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Airway pathology in COPD : smoking cessation and pharmacological treatment intervention. Results from the GLUCOLD study

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Chapter 2

Dissociation of lung function and airway inflammation in chronic obstructive pulmonary disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is defined by progressive, irreversible airflow limitation and an inflammatory response of the lungs, usually to cigarette smoke. However, COPD is a heterogeneous disease in terms of clinical, physiological, and pathological presentation. We aimed to evaluate whether airflow limitation, airway responsiveness, and airway inflammation are separate entities underlying the pathophysiology of COPD by using factor analysis. A total of 114 patients (99 males/15 females, age 62 ± 8 years, 42 pack-years smoking, no inhaled or oral steroids >6 months) with irreversible airflow limitation (postbronchodilator FEV_1 $63 \pm 9\%$ predicted, FEV_1 /inspiratory vital capacity [IVC] $48 \pm 9\%$) and symptoms of chronic bronchitis or dyspnea were studied in a cross-sectional design. Postbronchodilator FEV_1 and FEV_1 /IVC, reversibility to inhaled β_2 -agonists, diffusing capacity, provocative concentration of methacholine required to produce a 20% drop in FEV_1 , total serum IgE, exhaled nitric oxide, and induced sputum cell counts (% eosinophils, % neutrophils) were collected. Factor analysis yielded 4 separate factors that accounted for 63.6% of the total variance. Factor 1 was comprised of FEV_1 , FEV_1 /IVC, and residual volume/total lung capacity. Factor 2 included reversibility, IgE, provocative concentration of methacholine required to produce a 20% drop in FEV_1 , and diffusing capacity. Factor 3 contained exhaled nitric oxide and factor 4 included sputum % neutrophils and % eosinophils. We conclude that airflow limitation, airway inflammation, and features commonly associated with asthma are separate and largely independent factors in the pathophysiology of COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a disease characterized by progressive airflow limitation, which is not fully reversible (1). However, COPD has been recognized as a heterogeneous disorder (2), with components of chronic bronchitis, small airways disease, emphysema, and in some patients perhaps, features of asthma (3). This is accompanied by pathophysiological characteristics, such as partial reversibility to bronchodilators, air trapping, impaired diffusing capacity, and airway hyperresponsiveness (4). The presence and contribution of these features to the severity of COPD varies between patients and may reflect distinct pathophysiological mechanisms in development, clinical presentation, and course of the disease. It is increasingly recognized that such disease heterogeneity provides opportunities for targeted interventions (3;5).

Airway inflammation is thought to play an important role in the pathogenesis of COPD (6). The cellular inflammatory response is characterized by an increase in neutrophils, macrophages, and CD8-positive T-lymphocytes in small and large airways as well as in lung parenchyma (7). The major cell type in induced sputum is the neutrophil, the quantity of which is associated with the severity of airflow limitation (8;9). Although induced sputum does not cover all the inflammatory and structural changes of the lungs in patients with COPD, it does represent a non-invasive marker of inflammation that is potentially useful for disease monitoring. Sputum eosinophilia has also been observed in patients with stable COPD, but its relationship to airflow limitation is controversial (8;10;11). It has been argued that sputum eosinophilia is related to concomitant features of asthma (12). This link would indicate that the pathophysiological entities underlying the clinical phenotypes in COPD may be diverse and are still largely unknown.

The aim of this study was to objectively specify the heterogeneity of COPD by categorizing various functional and inflammatory features of COPD into separate, complementary domains without *a priori* assumptions. Factor analysis allows reducing multiple disease characteristics to a few independent factors, in which each factor groups associated parameters. Because this is essentially accomplished free of predetermined hypothesis on any interrelated parameters, this technique can be considered as a hypothesis-generating analysis.

Factor analysis has been applied previously in studies of patients with asthma (13-16) and COPD (17). In patients with asthma, it has demonstrated that lung function, baseline airway hyperresponsiveness, and eosinophilic inflammation in sputum are nonoverlapping dimensions (13), suggesting that evaluation of patients with asthma should include measurement of all these parameters. In COPD, factor analysis has been applied to study the relationship between dyspnea ratings, exer-

cise capacity, lung function and hyperinflation (17-23). However, these studies did not include inflammatory markers and were unable to study the interrelationships between airway inflammation and the functional features of COPD. Therefore, in the present study, we performed factor analysis, including lung function indices and markers of inflammation in induced sputum and exhaled air, in 114 patients with clinically stable COPD. Some of the results of this study have been previously reported in the form of an abstract (24).

