

Sputum Eosinophilia and Maximal Airway Narrowing in *Dermatophagoides pteronyssinus* Allergic Rhinitis Patients*

Only Rhinitis or Rhinitis Plus Mild Asthma?

María J. Alvarez Puebla, MD, PhD; Rodolfo Castillo, MD, PhD; Agustin Rey, MD, PhD; Nancy Ortega, MD; Carlos Blanco, MD, PhD; and Teresa Carrillo, MD, PhD

Study objective: To study the existence of bronchial disease among rhinitis patients. To evaluate the laboratory test or set of tests (ie, symptoms, exposure, and sensitization to the allergen, and the provocative dose of methacholine [Mth] causing a 20% fall in FEV₁ [PD₂₀] and the maximal response plateau [MRP] to Mth) that best identifies a case of mild asthma.

Design: Cross-sectional analysis in 52 *Dermatophagoides pteronyssinus*-monosensitized patients who were consulting a physician for perennial rhinitis.

Setting: Allergy Department, Hospital Doctor Negrín, Las Palmas, Grand Canary Island, Spain.

Interventions and measurements: Patients filled out a standardized asthma symptom questionnaire, and underwent sputum induction and Mth challenge in which 40% falls in FEV₁ were attained. Dose-response curves were expressed in terms of both PD₂₀ values and the level of the MRP. *D pteronyssinus* allergen exposure was assessed in dust samples from patients' beds.

Results: No difference between patients who positively responded to the questionnaire and those who did not was observed. Mth-PD₂₀ values were not detected in 13% of the patients reporting bronchial symptoms, and an MRP was not identified in 59% of the subjects who did not respond positively. A higher degree of allergen sensitization (ascertained from skin test results, and total and specific serum IgE levels) and higher degree of sputum eosinophilia were detected in subjects in whom an MRP had not been identified. The presence of sputum eosinophilia provided the best differentiation between those patients who presented with an MRP and those who did not.

Conclusion: The individual perception of bronchial symptoms is highly variable among perennial allergic rhinitis patients. The lack of a maximal airway-narrowing plateau is related to the presence of sputum eosinophilia, which might be useful in the detection of patients susceptible to anti-inflammatory therapy. Prospective studies evaluating whether these patients are more likely to develop symptomatic asthma in the future and if the early anti-inflammatory treatment prevents its development are needed. (CHEST 2002; 122:1560-1565)

Key words: allergic rhinitis; bronchial hyperresponsiveness; eosinophils; maximal airway narrowing; maximal response plateau; methacholine; mild asthma; sputum

Abbreviations: AS- = asthma symptoms negative; AS+ = asthma symptoms positive; BHR = bronchial hyperresponsiveness; BPT = bronchial provocation test; GM = geometric mean; IQR = interquartile range; MRP = maximal response plateau; MRP- = maximal response plateau not found; MRP+ = maximal response plateau found; Mth = methacholine; PD₂₀ = provocative dose of a substance causing a 20% drop in FEV₁

Asthma is a chronic inflammatory airways disease that, in some instances, leads to permanent changes in the bronchial architecture secondary to

the remodeling process.¹ Consequently, inhaled steroids have become the first-line therapy. In patients with mild asthma, therapy with inhaled steroids reduces symptoms and bronchial hyperresponsiveness (BHR), increases lung function, and may improve the long-term outcome of the disease.² Such effects are more prominent when therapy is administered during the early stages of asthma, and, thus, early detection of the disease has been encouraged.³

Patients with mild asthma often exhibit normal lung function test results, and the diagnosis of their condition lies mainly with historical data. It has been reported that the perception of airway narrowing

*From the Department of Allergy (Dr. Alvarez Puebla), Hospital de León, León, Spain; and the Departments of Allergy (Drs. Castillo, Ortega, Blanco, and Carrillo) and Pathology (Dr. Rey), Hospital Doctor Negrín, Las Palmas, GC, Spain.

This study was supported by a research grant from the Government of Canarias (FUNCIS).

Manuscript received November 8, 2001; revision accepted May 1, 2002.

