

# **Lung function fluctuation patterns unveil asthma and COPD phenotypes unrelated to type 2 inflammation**

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## Abstract

**Background:** In all chronic airway diseases, the dynamics of airway function are influenced by underlying airway inflammation and bronchial hyperresponsiveness along with limitations in reversibility, due to airway and lung remodeling as well as mucous plugging. The relative contribution of each component translates into specific clinical patterns of symptoms, quality of life, exacerbation risk, and treatment success.

**Objective:** We aimed to evaluate whether subgrouping of patients with obstructive airway diseases according to patterns of lung function fluctuation allows identification of specific phenotypes with distinct clinical characteristics.

**Methods:** We applied the novel method of fluctuation-based clustering (FBC) to the twice-daily FEV<sub>1</sub> measurements recorded over a one-year period in a mixed group of 134 adults with mild-to-moderate asthma, severe asthma, or COPD from the European BIOAIR cohort.

**Results:** Independent of clinical diagnosis, FBC divided patients into 4 fluctuation-based clusters with progressively increasing lung functional alterations that corresponded with patterns of increasing clinical severity, risk of exacerbation and lower quality of life. Clusters of patients with airway disease were identified with significantly elevated biomarkers relating to remodeling (osteonectin) and cellular senescence (plasminogen activator inhibitor-1), accompanied by a loss of airway reversibility, pulmonary hyperinflation and loss of diffusion capacity. The 4 clusters generated were stable over time and revealed no differences in markers of type 2 inflammation (blood eosinophils and periostin).

**Conclusion:** FBC-based phenotyping provides another level of information, complementary to clinical diagnosis, and unrelated to eosinophilic inflammation, that could identify patients who may benefit from specific treatment strategies or closer monitoring.

## Key Messages

- We identified patients with features of lung remodeling independent of clinical diagnosis and eosinophilic inflammation.
- We found 3 distinct severe asthmatic sub-phenotypes with various degrees of lung remodeling.
- Identifying such clusters of patients might be useful for future phenotype specific treatments or risk characterization requiring closer monitoring or preventative measures.

**Capsule summary:** Daily lung function fluctuations contain diagnostic information, independent of clinical diagnosis (asthma, COPD) and eosinophilia, revealing distinct phenotypes differing in severity, exacerbation risk and biomarker profiles related to airway remodeling.

**Key words:** Asthma; Chronic Obstructive Pulmonary Disease; Cluster analysis; Phenotyping; Remodeling.

## Abbreviations:

ACQ, Asthma control questionnaire

ANOVA, Analysis of variance

BAFF, B-cell activating factor

BMI, Body mass index

C9, Complement factor 9

CCL23, Chemokine ligand 23 (or Macrophage inflammatory protein 3)

CD-40L, Cluster of differentiation 40 ligand

COPD, Chronic obstructive pulmonary disease

137	CV, Coefficient of variation
138	DLCO, Diffusion capacity for carbon monoxide
139	DPP-4, Dipeptidyl peptidase-4
140	ECM, Extracellular matrix
141	ELISA, Enzyme-linked immunosorbent assay
142	FBC, Fluctuation-based clustering
143	FEV1, Forced expiratory volume in one second
144	FRC, Functional residual capacity
145	FVC, Forced vital capacity
146	hsCRP, High sensitivity C-Reactive Protein
147	ICS, Inhaled corticosteroids
148	IgE, Immunoglobulin E
149	IL, Interleukin
150	IVC, Inspiratory vital capacity
151	MD, Missing data
152	MMP-3, Matrix metalloproteinase-3
153	OCS, Oral corticosteroids
154	PAI-1, Plasminogen activator inhibitor-1
155	PEF, Peak Expiratory Flow
156	QoL, Quality of life
157	RFU, Relative Fluorescence Units
158	RV, Residual volume
159	SGRQ, St George's respiratory questionnaire
160	sRAGE, Soluble receptor for advanced glycosylation end products
161	TLC, Total lung capacity
162	TSLP, Thymic Stromal Lymphopoietin

## Introduction

Asthma and COPD are increasingly recognized as entities in a continuum of heterogeneous obstructive airway disease with distinct phenotypes.<sup>1,2</sup> For clinicians, there is great need to identify phenotypes for treatment optimization and to predict risk of worsening. Progress has been made in identifying phenotypes of patients with predominantly type 2 inflammation who may benefit from novel biological therapies targeting cytokines such as IL-4, IL-5 and IL-13.<sup>3</sup> However, the unpredictable nature of exacerbations and heterogeneity of responses to therapy, especially in patients with severe asthma, COPD and transition forms between these entities, still present a major clinical challenge.<sup>4,5</sup>

The information held in airway function dynamics is largely underestimated and may be highly relevant for understanding the phenotypic overlap between asthma, severe asthma and COPD, as well as treatment response. It is recognized that the dynamics of airway function are influenced by airway inflammation and bronchial hyperresponsiveness, and also independently by airway remodeling.<sup>6</sup> These structural changes can be induced by sheer stress and mechanical factors and involve loss of elastic recoil, mucous plugging, alterations in the properties of smooth muscle, tissue, or extracellular matrix and premature cellular senescence.<sup>7-10</sup> For instance, rapid bronchial obstruction, due to exaggerated bronchial responsiveness, contributes to a specific dynamic behavior, and such patients might be clinically characterized by a high exacerbation risk. On the other hand, irreversible obstruction, due to mechanical impairments, contributes to another specific dynamic behavior, and such patients might be clinically characterized by a poor response to bronchodilator therapy. Thus, the relative contribution of hyperresponsiveness and structural changes to overall lung function dynamics translates into specific clinical patterns or treatment responses. Accordingly, lung function fluctuation has been found to associate with

disease progression and control, risk of exacerbations, and treatment response (see online supplement).<sup>11-13</sup>

The aim of the current study was therefore to assess whether the clustering of patients with obstructive airway diseases based on lung function fluctuation dynamics alone unveils subgroups of patients with specific functional phenotypes, partly independent of their clinical diagnosis, with a view to uncover potential treatable traits. To do this, we conducted fluctuation-based clustering (FBC),<sup>14</sup> in a mixed group of 134 well-characterized adults with mild-to-moderate asthma, severe asthma, or COPD, from the unique longitudinal European BIOAIR multicenter study.<sup>15</sup> Lung function data was collected twice daily over a one-year period, generating an extensive database of lung function measurements alongside clinical characteristics and biomarker measurements. This method identifies groups of patients with similar patterns of lung function fluctuation over a predetermined window of time in an observer-independent manner. Using this approach, while purposefully making no distinction between asthma and COPD, we could investigate whether patients with similar patterns of lung function fluctuation also share patterns of clinical and inflammatory characteristics, lung mechanics and of particular interest, evidence of lung remodeling or cellular senescence by comparing associated biomarker profiles. Furthermore, the exceptional longitudinal design enabled us to examine phenotype stability by comparing cluster characteristics at study entry and exit. We are not aware of any other study that has followed patients with asthma and COPD with twice daily lung function measurements over a whole year; hence we cannot test our original findings in a comparable validation cohort.

## Methods

### *Ethics approval*

Ethics committees of the corresponding participating study centers and all study participants provided written informed consent.

