Persistent Airflow Obstruction: A Marker of a Severe Asthma Cohort – Inflammatory, Functional and Pathological Features

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Research Article

Keywords: Persistent airflow, asthma, inflammatory, pathological features

Posted Date: December 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1110535/v1

Abstract

In our previous severe asthma cohort, 82% had fixed obstruction. Although they had greater airway smooth muscle area with decreased periostin, inflammation and remodeling weren't associated with symptom control. High-resolution computed tomography (HRCT) and measures of small airways could be important tools for exploring asthma severity. Our aim was to describe characteristics associated to airflow obstruction in our non-controlled severe asthmatics according to obstruction profile. Persistent obstruction subgroups were also evaluated comparing disease severity.

Methods: Patients were evaluated using asthma control questionnaire, induced sputum, spirometry, plethysmography, and Single Breath N2 washout test, at baseline, after oral corticosteroid (OC) and at the end of the treatment. They also underwent thorax HRCT and bronchoscopy with endobronchial biopsy.

Results: Sixty-two were included and 77.4% classified as having persistent obstruction; 75% and 25% with moderate and severe obstruction, respectively. Pulmonary function values (FEV1) improved in both subgroups, except in severe. Patients with bronchial thickening, according to RB1 WA% and pi10, had significantly higher airway smooth muscle area.

Conclusion: Patients with severe obstruction had greater lung function impairment, no response to OC or bronchodilator. This could be explained by airway remodeling characterized by higher airway smooth muscle area and bronchial thickness assessed by thorax HRCT.

Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. Symptoms vary over time and in intensity, together with variable airflow limitation [1]. Severe asthmatic patients are a substantial subset who remain difficult-to-treat, requiring high-dose inhaled corticosteroid (IC) in addition to other controllers, including systemic steroids [2]. Despite intense treatment, patients with severe asthma usually suffer with poor control, frequent exacerbations, and low health-related quality of life [3,4].

One main goal of asthma treatment is to reduce exacerbations as well as fixed airflow limitation, surrogates of future risk [1]. Early-life events, such as age-related symptoms, environmental exposures [5], and sensitivities to inhaled irritants (atopy), are linked to early loss of lung function and can manifest later as dyspnea on exertion, impaired quality of life or asthma onset [6]. Persistent airflow limitation has been considered common in adults with severe asthma (SA) [7,8].

In the last 10 years, asthma heterogeneity has been explained and categorized according to phenotypes based on one or more features. Considering clinical features, 3 categories of patients with SA seem to be of importance: exacerbation phenotype; fixed airflow obstruction, and steroid-dependent asthma [9]. The Brazilian Severe Asthma Sao Paulo (BRASASP) cohort described 5 possible clusters based on hierarchy classification, including sputum eosinophilia, atopy, airflow obstruction and asthma onset [10]. Most baseline characteristics of this population were similar to characteristics of other international cohorts.

However, 82% of our patients have fixed obstruction, independently of other clinical and inflammatory characteristics [10]. Increased fixed airway obstruction has been recognized in children with asthma exacerbations compared with those without exacerbations, from infancy throughout childhood, suggesting that exacerbations are a consequence rather than a cause of diminished airway caliber in childhood [11,12].

Fixed airflow obstruction phenotype indicates a worse prognosis and its physiopathology is poorly understood. Studies have shown that fixed obstruction is partially explained by airway remodeling [13]. Airways with lower compliance due to collagen deposition, proliferation of airway smooth muscle, and excess mucus production are suggested markers of fixed obstruction phenotype. Contributing factors to airway remodeling include smoking, SA in childhood, mucus hypersecretion, bronchiectasis, and possibly untreated type 2 (T2) asthma [14], among others. In the BRASASP cohort [10] 50 patients underwent endobronchial biopsies [15]. Asthmatic patients with persistent airflow obstruction had greater airway smooth muscle (Asm) area with decreased periostin; however, airway tissue inflammation and remodelling were not associated with symptom control [15]. Therefore, it is important to recognize the fixed airflow obstruction as a patient's characteristic in clinical practice.

High-resolution computed tomography (HRCT) has been used as an important noninvasive tool to assess airway features in severe asthma, including its structural changes. Consistent data had already shown that airway thickness and air trapping correlates to asthma severity [16–18]. Another important tool to explore asthma severity includes assessment of small airways by different lung function methods. Small airway impairment can be measured by both HRCT and airflow heterogeneity. It is observed in more severe asthmatic patients and can be improved after corticosteroid treatment [16, 19].

The high prevalence of small airways involvement in people with asthma observed in the ATLANTIS study [20] highlights the need to increase the knowledge of possible structural and functional alterations observed on the small airways in asthma [21].

Our hypothesis is that a portion of patients with severe asthma will persist with airway obstruction despite treatment with high IC dose associated with long-acting beta2-agonist bronchodilator (LABA) and could characterize a specific subgroup of patients. The aim of this study is to describe the characteristics associated to the airflow obstruction profile in our cohort of severe asthma, comparing the characteristics of non-controlled severe asthma patients with persistent obstruction (PO) to those with non-persistent obstruction (NPO) after standardized treatment follow-up. In addition we evaluate the subgroup of PO, comparing patients according to the severity of the disease.

Methods

Setting and Participants

This was a secondary analysis from a previously published interventional prospective study with participants from the BRASASP cohort [10]. Inclusion and exclusion criteria were defined based on severe asthma knowledge at that time [10]. The study was approved by the local institutional review board Comissão de Ética para Análise de Projetos de Pesquisa – CAPPesq da Diretoria Clínica do Hospital das Clínicas e da Faculdade de Medicina da Universidade de São Paulo (nº 757/05) and the study is registered on ClinicalTrials.gov (NCT01089322). All methods were performed in accordance with relevant guidelines and regulations. All patients provided written informed consent in accordance with local requirements.