Correspondence to: María J. Alvarez Puebla, MD, PhD, C/Señor de Bembibre No. 8, 6°B, 24005 León, Spain; e-mail: malvarez@meditex.es

both by patient⁴ and physician⁵ may be highly variable. Allergic asthma often is associated with rhinitis, which constitutes an independent risk factor for its development.⁶ Rhinitis symptoms can induce a higher degree of disability in the patient than mild bronchial symptoms,⁷ leading to serious underdiagnosis and, consequently, to the undertreatment of patients with mild asthma.⁸

Epidemiologic studies have clearly demonstrated a close relationship between asthma and the exposure to the allergens of the house dust mite *Dermatophagoides pteronyssinus*.^{9,10} In the present report, we have studied 52 recently diagnosed patients who were allergic to *D pteronyssinus* and who consulted physicians for symptoms of perennial rhinitis. Patients were grouped first according to their positive or negative responses to a standardized asthma symptom questionnaire, and second according to the whether a maximal response plateau (MRP) could be identified. A number of tests that are usually utilized in asthma assessment (*ie*, allergen exposure, skin tests, total and specific IgE quantitation, sputum induction, and, a methacholine [Mth] bronchial provocation test [BPT] in which FEV₁ drops by 40% were attained) were carried out. We evaluated whether some of the former indexes could contribute to detect the subgroup of patients susceptible to bronchial steroid therapy.

MATERIALS AND METHODS

Subjects and Study Design

We selected 52 nonsmoking *D pteronyssinus*-allergic patients who consecutively attended our department reporting nonseasonal rhinitis. *D pteronyssinus* sensitization was established by means of positive skin-prick test results with a standardized extract (ALK-Abelló; Madrid, Spain) and detection of a specific IgE to this allergen in the context of a compatible clinical history. Subjects who had been sensitized to other perennial or seasonal allergens were excluded. Patients underwent clinical and physical evaluations and filled out a standardized asthma symptoms questionnaire. According to their responses to the questionnaire, they were matched in the asthma symptoms-positive (AS+) group or the asthma symptoms-negative (AS-) group.¹¹ Mth-BPT, until FEV₁ values fell by 40% from baseline, was performed, and blood and sputum samples were collected afterward. The exposure to the major allergen of *D pteronyssinus* (Der p 1) was assessed in dust samples from patients' beds. During the 2 months before entry into the study, subjects had not presented with airways infections, and only short-acting β -agonist drugs used "as needed" were allowed. No antiallergy drugs were taken in the 2 months before study entry. Every subject was informed about the study and gave their consent before starting the study. The ethics committee of our hospital approved the study protocol.

Methods

Asthma Symptoms Questionnaire: Patients completed a standardized asthma symptoms questionnaire for which the repro-

ducibility and validity had been demonstrated.¹¹ The frequency of presentation of 10 bronchial symptoms (*ie*, dyspnea during exercise, diurnal resting dyspnea, diurnal wheezing, diurnal cough, nocturnal wheezing, nocturnal dyspnea, nocturnal cough, sleep disturbance, fear because of asthma, and chest tightness) during the last month was scored as follows: 0, never; 1, on 1 day or a few days; 2, on several days; 3, on most days; and 4, every day. The total symptoms score ranged from 0 to 40 points.

Collection of Dust Samples and Quantitation of Mite Allergens: Dust collection was carried out as described.¹² A portable vacuum cleaner provided with a prefilter (ALK-Abelló) that retains 74% of particles 0.3 to 0.5 μ m in size, 81% of particles 0.5 to 1.0 μ m in size, 95% of particles 1.0 to 10 μ m in size, and 100% of larger particles¹³ was used. The layer of bedding below the lower sheet, the layer above the upper sheet, and inside the pillow case were vacuumed for 2 min per m². The filter box then was removed and stored in a plastic bag at -20°C until further analysis could be conducted. The content of major mite allergen (Der p 1) was determined in duplicate by a commercial enzyme-linked immunosorbent assay based on monoclonal antibodies (ALK). Standard curves for these assays (1 to 200 ng allergen/mL) were routinely obtained from freeze-dried whole mite extracts that were titrated against purified allergens. Allergen concentrations were estimated from three independent experiments. The mean intra-assay coefficient of variation was < 3%, while the interassay coefficient of variation from control subjects tested with all the assays was < 15%. The detection limit of the assays was 1 ng/mL.