Material and Methods

Patients

Hundred fourteen patients (15 women) aged between 45 and 75 years with COPD, participating in a multi-center trial (Groningen Leiden Universities and Corticosteroids in Obstructive Lung Disease; GLUCOLD study), were included in this study. Patients were recruited from our outpatient clinics, as well as by advertisements in local newspapers, and by screening lung functions from patients in general practice in and around Leiden and Groningen, The Netherlands. All patients were current or ex-smokers and had a history of at least 10 pack years of smoking. They had irreversible airflow limitation (postbronchodilator FEV_1 and $FEV_1/IVC < 90\%$ *confidence interval* (CI) of the predicted value (25), $FEV_1 \geq 1.3$ liter and $> 20\%$ of the predicted value) and at least one of the following symptoms: chronic cough, chronic sputum production, or dyspnea on exertion. In choosing the 90% CI of predicted values as selection criterion, as opposed to percentage of predicted values per se, we followed the recommendation by the European Respiratory Society (25). Patients with a prior or concomitant history of asthma, alpha-1 antitrypsin deficiency (SZ, ZZ, zero phenotype), or other active lung disease except for mild bronchiectasis were not permitted to the study. The patients did not use a course of oral or inhaled steroids during the last three months, and did not have maintenance treatment with inhaled or oral steroids during the last six months. Maintenance drug therapy of non-selective beta-antagonists, long-acting bronchodilators, methylxanthines, N-acetylcysteine or NSAID's was not permitted. Patients were allowed to use short acting β_2 -agonists and ipratropium bromide as rescue medication. The patients were in clinically stable condition and had no symptoms of respiratory tract infection for at least two weeks prior to the study. The Medical Ethics Committees of each center approved the study and all patients gave their written informed consent.

Study Design

The present study had a cross-sectional design, consisting of baseline measure-

ments of the GLUCOLD Study. At visit 1 spirometry was performed before and after inhalation of salbutamol and blood was collected for measurement of total IgE. Body plethysmography, CO-diffusing capacity, and bronchial responsiveness to methacholine were obtained at a second visit. Finally, at the third visit exhaled NO was measured and hypertonic saline-induced sputum was collected. Inhaled bronchodilators were withheld for at least 8 hrs prior to visit day 1 and 2. All visits were performed within six weeks.

Lung Function

Spirometry was performed according to international guidelines (26). A daily-calibrated pneumotachograph (Masterscreen Pneumo or Masterscreen IOS; Jaeger, Würzburg, Germany) was used throughout the study. First, 3 slow inspiratory vital capacity maneuvers (IVC, largest value used) were carried out. Second, maximally 8 forced expiratory vital capacity (FVC) maneuvers were performed to obtain at least 3 technically satisfactory expiratory flow-volume curves from which the forced expiratory volume in 1 second (FEV_1) and FVC did not deviate $> 5\%$ or 100 ml from the largest FEV_1 and FVC. From these curves, we used values of the curve with the largest sum of FEV_1 and FVC (26).

Reversibility of airflow limitation (ΔFEV_1) was measured 15 min after administration of 400 μg salbutamol per metered dose-inhaler connected to a spacer (27). The response was expressed as change in FEV_1 as percentage of predicted value (27).

Total lung capacity (TLC) and residual volume (RV) were measured using a constant volume body plethysmograph (Masterscreen body or Masterlab body; Jaeger, Würzburg, Germany), using panting at 0.5 Hz (25).

The diffusing capacity (transfer factor) for carbon monoxide (TL_{CO}) and TL_{CO} per liter alveolar volume (K_{CO}) were measured using the single breath holding method with a rolling seal closed system (Masterscreen MS-CS-FRC, Masterlab transfer or Compactlab transfer; Jaeger, Würzburg, Germany) (28). Reference values for all lung function measurements were obtained from Quanjer *et al.* (25).

Methacholine challenge tests were performed according to the tidal breathing method (29), using serial doubling concentrations methacholine-bromide (0.038 to 39.2 mg/ml) in saline. Methacholine was aerosolized (DeVilbiss 646, Somerset, PA) and inhaled by the 2-min tidal breathing method at 5-minute intervals until FEV_1 dropped by $\geq 20\%$ (lowest of two FEV_1 values; at 30 and 90 seconds) from baseline. The response was expressed as the provocative concentration causing 20% fall in FEV_1 (PC_{20}).

Sputum induction and processing

Sputum was induced and processed according to a validated technique (30), with

some modifications. Prior to sputum induction, 200 µg salbutamol was administered and baseline FEV₁ was recorded. Hypertonic sodium chloride aerosols (4.5 w/v %) were generated by a DeVilbiss Ultraneb 2000 ultrasonic nebulizer with a calibrated particle size (MMAD 4.5 µm) at maximal output (2.5 ml/min). The aerosols were inhaled via the mouth through a two-way valve (No. 2700; Hans-Rudolph, Kansas City, MO, USA), with the nose clipped. Subsequently, the patients inhaled hypertonic saline aerosols during 3 periods of 5 min and sputum was expectorated after each inhalation. Salbutamol was administered when FEV₁ dropped by > 10% from post-salbutamol baseline value and the procedure was interrupted when FEV₁ dropped by > 20%.

Whole sputum samples were processed. The sample was then mixed with an equal volume of dithiothreitol 0.1% w/v (Sputolysin, Calbiochem, USA) and placed in a shaking water bath at 37°C for 15 min. Then sputum was filtered through a nylon gauze (pore-size approximately 48 µm) and centrifuged (450 x g) for 10 minutes at 20°C. The cell pellet was then resuspended in phosphate-buffered saline (PBS) containing 1 % (w/v) human serum albumin (HSA), pH 7.4. Cell viability and total cell counts were performed by Trypan blue exclusion using a hemacytometer. Subsequently, cytocentrifuge slides were prepared (450 rpm, 6 minutes, 100 µl/slide, Cytospin 3, Shandon, Life Sciences International, Veldhoven, NL) and differential cell counts were performed on May-Grünwald-Giemsa-stained cytospins by a qualified technician. Differential leucocyte and cylindrical epithelial cell counts were expressed as a percentage of nucleated cells excluding squamous cells. A sputum sample was considered adequate when the percentage squamous cells was less than 80% (30).