Study Design

The study design is shown in Figure 1; the cohort clinic, spirometry, and quality of life baseline results are published elsewhere [4]. Briefly, following a 2-week run-in period, uncontrolled SA was treated with 40 mg/day oral prednisone for 2 weeks, in addition to inhaled maintenance therapy with budesonide/formoterol 400/12 µg twice daily, and rescue 200/6 µg as needed for 12 weeks. Patients were evaluated with an asthma control questionnaire (ACQ-7), induced sputum, spirometry, plethysmography and Single Breath N2 washout test (SBN2), at baseline (B), after an oral corticosteroids (OC) trial (W2), and at the end of the study (W12). In the last visit, patients also underwent a thorax HRCT and bronchoscopy with endobronchial biopsy.

Airflow Obstruction by Subgroups

At W2, patients with post bronchodilator (BD) FEV1/FVC > 0.70 and FEV1 ≥ 80% predicted were classified as having no persistent obstruction (NPO). SA patients who did not fulfill the above criteria were classified as having persistent obstruction (PO). Furthermore, PO patients were categorized according to obstruction severity: a) moderate persistent obstruction (MPO): FEV1 after BD > 50% and < 80%; b) severe persistent obstruction (SPO): FEV1 after BD ≤ 50%.

Pulmonary Function

Spirometry was performed according to ATS/ERS guidelines [22] and ECSC prediction equations [23]. Lung volumes were measured with a body plethysmography [24]. Carbon monoxide-diffusing capacity (DLCO) was measured using a single-breath technique. SBN2 was performed by a spirometer with a nitrogen (N2) measurer. FEV1 improvement/response between B and W2 (regular treatment: IC+LABA+SABA+OC, available at this time point was determined by the ratio: [after BD FEV1% (W2) - before BD FEV1% (B)] / before BD FEV1% (W2) [4].

Inflammatory Markers

Induced sputum, process, and measurements were previously described [10].

CT Imaging (HRCT)

Volumetric whole-lung scans were acquired at full inspiration at the end of the study (W12). Fully automated software (vidadiagnostics.com workstation) was used to quantify airway morphometry. Right

upper lobe apical segmental bronchus (RB1) as the standard segmentation was used for comparison with function and clinical characteristics [17,18]. Specific CT scan measurements included airway wall thickness (WT), wall area (WA), percentage of WA (WA%) and airway wall thickening by the square root of wall area for a theoretical airway with 10mm lumen perimeter (Pi10). WA% calculation was determined as (WA/total area) x 100.

Endobronchial Biopsies and Tissue Processing

Four to 6 endobronchial biopsies per patient were obtained on carina and right lower bronchus, at the last study visit (W12). Histological sections were stained using histochemical and immunohistochemical methods. Analysis included basement membrane thickness; intra-epithelial mucus; elastic fibers; collagens I, III, and IV; decorin; versican; fibronectin; laminin; tenascin; periostin; Asm area; bronchial vessels; vascular endothelial growth fator (VEGF); eosinophils; neutrophils; tryptase+ and chymase+ mast cells; CD4 and CD8 lymphocytes; IL-17+ and TGF-b1+ cells. Detailed description has already been published [15]. Quantification of α-smooth muscle actin (α-SMA) was performed within the airway smooth muscle (Asm) area and in the whole biopsy area, excluding the epithelium. Airway smooth muscle area is the fractional area of α-SMA within the Asm bundles per total biopsy area, and total smooth muscle area is the fractional area of α-SMA in the whole biopsy per total biopsy area [15].

CT Imaging and Endobronchial Biopsies

To evaluate bronchial wall thickening through tomography features using pi10 (mm) and RB1 wall area % values as markers, we stratified biopsy measurements according to lung function response at W2. Because a cutoff point to characterize bronchial thickening measured by CT is not clearly defined, we used the ROC curve analysis to determine the best cutoff point to discriminate SPO patients based on airway thickness. Cutoff values for pi10 and RB1 wall area % were used to divide groups into higher versus lower bronchial thickening.

Statistical Analysis

Continuous variables were evaluated for normality by Kolmogorov-Smirnoff or Shapiro-Wilk tests and are shown as mean ± standard deviation or median (IQR 25% - 75%). Paired *t* and unpaired *t* tests were used to analyze 2 groups whenever appropriate. ANOVA repeated measures or on ranks were used to compare variables in 3 moments (B, W2, and W12) and airway thickening at HCRT according to airflow obstruction subgroups. Wilcoxon signed rank test and Mann-Whitney test were also used for group analysis. Categorical variables are presented as numbers and percentages and were analyzed by chi-square test. Correlations were assessed using Pearson or Spearman coefficient tests. The statistical package Sigma Stat ® version 3.5, Sigma Plot ® version 10 and PASW Statistics (SPSS) ® version 18 were used for statistical analysis. Statistical significance was considered $p < 0.05$.

Results

Seventy-four patients were screened according to the severe asthma definition. Of those, 12 were excluded and 62 (83.8%) were selected and analyzed in W2 (Figure 2). After splitting into subgroups, 48 (77.4%) were classified as PO. Of these, 75% were stratified as MPO and 25% as SPO (Table 1).

Independently of airflow obstruction subgroups, patients were mainly female and overweight or obese. As expected, the PO group had a significantly lower FEV1%, but with no difference between clinical parameters compared with NPO (Table 1).

However, when analyzing subgroups of PO, body mass index (BMI) was significantly higher in the SPO compared with NPO group. SPO asthma duration was significantly higher than that in NPO although no significant difference occurred in age or in asthma onset. No difference was found between subgroups related to atopy, percentage of former smokers, number of hospitalizations in the previous year, and dose of inhaled/oral corticosteroids. SPO subgroup patients had significantly lower baseline FEV1 than NPO and MPO had (Table 1).

Although all patients were uncontrolled at baseline, SPO subgroup presented with higher ACQ-7 scores than MPO and NPO. ACQ-7 score improved significantly after OC use (W2), both in NPO and MPO subgroups. In all subgroups, ACQ-7 score variation was higher than the clinically significant value (0.5 points) (Table 2).

