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Clinical characterization and predictors of IOS-defined small airway dysfunction in asthma.

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The authors have no financial or non-financial potential conflicts of interest to declare related to this project. Mrs. Licini acquired the data. Dr. Cottini and Dr. Lombardi conceived the study, while Dr. Cottini and Dr. Berti designed the study. Dr. Cottini had full access to all of the data in the study and takes responsibility for the integrity of the data; Dr. Berti takes responsibility for the accuracy of the data analysis. Dr. Cottini and Dr. Berti drafted the article. All the authors were involved in the writing and editing of the manuscript, and approved the final version to be published

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ABBREVIATIONS: SAD: Small airway dysfunction; FeNO: Fractional exhaled nitric oxide; BMI: Body Mass Index; FEV1: Forced expiratory volume in the first second; FVC: forced vital capacity; FEF25-75: Forced Expiratory Flow Between 25% and 75%; R5-R20: Resistances at 5 and 20 Hz; X5: Reactance at 5 Hz; Ax: Reactance area; FRes: Resonant frequency in Hz; ICS: inhaled corticosteroids; LABA: Long-acting β adrenoceptor agonist; LAMA: Long-acting muscarinic antagonist.

50

51 **ABSTRACT**

52 **Background:** The involvement of small airways has recently gained greater recognition in asthma.
53 Impulse oscillometry (IOS) is a simple and noninvasive method based on the forced oscillation
54 technique, for the detection of small airway dysfunction (SAD).

55 **Objective:** We aimed to identify the predictors of SAD in an unselected sample of 400 patients with
56 physician-diagnosed asthma.

57 **Methods:** All patients underwent standard spirometry and IOS at the first visit, and were stratified
58 by the presence of SAD defined by IOS (fall in resistance from 5 to 20 Hz [$R5-R20$] $>0.07\text{kPa}\times\text{s}\times\text{L}^{-1}$). Univariable and multivariable analyses and classification-tree method were
59 used to analyze cross-sectional relationships between clinical variables and outcome (SAD).
60

61 **Results:** SAD was present in 62% of the cohort. Subjects with SAD showed a less well-controlled
62 asthma, according to the Global Initiative for Asthma (GINA) definition, and a higher mean inhaled
63 corticosteroid (ICS) dosage use compared to subjects without SAD (both $p<0.001$).

64 Increased FeNO (OR 2.05, 95% CI 1.14-3.70), female sex (OR 2.27, 95% CI 1.29-4.06), smoking
65 (OR 3.06, 95% CI 1.60-6.05), older age (OR 3.08, 95% CI 1.77-5.49), asthma-related night
66 awakenings (OR 3.34, 95% CI 1.85-6.17), overweight (OR 3.64, 95% CI 1.99-6.85) and exercise-
67 induced asthma symptoms (EIA) (OR 6.39, 95% CI 3.65-11.45) resulted independent predictors of
68 SAD. Classification-tree analysis confirmed that EIA, overweight, asthma-related night
69 awakenings, smoking and older age have potential for clinical use in distinguishing patients with
70 SAD from those without it.

71 **Conclusion:** We identified predictors of SAD and showed that especially EIA, overweight, asthma-
72 related night awakenings, smoking, and older age were strongly associated with SAD.

73

HIGHLIGHTS BOX**What is already known about this topic?**

Small-airways dysfunction (SAD) is associated with worse control of asthma, higher numbers of exacerbations, the presence of nocturnal asthma, more severe bronchial hyperresponsiveness, exercise-induced asthma, and the late-phase allergic response.

What does this article add to our knowledge?

Patients with SAD have a worst asthma control and use higher inhaled corticosteroid dosage. Among others, exercise-induced asthma symptoms, overweight, asthma-related night awakenings, smoking and older age are strong predictors of SAD in patients with community-managed asthma.

How does this study impact current management guidelines?

SAD is present in almost two-third of community-treated patients, especially in those with worst asthma control. IOS for the detection of SAD should be performed; if not available, risk factors for SAD should be investigated during clinical history collection.

INTRODUCTION

Asthma is a chronic and heterogeneous condition affecting the airways [1], characterized by inflammatory infiltration and remodeling of the bronchial tree [1-5]. Recently, the involvement of small airways (internal diameter less than 2 millimeters), gained greater recognition in this disease [6-8]. It has been estimated that approximately 75% of the small airways must be obstructed before these changes can be detected using standard spirometry [9,10]. Moreover, recent studies suggest that the peripheral airway obstruction, usually known as small airways dysfunction (SAD), can greatly worsen the clinical expression of asthma [11-13], and associate with more severe bronchial hyper-responsiveness, worse asthma control and a higher number of exacerbations [14]. SAD seems to be common among asthmatic patients, and has been linked to the presence of nocturnal symptoms, active smoking, older age, fixed airflow obstruction, allergic or exercise-induced asthma [15-17].