Exhaled nitric oxide

Exhaled NO (eNO) levels were determined according to a standardized procedure (31) with some modifications, using a chemiluminescence analyzer (Sievers NOA 270B or ECO physics CLD 700 AL). Patients were asked not to smoke for at least 1 hour prior to the test. A slow vital expiratory capacity maneuver with a constant expiratory flow of 100 mL/sec against an expiratory resistance of 5 cm H₂O was performed. Expiratory NO concentration was sampled continuously from the center of the mouthpiece and the average concentration was determined during a period of 10 seconds. Three reproducible successive recordings were made at 30-s intervals, from which the mean values of exhaled NO were used in the analysis.

Peripheral blood measurements

Total serum IgE concentrations were measured by fluoroimmunoassay (FEIA) using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden).

Statistical analysis

Mean values and SD were computed. When appropriate variables were logarithmically transformed before statistical analysis and presented as median with interquartile range.

Exploratory factor analysis included the following variables: postbronchodilator FEV₁ (%pred), postbronchodilator FEV₁/IVC (%), ΔFEV₁ (%pred), K_{CO} (%pred), PC₂₀ (mg/ml), RV/TLC (%), eNO (ppb), sputum % neutrophils and % eosinophils and total serum IgE (IU/ml). The possibility to perform factor analysis was tested by Bartlett's test of sphericity. The Kaiser-Meyer-Olkin (KMO), a measure of sampling adequacy based on correlation and partial correlation, was also evaluated. A high KMO (maximum 1.0) indicates that data are likely to factor well since correlations between pairs of variables can be explained by the other variables (low partial correlation coefficients). Correlation coefficients were analyzed by principal component factor analysis and subsequent rotation according to the standard Varimax criterion (32). In this type of analysis, the correlation between parameters is attributed to their common dependence on independent entities called "factors". The parameters are separated into independent subgroups, and the correlation of parameters within subgroups is due to their common factor. The coefficients that link parameters to factors are called "factor loadings", and are the correlation coefficient between parameters and factors. The number of factors is chosen to be as small as possible but large enough to account for most of the variation within the data. The number of factors was determined by the number of eigenvalues, an index of the proportion of variance explained by successive factors, whose magnitude was ≥ 1 on the Scree plot. However, also the percentage of total variance explained by the factors was taken into account. The Varimax rotation procedure aims to increase the interpretability of the factors by rotation to a simple structure with optimal loadings, such that they are high or low. Ideally, each variable would have a high loading on one factor, whereas its loadings on other factors would be low. In the Varimax rotation this is technically achieved by maximalizing the variation within a factor. Finally, we conducted three additional factor analyses to determine the stability of the factor structures, and thus the robustness of our findings. First, we excluded outliers from the data set, defined as data outside the range of mean ± 3 × SD, and repeated the factor analysis. Next, the analysis was repeated with number of neutrophils and eosinophils per ml sputum, instead of % neutrophils and eosinophils. Since smoking is the main risk factor for development of COPD, an additional factor analysis was performed in which the number of pack years was added. Univariate correlations were evaluated using Pearson correlation coefficient. All analyses were performed with the Statistical Package of Social Sciences (SPSS 11.0).

Results

Patient characteristics

Patient characteristics of the 114 participants are presented in Table 1. The mean (SD) postbronchodilator FEV₁ was 63.0 (8.8) %pred, with a range from 40.8 to 77.7 %pred. This result indicates that all patients were classified as having moderate to severe COPD according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (GOLD stage II and III) (1). The patients were heavy smokers with a median of 41.8 pack-years and most of them were current smokers (63.2%).

Table 1. Patient characteristics.

| | n | Mean (SD or IQR) |
|--|-----|------------------|
| Clinical characteristics | | |
| Sex, male/female | 114 | 99 / 15 |
| Age, yr | 114 | 62 (8) |
| Smoking history, pack-years * | 114 | 41.8 (31.2-54.8) |
| Current smoker, yes/no, n | 114 | 72 / 42 |
| IgE, IU/ml* | 113 | 40 (11.5-125) |
| Lung Function | | |
| Post FEV ₁ , %pred | 114 | 63.0 (8.8) |
| Post FEV ₁ /IVC, % | 114 | 48.2 (8.5) |
| ΔFEV ₁ , %pred | 113 | 6.9 (4.9) |
| K _{CO} , %pred | 112 | 75.9 (25.5) |
| PC ₂₀ , mg/ml† | 110 | 0.60 (2.76) |
| RV/TLC, % | 113 | 48.5 (8.8) |
| Airway inflammation | | |
| eNO, ppm | 92 | 13.1 (12.7) |
| Sputum eosinophils, %* | 106 | 1.1 (0.3-2.2) |
| Sputum neutrophils, % | 106 | 69.4 (16.0) |
| Sputum eosinophils, n (x 10 ⁴ /ml)* | 106 | 1.4 (0.4-4.5) |
| Sputum neutrophils, n (x 10 ⁴ /ml)* | 106 | 102 (47-229) |