### *Study design*

This analysis was performed based on lung function measurements assessed twice-daily and collected over a one-year period in the BIOAIR study (ClinicalTrials.gov Identifier: NCT00555607).<sup>15</sup> Originally, 169 adults with asthma, and 64 with COPD were recruited. Patients with asthma were screened at visit 1 and allocated to mild-to-moderate asthma (n=76) and severe asthma (n=93) groups according to established criteria.<sup>16</sup> Initially patients underwent a treatment optimization period of 4 weeks (from visit 1 to visit 2), further details regarding the specific treatment regimes followed in each patient group are presented in the online supplement. Unless otherwise stated, statistical comparisons were performed using data collected at visit 2, after optimization, as this data was considered less prone to variability caused by differences in medication. This period was followed by a 2-week, double-blind, placebo-controlled oral prednisone intervention (0.5 mg/kg body weight, from visit 2 to visit 3) enabling assessment of lung function and biomarker responses to oral corticosteroid intervention. Finally, patients were followed up for 12 months (up to visit 6). Lung function measurements, including FEV<sub>1</sub> and PEF were measured twice-daily, morning and evening, using a portable, hand-held spirometer (Vitalograph Electronic PEF/FEV<sub>1</sub> Diary, Version XM, Vitalograph Ltd. Buckingham, UK), which also recorded clinical symptoms and medication use daily throughout the entire study.<sup>15</sup> Fluctuations in both of the lung function parameters recorded, FEV<sub>1</sub> and PEF, can contain similar disease information,<sup>14</sup> but the current

analysis focused on one parameter for simplicity, FEV<sub>1</sub>, a measurement less dependent on patient co-operation. Inclusion in the FBC model required a minimum number of daily FEV<sub>1</sub> recordings to ensure high quality input data, as described in the online supplement (Figure E1). This resulted in a total of 134 BIOAIR patients being eligible for inclusion, and 99 being excluded due to incomplete data.

#### *Clustering of patients by means of fluctuation-based clustering (FBC) of FEV<sub>1</sub>*

FBC methodology is described in more detail in the online supplement.<sup>14</sup> Briefly, FEV<sub>1</sub> was expressed as age, sex, height and ethnicity adjusted z-score (denoted zFEV<sub>1</sub>), and patients with similar fluctuation behavior in twice-daily measurements of zFEV<sub>1</sub> during the one-year follow-up (visit 3 to visit 6) were grouped into clusters. FBC takes into account the entire distribution of zFEV<sub>1</sub> values during the window of observation. Thus, FBC not only considers the mean lung function, but also the magnitude and frequency of the fluctuations around this mean. FBC measures the dissimilarity of the zFEV<sub>1</sub> distributions of individual patients, using a well-established mathematical tool called Earth Mover's Distance.<sup>17</sup> Based on these dissimilarities (i.e. distances), clusters were constructed and their stability was verified using suitable statistical methods.<sup>14</sup> The use of the age, sex, height and ethnicity adjusted z-scores of FEV<sub>1</sub> ensures that age, and declining lung function that results from increasing age are taken into account in the calculations that led to determining the clusters.

#### *Biomarker measurements*

Serum CRP levels were measured using a standardized high sensitivity assay with a clinical Cobas c502 (8000) instrument (Roche Diagnostics). Serum periostin was measured using an in-house developed ELISA with two rat anti-human periostin monoclonal antibodies (clones SS18 and SS17B) as previously described.<sup>18</sup> Other circulating biomarkers were selected from larger multiplex panels of mediators based on putative involvement in processes of relevance

to inflammation, obesity or remodeling. Serum CD-40L, sRAGE, MMP-3, DPP-4, YKL-40, osteonectin, BAFF and PAI-1, were measured using Luminex® technology with screening assay reagents from R&D Systems (Bio-Techne, Abingdon, UK) and analyzed according to the manufacturers' instructions. Plasma IL-6, CCL23, TSLP,  $\alpha$  1-antichymotrypsin, C9 and chymase were measured using antibody suspension bead arrays. This in-house developed affinity proteomics method was performed using Human Protein Atlas antibodies ([www.proteinatlas.org](http://www.proteinatlas.org)) as previously described.<sup>19</sup>

### *Statistical analysis*

Statistical methods are described in more detail in the online supplement. Briefly, in order to explore the characteristics of a given phenotype, means and standard deviation (or median with interquartile range where relevant) of various clinical, baseline lung functional and molecular biomarkers measured were compared using relevant parametric or non-parametric multiple group tests (ANOVA or Kruskal Wallis). For categorical variables, Chi<sup>2</sup> or Fisher's exact test were performed. Alternatively, enrichment analysis was performed using the hypergeometric test.<sup>20</sup>

## **Results**

### *Description of the analysis population*

Among the 233 patients included in the BIOAIR study, 99 patients were excluded due to incomplete data, characteristics of this group are shown in the online supplement (online Table E1, Figure E1). Among the 134 patients analyzed, there were 53 (39.6%) mild-to-moderate asthmatics, 54 (40.3%) severe asthmatics, and 27 (20.1%) patients with COPD. Clinical characteristics of these disease groups based on BIOAIR inclusion criteria are summarized in Table 1, and biomarkers in online supplement Table E2. The three subject

groups differed significantly with respect to several measures of disease severity, lung function and potential biomarkers of inflammation and remodeling. The mean number of FEV<sub>1</sub> measurements per patient during follow-up was 428±170.

#### *Fluctuation-based clustering analysis identifies four clusters*

The FBC analysis identified four specific clusters. Figure 1 shows a heatmap of the four lung function fluctuation-based clusters with representative examples. Cluster 1 (‘mild fluctuation phenotype’) consists of patients with mildly decreased zFEV<sub>1</sub> values and low-scale FEV<sub>1</sub> fluctuations quantified using the coefficient of variation (CV) of the FEV<sub>1</sub> as measured (median coefficient of variation [25<sup>th</sup> percentile; 75<sup>th</sup> percentile] 7.4% [5.3%;9.7%]), cluster 2 (‘moderate fluctuation phenotype’) with lower zFEV<sub>1</sub> and low-scale fluctuations (9.1% [6.8%;12.2%]), cluster 3 (‘severe fluctuation phenotype’) with lower zFEV<sub>1</sub> and medium-scale fluctuations (13.0% [11.0%;16.2%]), and cluster 4 (‘very severe fluctuation phenotype’) with very low zFEV<sub>1</sub> and large-scale fluctuations (17.8% [13.2;20.7%]). CV differed significantly according to cluster (p<0.001).

Mild-to-moderate asthmatics (M) were found in all 4 clusters, though were significantly overrepresented in clusters 1 (p<0.001) and 2 (p<0.001) (Table 2, online Table E3). Severe asthmatics were found in all four clusters, though they were significantly overrepresented in cluster 3 (p=0.048) (online Table E3). COPD patients were found in clusters 2, 3, and 4, but were significantly overrepresented in cluster 4 (p<0.001) (online Table E3).

#### *Cluster-specific characteristics*

Tables 3 and 4 show the clinical and inflammatory characteristics of the patients in the four clusters and indicate their differences in multiple comparison tests. Progressing from cluster 1 to 4, patients were typically older and exhibited continuously worsening lung mechanics with decreasing airway reversibility and greater evidence of systemic inflammation (summarized

in Figure 2 and Figure 4). The levels of several potential biomarkers of inflammation and remodeling also increased from 1 to 4, of which osteonectin, CD40 ligand and PAI-1 were unique to the fluctuation-based clusters but not significantly different among the three clinical disease groups recruited per protocol. Generally, the presence of clinical comorbidities was heterogeneously spread among the four clusters (Table E4).

**Cluster 1** was the smallest cluster and consisted of 12 (9.0% of study cohort) patients. Compared with the other three clusters, the patients were younger, had normal baseline lung function and quality of life and no significant signs of airway inflammation or remodeling. Several biomarkers related to remodeling, extracellular matrix and cellular senescence (alpha 1-antichymotrypsin, PAI-1, osteonectin and YKL-40) and inflammation (CD40 ligand, hs-CRP and C9) were lowest in this group.

**Cluster 2** was the largest cluster and consisted of 47 (36.7%) patients. Quality of life was slightly lower than in cluster 1, although not significantly, and more than double the number of patients experienced at least one asthma exacerbation during the one-year follow-up compared to those in cluster 1 (42.9% vs 16.7%). Reversibility was similar to cluster 1, but baseline lung function (zFEV<sub>1</sub>, TLC) was significantly lower. Biomarker levels in this group tended to be intermediate between clusters 1 and 4.

