

Sputum analysis, bronchial hyperresponsiveness, and airway function in asthma: Results of a factor analysis

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Background: Recent studies have shown weak associations among FEV₁, bronchial hyperresponsiveness (BHR), sputum eosinophils, and sputum eosinophil cationic protein (ECP), suggesting that they are nonoverlapping quantities. The statistical method of factor analysis enables reduction of many parameters that characterize the disease to a few independent factors, with each factor grouping associated parameters.

Objective: The purpose of this study was to demonstrate, by using factor analysis, that reversible airway obstruction, BHR, and eosinophilic inflammation of the bronchial tree, as assessed by cytologic and biochemical analysis of sputum, may be considered separate dimensions that characterize chronic bronchial asthma.

Methods: Ninety-nine clinically stable patients with a previous diagnosis of asthma underwent spirometry, sputum induction, and histamine inhalation tests.

Results: Most patients were nonobstructed (FEV₁, 91% ± 20%); a low level of bronchial reversibility (FEV₁ increase after β₂-agonist, 7.8% ± 9.2%) and BHR (histamine PC₂₀FEV₁ geometric mean, 0.98 mg/mL) were found. Sputum eosinophil differential count (12.4% ± 17.7%) and sputum ECP (1305 ± 3072 μg/mL) were in the normal range of our laboratory in 38 and 22 patients, respectively. Factor analysis selected 3 different factors, explaining 74.8% of variability. Measurements of airway function and age loaded on factor I, PC₂₀FEV₁ and β₂-response loaded on factor II, and sputum ECP and eosinophils loaded on factor III. Additional post hoc factor analyses provided similar results when the sample was divided into 2 subgroups by randomization, presence of airway obstruction, degree of BHR, percentage of sputum eosinophils, or concentration of sputum ECP. **Conclusions:** We conclude that airway function, baseline BHR, and airway inflammation may be considered separate dimensions in the description of chronic asthma. Such evidence supports the utility of routine measurement of all these dimensions. (*J Allergy Clin Immunol* 1999;103:232-7.)

Key words: Asthma, factor analysis, bronchial hyperresponsiveness, airway inflammation, induced sputum

Abbreviations used

BHR: Bronchial hyperresponsiveness
ECP: Eosinophil cationic protein
FVC: Forced vital capacity
IVC: Inspiratory vital capacity

Asthma is characterized by episodes of dyspnea with wheezing, variable airflow limitation, and bronchial hyperresponsiveness (BHR).¹ Airway inflammation plays an important part in the pathogenesis of asthma.²⁻⁹ The cellular inflammatory response of the bronchial mucosa is characterized by infiltration of eosinophils,^{4-7,9} and activated eosinophils are able to release many cytotoxic proteins, including eosinophil cationic protein (ECP), which plays a central role in bronchial epithelial damage.¹⁰⁻¹¹

Various outcomes are being used to evaluate the severity of disease and to assess response to therapy in chronic bronchial asthma. Traditionally, measurement of airway function, especially spirometry, has been the primary means of evaluating and assessing patients with asthma. BHR has been used for assessing clinical variability in patients with asthma in addition to standard spirometric measures. Recent studies have indicated that eosinophilic inflammation (both number and activation of eosinophils) is an important outcome measure in the diagnosis and management of asthma.¹²⁻¹⁵ In many studies, however, the level of significance of the interrelations among all these outcome measures has been found to be clinically irrelevant.¹⁶⁻²⁰ In this regard we have recently found that sputum measurements of eosinophilic inflammation do not accurately reflect current clinical indices of asthma severity in patients with chronic, stable asthma¹² and might therefore provide complementary data for monitoring of the disease.

The purpose of this study was to demonstrate that measures of airway obstruction, BHR, and airway inflammation reflect separate nonoverlapping dimensions underlying the pathophysiology of patients with chronic, stable asthma. Such statistical evidence would further support the utility of routine measurement of all these dimensions. In this investigation we used factor analysis to analyze data collected in 99 patients with stable, chronic asthma. Briefly, the aim of factor analysis is to condense as much of the variability in the data to as few factors as possible.