At baseline, all subgroups had sputum eosinophilia (eosinophils $\geq$ 3%), but NPO patients had significantly higher values than MPO and SPO had. All subgroups had a statistically significant sputum eosinophil reduction after the OC trial (Table 2) although they did not achieve the threshold of less than 3%.

Functional values throughout the study are shown in Table 2 and Figure 3. Spirometry and plethysmography baseline values were significantly different in MPO and SPO subjects from those in NPO. After OC use, most pulmonary function values, particularly FEV1, showed improvement in NPO and MPO subgroups. This improvement was particularly important in NPO patients, achieving normal values in some parameters, such as FVC, FEV1, and airway resistance (Raw %). However, impairment in lung function was observed at the end of the study in this group but was still better than that at baseline. Interestingly, Raw % was 2 and 3 times higher in MPO and SPO, respectively, than in the NPO group, even after the OC trial. In SPO, there was no improvement in lung function parameters even after the OC trial. The mean difference between FEV1% predicted at baseline (B) and the regular treatment (W2) for NPO, MPO, and SPO, respectively, was 19 ± 5 ($p = 0.007$), 11 ± 3 ($p = 0.001$), and 4 ± 4 ($p = 0.405$). DLCO was within the normal range in all groups during the entire trial.

SPO subgroup's nitrogen washout (dN2) % at baseline was significantly higher than that in NPO and MPO ($p < 0.05$). After the OC trial, dN2 values (% predicted) showed a significant reduction only in NPO and MPO ($p < 0.05$) subgroups, although they returned to baseline values at W12. SPO dN2% after OC trial stands out due to significantly higher values compared with other subgroups (Figure 4).

Wall airway thickness measured by HRCT scan differed between groups after treatment phase. Patients with PO had higher RB1 WA% than NPO, with no difference between MPO *versus* SPO obstruction severity subgroups. Furthermore, SPO had higher RB1 WA% and pi10 measurements compared with that in NPO patients. There was no significant difference in other CT measurements like RB1 wall thickness and wall area (Table 3).

When the groups were evaluated according to RB1 wall area% (RB1 WA%), patients with higher bronchial thickening (RB1 WA% ≥ 69.74) have significantly higher Asm area (0.22 ± 0.12 vs 0.16 ± 0.77; p = 0.023), but there was no difference in α-SMA total biopsy (0.13 ± 0.12 vs 0.09 ± 0.06; p = 0.113) between groups (Figure 5). No difference in Asm and α-SMA total biopsy was observed when comparing groups stratified by pi10 values. Extracellular matrix and inflammatory components were evaluated but showed no relationship with bronchial thickening.

Discussion

In the present study, 14 (22.6%) patients had an improvement in lung function, although 48 (77.4%) patients remained with persistent obstruction despite high-dose CI plus LABA plus an oral corticosteroid trial (regular treatment at W2). Among the patients who remained with persistent airflow obstruction, we were able to identify 2 distinct functional behaviors: milder lung function impairment and response to anti-inflammatory treatment (MPO) *vs*. severe lung function impairment with no response to anti-inflammatory treatment (SPO). This last subgroup (25%) was markedly different from the other groups, mainly in relation to pulmonary function parameters, with greater airway resistance, air trapping, and greater heterogeneity of the small airways. Similarly, HRCT measures showed higher bronchial thickness in the SPO group, which was associated with higher airway muscle components in bronchial biopsies.

Our clinical, functional, and inflammatory findings are similar to those of other studies published from SA cohorts [4,25,26], but we identified a subgroup with a more severe fixed airflow obstruction. This trait is associated with severe asthma despite the inflammatory profile, challenging the main target of the asthma treatment concept [27]. The heterogeneous response to conventional anti-inflammatory treatment indicates that multiple factors may influence SA evolution. A cluster analysis of the Severe Asthma Research Program (SARP) cohort [28] identified that as asthma severity increases, sputum neutrophil increases, and sputum eosinophil persists, indicating that therapies might target distinct inflammatory cell types. High levels of induced sputum eosinophil were an inflammatory marker in our cohort and did not differentiate patients according to the pattern of airway obstruction. Sputum eosinophilia was found at baseline and persisted after oral corticosteroid burst. The development of persistent obstruction in our asthmatic patients does not seem to depend exclusively on the severity of inflammation.

Some factors that may lead to persistent obstruction phenotype include asthma duration, amount of anti-inflammatory treatment delivered, and some patient characteristics responsible for different inflammatory and remodeling behavior to asthma triggers [29]. Our SPO patients have significantly longer asthma duration with at least 3 decades from disease onset. Evidence recognizing that most adult asthma starts

in childhood [30,31] is increasing as are data suggesting that exacerbations are a consequence rather than a cause of diminished airway caliber in childhood [11,12], which means that influences of asthma duration on lung function are still poorly understood. There is a clear “black box period” that includes transition between childhood and adulthood [32], with multiple factors that may interfere with asthma behavior. Poor lung development intra utero or irreversible structural changes during longer follow-up could decline lung function at age 21 [33], defining an irreversible worsening marker [34]. Therefore, lifetime non-controlled asthma may correlate to irreversible airway damage and, ultimately, persistent airflow obstruction.

Higher inhaled corticosteroid doses are associated with better symptoms and inflammation control as well as an impact on better disease progression [35]. However, this does not seem to play any important role in our population because no difference in oral or inhaled corticosteroid doses was found at baseline among our subgroups. We are not able to determine the influence of previous treatment in the lives of these patients [10].