**Cluster 3** consisted of 31 (23.1%) subjects. Compared to cluster 2, cluster 3 included patients with significantly lower quality of life, poorer asthma control, and 51.6% of patients experienced exacerbations during the one year follow-up period. Lung function was decreased, including poorer reversibility and a significantly higher degree of pulmonary hyperinflation compared to patients in cluster 2. Certain circulating biomarkers were notably different in cluster 3 compared to other clusters including IL-6 and YKL-40 which were

highest in this group (and which correlated with each other, Spearman  $r=0.3$ ,  $p=0.003$ ), and chymase which was lowest. Oral steroid use was also highest in cluster 3 compared to the other clusters.

**Cluster 4** was the most distinct, consisting of 42 (31.3%) patients. These, typically older, patients displayed significantly lower disease control and quality of life. Similar to cluster 3, more than half of patients suffered at least one exacerbation during the one year follow-up period (52.4%). Baseline lung function for these patients was not only markedly depressed, including poor reversibility and pulmonary hyperinflation as well as significantly decreased diffusion capacity. Furthermore, bronchodilator response was poorest in this cluster of patients with the lowest  $zFEV_1$  and highest degree of fluctuation and exacerbation risk. Although eosinophil numbers did not differ significantly among the four clusters, white blood cells and neutrophils tended to progressively increase from cluster 1 to 4. Several biomarkers related to remodeling (alpha 1-antichymotrypsin, osteonectin) and inflammation (CD40 ligand, hs-CRP and C9) were highest in this group, also increasing progressively from cluster 1 to cluster 4.

Clinical characteristics and biomarkers that did not show significant differences among the four clusters included the prevalence of atopy, blood eosinophil numbers and circulating periostin levels.

### *Phenotype stability*

Comparing the clinical characteristics of the four clusters at study entry and study exit revealed that the clusters were relatively stable over time, with measures of lung function, quality of life and asthma control being similar at the two time points, Figure 3. Also, oral corticosteroid use was not different in any of the clusters when comparing visits at entry and exit (online supplement Table E5). However, the clusters showed more variability between study entry and exit regarding circulating biomarkers such as osteonectin and YKL-40. Blood

eosinophils were not different among the clusters, neither at the beginning nor end of the study.

#### *Severe asthma subgroups, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub>*

The FBC method unveiled heterogeneity among severe asthmatics with patients being evenly distributed across three lung function-based clusters (Table 2). Consequently, we investigated whether a subgroup analysis based on the combination of severe asthma diagnosis (S) with lung function fluctuation-based cluster (2-4) could provide additional information when compared to patients with milder asthma. As shown in online supplement Table E6, asthma control was poorest in S<sub>4</sub> and accordingly, the number of exacerbations experienced during the one-year follow-up was also highest in this group. However, oral corticosteroid use was highest in S<sub>3</sub>. The subgroups also differed with respect to BMI which was highest in S<sub>4</sub>. Measures of lung function progressively worsened from S<sub>2</sub> to S<sub>4</sub>.

Regarding markers of inflammation among the asthma subgroups, white blood cells and neutrophil numbers, but not eosinophils, were highest in S<sub>3</sub> and S<sub>4</sub> (online supplement Table E6). Certain biomarkers were also particularly high in these subgroups including MMP-3,  $\alpha$ 1-antichymotrypsin, hs-CRP and TSLP which were highest in S<sub>4</sub>, and IL-6 and MIP-3 which were highest in S<sub>3</sub>.

A similar sub-group analysis was performed for the mild-to-moderate asthma group who were predominantly distributed in clusters 1, 2 and 3 (Table 2, online supplement Table E3). A comparison of characteristics found in sub-groups M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> is shown in supplemental Table E7. Again, differences were observed between the sub-clusters with asthma control and measures of lung function progressively worsening from M<sub>1</sub> to M<sub>3</sub>, and osteonectin levels increasing from M<sub>1</sub> to M<sub>3</sub>.

## Discussion

Asthma and COPD are heterogeneous diseases with varying degrees of fluctuating airflow limitation that may be caused by different underlying mechanisms. In the current investigation we focus on the target organ itself and whether patterns of lung function fluctuation provide clinically relevant information to aid asthma phenotyping, diagnosis and treatment. Lung function fluctuations are influenced by airway obstruction, but also bronchial reactivity and reversibility. Using this novel clustering method, we clustered a group of patients with obstructive airway diseases based on their lung function fluctuation dynamics and found four fluctuation-based clusters, with progressively increasing functional alterations. These clusters reflect distinct patterns of dynamic lung function characteristics, partly independent of clinical diagnosis, as summarized in Figure 4.

Our analysis was inspired by Pavord et al, in particular, by the idea that the terms asthma and COPD are, respectively, descriptive labels for a collection of respiratory symptoms with various and possibly overlapping underlying pathophysiological mechanisms.<sup>2</sup> Consequently, we pursued an unassuming, data-driven approach when analyzing together the data of both asthma and COPD patients. We believe such an approach may potentially help unveil two types of pathophysiological mechanisms: Those that may be characteristic of asthma and COPD, respectively, and those that these two obstructive airway diseases may have in common, as discussed below.

Remarkably, the significant decrease in quality of life associated with increasing cluster number was accompanied by increasing lung functional abnormalities, loss of airway reversibility (despite large day-to-day lung function fluctuations), pulmonary hyperinflation and altered diffusion capacity. Patients in clusters 3 and 4 tended to have more exacerbations and generally displayed a lower bronchodilator response but larger day-to-day fluctuations in

lung function. This may explain why in many patients with respiratory symptoms single reversibility testing is not particularly useful for differential diagnosis or for the assessment of response to treatment.<sup>21-24</sup> Indeed, previous research suggests that short and long term lung function fluctuations are not necessarily correlated.<sup>25</sup> Thus, day-to-day fluctuations may not simply be an expression of bronchodilator response, but instead reflect the influence of other mechanical factors on responses to environmental stimuli. These functional abnormalities, which might be due to progressive structural changes, can be seen in both asthma and COPD. Generally speaking, reversible airway obstruction is classically more associated with asthma, and fixed airway obstruction more with COPD, so this cross-border clustering approach may improve our understanding of functional abnormalities within the context of a continuum of airway diseases with variations in phenotypic expression.<sup>2</sup>

The additional diagnostic value of FBC is exemplified by patients with the common clinical diagnosis of severe asthma being distributed relatively evenly across three different lung function clusters. These three subgroups (S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub>), showed clear differences in lung functional abnormalities and dynamics, and distinct clinical phenotypes consistent with findings in the literature. Persistent airflow obstruction is increasingly common as asthma severity increases, likely due to the manifestation of progressive structural changes in the airway walls and mucous plugging.<sup>9,11,26</sup> Sorkness et al. found that severe asthmatics have a greater component of air trapping relative to airflow limitation, contributing to airway obstruction.<sup>27</sup> Therefore, the greater airway obstruction combined with the hyperinflation found in groups S<sub>3</sub> and S<sub>4</sub> is in accordance with features of persistent airflow obstruction, air trapping, and airway remodeling indicative of distal or small airways disease, and characteristic of more severe asthma phenotypes. Interestingly, Choi et al. identified four clusters very similar to the current mild asthma population and S<sub>2</sub>, S<sub>3</sub>, and S<sub>4</sub>, using an imaging-based clustering approach.<sup>28</sup> In particular, S<sub>3</sub> showed similar clinical characteristics

to their luminal narrowing-dominant cluster, and S<sub>4</sub> was similar to their wall-thickening-dominant cluster.<sup>28</sup>

We also identified a subgroup of severe asthmatics (S<sub>4</sub>) characterized by an overrepresentation of obese subjects. There is strong evidence that obesity is associated with reduced asthma control and increased asthma severity.<sup>29</sup> It is also associated with airway obstruction, hyperinflation, and low diffusion capacity, reduced lung volumes, and low-grade systemic inflammation.<sup>29,30</sup>