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METHODS

Patients

Ninety-nine consecutive patients (53 males) aged 16 to 75 years (median age, 42 years) with stable, chronic bronchial asthma according to the criteria of the National Heart, Lung and Blood Institute²¹ participated in the study. Asthma was characterized by a history of episodes of dyspnea with wheezing and by BHR to histamine ($PC_{20}FEV_1$, <8 mg/mL). In 81 patients skin prick test responses were positive for the common aeroallergen extracts. Subjects sensitized to pollens were studied out of the relevant season. The duration of disease ranged from 1 month to 40 years. Each patient was in clinically stable condition at the time of the study. At the time of the study, 50 patients received daily inhaled corticosteroids and bronchodilators. All bronchodilators were withheld for at least 12 hours before each study. All subjects had been free from acute respiratory infections within the preceding 4 weeks. Informed consent was given by each subject, and the study was approved by the Local Ethics Committee.

Protocol

Spirometry, sputum induction, and skin prick tests were performed on day 1. Twenty-four hours later, each patient underwent histamine bronchoprovocation test.

Diagnosis of immediate-type hypersensitivity

Allergy tests, including a battery of common aeroallergen extracts, were performed with the skin prick method. Cutaneous positivity to allergen was defined by wheal (>3 mm) and erythema with pseudopods.

Lung function

Baseline pulmonary function testing was performed by measuring static and dynamic lung volumes with a water-sealed spirometer (Pulmonet Godart; Sensormedics Corp, Yorba Linda, Calif) as previously reported.²² The normal values for lung volumes are those proposed by the European Community for Coal and Steel.²³

Induction and analysis of sputum

Induction of sputum was performed according to the method of Pin et al.¹⁴ Briefly, 10 minutes after fenoterol inhalation (200 μ g), hypertonic saline was nebulized with an ultrasonic nebulizer (Fisoneb; Fisons Corp, Rochester, NY) and was inhaled for 5-minute periods up to 20 minutes. The concentration of saline was increased at intervals of 10 minutes from 3% to 4%. FEV_1 was measured every 5 minutes during inhalation of hypertonic saline solution. The sputum induction procedure did not cause troublesome symptoms, and the FEV_1 did not decrease by more than 20% in any subject. Every 5 minutes subjects were asked to try to cough sputum into a Petri dish and to collect saliva in a separate container. Cytologic analysis and ECP measurement were performed according to the method of Ronchi et al.¹² Two or 3 plugs free of salivary contamination were suspended in dithiothreitol solution (0.1%) and incubated for 30 minutes at 37°C for slide making. Cells were centrifuged at 1500 rpm for 10 minutes and then resuspended in saline. Three sputum slides were then prepared for cytologic examination by cytocentrifugation. Cells were air dried and stained with May-Grünwald-Giemsa stain. Cell differentials were determined by counting 200 non-squamous cells on each sputum slide. The volume of the remaining

portion of sputum samples was determined, and an equal volume of dithiothreitol (0.1%) was added. The samples were mixed by vortex and incubated at 37°C for 20 minutes. The samples were then centrifuged at 1000g for 10 minutes. The supernatants were aspirated and frozen at -70°C for later ECP analysis. ECP was assessed by a fluoroimmunoassay (CAP ECP FEIA Kabi Pharmacia; Pharmacia Diagnostics AB, Uppsala, Sweden). Anti-ECP, covalently coupled to immunoCAP, reacted with the ECP in the specimens. After washing, enzyme-labeled antibodies against ECP were added to form a complex. After incubation, unbound enzyme anti-ECP was washed away, and the bound complex was then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate was measured in Fluoro-Count 96 (Kabi Pharmacia). The sensitivity of this technique is less than 0.5 μ g/L. ECP was determined in duplicate.