Definition and abbreviations: ΔFEV₁ = change in FEV₁ as percentage of predicted (reversibility to salbutamol); eNO = exhaled nitric oxide; IQR = interquartile range (25th and 75th percentile); IVC = inspiratory vital capacity; K_{CO} = diffusing capacity for carbon monoxide per liter alveolar volume; %pred = percentage of predicted; PC₂₀ = provocative concentration of methacholine causing a 20% fall in FEV₁; RV = residual volume, TLC = total lung capacity. * Median (IQR). † Geometric mean ± doubling dose.

Table 2. Varimax Rotated Factor-loading Matrix from Factor Analysis.

| | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|----------------------------------|--------------|--------------|-------------|--------------|
| Postbr. FEV ₁ , %pred | 0.89 | -0.16 | -0.09 | -0.07 |
| Postbr. FEV ₁ /IVC, % | 0.82 | 0.04 | -0.23 | 0.22 |
| RV/TLC, % | -0.59 | -0.36 | -0.03 | 0.15 |
| ΔFEV ₁ , %pred | -0.06 | -0.72 | 0.08 | 0.11 |
| IgE, IU/ml | -0.09 | 0.61 | 0.10 | -0.07 |
| PC ₂₀ , mg/ml | 0.36 | 0.51 | -0.35 | 0.14 |
| K _{CO} , %pred | 0.44 | 0.49 | 0.25 | 0.31 |
| eNO, ppb | -0.09 | 0.10 | 0.89 | -0.11 |
| Sputum eosinophils, % | -0.09 | 0.02 | 0.19 | -0.84 |
| Sputum neutrophils, % | -0.18 | -0.25 | 0.47 | 0.54 |
| Eigenvalue | 2.67 | 1.50 | 1.20 | 0.99 |
| Total variance explained, % | 26.7 | 15.0 | 12.0 | 9.9 |

Definition and abbreviations: ΔFEV₁ = change in FEV₁ as percentage of predicted (reversibility to salbutamol); eNO = exhaled nitric oxide; IVC = inspiratory vital capacity; K_{CO} = diffusing capacity for carbon monoxide per liter alveolar volume; %pred = percentage of predicted; PC₂₀ = provocative concentration of methacholine causing a 20% fall in FEV₁; RV = residual volume, TLC = total lung capacity. Bold values represent the highest loadings of a variable.

Factor analysis

Bartlett's test of sphericity indicated a correlation between the presently used variables because the correlation matrix was statistically different from an identity matrix (approximate $\chi^2 = 165.864$, degrees of freedom = 45, $p < 0.001$). The Kaiser-Meyer-Olkin value was 0.594. Factor analysis yielded 3 separate factors, explaining only 53.7% of the total variance in the data set when the eigenvalue 1 criterion was applied. Therefore, an additional factor analysis was performed with the same data in which 4 factors were selected. This resulted in an increase of total explained variance to 63.6%.

The correlations with the original variables obtained for each Varimax-rotated factor (called factor loadings) and the eigenvalues are displayed in Table 2. FEV₁, FEV₁/IVC, and RV/TLC loaded significantly on factor 1. Factor 2 included ΔFEV₁, total IgE, PC₂₀, and K_{CO}. eNO loaded on factor 3, whereas sputum % neutrophils and eosinophils loaded on factor 4. Interestingly, K_{CO} contributed also to factor 1, and % neutrophils contributed also to factor 3.

Additional Factor Analyses

Factor analysis without outliers in the data set resulted in a similar four-factor struc-

ture as the original one, accounting for 63.4% of the total variance, with the exception that % neutrophils loaded on factor 3 with eNO and not on factor 4.

Factor analysis with number of neutrophils and eosinophils per ml sputum, instead of % neutrophils and eosinophils, did not change the contents of the factors essentially. Again four factors, accounting for 66.0% of the total variance, were found using the eigenvalue 1 criterion. Factor 1 was the same as in the original analysis. Factor 2 included numbers of neutrophils and eosinophils, both with a positive factor loading. Factor 3 contained the variables that originally loaded on factor 2: ΔFEV_1 , total IgE, and PC_{20} . Finally, factor 4 included eNO and K_{CO} .

Factor analysis with inclusion of number of pack-years as an additional variable revealed four factors explaining 59.1% of total variance, according to the eigenvalue 1 criterion. All factors were similar to the original analysis described previously, with the exception that K_{CO} and PC_{20} switched from factor 2 to factor 1. Number of pack-years smoked loaded on factor 2 together with ΔFEV_1 and total IgE (Table 3).