Mth BPT: Testing was performed according to international guidelines.^{14,15} Dilutions of Mth (Provocholine; Roche Laboratories; Nutley, NJ) at 0.125, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 100.0, and 200.0 mg/mL in phosphate-buffered saline solution were made on the study day. Baseline spirometry was performed, and FEV₁ values $\geq$ 70% of predicted normal values were required. The reference values of Crapo et al¹⁶ were used. Patients had not inhaled β -agonist drugs or ingested caffeine on the day of the test. Diluent (phosphate-buffered saline solution) and Mth at increasing concentrations were administered using a dosimeter (MEFAR srl; Borezzo, Italy) that had been programmed to deliver five inhalations of 1 s each, with 10 μ L solution administered in each inhalation. FEV₁ values were measured by spirometry 3 min following inhalation. A variability rate lower than 5% among basal and postdiluent FEV₁ values was required to start the test. The challenge finished when a fall in FEV₁ values $\geq$ 40% from the postdiluent value was achieved or when the highest Mth concentration was inhaled. Results were expressed in terms of the Mth-provocative dose of a substance (Mth) causing a 20% fall in FEV₁ (PD₂₀) from baseline values. The level MRP, which was considered to be present if three or more of the highest doses of the agonist fell within a 5% response range, was obtained by averaging the data points on the plateau.

Sputum Induction: Sputum samples were obtained by hypertonic saline solution inhalation, as has been described.¹⁷ Subjects were pretreated with four puffs of inhaled salbutamol. To avoid the contamination of the sample, patients rinsed their mouths and cleaned their noses before induction and, when possible, before expectoration. An ultrasonic nebulizer (Ultraneb 2000; DeVilbiss; Somerset, PA) was used to administer the saline solution at 5% for three periods of 10 min each. After each period, patients were asked to cough and expectorate into a sterile container, and, when possible, saliva was put into a different receptacle. The test finished when a macroscopically adequate sputum sample was obtained or when the three periods of inhalation had been completed.

The volume of the entire sputum sample was measured, and 3 mL of the sample was mixed with an equal volume of dithiotreitol (Sputasol; Unipath LTD; Basingstoke, Hampshire,

UK) at 1:100 and was stirred at room temperature for 15 min. The mixture was filtered through one 0.45- μ m filter (Millipore; Somerset, PA) and was centrifuged at 1,500g for 10 min. The pellet was suspended in a 0.9% saline solution serum, was cytocentrifuged, and was stained either with Papanicolaou or Giemsa stains for differential cell counts. The sample was considered to be adequate for analysis when macrophages could be visualized and squamous cell contamination was < 20%.¹⁸ The percentage counts of macrophages, eosinophils, neutrophils, mast cells, lymphocytes, and ciliated cells were made over a total count of 400 cells. A single person, blinded to the patients' clinical conditions, performed the evaluation.

Soluble Markers in Serum: The concentrations of serum total and specific IgE were measured by fluoroenzyme immunosorbent assay (UNICAP; Pharmacia Diagnostics; Uppsala, Sweden). The limits of detection for the assays were 0.35 kilounits/L for specific IgE and 2.0 kilounits/L for total IgE concentrations.

Statistical Analysis

Statistical analysis of the data was performed using a statistics program (SPSS for Windows, version 8.0; SPSS; Chicago, IL). Descriptive statistics summarized the demographic and clinical features of the patients. Results were expressed as the median and interquartile range (IQR). Mth-PD₂₀ values were log-transformed for analysis, and the results were expressed in terms of the geometric mean (GM). Differences between groups were analyzed by the Mann-Whitney *U* test, and correlation between variables was determined using the Spearman rank correlation coefficient. We used a model of multiple linear regression in which the variables that differed between groups were sequentially entered and applied to the dependent variable until a model in which no more variables were eligible for entry or removal was arrived at. The value of *F* was the criterion for entering variables (*F* < 0.05) or removing variables (*F* > 0.1). A *p* value of < 0.05 was considered to be significant.