Bronchial challenge

Each patient was administered a histamine aerosol inhalation test. Increasing concentrations of histamine-acid phosphate in normal PBS (prepared by the University Hospital Pharmacy) were inhaled from a DeVilbiss 646 nebulizer (DeVilbiss Co, Somerset, Pa) driven at an airflow rate of 6 L/min, resulting in mean (SD) output of 0.31 mL/min (0.03), by using the tidal breathing method. With this method, 4 mL of solution was placed in the nebulizer, and inhalation continued during tidal breathing for 2 minutes. Histamine solution was stored at 4°C and nebulized at room temperature. Normal PBS was inhaled first, followed at 5-minute intervals by histamine in 2-fold increasing concentrations from 0.031 to 16 mg/mL. The test was withheld at the concentration of histamine that caused a decrease in FEV_1 of 20% or greater than that caused by saline (provocative concentration). From the log dose-response curve, the concentration of histamine required to produce $PC_{20}FEV_1$ was noted. Details of the technique have previously been described.²⁴

Statistical analysis

The $PC_{20}FEV_1$ values were logarithmically transformed for statistical analysis and presented as geometric means. All the correlations were evaluated by using Pearson's correlation coefficient (r). Differences between groups were evaluated by using the unpaired t test, and differences in gender between groups were examined by using the chi-squared test. Probability (P) values less than .05 were considered statistically significant.

Factor analysis was used to determine the dimensions underlying the pattern of interrelationships.²⁵ Factor analysis is a statistical technique applied to a single set of variables to discover which sets of variables form coherent subsets that are relatively independent of one another. Variables that are correlated with one another, which are also largely independent of other subsets of variables, are combined into factors. Briefly, the purpose of the analysis is to obtain a small number of factors that account for most of the variability. Accordingly, it is important to recognize that factor analysis does not merely regroup variables that are highly correlated with one another. In the final solution, correlations are obtained among all variables entered in the analysis and the virtual factors. Each of the original variables used in the analysis are said to "load" on the factors, to greater or lesser extent, on the basis of the magnitude of the obtained correlations between each variable and the factor. In general, each variable loads (ie, is correlated most highly with) a single factor. Factors are labeled, conveniently, in terms of the pattern of these loadings. Thus the purpose of using factor analysis in this

TABLE I. Clinical characteristics, functional data, and sputum outcomes in 99 patients with chronic, stable asthma*

	Mean $\pm$ SD	Range
Respiratory function		
FEV ₁ , L (% predicted value)	2.97 $\pm$ 1.07 (91.2 $\pm$ 20.0)	1.19-5.81 (43-130)
FVC, L (% predicted value)	3.9 $\pm$ 1.1 (101.4 $\pm$ 14.9)	1.5-6.39 (67-136)
IVC, L (% predicted value)	4.0 $\pm$ 1.1 (100.3 $\pm$ 16.2)	1.98-6.21 (84-127)
BHR†		
Histamine PC ₂₀ FEV ₁ , mg/mL	0.98	0.016-16
Bronchial reversibility		
β_2 -Response (% FEV ₁ increase from baseline)	7.81 $\pm$ 9.23	0-36
Airway inflammation		
Sputum eosinophils (% differential count)	12.4 $\pm$ 17.7	0-71.94
Sputum ECP, mg/L	1305 $\pm$ 3072	7-21,636

*Subjects were 53 males and 46 females, with a median age of 42 years.

†Geometric mean.

study was to determine whether, in subjects with clinically stable asthma, measurements of airway function, BHR, and airway inflammation would reduce to similar or different factors.

To determine the number of factors to extract, we used a combination of methods. Scree plot and eigenvalues were examined, and no fewer factors than indicated by the Scree plot and no more than indicated by the use of the "eigenvalues one" criterion were extracted. Once the factors are extracted (at the end of the process), factors are frequently rotated in multidimensional space to obtain the simplest and most interpretable factors and to preserve the independence among factors. Several rotation options are available. We chose to rotate the initial solution to simplify the factors themselves (Varimax rotation option). We tried to rotate factors by an oblique method (Oblimin), but this rotation option did not show any differences compared with the Varimax rotation. Factor loadings are reported for the rotated factor matrix. Because no procedure is known for computing the standard errors of factor loadings, no test of statistical significance is possible, and therefore loadings are compared with some conventional rules of thumb. One common rule of thumb is to consider loadings of 0.45 or larger to be "high"; this convention has been used in this study. Finally, the possibility to perform the factor analysis was tested by means of Bartlett's test of sphericity.