The complexity and heterogeneity of asthma is characterized by multiple and distinct inflammatory pathways [27]. It is unlikely that all patients have a similar response when exposed to the same trigger. Current data cannot explain all asthma characteristics based purely on genetic data [36]. It is well known that asthma phenotypes depend on the interaction of individual genetic profiles with multiple exposures. Increased data on epigenetics are emerging to contribute to the understanding of actual asthma pathophysiology [27,37]. Individual characteristics are certainly a determinant of severity and how asthma manifests. Inflammatory predisposition profiles, such as higher expression of IL-13 genes [38], and TNF-alpha, IL-4R-alpha, and IL-4 polymorphisms [39] were linked to severity. The development of persistent obstruction in asthmatic patients should also be influenced by individual predisposing factors. Specific inflammatory profiles may contribute to a subset of patients with a greater component of muscle hypertrophy and remodeling leading to fixed obstructive disorder [15,40].

Thickened Asm, a finding usually identified in early life [41], is a distinguishing feature in this group. Our data [15] show that symptom control was not associated with airway tissue inflammation and remodeling. It is still a controversy whether these features are inflammation independent or arise as a proliferative response. Increase in the proliferating and higher airway smooth muscle cell levels were not related to inflammation, supporting the idea that evolution in smooth muscle in asthma is independent of airway inflammation [42].

Our patients had high BMI, especially in the severe persistent obstruction group. Obesity is a well-recognized phenotype in severe asthma with growing evidence relating to a T2 low inflammatory profile [43]. Otherwise, it may also be a consequence of long-term corticosteroid treatment, psychiatric disorders, and physical activity impairment [44]. This phenotype is commonly observed in other international cohorts [45,46]. Obesity may influence asthma severity and progression due to some factors like gastroesophageal reflux disease (GERD) [47] and static lung volume changes, mainly lower functional residual capacity [48,49].

Our SPO was the group with the worst response to both oral corticosteroid treatment and bronchodilator. The lung function impairment in this group was characterized, not only by large airway involvement (FEV1), but also by significant small airway commitment (airway resistance, airway trapping, and heterogeneity of lung function). Air trapping measured both by HRCT [16] and plethysmography [50], had already been related to persistent obstruction in severe asthma. We were able to go further in the assessment of distal airways by using single breath washout nitrogen maneuvers. These are important data because several studies had already demonstrated the relationship between small airway involvement and poor asthma control as well as high exacerbation rates [51–53].

The SPO subgroup had thicker bronchi, suggesting a more pronounced remodelling process. HRCT imaging may reflect histopathological features related to airway remodeling. We were able to demonstrate that bronchial thickness was associated with airway smooth muscle hypertrophy. We had previously shown the relationship between persistent obstruction phenotype and a more proliferative airway smooth muscle behavior, induced by the evidence of less periostin, TGF-b+ cells and mucosal chymase+ mast cells in bronchial biopsies [15]. In this scenario, thorax HRCT emerges as a non-invasive, informative, and useful tool to assess severe asthmatic patients. Kaminska et al [54] and Zhang et al [55] also demonstrated the correlation between persistent obstruction phenotype with airway smooth muscle hypertrophy. However, they were not able to discriminate this phenotype by using CT imaging and showed distinguishable inflammatory sputum profile compared with severe asthmatic patients with reversible airflow obstruction. In our study, the pathological features related to persistent obstruction were unaccompanied by increases in extracellular matrix structural components or a different inflammatory cell profile. Berair et al [40] recently published similar results confirming the association of the fixed obstruction phenotype with airway smooth muscle hypertrophy, lack of significant inflammation, and the possibility of using HRCT as a surrogate marker of airway remodeling.

Our study has some limitations. The small sample size in the subgroups could make the study underpowered to demonstrate a statistical difference in all variables according to their severity. This was a single-center study, and we caution extrapolating our data. Particular features of our population may not be reflected in other cohorts, although our baseline patient profiles are similar to other severe asthmatic populations [25,40,55]. To delineate a systematic protocol, we chose a fixed dose of prednisone, which may not have achieved the higher anti-inflammatory effect, related to some patients' particularities, such as obesity or corticosteroid resistance [46,48]. Another point that stands out refers to the fact that, at the time when the study was designed, additional treatments for severe asthmatic patients, such as *long-acting muscarinic* receptor *antagonists (*LAMAs), and none of the biologicals were approved for use in asthmatic patients. We used quantitative HRCT measurements and biopsy samples, which are not used in clinical practice. However, these consistent results provide knowledge for severe asthma characterization that may be translated into valuable tools in clinical practice. Finally, we were not able to assess air trapping by HRCT because we did not perform expiratory volumetric scans.

Conclusion

In summary, a high proportion of our patients remained with persistent airflow limitation and poor symptom control after regular treatment and close follow-up. Stratification of patients according to the degree of airway obstruction, as non-persistent, moderate, or severe, was able to not only discriminate severity according to lung function but also to demonstrate treatment response, which has great value in clinical practice. Interesting features marked the severe persistent obstruction patients characterized by greater airway resistance and air trapping, higher small airway heterogeneity, and no oral corticosteroid or bronchodilator response. The main component that explains our findings is airway remodeling characterized by bronchial thickness that could be assessed by thorax HRCT, a specific phenotype. This may have an important impact in the clinical decision when to treat a patient with severe asthma, guided by previous risk factors related to obstructive phenotype, indicting worse clinical evolution and acquired exacerbation, and that possibly does not change but may be linked to a worse prognosis. New studies to evaluate the response to different available therapeutic options should be performed in this specific phenotype to confirm our results.

Declarations

Acknowledgments:

The authors thank Dr. Dany Jasinowodolinski for collaboration in HRCT data analysis.