RESULTS

First Criterion of Grouping: Response to the Asthma Symptom Questionnaire

The 52 mite rhinitis patients were matched according to their positive (*n* = 30) or negative

(*n* = 22) responses to the questionnaire (Table 1). Groups did not differ in age, sex, allergen sensitization, Der p 1 exposure, or baseline lung function. Sputum induction was safe in every patient. Adequate samples for analysis were obtained in 93% of the AS+ patients and in 68% of the AS- patients. No difference in sputum composition was detected between groups. The results of the Mth-BPT were positive (*ie*, the induced FEV₁ falls $\geq$ 20%) in 26 of the AS+ patients (86%) and in 11 of the AS- patients (50%). An MRP was identified in 14 patients (47%) and 9 patients (41%), respectively. No differences between groups were detected in either Mth-PD₂₀ values (GM, 2.63 vs 0.65 mg, respectively) or Mth-MRP values (median, 21% [$\leq$ IQR, 17 to 27] vs 10% [IQR, 9 to 30], respectively).

Second Criterion of Grouping: Identification of an MRP

Patients were grouped according to whether an MRP was found (MRP+; *n* = 23) and or was not found (MRP-; *n* = 29) [Table 2]. Asthma symptoms were reported by 16 of the MRP+ patients (69%) and by 14 of the MRP- patients (48%). When groups were compared, the magnitude of the skin test response, and the levels of total and *D pteronyssinus*-specific serum IgE were higher in the MRP- group. Adequate sputum samples were obtained in 14 patients (61%) from the MRP+ group and in every patient from the MRP- group. The MRP- group exhibited higher percentages of eosinophils and neutrophils, and lower numbers of ciliated cells in the sputum samples than did the MRP+ group. Mth-PD₂₀ values, detected in eight patients (35%) from the MRP+ group and in every patient from the MRP- group, were lower (*p* < 0.001) in the former group (GM, 6.97 vs 0.14 mg, respectively).

Table 1—Data From the Patients Grouped According to Their Positive or Negative Responses to the Asthma Symptoms Questionnaire*

Variables	AS+	AS-
Asthma score, points	10 (4.25–15.75)	0
Age, yr	21 (18–24)	21 (16–31)
Male sex, %	58	59
Skin-prick test, mm ²	60 (36–88)	81 (35–130)
Total serum IgE, KU/L	438 (113–1495)	502 (162–1391)
<i>D pteronyssinus</i> serum IgE, KU/L	89 (32.2–100)	90 (66.5–98)
Der p 1 exposure, μ g/g dust	8.83 (3.8–20.0)	16.35 (3.13–38.4)
Baseline FEV ₁ , %	90 (77.7–99.7)	90 (67–98)
Mth-PD ₂₀ , † mg	2.62	0.65
Eosinophils, %	7.0 (3.0–11.7)	9.4 (4.0–20.5)
Lymphocytes, %	7.0 (1.8–13.2)	6.7 (2.3–17.0)
Macrophages, %	46.5 (20.0–68.0)	53.0 (30.0–60.0)
Neutrophils, %	13.0 (4.0–26.0)	16.0 (8.5–30.5)
Ciliated cells	14.8 (9.5–30.5)	7.3 (3.0–20.0)

*Values given as median (IQR), unless otherwise indicated.

†Values given as GM.

Table 2—Data From the Patients Grouped According to the Identification of an MRP*

Variables	MRP+	MRP–	p Value†
Asthma score, points	8 (1–12.7)	10 (2.5–16.5)	NS
Age, yr	20 (17–22)	21 (18–25)	NS
Male sex, %	65	52	
Skin-prick test, mm ²	56 (28–84)	102 (68–151)	0.003
Total serum IgE, KU/L	237.5 (113–492)	640 (313–1379)	0.021
<i>D pteronyssinus</i> serum IgE, KU/L	57.4 (31.5–94.5)	100 (87.5–100)	0.038
Der p 1 exposure, µg/g dust	14.1 (3.3–23.2)	13.9 (4.3–41.6)	NS
Baseline FEV ₁ , %	94 (89–100)	91 (78–100)	NS
Mth-PD ₂₀ ‡, µmol	6.97	0.14	< 0.001
Eosinophils, %	4.4 (3.0–8.7)	15.0 (12.2–19.2)	< 0.001
Lymphocytes, %	5 (1.5–14.7)	7.5 (5.5–14.5)	NS
Macrophages, %	52.5 (34.2–63.7)	55.0 (22.0–69.0)	NS
Neutrophils, %	8.0 (1.5–20)	21.0 (10.5–25)	0.04
Ciliated cells	22.0 (10.0–30.5)	6.2 (2.2–14.0)	0.011

*Values given as median (IQR), unless otherwise indicated. NS = not significant.