For both severe asthma and COPD, patients in clusters 3, and, particularly, 4, had very low airway reversibility, pulmonary hyperinflation and alterations in diffusion capacity. One reason may be tissue remodeling which can also explain a lack of responsiveness towards anti-inflammatory or bronchodilator treatment. In accordance, biomarkers associated with remodeling, alterations in extracellular matrix biology or cellular senescence were also greatest in clusters characterized by the lowest reversibility and severe impaired functional abnormalities. Especially noteworthy is the newly discovered ECM-glycoprotein osteonectin, that is associated with high rates of collagen turnover, lung fibrosis and also regulates the expression and activity of various growth factors and matrix metalloproteinases (MMPs).<sup>31</sup> MMP-3, which is believed to play a role in airway disease via effects on tissue remodelling,<sup>31</sup> was highest in cluster 3. However, we examined possible confounding effects of oral steroid use on all biomarker profiles and it should be noted that MMP-3 levels in particular are greatly increased by oral corticosteroids.<sup>32</sup> As oral steroid use was greatest in cluster 3 (and subcluster S<sub>3</sub>) findings regarding MMP-3 may need to be interpreted with caution. Plasminogen activator inhibitor-1 (PAI-1), an inhibitor of the fibrinolytic system and key marker of cellular senescence, has also been found to regulate airway remodeling in asthma and in the current study was highest in clusters 3 and 4.<sup>10,33</sup>

One more proposed marker of remodeling, YKL-40, was also highest in cluster 3. YKL-40 is emerging as a potential biomarker of non-type-2 driven inflammation that associates with IL-6 and IL-8, increasing asthma severity, basement membrane thickness and more neutrophilic airway inflammation.<sup>34,35</sup> In agreement, both IL-6 and YKL-40 levels were greatest in cluster 3 and correlated with each other.

Inflammatory processes may also differ between the four clusters. Although blood eosinophil numbers did not differ significantly among the four clusters, neutrophils tended to progressively increase from cluster 1 to 4, highlighting the relationship between non-type-2 inflammation and more severe airway disease. Interestingly, although circulating blood neutrophil numbers were highest in clusters 3 and 4, blood eosinophils were not lowest in these most severe clusters as may be expected, possibly reflecting the presence of patients with type 2 inflammation that is refractory to corticosteroids. Several circulating biomarkers of relevance to inflammation showed higher levels in clusters with a more severe dynamic profile including  $\alpha$  1-antichymotrypsin (or SERPINA3), a protease inhibitor known to be elevated in inflammatory conditions and which may also have protective effects in the airways by inhibiting tissue-damaging proteases.<sup>36</sup> Complement component 9 (C9) and C reactive protein (CRP), both components of innate immune processes known to be elevated during inflammatory processes were also highest in cluster 4, as well as CD40L which is expressed by activated T cells and involved in the antibody isotype switching process of B cells.<sup>37-39</sup> CRP in particular may be reflecting more non-type 2 inflammation. Taken together, several novel hypothesis-generating findings emerged in this analysis, which all will require future validation.

Strengths of the current investigation include the unique design of the BIOAIR study, enabling a first comparison of lung function fluctuations over a one-year period in extensively characterized patients with asthma or COPD. As one single, simple lung function parameter was used to perform the clustering analysis, the issue of variable selection was circumvented which renders many clustering approaches subjective. A further strength is the use of a time-related variable for clustering, thereby accounting for the fluctuating nature of these diseases, instead of parameters measured at one single time point. The four clusters that emerged were also robust, being characterized by similar lung mechanical properties at the beginning and end of the observation period (Figure 3). Mediator levels however were more variable, in line with previous findings in the BIOAIR cohort.<sup>40</sup> Finally, the four clusters revealed unique changes in several potentially useful biomarkers that were not apparent when comparing disease groups only.

The main drawbacks of the current investigation are the relatively small sample size and lack of a validation cohort. The unique one-year observation period and twice-daily lung function measurements, in combination with the magnitude of clinical characteristics and specific biomarkers measured, hamper the availability of suitable material for replication. This is therefore a single, proof-of-concept study, requiring future studies for validation. Another drawback is that not all BIOAIR participants could be included in the cluster analysis due to missing data points. The excluded patients were mostly those with severe asthma and COPD, and with somewhat lower lung function measurements than those included, which may need to be considered when interpreting the results. Nevertheless, we were able to provide an estimate of the number of observations required for cluster stability, and following exclusion of those with insufficient data could generate a high quality dataset.

The current findings are clinically relevant as they identify potentially treatable traits, regardless of an asthma or COPD diagnosis,<sup>2</sup> and can improve disease management and monitoring. As currently available novel treatment options are primarily biological therapies, which are mostly beneficial to subgroups of patients with evidence of type-2 inflammation, it is of value to know that monitoring of daily lung function can reveal subgroups of patients with markedly different lung function dynamics but no differences in blood eosinophils, the most established marker of type-2 asthma to date.<sup>3</sup> In accordance, the prevalence of atopy and levels of periostin were similar among the four clusters. Potential biomarkers of remodeling, extracellular matrix biology or cellular senescence, as well as blood neutrophils, are particularly altered in cluster 4. Since novel therapeutic approaches targeting cellular senescence and aiming to reverse remodeling are under development, FBC may identify patients eligible for such treatment strategies.<sup>10</sup> Furthermore, lung function fluctuation-based phenotyping could be a simple and cost-effective way to aid disease monitoring, perhaps in a telemonitoring setting by remotely identifying severely ill patients at risk (e.g., in the context of the COVID-19 pandemic), thus avoiding the burden of travelling to a specialized asthma centre. This may be particularly relevant for patients with an unclear diagnosis, co-morbidities such as obesity, poor response to treatment, or those with high exacerbation risk.

In summary, this novel clustering approach identifies four phenotypes of lung function fluctuations, in which the progressive functional alteration corresponds to gradually increasing clinical severity and may relate to increasing remodeling, regardless of an asthma or COPD diagnosis, and independent of eosinophilic inflammation. This in turn may translate into treatable traits such as specific components of remodeling pathways, and suggest monitoring strategies, particularly for those whose airway function is least stable and who may be at increased risk of virus-induced complications. The value of this clustering approach

may also be enhanced when combined with clinical diagnosis by increasing our understanding of the different biological mechanisms that lead to similar effects on airway function.

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## Figure legends

**Figure 1:** Heat map representation of the fluctuation-based clustering analysis. Four meaningful clusters (1-4) of patients with a similar fluctuation pattern of zFEV1 were identified. Representative examples of lung function fluctuation dynamic abnormalities are shown in cluster 1 ('mild fluctuation phenotype') with typically mildly decreased zFEV1 values and low-scale fluctuations, cluster 2 ('moderate fluctuation phenotype') with lower zFEV1 and low-scale fluctuations, cluster 3 ('severe fluctuation phenotype') with low zFEV1 and large-scale fluctuations, and cluster 4 ('very severe fluctuation phenotype') with very low zFEV1 and large-scale fluctuations.

**Figure 2.** Selected patient characteristics according to clusters 1 to 4. Results are displayed as median with interquartile range for A) patient reported outcomes (St George's Respiratory Questionnaire score, Asthma Control Questionnaire score, and frequency of patients who experienced at least one exacerbation during 1-year follow-up), B) lung function measurements (FEV1, DLCO and reversibility) as well as biomarkers related to C) inflammation (blood neutrophils, eosinophils and hs-CRP) and D) remodelling (osteonectin, PAI-1 and  $\alpha 1$  antichymotrypsin). Of the three biomarkers uniquely associated with the four clusters, only osteonectin correlated positively with age, although the between-cluster differences remained significant and followed the same pattern following adjustment for age (data not shown). P values reflect Kruskal Wallis multigroup comparisons (or for continuous variables the  $\chi^2$  or the Fisher's Exact test). Abbreviations: ACQ=Asthma Control Questionnaire; CD-40L=cluster of differentiation-40 ligand; DLCO=diffusion capacity of the lung for carbon monoxide; FEV1=forced expiratory volume in one second; hs-CRP=high sensitivity-C-reactive protein; PAI-1=Plasminogen Activator Inhibitor-1; SGRQ=St George's Respiratory Questionnaire.