The study was funded by the FAPESP (grant No. 05/58757-3)

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions:

R.M.C.P., A.C., K.F.R., R.S., T.M. and M.D. equally contributed to the study conception and design. Data collection and analysis were performed by R.M.C.P. Data analysis and interpretation were performed by R.M.C.P., A.C., K.F.R., R.S. Analysis and interpretation of clinical and HRCT data was performed by R.A.A.. S.R. performed the analysis and interpretation of clinical data. F.L.A.F. performed analysis and interpretation of clinical and pulmonary functional data. T.M. and M.D. contributed with acquisition, analysis and interpretation of pathology data. D.S.F. contributed with analysis and interpretation of pathology data. R.M.C.P., A.C., K.F.R., R.S., T.M., M.D., R.A.A., S.R., F.L.A.F., D.S.F. contributed drafting the article and revising it critically. R.M.C.P., A.C., K.F.R., R.S., T.M., M.D., R.A.A., S.R., F.L.A.F. approved the final version.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1
Demographic and clinical characteristics at baseline.

	NPO	PO		
	(n=14)	All (MPO + SPO - n=48)	MPO (n=36)	SPO (n=12)
Sex, F/M, n (%)	12/2 (86/14)	34/14 (70/30)	27/9 (75/25)	7/5(58/42)
Age – yr #	42.0 ± 2.9	45.5 ± 1.5	44.7 ± 1.9	48.5 ± 3.6
Onset age #	13.2 ± 3.5	13.2 ± 2.0	13.8 ± 2.4	8.7 ± 3.8
Asthma duration #	29.1 ± 4.0	32.6 ± 2.3	31.2 ± 2.8	40.2 ± 3.7 §
BMI kg/m2 ¶	26.9 ± 5.5	30.7 ± 6.1	30.3 ± 6.6	31.9 ± 5.1 ***
Baseline FEV1 (L)	1.96 ± 0.51	1.55 ± 0.59	1.69 ± 0.55	1.14 ± 0.49 ***†
Baseline FEV1 (%)	65.2 ± 18.4	48.6 ± 14.8*	51.8 ± 13.9**	34.9 ± 12.2 ***†
IC dose mcg ¶	1429 ± 302	1396 ± 319	1333 ± 313	1533 ± 334
OC n (%) / dose mg ¶	4 (21.1) /20 ± 0	15 (31.2) / 22 ± 13	9 (47.4) / 28 ± 16	6 (31.6) / 15 ± 4.5

PO: persistent obstruction; NPO: Non Persistent Obstruction; MPO: Moderate Persistent Obstruction; SPO: Severe Persistent Obstruction; BMI: body mass index; IC: inhaled corticosteroid (budesonide); OC: oral corticosteroid (prednisone); $p < 0.05$ *NPO vs. PO; **NPO vs. MPO; ***NPO vs. SPO; † MPO vs. SPO; § $p = 0.05$ NPO vs. SPO; # mean ± Std Error; ¶ mean (SD).

Table 2
Asthma control, inflammatory (induced sputum eosinophils) and functional values of NPO, MPO, and SPO subgroups during standard treatment.

	NPO (n=14)			MPO (n=36)			SPO (n=12)		
	B	W2	W12	B	W2	W12	B	W2	W12
ACQ-7 score	2.89 ± 1.05	1.75 ± 0,94 *	2.11 ± 1.09	3.36 ± 0.94 §	2.39 ± 1.09 * §	2.07 ± 0.88 ‡ §	3.68 ± 1.34 ¶ #	3.10 ± 1.13 ¶ #	2.96 ± 1.22 ¶ #
EOS - IS (%)	28 ± 6	7 ± 2 *	18 ± 4 **	13 ± 2 §	5 ± 1 * §	14 ± 3 ** §	8 ± 3 ¶ #	10 ± 3 ¶ #	7 ± 3 ¶ #
FVC (%)	79.21 ± 17.10	95.64 ± 10.62 *	91.36 ± 11.55	66.00 ± 16.14 §	75.14 ± 13.50 * §	70.84 ± 17.05 §	51.58 ± 16.40 ¶ #	55.17 ± 19.08 ¶ #	54.25 ± 13.12 ¶ #
FEV1 (L)	1.96 ± 0.51	2.34 ± 0.45*	2.14 ± 0.50**	1.40 ± 0.50	1.70 ± 0.50* §	1.61 ± 0.64‡ §	0.96 ± 0.39 ¶ #	1.08 ± 0.52¶	1.07 ± 0.48¶#
Raw (%)	208 ± 82	105 ± 38 *	167 ± 76 **	326 ± 124 §	246 ± 119 * §	308 ± 107 §	343 ± 107 ¶	330 ± 156 ¶	375 ± 164 ¶
RVTLC	43 ±11	36 ± 9 *	43 ± 9 **	51 ± 8 §	46 ± 10 *	49 ± 12	59 ± 7 ¶ #	56 ± 10 #	59 ± 9 #
DLCO (%)	108 ± 26	115 ± 18 *	106 ± 11	107 ± 20	111 ± 15	106 ± 24	93 ± 15	96 ± 24	108 ± 30

ACQ: asthma control questionnaire; DLCO: carbon monoxide-diffusing capacity; EOS: eosinophils; IS: induced sputum; FVC: forced vital capacity; FEV1: forced expiratory volume in first second; Raw: airway resistance; RV: residual volume; RV/TLC: residual volume/total lung capacity relation; TLC: total lung capacity. B: baseline; W: week

$p < 0.05$; * B vs. W2; ** W2 vs. W12; ‡ B vs. W12; § NPO vs. MPO; ¶ NPO vs. SPO; # MPO vs. SPO.

B vs. W2 vs. W12: Wilcoxon Signed Rank Test; NPO vs. MPO vs. SPO: ANOVA - Tukey HSD& Std Error

Table 3
HRCT measurements stratified according to lung function response.

	NPO	PO		
	(n=14)	All (MPO + SPO n=48)	MPO (n=36)	SPO (n=12)
RB1 wall thickness (mm)	1.6 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	1.7 ± 0.1
RB1 wall area (mm2)	36.0 ± 14.4	32.2 ± 9.7	31.7 ± 10.0	36.1 ± 6.6
RB1 wall area %	64.7 ± 3.7	68.2 ± 4.4*	67.7 ± 4.9	69.4 ± 2.7§
Pi10 (mm)	3.9 ± 0.1	4.0 ± 0.2	4.0 ± 0.2	4.1 ± 0.2§
RB1: Right upper lobe apical segmental bronchus; Pi10: airway with 10mm lumen perimeter. $p < 0.05$; *NPO vs. PO; §NPO vs. SPO.				