Overall, conventional spirometry measurements, as forced expiratory volume in 1 second (FEV1), peak expiratory flow, and FEV1/forced vital capacity (FVC) ratio, reflect mostly variability or reversibility of airway obstruction and are unable to sensitively evaluate small airways [9,10,13,17]. There has been an extensive search for physiological methods able to accurately detect changes in the small airways compartment. IOS is a simple and noninvasive method based on the forced oscillation technique, requiring minimal patient cooperation [18-20]. Essentially, IOS perturbs the respiratory system during tidal breathing by using a series of pressure oscillations over a range of frequencies (typically 5-35Hz) applied to the mouth [18-20]. Recently, a multinational study of small airways dysfunction markers in adult asthma identified that the difference between the R5 and R20 (R5-R20) was the IOS-measured marker that, among several small airway physiological markers, most strongly correlated with small airways disease. [15]. Thus, the contribution of the distal airways may be appropriately determined by the R5-20, as already performed in asthmatic patients in clinical trials and hospital cohorts [15, 21-23].

However, IOS is not routinely performed in clinical practice, and this may result in a reduced identification of SAD in asthmatic patients. Therefore, in this study we aimed to better characterize SAD, assessed by IOS, in an unselected sample of patients with physician-diagnosed, community-treated asthma, by the identification of the baseline clinical predictors of this condition.

MATERIAL AND METHODS

Patients and study design

This is a cross-sectional analysis of a single-centered, observational study on 400 adult (≥ 18 years old) asthmatic community-treated patients, consecutively recruited between January 1st, 2017 and March 1st, 2018. In this context, all the patients with stable asthma (without new or worsening symptoms of wheezing, breathlessness, chest tightness, and coughing) at the time of the visit and during the 4 weeks before were recruited. Patients fulfilling the criteria for asthma/chronic obstructive pulmonary disease overlap syndrome were excluded (24). All patients underwent to standard spirometry, IOS and Fractional exhaled nitric oxide (FeNO) measurements at the same initial screening visit in our secondary care asthma clinic. Demographic parameters, clinical features (i.e. concomitant atopy, asthma duration, eosinophil count, etc.), asthma therapy and control as defined by Global Initiative for Asthma (GINA) guidelines [24] at first visit were recorded. The classification in GINA stepwise categories was based for each patient on controller medications and doses [24]. GINA assessment of asthma control includes the assessment of the following symptoms in the 4 preceding weeks: presence of daytime asthma symptoms more than twice a week, nighttime asthma symptoms, activity limitation and use of short acting β_2 -agonist more than twice a week. The resulting asthma control is classified as well controlled (WC; no symptoms), partially controlled (PC; 1-2 symptoms), and uncontrolled (UC; ≥ 3 symptoms) [24]. The most recent eosinophil count during the 2 years before the first assessment was also collected.

Patients were defined atopic if a positive skin prick test to aeroallergens or specific IgE on peripheral blood and symptoms consistent with allergic asthma were present (i.e. asthma triggered by aeroallergens). If atopic background was not known, patients underwent skin testing with a common standardized allergen battery of indoor and outdoor allergens, as recommended for clinical use and research in Europe [25]. For details, see the **Online Repository**.

Extra-fine inhaled corticosteroids (ICS) reported were (with particle mass median aerodynamic diameter): Beclomethasone dipropionate/Formeterol pMDI (1.5 μm), Beclomethasone dipropionate/Formeterol DPI (1.5 μm), Ciclesonide pMDI HFA solution (1.1 μm), Beclomethasone dipropionate pMDI HFA solution (1.1 μm), Tiotropium Respimat SMI (2 μm) [26,27]. This study was approved by the local Institutional Review Board (IRB number NP3364). All patients gave written informed consent for their data to be stored electronically.

The study was conducted according to STROBE guidelines (STrengthening the Reporting of Observational Studies in Epidemiology) for cohort, case-control, and cross-sectional studies.

Spirometry and Oscillometry Measurements

A Vyntus™ PNEUMO - PC Spirometer (VyAire Medical) was used to perform spirometry in triplicate in accordance with European Respiratory Society guidelines [28]. For details, see **Online Repository**.