RESULTS

Table I shows that most patients were not obstructed, according to their stable clinical conditions. The low level of bronchial reversibility observed in many patients was probably due to the normal, or in some cases super-normal, values of baseline FEV₁. All patients but 2 had positive histamine test results. In 2 patients the provocative concentration of histamine was 16 mg/mL. However, their clear history of asthma, with dyspnea, cough, and wheezing; the high level of eosinophils in sputum in 1 of the patients; and a greater than 25% increase in FEV₁ after bronchodilator in the other allowed us not to exclude them. Sputum eosinophils and sputum ECP were in the normal range for our laboratory in 38 patients and 22 patients, respectively.

Correlations

FEV₁, forced vital capacity (FVC), and FEV₁/vital capacity ratio were significantly related to β_2 -response

and PC₂₀FEV₁; indexes of airway inflammation (sputum eosinophils and ECP) were related to each other. In contrast, no relationship was found among sputum outcomes and functional variables or BHR.

Factor analysis

First we performed factor analysis on the 99 patients by including age, FEV₁, FVC, inspiratory vital capacity (IVC), β_2 -response, PC₂₀FEV₁, eosinophils, and ECP as variables. The Bartlett's test of sphericity showed that the variables used were correlated (approximate chi-square 624, *df* 28, *P* < .0001). The factor analysis yielded 3 factors that accounted for 74.8% of the total variance in the data. The Varimax rotation yielded 3 interpretable factors, and the correlations with the original variables obtained for each rotated factor (factor loadings) are displayed in Table II. The results did not change when PC₂₀FEV₁, eosinophils, and ECP were expressed as log. The square of the factor loading provides the proportion of the estimated common variance in a variable that is explained by the factor. The sum of the squared factor loadings, the eigenvalue, indicates how much the factor contributes to explaining the common variance underlying the variables. As shown, functional data loaded on factor I, BHR with bronchial reversibility loaded on factor II, and inflammatory parameters loaded on factor III.

Post hoc analysis

We conducted additional analysis by randomly dividing patients into 2 subgroups and including the same variables previously described in each subgroup. The subgroups were matched in terms of age, gender, lung function, sputum analysis, and BHR as indicated by the unpaired *t* tests and the chi-squared test conducted between subgroups. We obtained similar results for each subgroup, and the analysis was comparable to the analysis of the entire group of patients. In fact, the factor analysis yielded 3 factors with similar factor loadings, accounting for 75.27% of the variance in one subgroup and 75.99% in the other. Then we conducted several analyses by dividing patients into 2 subgroups on the basis of (1) airway obstruction, (2) severity of BHR, (3)

TABLE II. Varimax rotated factor-loading matrix from a factor analysis of functional data, BHR, and sputum outcomes in 99 patients with chronic, stable asthma*

	Factor I	Factor II	Factor III
Age	-0.71	-0.05	0.32
FEV ₁	0.90	0.38	-0.01
FVC	0.96	0.13	-0.01
IVC	0.96	0.09	0.01
PC ₂₀ FEV ₁	0.09	0.82	-0.03
β ₂ -Response	-0.20	-0.78	0.01
Eosinophils	-0.01	0.05	0.81
ECP	-0.11	-0.09	0.73
Eigenvalue	3.62	1.26	1.11
Percent of total variance	45.29	15.71	13.85

*Bold values represent the highest loadings.

entity of sputum eosinophilia, (4) concentration of ECP in the sputum, and (5) inhaled corticosteroid therapy.

In the category of airway obstruction, patients were divided into the following groups: patients with airway obstruction (FEV₁/VC < 70%, n = 38) and those without airway obstruction (FEV₁/VC > 70%, n = 61). The obstructed patients were older ($P < .0001$) and exhibited a significantly lower PC₂₀FEV₁ ($P < .0001$) compared with the patients with normal airway function. The factor analysis yielded 3 factors in both subgroups, accounting for 74.4% and 74.1% of the variance, respectively. Results similar to the entire group were observed.