**Figure 3.** Stability of phenotypes, comparison of selected patient characteristics at start (visit 1 or visit 2) and end (visit 6) of study. Results are displayed as median with interquartile range for A) patient reported outcomes (St George's Respiratory Questionnaire score and Asthma Control Questionnaire score), B) lung function measurements (FEV1 and DLCO) as well as biomarkers related to C) inflammation (blood neutrophils and eosinophils) and D) remodelling (osteonectin and YKL-40). P values reflect Kruskal Wallis multigroup comparisons for clusters 1-4 at study start and study end.

**Figure 4.** Summary of how clinical, pulmonary, inflammatory, remodelling and senescence features differ among the four lung function fluctuation-based clusters.

Table 1. Clinical characteristics of patients at inclusion according to airway disease (n=134).

	Mild-to-moderate asthma (N=53)	MD	Severe asthma (N=54)	MD	COPD (N=27)	MD	p-value*
<b><u>Clinical characteristics</u></b>							
Age, years	42.6±12.6	-	50.6±10.5	-	64.8±7.9	-	<0.001
Gender, male	20 (37.7%)	-	21 (38.9%)	-	19 (70.4%)	-	0.01
BMI, kg/m <sup>2</sup>	25.1±4.0	-	28.5±5.1	-	27.0±4.7	-	<0.001
Obese (BMI≥30 kg/m <sup>2</sup> )	7 (13.2%)	-	15 (27.8%)	-	5 (18.5%)	-	0.17
Age of disease onset, years	18.0 [5.5;33.0]	2	33.0 [20.3;43.3]	4	60.0 [51.0;66.0]	2	<0.001
ACQ, Juniper	0.9 [0.4;1.3]	2	2.0 [1.2;2.7]	4	NA	NA	<0.001
QoL, SGRQ	15.4 [10.5;29.6]	11	41.6 [31.9;57.1]	5	39.6 [32.5;50.6]	4	<0.001
Atopy	24 (47.1%)	2	20 (40.0%)	4	0 (0%)†	1	0.47‡
IgE (kU/L)	123 [43;320]	3	153 [57;314]	-	55 [29;148]	-	0.02
Blood eosinophils, x10 <sup>9</sup> /L	0.24 [0.13;0.35]	1	0.26 [0.10;0.46]	1	0.21 [0.14;0.35]	-	0.84
Blood neutrophils, x10 <sup>9</sup> /L	3.4 [3.0;4.4]	1	4.9 [3.1;6.4]	1	4.4 [3.7;5.5]	-	<0.001
Current ICS dose, µg <sup>£</sup>	500 [400;775]	-	1500 [1000;2000]	-	800 [500;1000]	-	<0.001
Current OCS use	0 (0%)	-	14 (25.9%)	-	0 (0%)	-	<0.001
Current smokers	1 (1.9%)	-	2 (3.7%)	-	9 (33.3%)	-	<0.001
Former smokers	13 (24.5%)	-	19 (35.2%)	-	18 (66.6%)	-	0.001
Never smokers	39 (73.6%)	-	33 (61.1%)	-	0 (0%)	-	<0.001
Pack years	0 [0;1]	-	0 [0;2.3]	-	32.5 [24.8;60.0]	-	<0.001
<b><u>Lung function</u></b>							
Reversibility, % change	10.5±6.0	1	8.5±6.0	1	3.0±3.8	-	<0.001
FEV <sub>1</sub> , z-score	-1.4±1.3	2	-2.0±1.3	1	-3.3±0.7	1	<0.001
FEV <sub>1</sub> , % predicted	82.2±16.9	2	71.5±19.5	1	45.9±10.8	1	<0.001
FVC, z-score	-0.2±0.9	2	-1.1±1.2	1	-1.4±0.8	1	<0.001
FVC, % predicted	97.0±12.4	2	85.2±16.6	1	78.2±11.8	1	<0.001
FEV <sub>1</sub> /FVC z-score	-1.7±1.2	2	-1.7±1.5	1	-3.5±1.1	1	<0.001
DLCO, % predicted	94.5±14.5	5	86.0±16.6	8	59.4±20.0	1	<0.001
FRC, % predicted	96.1 [82.4;119.7]	6	92.6 [82.2;113.5]	9	126.2 [104.0;147.1]	3	<0.001
IVC, % predicted	102.3±14.1	6	96.8±19.1	1	89.4±11.2	3	0.007
TLC, % predicted	104.3±12.5	2	103.1±15.4	1	109.2±18.0	-	0.22
RV, % predicted	104.6 [92.5;126.1]	3	118.0 [97.8;139.4]	1	150.7 [111.6;174.1]	-	0.001
RV/TLC z-score	1.0 [0.9;1.2]	3	1.2 [1.0;1.4]	1	1.4 [1.2;1.6]	-	<0.001

Values shown are mean ± standard deviation, median [25<sup>th</sup> percentile;75<sup>th</sup> percentile], and numbers (percentages). Abbreviations: ACQ, Asthma Control Questionnaire; BMI, body mass index; DLCO, diffusing capacity of the lung for carbon monoxide; FEV<sub>1</sub>, forced expiratory volume in one second; FRC, forced residual volume; FVC, forced vital capacity; IVC, inspiratory vital capacity; MD, missing data; NA, not applicable; QoL, quality of life; RV, residual volume; SGRQ, St George's Respiratory Questionnaire; TLC, total lung capacity. \*Comparison between groups using the one-way ANOVA or the Kruskal-Wallis test, as appropriate, for continuous variables, and the Chi<sup>2</sup> or Fisher's exact test, as appropriate, for categorical variables; †Inclusion criteria; ‡Comparison between mild-to-moderate asthmatics and severe asthmatics. £ Beclomethasone equivalent.

**Table 2. Distribution of clinical diagnoses (mild-to-moderate asthma, severe asthma, COPD) according to lung function fluctuation-based clusters (n=134).**

	<b>Cluster 1 (N=12) «mild fluctuation phenotype»</b>	<b>Cluster 2 (N=49) «moderate fluctuation phenotype»</b>	<b>Cluster 3 (N=31) «severe fluctuation phenotype»</b>	<b>Cluster 4 (N=42) «very severe fluctuation phenotype»</b>
<b>Mild-to-moderate asthma (N=53)</b>	10 (18.9%) <b>Subgroup M<sub>1</sub></b>	29 (54.7%) <b>Subgroup M<sub>2</sub></b>	10 (18.9%) <b>Subgroup M<sub>3</sub></b>	4 (7.5%)
<b>Severe asthma (N=54)</b>	2 (3.7%)	18 (33.3%) <b>Subgroup S<sub>2</sub></b>	16 (29.6%) <b>Subgroup S<sub>3</sub></b>	18 (33.3%) <b>Subgroup S<sub>4</sub></b>
<b>Patients with COPD (N=27)</b>	0	2 (7.4%)	5 (18.5%)	20 (74.1%) <b>Subgroup C<sub>4</sub></b>

Data shows how many patients from each disease group (mild asthma (M), severe asthma (S) and COPD (C)) are found in each lung function fluctuation-based cluster, as absolute number (and percentage of total). Subgroups resulting from clinical diagnosis (M, S or C) combined with cluster number (1, 2, 3 or 4) are shown underneath. Severe asthma patients are primarily distributed in subgroups S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub>.

715 Table 3. Clinical characteristics of patients at visit 2 according to lung function fluctuation-based clusters 1 to 4 (n=134).