†Mann-Whitney U test.

‡Values given as GM.

Correlation and Linear Regression Study

When the whole sample of patients was analyzed, the number of sputum eosinophils correlated both with total serum IgE levels ($p = 0.003$; $r = 0.50$) and Mth-PD₂₀ values ($p = 0.035$; $r = -0.45$). Mth-PD₂₀ values correlated with the skin test area ($p < 0.001$; $r = -0.53$) and showed a trend toward correlation with the percentage of neutrophils in the sputum ($p = 0.059$; $r = -0.34$).

A linear regression test was performed by the stepwise entering of the variables that had differed between groups (*ie*, skin test; total and *D pteronyssinus*-specific IgE levels, and the numbers of eosinophils, neutrophils, and ciliated cells) and was applied to the dependent variable (*ie*, presentation or not of an MRP). Only sputum eosinophilia contributed to the identification of an MRP ($\beta = 0.463$; $p = 0.030$).

DISCUSSION

Allergic asthma and rhinitis are related disorders that frequently are associated and show a parallel development.⁶ Although less frequently than in patients with asthma, the lower airways of rhinitis patients exhibit eosinophilic infiltration^{17,19} and increased sensitivity to bronchoconstricting agonists,^{17,20} suggesting that both diseases are manifestations of the same disorder.⁶ Some authors³ have documented the benefits of the early detection and onset of inhaled steroid therapy in patients with mild asthma. However, these patients often exhibit normal baseline lung function test results and, given the low sensitivities of the biochemical tests,²¹ diagnosis of their conditions depends mainly on clinical data (*ie*, recurrent attacks of cough, chest tightness, dys-

pnea, and wheezing). The individual perception of airway-narrowing episodes is highly variable,⁴ leading to the misdiagnosis of asthma, implying long-term and unnecessary drug therapy, or, alternatively, leading to the underdiagnosis and consequent undertreatment of asthma.⁸

In this study, we classified a group of mite-allergic patients who consulted physicians for perennial rhinitis on the basis of their positive or negative responses to a validated asthma symptom questionnaire.¹¹ No different allergen exposure, sensitization, or sputum composition was detected between patients reporting asthma symptoms and those who did not. BHR, often expressed in terms of sensitivity (*ie*, PD₂₀ values), is a cardinal feature of asthma that also has been described among some rhinitis patients.²⁰ The values for Mth-PD₂₀ notably overlap between rhinitis and asthma, and thereby, a positive response to Mth is not diagnostic of asthma, but a negative response among patients currently exposed to the allergen rules out the diagnosis of the disease.²² We evaluated subjects who were sensitized to and currently exposed to the perennial indoor allergen *D pteronyssinus* and observed no different sensitivity to Mth between those reporting bronchial symptoms and those who did not. More importantly, Mth-PD₂₀ values were not detected in 14% of the subjects reporting recent bronchial symptoms, which brings into question the diagnosis of asthma in these subjects.⁴

BHR is a complex functional disorder of the airways that is defined by dose-response curves that provide different information.²³ The shift of the curve to the left indicates increased sensitivity and structurally correlates with a loss of integrity of the respiratory epithelium.²⁴ The severity of the re-

sponse, reflected by the shape of the curve, is evaluated in terms of the dose-response curve slope and the MRP.²² Bronchial response is secondary to both the reduction in the forces that limit airway narrowing and the increase in airway wall thickness.^{24,25} The inflammatory infiltrate and the edema of the submucosa as well as the deposition of collagen in the epithelial subbasement membrane increase the thickness of the bronchial wall and enhance the response to the agonist. The MRP, the point at which the inhalation of increasing concentrations of stimulus induces no further airflow limitation, is presumably the major pathophysiologic abnormality in asthma patients since its absence or detection in high degrees of airway narrowing puts subjects at risk from serious disease.²⁶ The plateau level also has a higher capacity than bronchial sensitivity to discriminate asthma from rhinitis.²⁷ In our study, a plateau level was not identified in more than half of the patients, and, strikingly, 44% of them did not report bronchial symptoms.