Table 3. Varimax Rotated Factor-loading Matrix from Factor Analysis with pack years.

| | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|-----------------------------|--------------|--------------|-------------|--------------|
| Postbr. FEV_1/IVC , % | 0.77 | -0.0002 | -0.33 | 0.20 |
| Postbr. FEV_1 , %pred | 0.72 | -0.19 | -0.24 | -0.03 |
| RV/TLC, % | -0.68 | -0.05 | -0.20 | 0.25 |
| K_{CO} , %pred | 0.59 | 0.25 | 0.37 | 0.24 |
| PC_{20} , mg/ml | 0.59 | 0.28 | -0.13 | -0.03 |
| Pack-years | -0.22 | 0.70 | -0.32 | 0.06 |
| ΔFEV_1 , %pred | -0.29 | -0.62 | -0.11 | 0.20 |
| IgE, IU/ml | 0.07 | 0.61 | 0.18 | -0.05 |
| eNO, ppb | -0.18 | 0.05 | 0.84 | 0.02 |
| Sputum eosinophils, % | -0.23 | 0.09 | 0.16 | -0.74 |
| Sputum neutrophils, % | -0.28 | -0.08 | 0.25 | 0.68 |
| Eigenvalue | 2.67 | 1.56 | 1.22 | 1.06 |
| Total variance explained, % | 24.2 | 14.2 | 11.1 | 9.6 |

Definition and abbreviations: ΔFEV_1 = change in FEV_1 as percentage of predicted (reversibility to salbutamol); eNO = exhaled nitric oxide; IVC = inspiratory vital capacity; K_{CO} = diffusing capacity for carbon monoxide per liter alveolar volume; %pred = percentage of predicted; PC_{20} = provocative concentration of methacholine causing a 20% fall in FEV_1 ; RV = residual volume, TLC = total lung capacity. Bold values represent the highest loadings of a variable.

Univariate correlations

The univariate relationships among physiological and inflammatory parameters (sputum neutrophils, eosinophils, and eNO) were as follows. ΔFEV_1 was not associated with inflammatory parameters; however, RV/TLC was associated with sputum % neutrophils ($r=0.203$, $p=0.04$), and postbronchodilator FEV_1 , RV/TLC, and PC_{20} were associated with number of neutrophils/ml sputum ($r=-0.246$, $p=0.01$; $r=0.213$, $p=0.03$ and $r=-0.338$, $p<0.001$, respectively). Postbronchodilator FEV_1 , PC_{20} , and FEV_1/IVC were associated with eNO levels ($r=-0.203$, $p=0.05$; $r=-0.207$, $p=0.05$ and $r=-0.304$, $p=0.003$, respectively). FEV_1/IVC was also related to sputum % eosinophils ($r=-0.219$, $p=0.02$), while postbronchodilator FEV_1 , K_{CO} , PC_{20} , and FEV_1/IVC were also associated with number of eosinophils/ml sputum ($r=-0.207$, $p=0.03$; $r=-0.204$, $p=0.04$; $r=-0.243$, $p=0.01$ and $r=-0.242$, $p=0.01$, respectively).

Discussion

The aim of this study was to objectively specify the heterogeneity of COPD, by categorizing the various functional and inflammatory features of COPD into separate, complementary domains without *a priori* assumptions. Therefore, we performed a factor analysis using physiological and inflammatory data of 114 patients with moderate to severe COPD, not treated with inhaled steroids. This resulted in a four-factor structure, explaining 63.6% of the total variance. Factor 1 included: FEV_1 , FEV_1/IVC and hyperinflation; factor 2: β_2 -response, total serum IgE, airway hyper-responsiveness and K_{CO} ; factor 3 included: eNO, and factor 4 included: sputum % neutrophils and eosinophils. These four factors indicate that airflow limitation, features commonly associated with asthma, and airway inflammation are separate, largely independent dimensions that characterize patients with COPD.

To our knowledge, this is the first study in patients with COPD combining functional parameters and markers of airway inflammation in a factor analysis. Previous studies have applied factor analysis on quality-of-life, symptoms scores, exercise capacity, and lung function parameters in patients with stable COPD, without evaluating inflammatory indices (17-23). However, the latter have been part of factor analysis in recent asthma research (13;15;16). In patients with mild to moderate asthma, Rosi and colleagues demonstrated by factor analysis that airway function, bronchial responsiveness with reversibility, and eosinophilic inflammation, as assessed in sputum, are independent dimensions (13). Our current results demonstrate that airway function, bronchial responsiveness with reversibility, and inflammation as assessed in sputum or exhaled are predominantly nonoverlapping dimensions in patients with COPD as well.

Interestingly, this study showed that measurements of airflow limitation, traditionally used to determine disease severity in COPD (33), and hyperinflation, a measure of air trapping, were combined in the first factor. According to the statistical method of factor analysis, these measurements represent an important, separate dimension in the assessment of patients with stable COPD. This result is consistent with some (17;23), but not all (21;22) previous factor analyses of lung function parameters in COPD. The second factor extracted from the data included reversibility of FEV₁, airway hyperresponsiveness, total serum IgE, and diffusing capacity. Similarly, Ries and colleagues reported that bronchodilator response and diffusing capacity of COPD patients were grouped into separate factors from expiratory flow rates and hyperinflation (17). To our knowledge, there are no other studies in patients with COPD that have included these variables in a factor analysis. Finally, the third and fourth factor included eNO and sputum % neutrophils and eosinophils, respectively. This is a novel finding, illustrating the partial independency of these markers of inflammation from the traditionally used functional disease markers in COPD.