	Cluster 1 (N=12)	MD	Cluster 2 (N=49)	MD	Cluster 3 (N=31)	MD	Cluster 4 (N=42)	MD	p-value*
<b>Clinical characteristics</b>									
Age, years	36.5 [31.5;55.5] ‡	-	47.0 [38.0;55.0]	-	52.0 [42.0;62.0]	-	54.5 [46.3;67.5] †	-	<b>0.008</b>
Gender, male	0 (0%)	-	24 (49.0%)	-	14 (45.2%)	-	22 (52.4%)	-	<b>0.01</b>
BMI, kg/m <sup>2</sup>	24.0 [23.8;26.0]	-	25.0 [24.0;28.0]	-	27.0 [23.5;30.0]	-	27.5 [25.0;31.8]	-	<b>0.03</b>
Obese (BMI≥30 kg/m <sup>2</sup> )	0 (0%)	-	8 (16%)	-	6 (19%)	-	13 (31%)	-	0.09
Age of disease onset, years	27.5 [3.8;38.0]	-	24.5 [13.8;37.3] ‡	1	32.5 [13.5;44.5]	3	48.5 [33.3;60.0]	4	<b>&lt;0.001</b>
Atopy	5 (45.5%)	1	18 (40.9%)	5	11 (36.7%)	1	10 (23.8%)	-	0.30
IgE (kU/L)	125 [35;320]	-	149 [54;292]	3	142 [36;240]	-	118 [33;190]	-	0.62
QoL, SGRQ	9.5 [9.2;24.9] ‡	3	21.2 [9.2;39.0] ‡¥	8	41.7 [34.6;46.4]	7	46.1 [32.5;58.1] †	8	<b>&lt;0.001</b>
ACQ, Juniper	0.4 [0.1;0.4] ‡¥	-	0.7 [0.3;1.2] ‡¥	1	1.6 [1.3;2.3] †	3	2.1 [1.3;2.5] †	6	<b>&lt;0.001</b>
Number of exacerbations during follow-up	0 [0;0]	-	0 [0;1]	-	1 [0;2]	-	1 [0;2]	-	0.11
At least one exacerbation during follow-up	2 (16.7%)	-	21 (42.9%)	-	16 (51.6%)	-	22 (52.4%)	-	0.14
Current ICS dose, µg <sup>£</sup>	550 [213;800] ¥	-	500 [400;1000]	-	1000 [640;1600] †	-	900 [500;1050]	-	<b>0.006</b>
Current OCS use	1 (8%)	-	1 (2%)	-	7 (23%)	-	6 (14%)	-	<b>0.03</b>
Current smokers	0 (0%)	-	2 (4%)	-	3 (10%)	-	7 (16.5%)	-	0.13
Former smokers	3 (25%)	-	18 (37%)	-	9 (29%)	-	20 (47.5%)	-	0.31
Never smokers	9 (75%)	-	29 (59%)	-	19 (61%)	-	15 (36%)	-	<b>0.03</b>
Pack years	0 [0;0.8] ‡	-	0 [0;3.0] ‡	-	0 [0;6.3]	1	5 [0;31.5] †	2	<b>0.001</b>
<b>Lung function</b>									
Reversibility, % change	10.1 [7.2;11.9]	-	10.0 [7.3;13.8] ‡	1	6.2 [3.2;9.9]	1	4.8 [2.4;8.0]	-	<b>&lt;0.001</b>
FEV <sub>1</sub> , z-score	0.6 [0.0;1.3] ‡¥	-	-1.2 [-1.7;-0.8] ‡†¥	-	-2.4 [-2.8;-1.9] †	1	-3.2 [-3.6;-2.4] †	-	<b>&lt;0.001</b>
FEV <sub>1</sub> , % predicted	111.5 [104.7;118.2] ‡¥	-	88.9 [80.6;95.6] ‡†¥	-	70.3 [61.2;78.3] †	1	54.3 [42.1;66.5] †	-	<b>&lt;0.001</b>
FVC, z-score	0.6 [0.1;1.5] ‡¥	-	-0.4 [-1.2;0.3] ‡¥	-	-1.4 [-1.9;-0.5] †	1	-1.7 [-2.2;-1.6] †	-	<b>&lt;0.001</b>
FVC, % predicted	107.1 [101.8;119.9] ‡¥	-	93.7 [83.4;103.9] ‡¥	-	82.2 [71.9;93.6] †	1	75.1 [68.3;85.0] †	-	<b>&lt;0.001</b>
FEV <sub>1</sub> /FVC z-score	-0.1 [-0.7;0.2] ‡¥	-	-1.4 [-1.8;-0.3] ‡	-	-1.8 [-2.7;-1.2] ‡†	1	-3.0 [-3.9;-2.3] †	-	<b>&lt;0.001</b>
DLCO, % predicted	95.4 [88.3;99.6] ‡	1	90.4 [83.0;102.4] ‡	9	86.8 [78.2;97.0] ‡	2	73.9 [48.9;86.2] †	2	<b>&lt;0.001</b>
FRC, % predicted	96.8 [90.7;117.9]	1	94.0 [81.1;115.1]	9	102.0 [83.5;127.6]	2	105.3 [88.7;132.6]	6	0.20
IVC, % predicted	115.1 [108.9;127.3] ‡¥	1	101.1 [93.8;110.2] ‡¥	3	91.4 [84.0;97.8] †	2	88.1 [80.5;99.2] †	4	<b>&lt;0.001</b>
TLC, % predicted	113.4 [108.8;118.0]	-	98.1 [93.3;108.7] †	2	105.6 [94.8;114.3]	-	103.1 [93.8;117.3]	1	<b>0.008</b>
RV, % predicted	115.3 [96.9;123.6]	-	101.7 [85.9;123.2] ‡¥	3	120.3 [100.2;153.9]	-	134.4 [109.6;163.4]	1	<b>&lt;0.001</b>
RV/TLC z-score	1.0 [0.8;1.0] ‡¥	-	1.0 [0.9;1.2] ‡¥	3	1.2 [1.1;1.4] †	-	1.3 [1.1;1.5] †	1	<b>&lt;0.001</b>

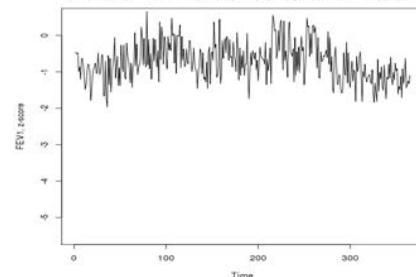
716 Values shown are median [25<sup>th</sup> percentile;75<sup>th</sup> percentile], and numbers (percentages). Abbreviations: ACQ, Asthma Control Questionnaire; BMI, body mass index; D<sub>LCO</sub>,  
717 diffusing capacity of the lung for carbon monoxide; FEV<sub>1</sub>, forced expiratory volume in one second; FRC, forced residual volume; FVC, forced vital capacity; IVC, inspiratory  
718 vital capacity; MD, missing data; QoL, quality of life; RV, residual volume; SGRQ, St George's Respiratory Questionnaire; TLC, total lung capacity. \*Comparison between  
719 groups using the one-way ANOVA or the Kruskal-Wallis test, as appropriate, for continuous variables, and the Chi<sup>2</sup> or the Fisher's exact test, as appropriate, for categorical  
720 variables. † significant difference as compared to cluster 1; ‡ significant difference as compared to cluster 4; ¥ significant difference as compared to cluster 3. Note: Certain lung  
721 function measurements were not performed at visit 2 and therefore reflect visit 1 (DLCO, FRC, IVC, TLC and RV). £ Beclomethasone equivalent.