In the severity of BHR category, patients were divided into the following groups: patients with histamine PC₂₀FEV₁ less than 1 mg/mL (n = 49) and patients with histamine PC₂₀FEV₁ of 1 mg/mL or greater (n = 50). We chose this arbitrary cut-off to obtain 2 groups with different degrees of BHR but with a similar number of patients. The first subgroup exhibited a lower level of airway function (FEV₁, FVC, and IVC, $P < .01$) than the other. The factor analysis again yielded 3 factors in both subgroups, accounting for 75.9% and 70.8% of the variance, respectively. Similar results between the 2 subgroups and the total group of patients were observed. Including or deleting the 2 subjects with PC₂₀FEV₁ values of 16 mg/mL did not modify the results.

In the entity of sputum eosinophilia category, patients were divided into those with normal sputum eosinophils (sputum eosinophil differential count <2.2%, n = 38) and patients with sputum eosinophilia (sputum eosinophil differential count >2.2%, n = 61). The patients with normal eosinophils were younger ($P = .02$) and had a lower level of sputum ECP ($P = .009$) than patients with eosinophilia. In the subgroup with a normal level of eosinophils, factor analysis yielded only 2 factors, accounting for 63% of the variance. Measurements of airway function loaded on Factor I, and sputum outcomes loaded on factor II. BHR and β₂-response loaded to a low extent on factor I. In the subgroup with high eosinophil levels, 3 factors that accounted for 75% of the variance were extracted, with results similar to the entire group (Table III).

TABLE III. Varimax rotated factor-loading matrix from a factor analysis of functional data, BHR, and sputum outcomes in the 2 subgroups of patients divided on the basis of the amount of sputum eosinophils*

	Eosinophils <2.2%		Eosinophils >2.2%		
	Factor I	Factor II	Factor I	Factor II	Factor III
Age	-0.70	-0.01	-0.69	-0.03	0.39
FEV ₁	0.98	0.03	0.92	0.23	-0.04
FVC	0.93	0.23	0.97	0.10	-0.02
IVC	0.89	0.26	0.96	0.08	0.01
PC ₂₀ FEV ₁	0.50	-0.41	0.07	0.86	0.08
β ₂ -Response	-0.53	0.23	-0.20	-0.78	0.18
Eosinophils	0.16	0.77	0.06	0.06	0.81
ECP	0.04	0.64	-0.17	-0.12	0.62
Eigenvalue	3.72	1.31	3.61	1.23	1.17
Percent of total variance	46.5	16.38	45.12	15.40	14.60

*Bold values represent the highest loadings.

In the concentration of ECP in sputum category, those patients with a low level of sputum ECP (<200 mg/L, n = 45) were younger ($P = .04$) and exhibited a lower level of sputum eosinophils ($P = .05$) than patients with a high level of sputum ECP (>200 mg/mL, n = 54). The factor analysis yielded 3 factors, with similar results between the 2 subgroups and the total group of patients. The 3 factors accounted for 76.77% of the variance in the low ECP group and 74.75% in the high ECP group.

Finally, in the inhaled corticosteroid therapy category, patients with inhaled corticosteroid therapy showed a higher response to β₂-agonist ($P = .015$) and a lower FEV₁ ($P = .01$). The factor analysis yielded 3 factors, with similar results in the subgroups and the total group of patients. The 3 factors accounted for 72.34% of the variance in the inhaled corticosteroid group and 77.27% in the other group.

DISCUSSION

An examination of our data by standard correlation analysis shows that inflammatory outcomes (sputum eosinophils and ECP) did not significantly relate to lung function or to baseline BHR. The levels of these relationships were somewhat consistent with the clinical irrelevance reported in other studies.^{12,16,18-20} The level of correlations we found indicates that physiologic and inflammatory outcome measures are not overlapping characteristics of asthma. In this investigation we used factor analysis to examine the independence of respiratory function, BHR, and bronchial inflammation in the assessment of patients with chronic, stable asthma. In factor analysis a set of variables is reduced or rearranged into smaller sets of separate dimensions or factors that account for significant portions of the variance in the interrelations among the variables. The solutions are characterized first by the number of independent factors extracted and second by the pattern of correlations between the original variables and the factors. In our application of the technique, we sought to determine

whether measures of respiratory function, baseline BHR, and bronchial inflammation would reduce to similar or different factors in patients with chronic, stable asthma. The results of our analysis indicate that airway function, baseline BHR, and sputum outcomes are independent factors that characterize or describe the status or condition of patients with chronic, stable asthma.