Bronchial challenge tests that employ high concentrations of an agonist to measure the maximal degree of airway narrowing are far from practical for routine clinical purposes due to the large falls in FEV₁ values that are induced and because they are extremely time-consuming. Therefore, we evaluated whether any other index could differentiate those patients who lacked an MRP from the remainder of rhinitis patients. Allergen sensitization (*ie*, skin test results, and total and *D pteronyssinus*-specific IgE levels) was higher among patients in whom no MRP was identified, suggesting a more intense systemic immunologic response to the allergen. Higher numbers of eosinophils and a trend toward higher numbers of neutrophils in sputum also were observed among patients without MRPs. In patients with severe asthma, sputum eosinophilia has been reported to be an independent factor for persistent airflow limitation.²⁸ Eosinophils are a major source of transforming growth factor- β 1,²⁹ a cytokine that is implied in the development of fibrosis within asthmatic airways.³⁰ Bronchial biopsy specimens from patients with severe asthma in whom eosinophilia persisted despite steroid therapy exhibited increased concentrations of transforming growth factor- β 1 coincident with a thicker subbasement membrane.³¹ These data suggest a close relationship between eosinophilic inflammation and the structural changes in the airways that, at least in part, are responsible for the increased bronchial response. In this study, the presence of eosinophilia in sputum and the level of the MRP correlated in allergic rhinitis patients.¹⁷ Our results showed that, even among subjects consulting physicians for rhinitis symptoms, sputum eosinophilia was the only factor correlated with the

lack of MRP, which supports the role for eosinophils in the initiation of the bronchial structural changes that lead to a higher degree of airway narrowing.

When using immunocytochemistry techniques,^{32,33} asthma patients exhibit higher numbers of epithelial cells in sputum than do nonasthmatic patients. The weak reproducibility of the sputum epithelial cells counts when standard optical microscopy is utilized³³ might explain the fact that in our study their numbers were strikingly higher in the patients in whom an MRP was detected, and also were higher than those described by other authors.^{32,33}

In summary, there is not a clear differentiation between allergic rhinitis alone and allergic rhinitis plus mild asthma. A degree of eosinophilic bronchial inflammation that relates to the increase or lack of increase in the level of the maximal airway narrowing plateau is detected even in some rhinitis patients who do not report asthma symptoms. Our results are preliminary findings that are in no way sufficient to predict whether these rhinitis patients without asthma symptoms and with normal lung function will develop symptomatic asthma in the future. However, given the close relationship between bronchial eosinophilia, airway fibrotic changes, and the lack of an MRP, long-term anti-inflammatory treatment in these patients might be considered. Prospective studies are needed to evaluate whether such treatment, based on the presence of sputum eosinophilia, might prevent the development of symptomatic asthma.

ACKNOWLEDGMENT: The authors thank Ms. Theresa Hetherington for her review of the English language of this manuscript.

REFERENCES

- 1 National Heart, Lung, and Blood Institute. Guidelines for the diagnosis and management of asthma: National Asthma Education and Prevention Program; expert panel report. *J Allergy Clin Immunol* 1991; 88:425-534
- 2 Haahtela T, Jarvinen M, Kava T, et al. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N Engl J Med* 1994; 331:700-705
- 3 Haahtela T. The long-term influence of therapeutic interventions in asthma with emphasis on inhaled steroids and early disease. *Clin Exp Allergy* 1998; 5:133-140
- 4 Salome CM, Xuan W, Gray EJ, et al. Perception of airway narrowing in a general population sample. *Eur Respir J* 1997; 10:1052-1058
- 5 Shim CS, Williams MH Jr. Evaluation of the severity of asthma: patients vs physicians. *Am J Med* 1980; 68:11-13
- 6 Leynaert B, Bousquet J, Neukirch C, et al. Perennial rhinitis: an independent risk factor for asthma in nonatopic subjects; results from the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1999; 104:301-304
- 7 Leynaert B, Neukirch C, Liard R, et al. Quality of life in allergic rhinitis and asthma: a population-based study of young adults. *Am J Respir Crit Care Med* 2000; 162:1391-1396