722 Table 4. Inflammatory characteristics of patients at visit 2 according to lung function fluctuation-based clusters 1 to 4 (n=134).  
 723

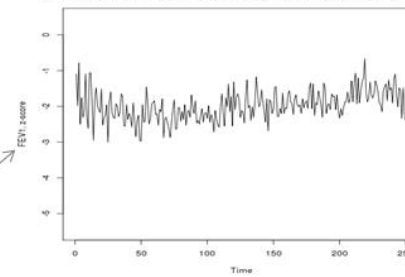
	Cluster 1 (N=12)	MD	Cluster 2 (N=49)	MD	Cluster 3 (N=31)	MD	Cluster 4 (N=42)	MD	p-value*
<b><u>Inflammatory biomarkers</u></b>									
hs-CRP, mg/L	0.74 [0.38;2.1] ‡	1	1.5 [0.38;3.2] ‡	10	2.2 [1.0;5.4]	2	3.9 [1.9;6.1] †	4	<b>0.0005</b>
White blood cells, ×10 <sup>9</sup> /L	6.0 [5.1;8.0]	-	6.2 [5.5;7.4] ‡	1	7.1 [6.0;9.5]	-	7.5 [6.1;9.4]	-	<b>0.017</b>
Blood eosinophils, ×10 <sup>9</sup> /L	0.16 [0.10;0.32]	-	0.27 [0.16;0.38]	1	0.24 [0.09;0.50]	1	0.22 [0.12;0.34]	-	0.44
Blood eosinophils ≥300 cells/μL	4 (33%)	-	19 (39%)	1	14 (45%)	1	12 (29%)	-	0.51
Blood neutrophils, ×10 <sup>9</sup> /L	3.5 [3.0;4.6]	-	3.4 [3.0;4.7] ‡	1	4.5 [3.2;6.1]	1	4.5 [3.7;6.5]	-	<b>0.0027</b>
Periostin ng/mL	72.5 [60;100.8]	-	75 [64.5;101]	8	78 [58.5;99.5]	2	85.5 [71.25;98.5]	2	0.69
CD40L, pg/mL	4080 [3530;5300] ‡	1	5730 [4120;6690]	9	5040 [3170;6240]	2	6370 [4060;7460] †	5	<b>0.014</b>
sRAGE, pg/mL	1650 [1170;1970]	1	1600 [1280;2030]	9	1400 [1010;2240]	2	1310 [876;1620]	5	0.075
BAFF, RFU	224 [190;263]	-	244 [200;278]	6	238 [214;281]	2	284 [193;352]	6	0.29
IL-6, RFU	700 [533;1016]	1	647 [598;862]	10	857 [688;1340]	7	830 [621;1110]	6	<b>0.045</b>
CCL23 (MIP-3), RFU	738 [600;1170]	1	737 [577;871]	10	886 [615;1130]	7	877 [656;1080]	6	0.10
TSLP, RFU	536 [322;641]	1	406 [342;496] ‡	10	419 [315;604]	7	662 [409;928]	6	<b>0.016</b>
<b><u>Biomarkers related to remodelling, matrix and senescence</u></b>									
MMP-3, ng/mL	12.7 [8.11;14.2]	1	13.2 [7.06;21.7]	9	17.7 [10.20;24.4]	2	17.5 [9.29;32.6]	5	0.090
DPPIV, ng/mL	184 [164;236]	-	226 [179;252] ‡	8	202 [165;225]	2	178 [160;208]	2	<b>0.013</b>
YKL-40 (or Chitinase 3-Like 1), ng/mL	27.1 [21.9;34.7] ¥	1	39.4 [25.9;54.3]	7	51.6 [28.0;86.3] †	1	38.6 [24.8;69.2]	3	<b>0.028</b>
Serpin E1 (PAI-1), ng/mL	97.1 [85.6;107.0] ‡	1	126 [98.2;162]	10	132.0 [100.6;156]	2	131 [114;162] †	5	<b>0.039</b>
α 1-antichymotrypsin, RFU	8450 [8280;8720]	1	8580 [8140;9370] ‡	10	8850 [8430;9370]	7	8990 [8620;9440]	6	<b>0.0097</b>
Osteonectin/SPARC, RFU	1180 [990;1560] ‡¥	-	1670 [1500;1970] †	6	1760 [1520;2080] †	3	1760 [1380;2090] †	6	<b>0.0063</b>
C9, RFU	7530 [6250;7960] ‡	1	7750 [7050;8760]	10	7880 [7300;8220]	7	8560 [7580;9220] †	6	<b>0.016</b>
Chymase, RFU	276 [220;313]	1	257 [227;311]	10	234 [214;273] ‡	7	289 [238;392] ¥	6	<b>0.042</b>

724 Values shown are median [25<sup>th</sup> percentile;75<sup>th</sup> percentile], and numbers (percentages). Abbreviations: hs-CRP, high sensitivity C-reactive protein; CD40 L, CD 40 ligand;  
 725 sRAGE, soluble receptor for advanced glycation end products; BAFF, B-cell activating factor; IL-6, Interleukin 6; CCL23, chemokine ligand 23; TSLP, thymic stromal  
 726 lymphopoietin; MMP-3, matrix metalloproteinase 3; DDPIV, dipeptidyl peptidase-4; SPARC, secreted protein acidic and rich in cysteine protein; C9, complement factor 9; RFU,  
 727 relative fluorescence units. \*Comparison between groups using the one-way ANOVA or the Kruskal-Wallis test, as appropriate, for continuous variables, and the Chi<sup>2</sup> or the  
 728 Fisher's exact test, as appropriate, for categorical variables. † significant difference as compared to cluster 1; ‡ significant difference as compared to cluster 4; ¥ significant  
 729 difference as compared to cluster 3.  
 730

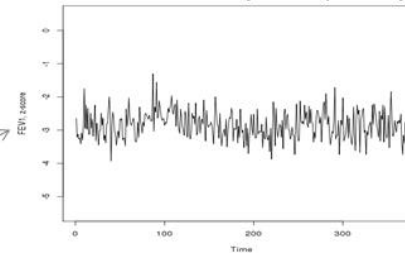
Cluster 1: mild dynamic phenotype



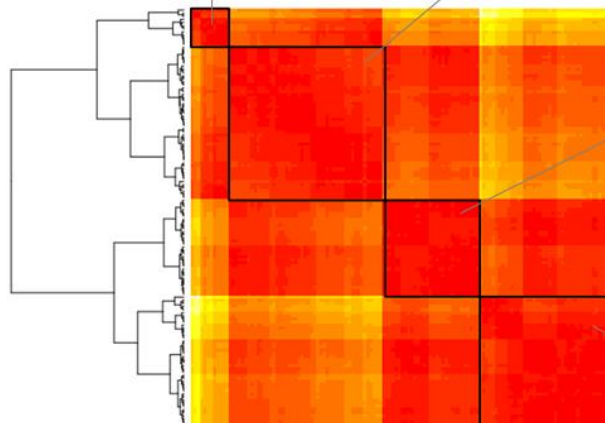
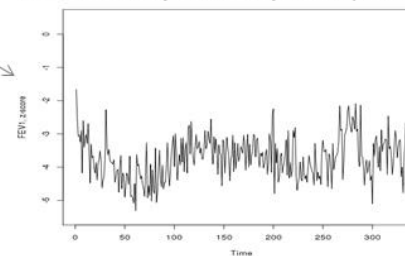
Cluster 2: moderate dynamic phenotype