The measurements of airway function were the first factor extracted in the analysis (Table II). The inclusion of age with spirometry is appropriate, given that age is an independent predictor variable of lung function. According to the statistical method of factor analysis, measurements of airway function represent an important separate dimension in the assessment of patients with chronic, stable asthma.

BHR is a cardinal feature of asthma and has been recognized to relate to severity of disease, frequency of exacerbation, and need for treatment.²⁶ In this study the second factor extracted from the data set was comprised of BHR and bronchial reversibility as assessed in terms of β_2 -response, indicating that BHR and airway function represent nonoverlapping quantities.

Airway inflammation outcomes (sputum eosinophils and ECP) represent the third factor extracted. Eosinophils and their activation products, such as ECP, have been considered crucial elements in the pathogenesis of asthma and therefore directly or indirectly involved in functional alterations of the bronchial tree. Recent studies have proposed the analysis of induced sputum as a reliable and noninvasive method to investigate airway inflammation in patients with asthma.^{14,15,27,28} Similar to the findings from bronchial biopsies and bronchoalveolar lavage fluid examinations, the sputum of patients with asthma is characterized by an increase in proportion of eosinophils and the level of ECP.²⁹ Moreover, sputum examination has been demonstrated to be a more accurate diagnostic test than measurement of blood eosinophils or serum ECP in detecting airway eosinophilic inflammation,²⁹ suggesting a relevant role of sputum analysis in the identification and monitoring of airway inflammation in asthma.^{12,14,30} Our results demonstrate that sputum eosinophils and ECP provide another unique dimension related to the status of patients with chronic bronchial asthma that was independent of both lung function and baseline BHR. These data confirm the results of previous studies in which the relationships between sputum outcomes and baseline airway obstruction were not clinically meaningful.¹²

Recent studies have found a relatively good correlation between sputum eosinophils and BHR when both normal subjects and smokers with bronchitis¹³ or patients with rhinitis³¹ were included in the correlation. Pizzichini et al¹³ argue that the high significance of the correlations between sputum markers and hyperresponsiveness partially reflects the significant differences between asthmatic and healthy subjects or smokers with bronchitis. When only asthmatic subjects were analyzed, the same correlations lost part of their clinical meaningfulness.¹³ Crimi et al¹⁸ have recently arrived at a similar conclusion. The present study seems to indicate that baseline BHR and reversibility provide data comple-

mentary to lung function and sputum outcomes in patients with stable, chronic asthma. In fact, other studies have suggested that BHR is a complex mechanism sustained not only by the recruitment of inflammatory cells but also by airway wall remodeling^{32,33} or autonomic dysfunction.³⁴

In our analysis, 3 factors (airway function, BHR, and eosinophilic inflammation of the bronchi) account for a significant proportion (about 74.8%) of the variance in the interrelationships among a set of variables designed to describe the status of 99 patients with chronic, stable asthma. Exploratory analyses of patient subgroups (by randomization, severity of airway obstruction, BHR, entity of sputum ECP, and therapy with inhaled corticosteroids) confirmed the results of the primary analysis. We need to point out that the subset sample sizes were relatively small considering that 8 variables were being analyzed, and this qualifies factor analysis on subsets. In the post hoc analysis we also divided patients according to sputum eosinophilia. On the basis of a recent study by Pizzichini et al²⁹ and a previous study of ours in which we observed that all normal subjects exhibited an eosinophil level of less than 2.2%,¹² we chose this cut-off limit for differentiating patients with normal values from those with pathologic values of eosinophils in the sputum. The factor analysis results seem to indicate that, in asthmatic patients with normal values of sputum eosinophils, baseline BHR does not represent an independent variable, thus losing its property as a valid descriptor of the disease. Further investigations will be needed to better explain this observation.

In conclusion, these results support the hypothesis that lung function, baseline BHR, and eosinophilic inflammation of the bronchi are nonoverlapping quantities in patients with chronic, stable asthma. Accordingly, clinical evaluation of asthmatic patients should include measurements of all these parameters.

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