Figures

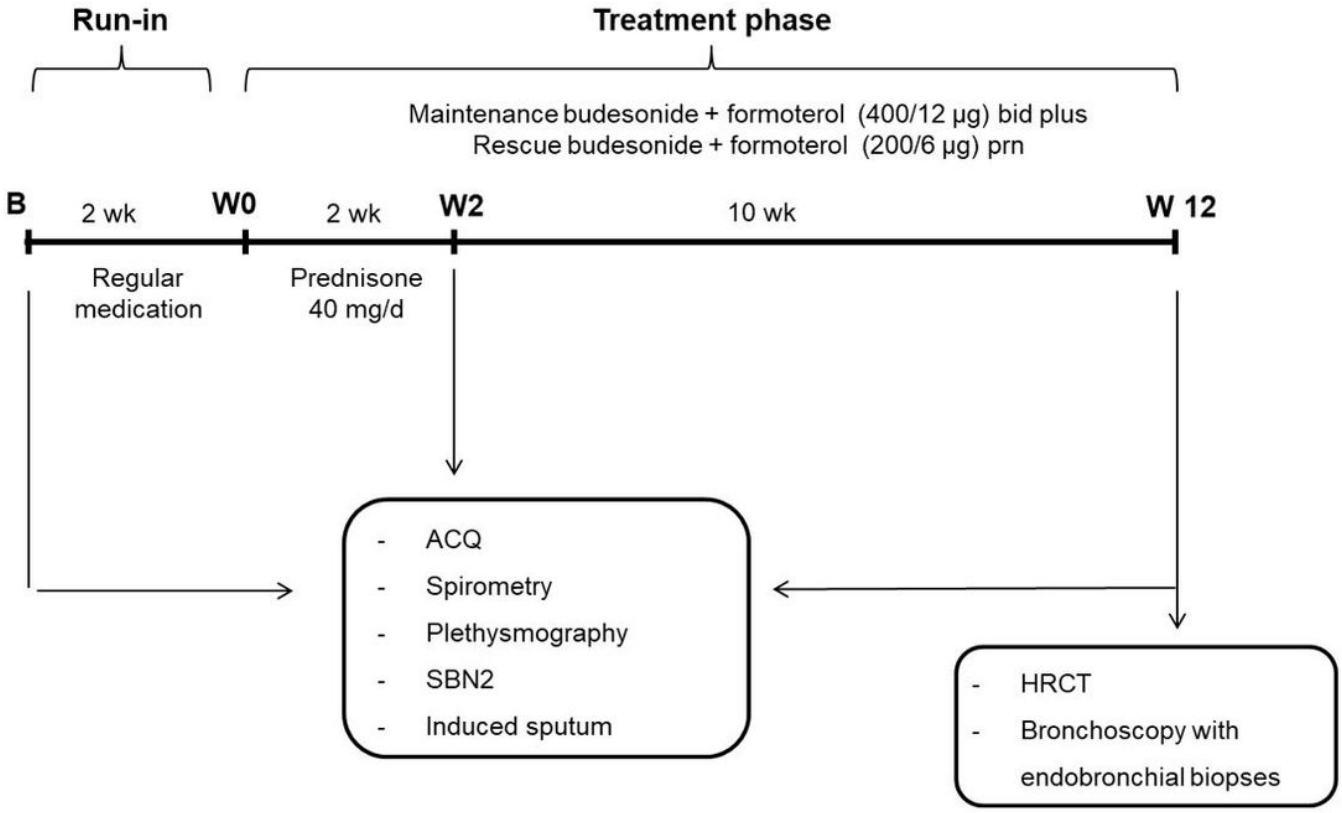


Figure 1

Study design. ACQ: asthma control questionnaire; SBN2: Single Breath N2 washout; HRCT: high resolution computed tomography; B: baseline; bid: twice a day; prn, as needed; W, week.