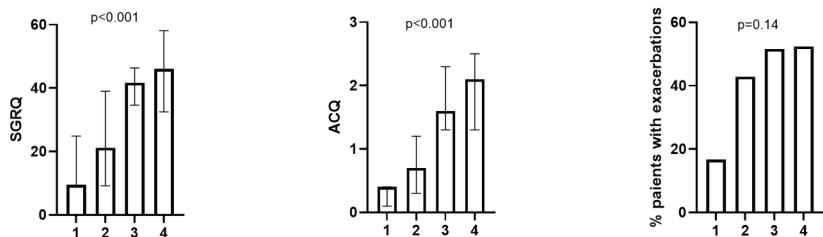
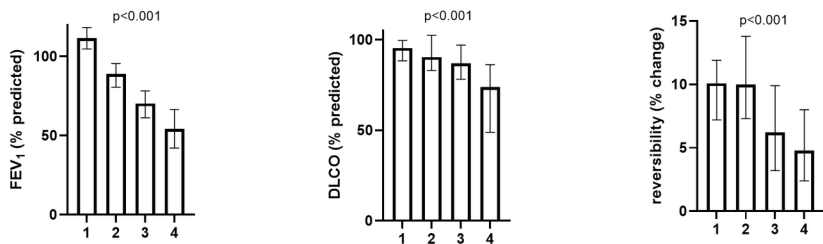
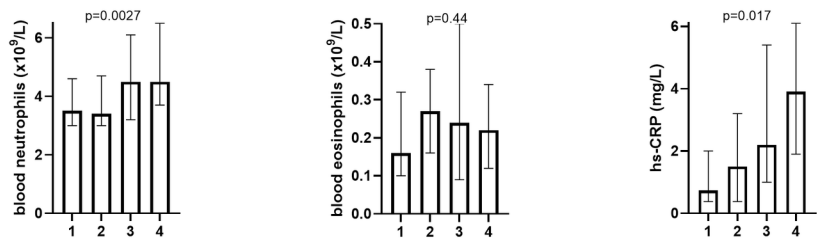
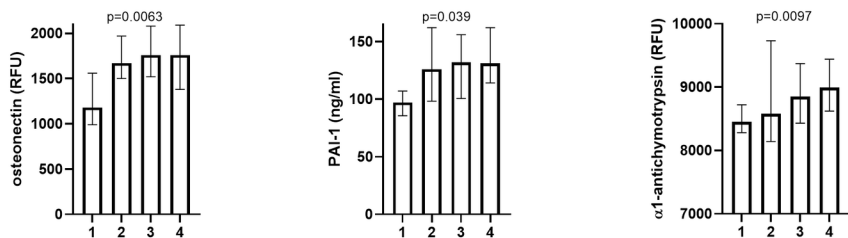


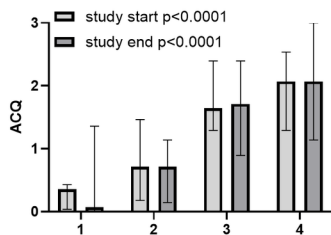
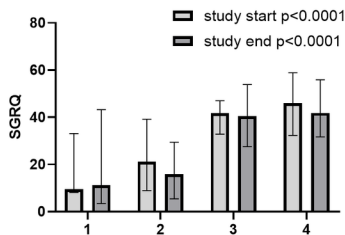
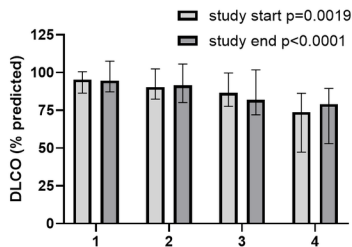
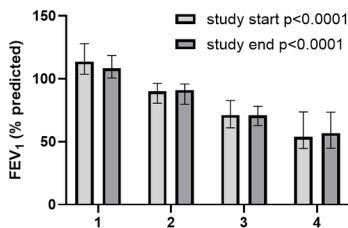
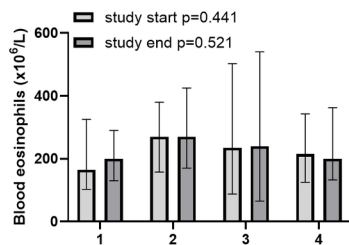
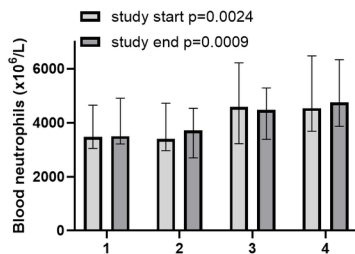
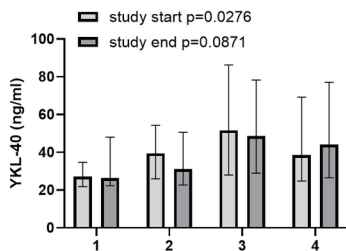
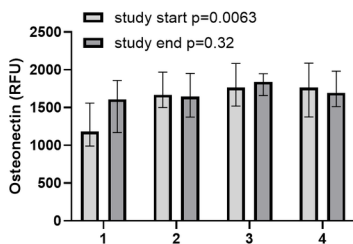
Cluster 3: severe dynamic phenotype

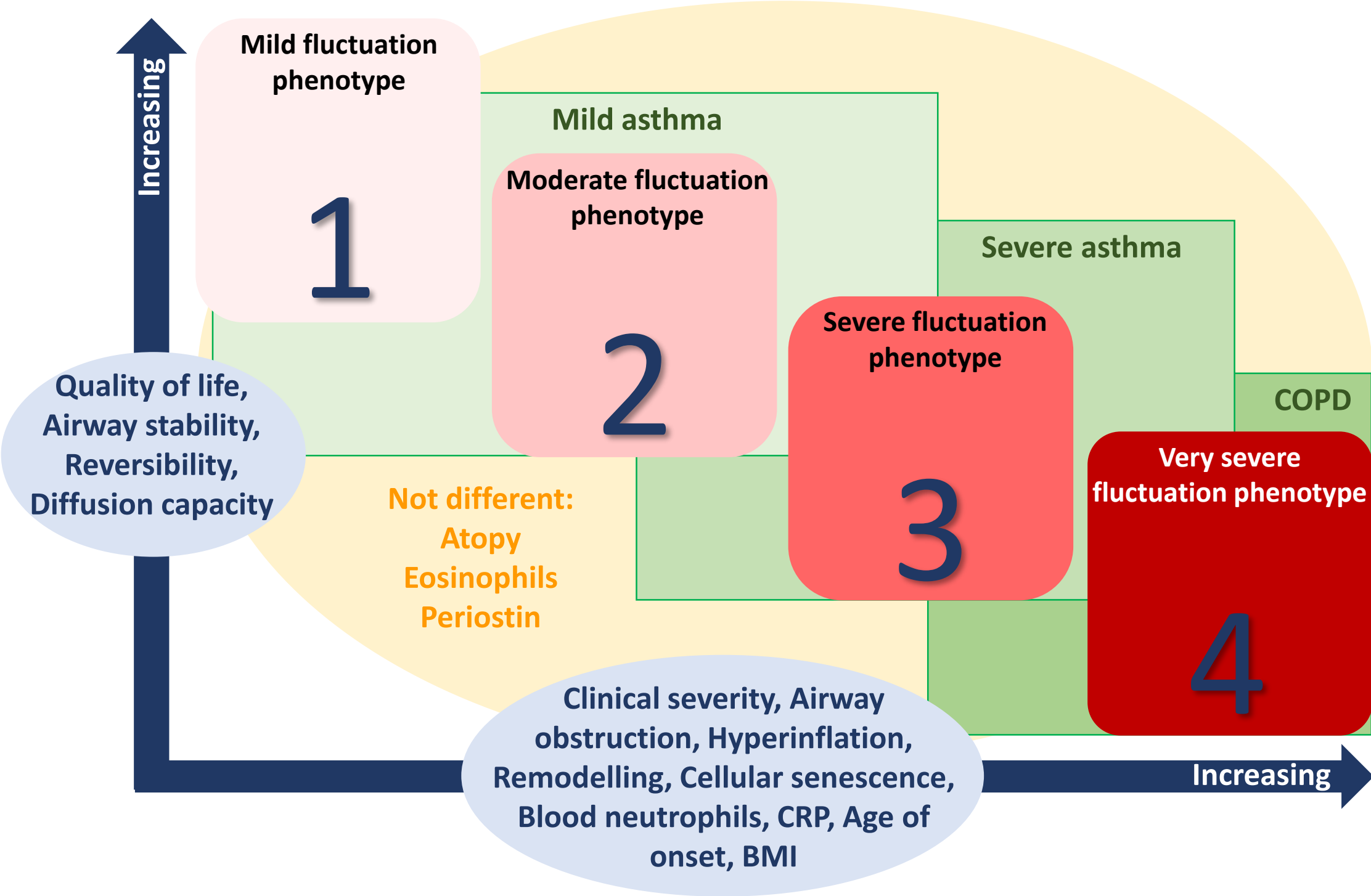


Cluster 4: very severe dynamic phenotype



**A****B****C****D**

**A****B****C****D**





# Patterns of lung function fluctuation in asthma and COPD patients reveal 4 clusters with distinct clinical characteristics

