Dissociation of Lung Function and Airway Inflammation in Chronic Obstructive Pulmonary Disease

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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, physiologic, and pathologic 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 FEV1, 63 ± 9% predicted, FEV1/inspiratory vital capacity [IVC] 48 ± 9%) and symptoms of chronic bronchitis or dyspnea were studied in a cross-sectional design. Postbronchodilator FEV1, and FEV1/IVC, reversibility to inhaled β2-agonists, diffusing capacity, provocative concentration of methacholine required to produce a 20% drop in FEV1, 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 comprised of FEV1, FEV1/IVC, and residual volume/total lung capacity. Factor 2 included reversibility, IgE, provocative concentration of methacholine required to produce a 20% drop in FEV1, 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.

Keywords: induced sputum; bronchial hyperreactivity; bronchodilator reversibility; nitric oxide; factor analysis

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 pathophysiologic 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 pathophysiologic 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 noninvasive 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 pathophysiologic entities underlying the clinical phenotypes in COPD may be diverse and 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 a 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, exercise 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 this 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).

METHODS

The extended version of the methods is available in the online supplement. A total of 114 Patients with COPD participating in a multicenter...
trial (Groningen and Leiden Universities and Corticosteroids in Obstructive Lung Disease [GLUCLOLD] study) were included. Patients were (ex-) smokers with 10 pack-years of smoking or more. They had irreversible airflow limitation (postbronchodilator FEV1, and FEV1/inspiratory vital capacity [IVC] < 90% confidence interval of the predicted value [25]. FEV1 ≈ 1.3 L and > 20% of the predicted value) and one or more of the following symptoms: chronic cough, chronic sputum production, or dyspnea on exertion. Patients did not use a course of steroids during the last 3 months, and did not have maintenance treatment with inhaled or oral steroids during the last 6 months. They were allowed to use short-acting bronchodilators, and were in clinically stable condition. The medical ethics committees of each center approved the study, and all patients gave their written informed consent.

The study had a cross-sectional design. IVC and FVC maneuvers were performed, and values of the curve with the largest sum of FEV1 and FVC were used (26). Reversibility of airflow limitation (ΔFEV1) was measured after administration of 400 μg salbutamol and expressed as change in FEV1 as percentage of predicted value (27). Total lung capacity (TLC) and residual volume (RV) were measured using a constant-volume body plethysmograph (25). The diffusing capacity for carbon monoxide per liter alveolar volume (KCO) was measured using the single breath holding method with a rolling seal closed system (28). Reference values for all lung function measurements were obtained from Quanjer and colleagues (25).

Methacholine challenge tests were performed according to the 2-minute tidal breathing method (29). The response was expressed as the provocative concentration of methacholine causing a 20% fall in FEV1 (PC20).

Sputum was induced and processed according to a validated technique (30). Whole samples were processed and differential cell counts were performed as a percentage of nucleated cells excluding squamous cells. Exhaled NO (eNO) levels were determined according to a standardized procedure (31). Total serum IgE concentrations were measured by fluoroenzymomassay.

Statistical analysis included assessment of normality, summarizing baseline data, factor analysis, and Pearson correlations. Exploratory factor analysis included the following variables: postbronchodilator FEV1 (% predicted [%pred]), postbronchodilator FEV1/IVC (%), ΔFEV1 (% predicted), KCO (%pred), PC20 (mg/ml), RV/TLC (%), eNO (ppb), sputum % neutrophils and % eosinophils, and IgE (IU/ml). For a detailed explanation of the method of factor analysis see the online supplement. Factor analysis is a data reduction technique that consists of clustering of variables into independent subgroups of variables called “factors”, and then simplifying the factor structure by Varimax rotation (32). The possibility to perform factor analysis was tested by Bartlett’s test of sphericity, and the Kaiser–Meyer–Olkin value was 0.594. Factor analysis yielded three 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 four factors were selected, resulting 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 KCO. eNO loaded on factor 3, whereas sputum % neutrophils and eosinophils loaded on factor 4. Interestingly, KCO 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 structure 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 positive factor loading. Factor 3 contained

### 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 of 40.8–77.7%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).

