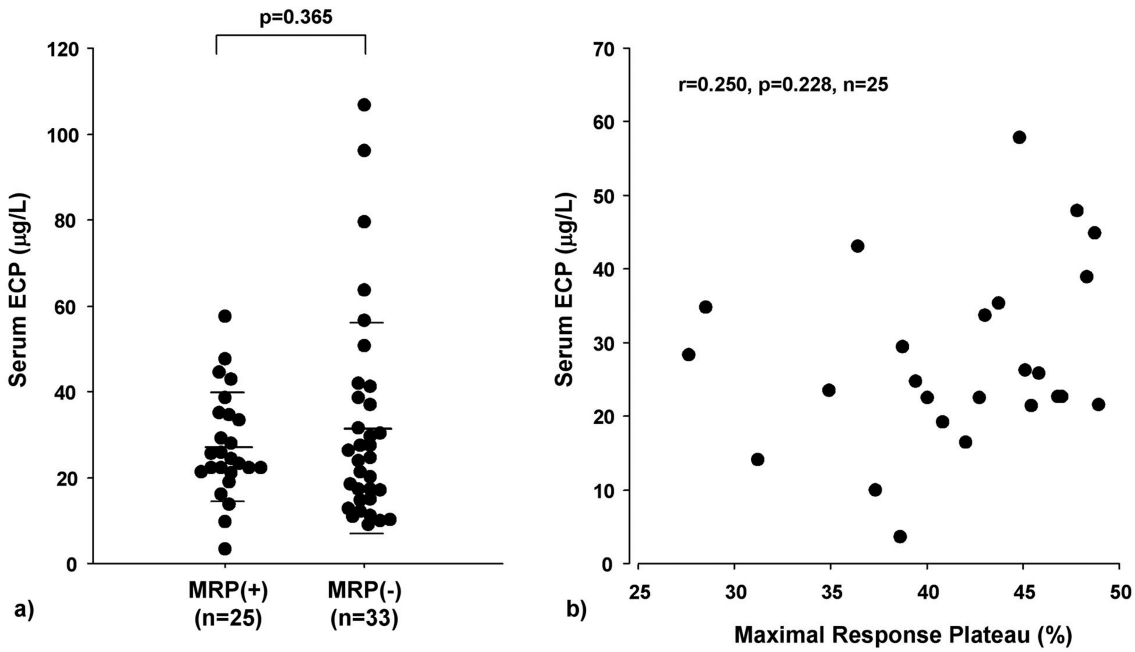

Figure 3. *(a) A comparison of serum ECP levels in subjects with and without a maximal response plateau (MRP). Horizontal bars represent mean ± SD. (b) The relationship between serum ECP concentration and the maximal response plateau level among the subjects with a plateau.*

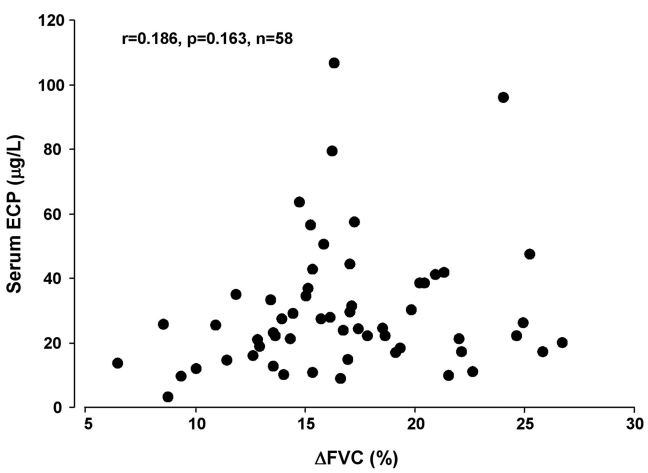

Figure 4. *Regression plots of serum ECP levels against ΔFVC in all subjects.*

matic patients without a maximal response plateau had a higher value of ΔFVC than asthmatic patients with a plateau. Furthermore, in the latter group, a significant correlation was found between the level of maximal response plateau and ΔFVC. These results substantiate the hypothesis proposed by Gibbons *et al.*[7] and suggest that ΔFVC may be used as a surrogate marker of maximal airway response in patients with mild asthma. Thus, ΔFVC has potential as a clinically useful parameter because its measurement carries no additional risk over that of a routine bronchial challenge test and avoids the problems inherent in provoking an excessive fall in FEV_1. On the other hand, there was no correlation between PC_{20} and ΔFVC, which also was the case in the patients described by other investigators.[7,26,28] These results suggest that the ease of bronchoconstriction, as reflected by the PC_{20}, and the degree of gas trapping, as reflected by the ΔFVC, represent two distinctive responses to methacholine inhalation.