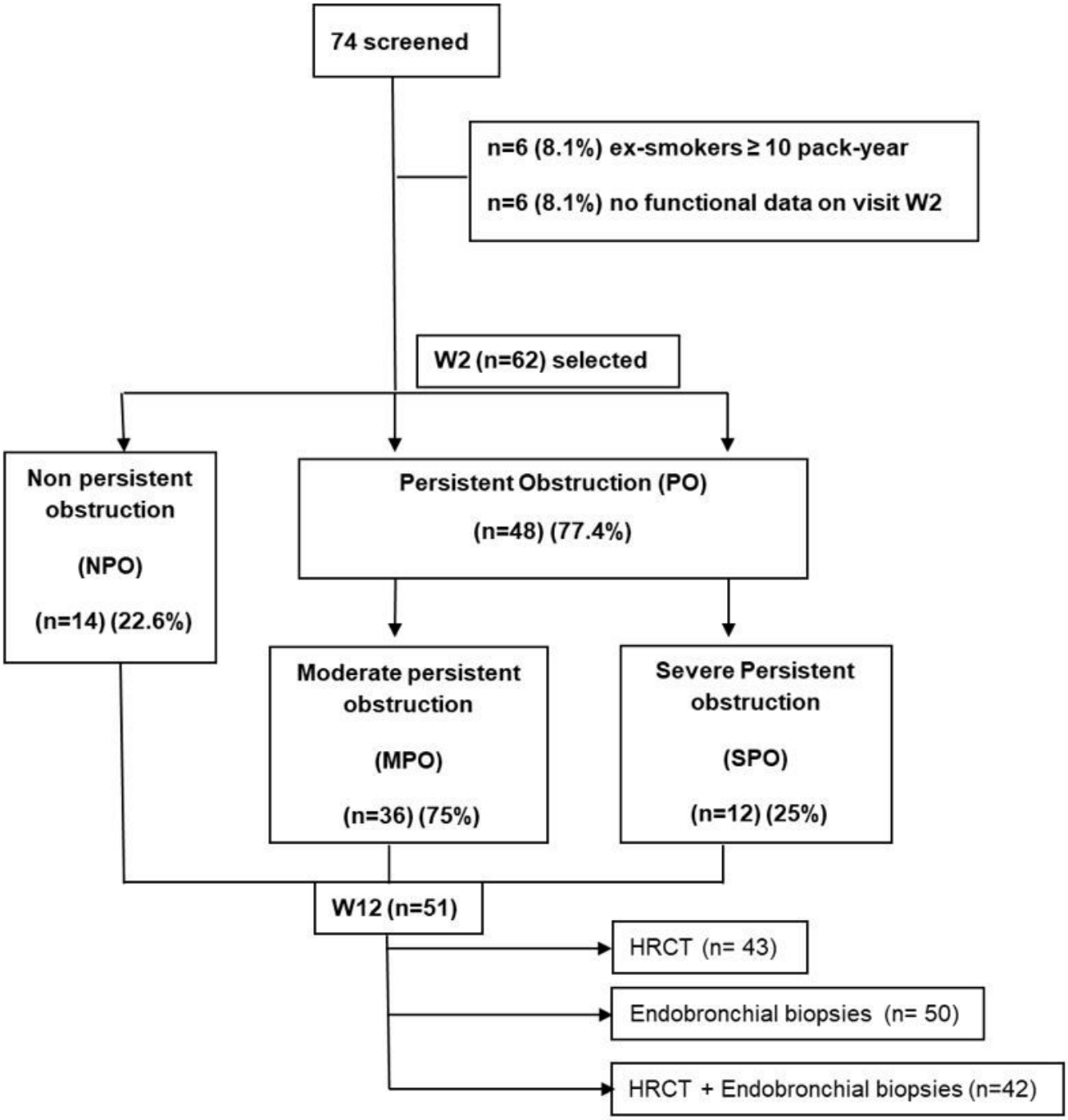
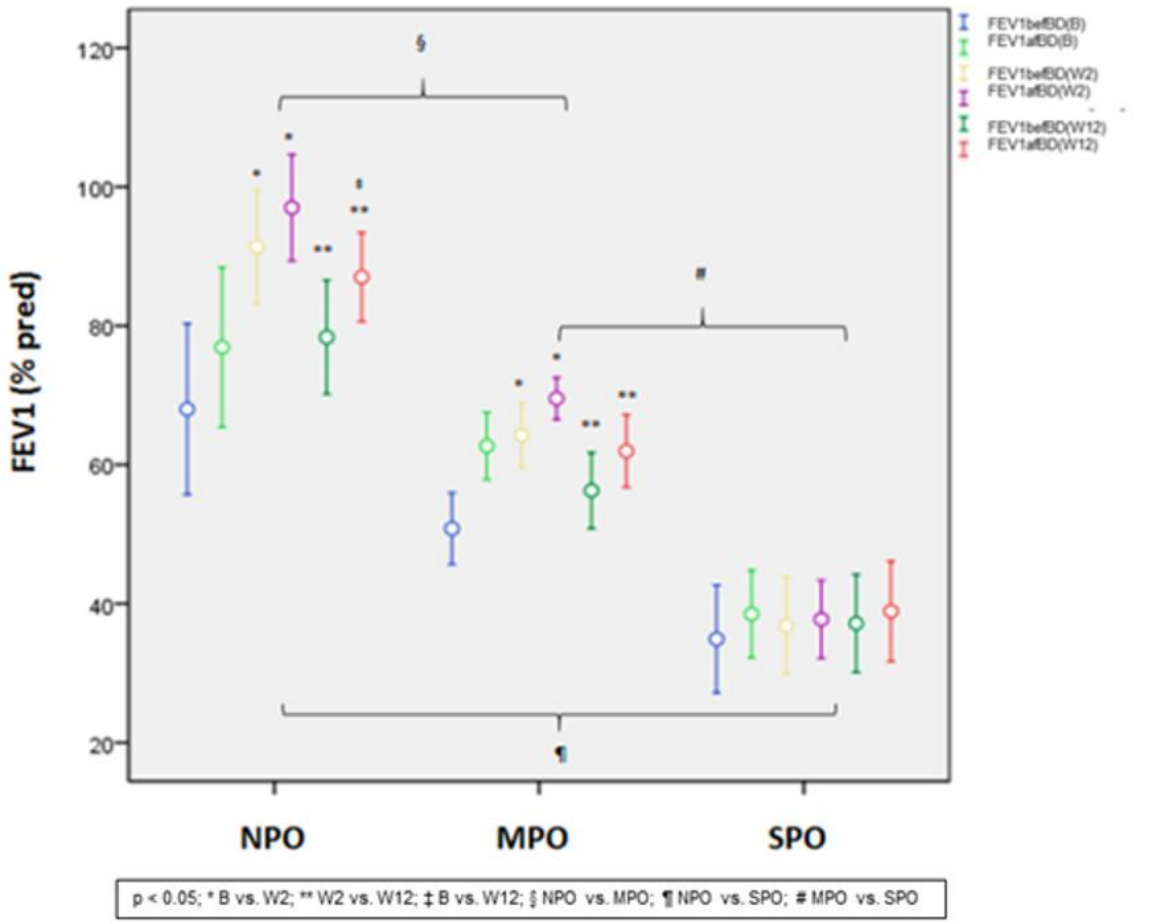