We included a large (n=114) group of patients with stable COPD, not using inhaled steroids for at least 6 months and without a clinical diagnosis of asthma. In terms of disease severity, patient characteristics included COPD patients of GOLD Stage II and III. Inclusion of very mild or very severe patients could have produced different results, and therefore our results potentially lack generalizability. In contrast to some other studies, we did not exclude patients with COPD who were partially reversible to a bronchodilator, because the selection of non-reversible patients only would have excluded a large group of patients with COPD (34-36). Furthermore, it needs to be emphasized that this is a cross-sectional analysis of patients with stable COPD, and that the results do not account for exacerbations and other temporal events. Sputum cell counts and eNO were measured as noninvasive markers of inflammation. Obviously, this does not cover all the inflammatory and structural changes of the airways in COPD, but it does represent the markers that are potentially useful for disease monitoring.

Factor analysis is not an approach that is widely applied, presumably because of its complexity. A simple example that clarifies its value and interpretation has recently been described by Juniper and colleagues (14). The purpose of this procedure is to reduce a large set of variables and to clarify (absence of) relationships between various parameters without reference to a specific criterion. Factor analysis does not regroup variables that are highly correlated, but factors are created based on calculated estimates of shared variance among variables, with the restriction that the factors reflect independent sources of variation. This procedure allows the user to determine whether associations between parameters are

attributable to noise of measurements. In clinical research, factor analysis allows the many parameters that characterize the disease to be reduced to a few, relatively independent factors. We applied standard procedures of exploratory factor analysis with respect to the number of variables used in the analysis, the selection of number of factors, and the factor rotation (32). Additional factor analyses with replacement of % neutrophils and % eosinophils by cell counts per ml sputum, exclusion of outliers from the data set, and addition of the cumulative amount of smoking resulted in similar factor structures as the original analysis. This demonstrates the robustness of the current findings.

The disease heterogeneity in COPD in terms of lung function and inflammation suggests that distinct pathophysiological pathways contribute to COPD. In agreement with this concept, we observed that multiple functional and inflammatory characteristics were categorized into four independent dimensions. Interestingly, none of the parameters of factors 1 or 2 showed significant additional loadings on factors 3 or 4, and vice versa, which strengthens the independence of functional and inflammatory dimensions. One exception to this was K_{CO} , which loaded together with eNO in the additional factor analysis using number of sputum cells instead of cell percentages. The value of using factor analysis in mapping disease heterogeneity is illustrated by our finding that some of the parameters were found to provide complementary information (loading on different factors) despite the existence of mutual correlation in univariate analyses.

The first factor can be interpreted as irreversible airflow limitation. The fact that diffusing capacity also had modest loading on this factor confirms earlier findings that the destruction of lung tissue is associated with increased airflow limitation and hyperinflation (37). Alternatively, diffusing capacity could also be a descriptor of the status of altered pulmonary circulation in COPD: another structural component of COPD. Hence, restructuring of airways as well as lung tissue seems to be an important mechanism resulting in airflow limitation. Interestingly, our data suggest that this process is greatly independent of neutrophilic and eosinophilic inflammation in the larger airways (grouped into factor 4). Neutrophils are able to induce tissue damage through the release of serine proteases and oxidants. However, this is not a prominent feature of other pulmonary diseases where chronic airway neutrophilia is even more prominent, such as cystic fibrosis and bronchiectasis (38). This suggests that other factors are involved in the generation of emphysema. In addition, increased neutrophil numbers are found in the airway lumen, but they are not a prominent feature of the airway wall or parenchyma in patients with COPD (7). Furthermore, the presence and role of eosinophils in COPD are uncertain (38). The observed increased levels of eosinophil cationic protein and eosinophil peroxidase in induced sputum from COPD

patients suggest the eosinophils are degranulated (39), which may be the result of the high neutrophil elastase levels in COPD (40). Our data supports this close relationship between neutrophils and eosinophils in COPD, but apparently, airflow limitation requires more than the presence of these granulocytes *per se*.

Parameters that are known to be associated with asthma predominantly grouped into the second factor. This may not be unexpected, since airway hyperresponsiveness, partial reversibility of airflow limitation, and increased serum IgE levels are not uncommon in COPD (41). An alternative interpretation would be that this second factor represents risk factors for progression of COPD, because bronchodilator response, airway hyperresponsiveness, and serum IgE levels have been associated with lung function decline (42). The finding that number of pack-years also loaded on factor 2 strengthens this concept, because smoking is known to be the main risk factor for progression of COPD (43). Although factor 2 also included K_{CO} , its parallel loading on factor 1 suggests that gas exchange impairment is associated with diverse pathophysiology.