It should be mentioned that the mean ΔFVC of this study is higher than those previously reported in adult asthmatic patients.[7,26] This is unlikely to be caused by differences in disease severity because only patients with mild asthma were included in this study. Children may be prone to muscle weakness and fatigue, and it is possible that the observed increase in ΔFVC may be a consequence of a reduction in FVC during bronchoprovocation testing, caused by a progressive shortening in expiration leading to incomplete emptying of the lungs. However, this can not be the case, because compliance with the American Thoracic Society criteria,[20] including the occurrence of an expiratory plateau, was checked on all occasions. Furthermore, the minimum duration of FVC maneuver was 6 seconds. FEV in 6 seconds has been suggested to be more reproducible than FVC and to be an acceptable surrogate for FVC in the diagnosis of airway obstruction.[29] The effects of lung elastic recoil on airway smooth muscle load play an important role in determining the degree of bronchoconstriction.[30] Cuttitta *et al.*[31] assumed that reduction in lung elastic recoil would account for a higher level of ΔFVC in elderly patients with asthma. Pulmonary elastic recoil was reported to be at a maximum in the late teens and decrease both with increasing and decreasing age.[32,33] We speculate that age-related lung elasticity factors may result in enhanced bronchoconstriction and thus a higher ΔFVC.

Most published studies on the relationship between BHR and serum ECP levels have treated airway hypersensitivity as BHR. Some authors found significant correlations between methacholine PC_{20} and serum ECP levels,[13,14] whereas others failed to find such relationships.[15,16] These result discrepancies probably reflect the multifactorial nature of airway hypersensitivity,[34] components of which vary between individuals, and therefore the results are strongly influenced by subject selection. Relatively few studies have examined the relationship between airway inflammation and maximal airway response. In patients with allergic rhinitis, Alvarez *et al.* reported that a higher degree of sputum eosinophilia was detected in subjects without a maximal response plateau[35] and that the presence of eosinophilia and the plateau level were significantly correlated.[17] However, no such relationship was found between sputum eosinophil count or ECP levels and methacholine PC_{20}.[17] Moller *et al.* found that the number of activated eosinophils in the bronchial mucosa was significantly related to the maximal response plateau value but not to methacholine PC_{20}.[18] These results raise the possibility that maximal airway response and implicitly ΔFVC, rather than methacholine PC_{20}, may be closely related to serum ECP levels.

In this study, not only methacholine PC_{20}, but also maximal airway response or ΔFVC, was found to be unrelated to serum ECP levels. This suggests that maximal airway response and ΔFVC do not have a higher capacity than bronchial sensitivity to discriminate blood eosinophil activation in mild asthma. This lack of correlation may be explained in several ways. First, patients with mild asthma were chosen for this study to detect a maximal response plateau. Subjects with mild asthma may not represent a whole spectrum of asthmatic patients and the small range of values of the parameters could, at least partly, account for our results. Second, the inflammatory process in asthma is primarily localized in the airways; thus, correlations between the parameters of BHR and eosinophil activation may be manifest in sputum or bronchoalveolar lavage fluid, rather than in peripheral blood, as used in this study. Third, a degree of airway remodeling, which can be found even in mild and short-evolution asthma,[36] would enhance BHR. This component of BHR may not be associated with increased serum ECP levels.

CONCLUSIONS

Patients with mild asthma without a maximal response plateau had a higher ΔFVC level than those with a plateau. Furthermore, in this latter group, a significant correlation was found between the plateau level and ΔFVC. These results suggest that ΔFVC may be used as a surrogate marker of maximal airway response in patients who have mild asthma. Not only methacholine PC_{20}, but also maximal airway response or ΔFVC, had no relationship with serum ECP levels. This suggests that neither maximal airway response nor ΔFVC reflect blood eosinophil activation anymore than methacholine PC_{20} in patients with mild asthma.

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