Figure 2

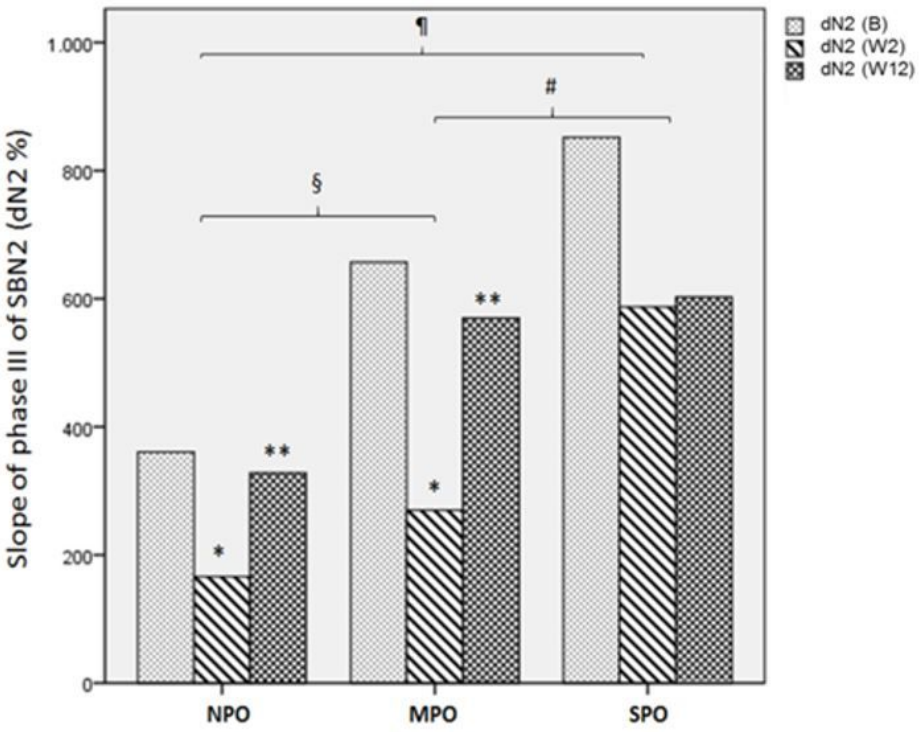
Flow chart showing the number of patients in each study group. B: baseline; W: week.



NPO: Non Persistent Obstruction; MPO: Moderate Persistent Obstruction; SPO: Severe Persistent Obstruction; FEV1: forced expiratory volume in 1 second; bef: before; aft:after; BD: bronchodilator; W:week

Figure 3

FEV1 (% predicted) before and after bronchodilator (BD) at baseline (B), regular treatment (CI + LABA + OC +SABA) (W2), and at the end of the treatment (W12).



NPO: Non Persistent Obstruction; MPO: Moderate Persistent Obstruction; SPO: Severe Persistent Obstruction
$p < 0.05$; * B vs. W2; ** W2 vs. W12; § NPO vs. MPO; ¶ NPO vs. SPO; # MPO vs. SPO
SBN2: Single Breath N2 washout test; dN2: nitrogen washout; W: week

Figure 4

Nitrogen washout (dN2) %.

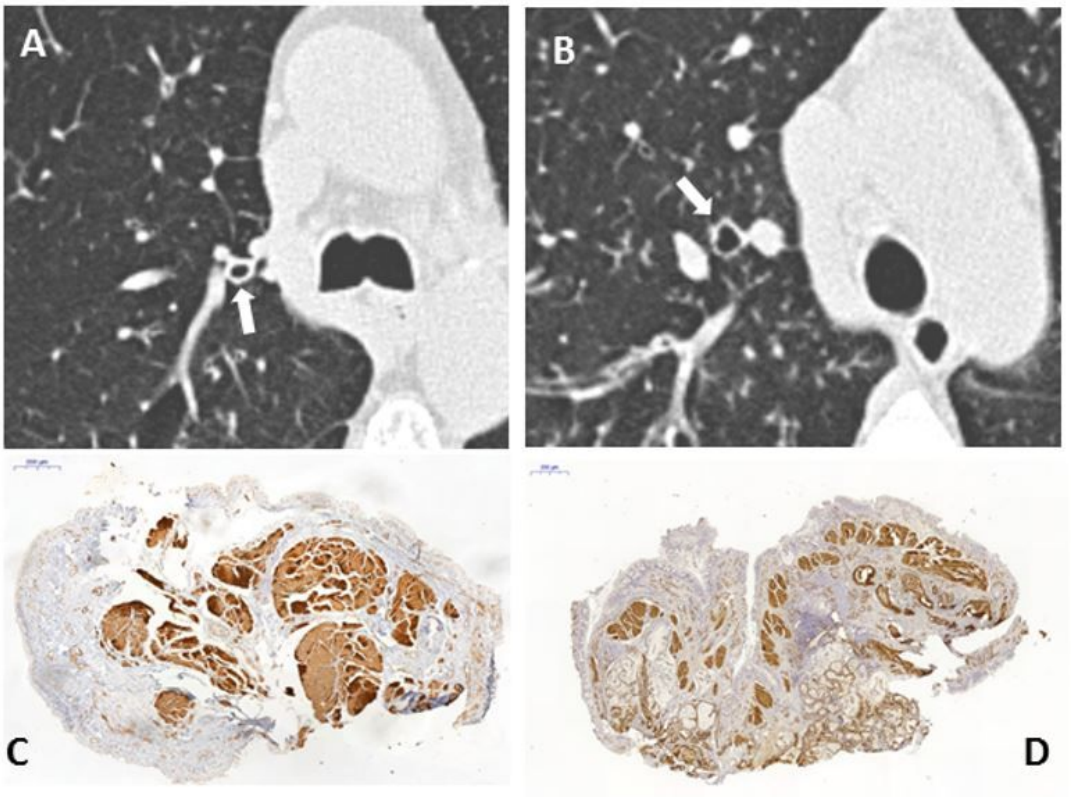


Figure 5

HRCT (A and B); endobronchial biopsies (C and D).