- 8 Siersted HC, Boldsen J, Hansen HS, et al. Population based study of risk factors for underdiagnosis of asthma in adolescence: Odense schoolchild study. *BMJ* 1998; 316:651–655
- 9 Sporik R, Holgate ST, Platts-Mills TA, et al. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood: a prospective study. *N Engl J Med* 1990; 323:502–507
- 10 Sporik R, Platts-Mills TA. Allergen exposure and the development of asthma. *Thorax* 2001; 56:II58–II63
- 11 Steen N, Hutchinson A, McColl E, et al. Development of a symptom based outcome measure for asthma. *BMJ* 1994; 309:1065–1068
- 12 Tovey ER, Chapman MD, Wells CW, et al. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981; 124:630–635
- 13 Dreborg S, Einarsson R, Lau S, et al. Dust sampling for determination of allergen content. *Allergy* 1995; 50:188–189
- 14 Sterk PJ, Fabbri LM, Quanjer PH, et al. Airway responsiveness: standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults; report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal—official statement of the European Respiratory Society. *Eur Respir J Suppl* 1993; 16:53–83
- 15 Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing: 1999; this official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000; 161:309–329
- 16 Crapo R, Morris A, Gardner R. Reference spirometric values using techniques and equipment that meets the ATS recommendations. *Am Rev Respir Dis* 1981; 123:659–664
- 17 Alvarez MJ, Olaguibel JM, Garcia BE, et al. Airway inflammation in asthma and perennial allergic rhinitis: relationship with nonspecific bronchial responsiveness and maximal airway narrowing. *Allergy* 2000; 55:355–362
- 18 Fahy J, Wong H, Liu J. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. *Am J Respir Crit Care Med* 1995; 152:53–58
- 19 Foresi A, Leone C, Pelucchi A, et al. Eosinophils, mast cells, and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to methacholine responsiveness. *J Allergy Clin Immunol* 1997; 100: 58–64
- 20 Prieto L, Gutierrez V, Morales C, et al. Differences in sensitivity, maximal response and position of the concentration-response curve to methacholine between asthmatics, patients with allergic rhinitis and healthy subjects. *Respir Med* 1998; 92:88–94
- 21 Metso T, Kilpio K, Bjorksten F, et al. Detection and treatment of early asthma. *Allergy* 2000; 55:505–509
- 22 Sterk PJ, Bel EH. The shape of the dose-response curve to inhaled bronchoconstrictor agents in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991; 143:1433–1437
- 23 Sterk PJ, Bel EH. Bronchial hyperresponsiveness: the need for a distinction between hypersensitivity and excessive airway narrowing. *Eur Respir J* 1989; 2:267–274
- 24 Moreno RH, Hogg JC, Pare PD. Mechanics of airway narrowing. *Am Rev Respir Dis* 1986; 133:1171–1180
- 25 Moore BJ, King GG, D'Yachkova Y, et al. Mechanism of methacholine dose-response plateaus in normal subjects. *Am J Respir Crit Care Med* 1998; 158:666–669
- 26 Bel EH, Zwinderman AH, Timmers MC, et al. The protective effect of a beta 2 agonist against excessive airway narrowing in response to bronchoconstrictor stimuli in asthma and chronic obstructive lung disease. *Thorax* 1991; 46:9–14
- 27 Prieto L, Marin J. Measurement of response to methacholine inhalation challenge in asthma and allergic rhinitis: PC20 or PC35FEV1? *Allergol Immunopathol (Madr)* 1990; 18: 285–289
- 28 ten Brinke A, Zwinderman AH, Sterk PJ, et al. Factors associated with persistent airflow limitation in severe asthma. *Am J Respir Crit Care Med* 2001; 164:744–748
- 29 Minshall EM, Leung DY, Martin RJ, et al. Eosinophil-associated TGF-beta1: mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997; 17:326–333
- 30 Vignola AM, Chanez P, Chiappara G, et al. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997; 156:591–599
- 31 Wenzel SE, Schwartz LB, Langmack EL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999; 160:1001–1008
- 32 Taha RA, Laberge S, Hamid Q, et al. Increased expression of the chemoattractant cytokines eotaxin, monocyte chemotactic protein-4, and interleukin-16 in induced sputum in asthmatic patients. *Chest* 2001; 120:595–601
- 33 Spanevello A, Migliori GB, Sharara A, et al. Induced sputum to assess airway inflammation: a study of reproducibility. *Clin Exp Allergy* 1997; 27:1138–1144