The current separation of airway hyperresponsiveness and FEV_1 into different factors supports epidemiological evidence that these disease characteristics provide complementary information on COPD (44), the PC_{20} in COPD not simply being a result of airflow limitation *per se*. However, the fact that PC_{20} also had moderate loading on the first factor and even highest loading the first factor in some of the additional factor analyses on, is in agreement with previous studies that suggest that PC_{20} is to some extent dependent on airway caliber in COPD (45). Although it has been reported previously that partial reversibility of airflow limitation is associated with sputum eosinophilia and elevated eNO in COPD (34), our factor analysis suggests that these features are largely independent. This confirms a previous factor analysis in asthma (13) and again may challenge the concept that eosinophilic airways inflammation is closely related to the “twitchiness” of the airways. Interestingly, we found that number of pack-years also loaded on the second factor with IgE and β_2 -response, and not with airflow limitation or sputum neutrophils. A significant relationship between total IgE and the degree of tobacco smoking has been reported previously (46), suggesting that the increase in IgE may be partly due to tobacco smoking. Although, in this study, univariate analysis revealed significant correlations between eNO and FEV_1 , FEV_1/IVC , and PC_{20} , eNO was extracted into a factor independent from functional parameters as well as sputum cell counts. This indicates that this exhaled marker might be a rather autonomic phenomenon and bodes ill for the use of eNO in monitoring of disease activity.

In conclusion, this analysis has categorized multiple disease features of COPD without *a priori* assumptions on their interrelationships. Our data suggest that

airflow limitation, asthma-like components, eNO and sputum inflammatory cell counts offer separate and additive information about the pathophysiological condition of COPD patients. This confirms the complex heterogeneity of the disease and may change some of the current concepts on the distinct pathophysiological pathways involved. Accordingly, it needs to be examined whether the clinical evaluation of patients with COPD should include each of these complementary entities.

References

1. Fabbri LM, Hurd SS. Global Strategy for the Diagnosis, Management and Prevention of COPD: 2003 update. *Eur Respir J* 2003;22:1-2; www.goldcopd.com.
2. Wedzicha JA. The heterogeneity of chronic obstructive pulmonary disease. *Thorax* 2000;55:631-632.
3. Mannino DM. COPD: epidemiology, prevalence, morbidity and mortality, and disease heterogeneity. *Chest* 2002;121:121S-126S.
4. Siafakas NM, Vermeire P, Pride NB, Paoletti P, Gibson J, Howard P, Yernault JC, Decramer M, Higenbottam T, Postma DS. Optimal assessment and management of chronic obstructive pulmonary disease (COPD). The European Respiratory Society Task Force. *Eur Respir J* 1995;8:1398-1420.
5. Croxton TL, Weinmann GG, Senior RM, Hoidal JR. Future research directions in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002;165:838-844.
6. Barnes PJ. Chronic obstructive pulmonary disease. *N Engl J Med* 2000;343:269-280.
7. Saetta M, Turato G, Maestrelli P, Mapp CE, Fabbri LM. Cellular and structural bases of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;163:1304-1309.
8. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996;153:530-534.
9. Peleman RA, Ryttila PH, Kips JC, Joos GF, Pauwels RA. The cellular composition of induced sputum in chronic obstructive pulmonary disease. *Eur Respir J* 1999;13:839-843.
10. Balzano G, Stefanelli F, Iorio C, De Felice A, Melillo EM, Martucci M, Melillo G. Eosinophilic inflammation in stable chronic obstructive pulmonary disease. Relationship with neutrophils and airway function. *Am J Respir Crit Care Med* 1999;160:1486-1492.
11. Rutgers SR, Postma DS, ten Hacken NH, Kauffman HF, van der Mark TW, Koeter GH, Timens W. Ongoing airway inflammation in patients with COPD who do not currently smoke. *Thorax* 2000;55:12-18.
12. Maestrelli P, Richeldi L, Moretti M, Fabbri LM. Analysis of sputum in COPD. *Thorax* 2001;56:420-422.
13. Rosi E, Ronchi MC, Grazzini M, Duranti R, Scano G. Sputum analysis, bronchial hyperresponsiveness, and airway function in asthma: results of a factor analysis. *J Allergy Clin Immunol* 1999;103:232-237.