### TABLE 1. PATIENT CHARACTERISTICS

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>n</th>
<th>Mean (SD or IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female, n</td>
<td>114</td>
<td>99/15</td>
</tr>
<tr>
<td>Age, yr</td>
<td>114</td>
<td>62 (8)</td>
</tr>
<tr>
<td>Smoking history, pack years*</td>
<td>114</td>
<td>41.8 (31.2–54.8)</td>
</tr>
<tr>
<td>Current smoker, yes/no, n</td>
<td>114</td>
<td>72/42</td>
</tr>
<tr>
<td>IgE, IU/ml*</td>
<td>113</td>
<td>40 (11.5–125.0)</td>
</tr>
</tbody>
</table>

#### Lung function

- **Postbronchodilator**
  - FEV1: 63.0 (8.8)
  - FEV1/IVC, %: 48.2 (8.5)
  - ΔFEV1, %pred: 6.9 (4.9)
  - KCO, %pred: 75.9 (25.5)
  - PC20, mg/ml: 110 0.60 (2.76)
  - RV/TLC, %: 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 (× 104/ml): 106 1.4 (0.4–4.5)
- Sputum neutrophils, n (× 104/ml): 106 102 (47–229)

**Definition of 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; KCO = 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.

The patients were heavy smokers with a median of 41.8 pack-years and most of them were current smokers (63.2%).

#### 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 χ2 = 165.864, degrees of freedom = 45, p = 0.001). The Kaiser–Meyer–Olkin value was 0.594. Factor analysis yielded three 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 four factors were selected, resulting 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 KCO. eNO loaded on factor 3, whereas sputum % neutrophils and eosinophils loaded on factor 4. Interestingly, KCO contributed also to factor 1, and % neutrophils contributed also to factor 3.
the variables that originally loaded on factor 2: ∆FEV₁, total IgE, and PC₂₀. Finally, factor 4 included eNO and KCO.

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 KCO and PC₂₀ switched from factor 2 to factor 1. Number of pack-years smoked loaded on factor 2 together with ∆FEV₁ and total IgE (see Table E1 online supplement).

Univariate Correlations

The univariate relationships among physiologic and inflammatory parameters (sputum neutrophils, eosinophils, and eNO) were as follows: ∆FEV₁ was not associated with inflammatory parameters; however, RV/TLC was associated with sputum % neutrophils (r = 0.203, p = 0.04), and postbronchodilator FEV₁, RV/TLC, and PC₂₀ 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 FEV₁, PC₂₀, and FEV₁/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). FEV₁/IVC was also related to sputum % eosinophils (r = −0.219, p = 0.02), while postbronchodilator FEV₁, KCO, PC₂₀, and FEV₁/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 physiologic 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: FEV₁, FEV₁/IVC, and hyperinflation; factor 2 included: β₂-response, total serum IgE, airway hyperresponsiveness, and KCO; 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, inflammatory indices 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 and as assessed in exhaled air, are independent dimensions (13). Our current results demonstrate that airflow function, bronchial responsiveness with reversibility, and inflammation as assessed in sputum or exhaled air 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 measures 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 independence 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 Stages II and III. Inclusion of patients with very mild or very severe COPD 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 nonreversible patients only would have excluded a large group of patients with COPD (34–36). Furthermore, it must 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 pathophysiologic 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 KCO, 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 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 patients with COPD suggest that the eosinophils are degranulated (39), which may be the result of the high neutrophil elastase levels in COPD (40). Our data support 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 be expected, because 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 KCO, its parallel loading on factor 1 suggests that gas exchange impairment is associated with diverse pathophysiology.

The current separation of airway hyperresponsiveness and FEV1 into different factors supports epidemiologic evidence that these disease characteristics provide complementary information on COPD (44), the PC20 in COPD not simply being a result of airflow limitation per se. However, the fact that PC20 also had moderate loading on the first factor and even highest loading on the first factor in some of the additional factor analyses, is in agreement with previous studies that suggest that PC20 is to some extent dependent on airflow caliper 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 β2-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 no significant correlations between eNO and FEV1, FEV1/IVC, and PC20, 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 pathophysiologic condition of patients with COPD. This confirms the complex heterogeneity of the disease and may change some of the current concepts on the distinct pathophysiologic pathways involved. Accordingly, it needs to be examined whether the clinical evaluation of patients with COPD should include each of these complementary entities.

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