14. Juniper EF, Wisniewski ME, Cox FM, Emmett AH, Nielsen KE, O'Byrne PM. Relationship between quality of life and clinical status in asthma: a factor analysis. *Eur Respir J* 2004;23:287-291.
15. Ottanelli R, Rosi E, Romagnoli I, Grazzini M, Stendardi L, Duranti R, Scano G. Do inhaled corticosteroids affect perception of dyspnea during bronchoconstriction in asthma? *Chest* 2001;120:770-777.
16. Gronke L, Kannies F, Holz O, Jorres RA, Magnussen H. The relationship between airway hyper-responsiveness, markers of inflammation and lung function depends on the duration of the asthmatic disease. *Clin Exp Allergy* 2002;32:57-63.
17. Ries AL, Kaplan RM, Blumberg E. Use of factor analysis to consolidate multiple outcome measures in chronic obstructive pulmonary disease. *J Clin Epidemiol* 1991;44:497-503.
18. Hajiro T, Nishimura K, Tsukino M, Ikeda A, Koyama H, Izumi T. Analysis of clinical methods used to evaluate dyspnea in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;158:1185-1189.
19. van Stel HF, Bogaard JM, Rijssenbeek-Nouwens LH, Colland VT. Multivariable assessment of the 6-min walking test in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;163:1567-1571.
20. Mahler DA, Harver A. A factor analysis of dyspnea ratings, respiratory muscle strength, and lung function in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992;145:467-470.
21. Wegner RE, Jorres RA, Kirsten DK, Magnussen H. Factor analysis of exercise capacity, dyspnoea ratings and lung function in patients with severe COPD. *Eur Respir J* 1994;7:725-729.
22. Taube C, Lehnigk B, Paasch K, Kirsten DK, Jorres RA, Magnussen H. Factor analysis of changes in dyspnea and lung function parameters after bronchodilation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;162:216-220.
23. Nguyen HQ, Altinger J, Carrieri-Kohlman V, Gormley JM, Stulberg MS. Factor analysis of laboratory and clinical measurements of dyspnea in patients with chronic obstructive pulmonary disease. *J Pain Symptom Manage* 2003;25:118-127.
24. Lapperre TS, Stroband JB, van Maaren MS, van der Veen H, Sont JK, Postma DS, Rabe KF, Sterk PJ, The Glucold Study Group. Pathophysiological pathways in COPD: a factor analysis of lung function, airway hyperresponsiveness (AHR) and sputum cell counts [abstract]. *Am J Respir Crit Care Med* 2002;165:A584.
25. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault J-C. Lung volumes and forced ventilatory flows. *Eur Respir J* 1993;6:5-40.
26. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;152:1107-1136.
27. Brand PL, Quanjer PH, Postma DS, Kerstjens HA, Koeter GH, Dekhuijzen PN, Sluiter HJ. Interpretation of bronchodilator response in patients with obstructive airways disease. The Dutch Chronic Non-Specific Lung Disease (CNSLD) Study Group. *Thorax* 1992;47:429-436.
28. Cotes JE, Chinn DJ, Quanjer PH, Rutgers SR, Yernault J-C. Standardization of the measurement of transfer factor (diffusing capacity). *Eur Respir J* 1993;6:41-52.

29. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo J-L. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. *Eur Respir J* 1993;6:53-83.
30. In 't Veen JCCM, de Gouw HW, Smits HH, Sont JK, Hiemstra PS, Sterk PJ, Bel EH. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. *Eur Respir J* 1996;9:2441-2447.
31. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-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* 1999;160:2104-2117.
32. Stevens J. Exploratory and confirmatory factor analysis. In: Stevens J, editor. Applied multivariate statistics for the social sciences Mahwah, NJ: Lawrence Erlbaum Associates; 1996. p. 362-428.
33. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. NHLBI/WHO workshop report. www.goldcopd.com, accessed June 2009.
34. Papi A, Romagnoli M, Baraldo S, Braccioni F, Guzzinati I, Saetta M, Ciaccia A, Fabbri LM. Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;162:1773-1777.
35. Sitkauskienė B, Sakalauskas R, Malakauskas K, Lotvall J. Reversibility to a beta2-agonist in COPD: relationship to atopy and neutrophil activation. *Respir Med* 2003;97:591-598.
36. Calverley PM, Burge PS, Spencer S, Anderson JA, Jones PW. Bronchodilator reversibility testing in chronic obstructive pulmonary disease. *Thorax* 2003;58:659-664.
37. Turato G, Zuin R, Miniati M, Baraldo S, Rea F, Beghe B, Monti S, Formichi B, Boschetto P, Harari S, Papi A, Maestrelli P, Fabbri LM, Saetta M. Airway inflammation in severe chronic obstructive pulmonary disease: relationship with lung function and radiologic emphysema. *Am J Respir Crit Care Med* 2002;166:105-110.
38. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur Respir J* 2003;22:672-688.
39. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med* 1997;155:449-453.
40. Liu H, Lazarus SC, Caughey GH, Fahy JV. Neutrophil elastase and elastase-rich cystic fibrosis sputum degranulate human eosinophils in vitro. *Am J Physiol* 1999;276:L28-L34.
41. Tracey M, Villar A, Dow L, Coggon D, Lampe FC, Holgate ST. The influence of increased bronchial responsiveness, atopy, and serum IgE on decline in FEV1. A longitudinal study in the elderly. *Am J Respir Crit Care Med* 1995;151:656-662.
42. Postma DS and Kerstjens HAM. Epidemiology and natural history of chronic obstructive pulmonary disease. In: Gibson GJ, Geddes DM, Costabel U, Sterk PJ, Corrin B, editors. *Respiratory Medicine*, Third ed. Saunders; 2003. p. 1109-1120.
43. Kanner RE. Early intervention in chronic obstructive pulmonary disease. A review of the Lung Health Study results. *Med Clin North Am* 1996;80:523-547.

44. Tashkin DP, Altose MD, Connett JE, Kanner RE, Lee WW, Wise RA. Methacholine reactivity predicts changes in lung function over time in smokers with early chronic obstructive pulmonary disease. The Lung Health Study Research Group. *Am J Respir Crit Care Med* 1996;153:1802-1811.
45. Yan K, Salome CM, Woolcock AJ. Prevalence and nature of bronchial hyperresponsiveness in subjects with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1985;132:25-29.
46. Sherrill DL, Halonen M, Burrows B. Relationships between total serum IgE, atopy, and smoking: a twenty-year follow-up analysis. *J Allergy Clin Immunol* 1994;94:954-962.