Impulse oscillometry (Masterscreen IOS/Sentry Suite, VyAire Medical) was performed in triplicate in accordance with manufacturer's and European Respiratory Society guidelines [20,29]. IOS is an effort-independent test: IOS propagates a train of bidirectional, harmonic sound waves along the bronchial tree. In essence, IOS can be considered a "bronchial sonar" [19,20]. We used the MasterScreen IOS Impulse Spirometry sold by VyAire Medical and the manufacturer's

recommended equations. For spirometry, we used the Global Lung Function (GLI) Network standardized lung function reference values. The IOS system is routinely calibrated, as suggested by the manufacturer [30]. For details, see **Online Repository**.

Respiratory resistances at 5 and 20 Hz (R_5 and R_{20} , in $\text{kPa} \times \text{s} \times \text{L}^{-1}$) were used as indices of total and proximal airway resistance, respectively. The fall in resistance from 5 to 20 Hz (R_5 – R_{20} , in $\text{kPa} \times \text{s} \times \text{L}^{-1}$) was considered to be an index for the resistance of peripheral airways, and an R_5 – R_{20} cutoff of $>0.07 \text{ kPa} \times \text{s} \times \text{L}^{-1}$ (a conservative upper limit of normal for R_5 – R_{20} as previously reported [21,22]) was chosen to define the presence of SAD. Moreover, the following variables were collected: reactance at 5 Hz (X_5 , in $\text{kPa} \times \text{s} \times \text{L}^{-1}$) reflecting elastic recoil of the peripheral airways; resonant frequency (F_{res} , in Hz), defined as the frequency at which the inertial properties of airway and the capacitance of lung periphery are equal; and reactance area (AX , the area under the reactance curve, in kPa/L), reflecting the elastic properties of the lung periphery and shown to be correlated with resistance at lower frequencies[19,20,22].

FeNO was performed in duplicate using HypAir FeNO (MediSoft Belgium), at a standard flow rate of 50 mL/second, according to manufacturer's and American Thoracic Society guidelines [31].

Statistical Analysis

Data were examined for normality of distribution, when appropriated. All ICS doses were converted to standard beclomethasone dipropionate potency equivalents for the purposes of statistical comparisons between groups.

Categorical data were summarized as percentages; significant differences or associations were analyzed using the X^2 test or Fisher exact tests. Continuous variables were presented as mean $\pm$ standard deviation (SD) or median and interquartile range (IQR), depending on normality

demonstrated by Kolmogorov–Smirnov test. Comparisons were performed with either Student’s t-test for independent samples (2-tailed) or with analysis of variance (ANOVA) comparisons with Bonferroni correction when more than 2 means were being compared. For SAD analyses, independent variables that appeared to have statistical significance in the univariable analysis were included in a multivariable logistic regression model using a backward stepwise method. The odds ratios (OR) and their 95% confidence interval (CI) obtained in the regression analysis were calculated. For the purpose of this analysis, continuous variables associated with SAD were converted in a binary variable (age>50 years old, BMI>25kg/m², asthma duration>15.0 years, eosinophils count>300mm³, FeNO>25 ppb, and inhaled corticosteroid dosage>1000 micrograms of Beclometasone equivalent). GINA levels of asthma control were dichotomized in uncontrolled *versus* well and partially controlled asthma categories.

Additionally, a classification tree analysis was performed in order to predict a categorical response (i.e. IOS-defined SAD yes/no) as a function of potential predictor variables (i.e. variables that appeared to have statistical significance in the univariable analysis) using recursive partitioning. A validation with an independent test sample within the database was then used to assess how many such partitions to adopt.

All the analyses were performed using JMP Pro package (SAS Institute Inc., Cary, North Carolina), and a *p-value of <0.05* was considered statistically significant.

RESULTS

Characteristics of the patients with and without SAD

Baseline demographics and clinical features for 400 asthmatic, community-treated subjects categorized according to the presence of SAD are shown in **Table 1**. Overall, subjects in this cohort were mostly Caucasians (n=397). Subjects with SAD were more likely to be female, older, overweight, non-atopic, current or former heavy smoker, to have longer asthma duration and higher levels of serum eosinophils and FeNO. Not only FEF₂₅₋₇₅ (p<0.0001), but also FEV1 and FEV1/FVC were reduced in higher proportions in patients with SAD (p<0.0001 and p=0.0181, respectively). Patients with SAD had a partially controlled asthma in 45.5% of cases and an uncontrolled asthma in 40.7% of cases, whereas most of the patients without SAD had well controlled (59.7%) or partially controlled asthma (37.0%). Furthermore, higher proportions of subjects with SAD had surrogates of a scarce asthma control compared to patients without SAD, namely exacerbation history (p<0.0001), emergency room visits (p<0.0001) and hospitalization for asthma symptoms (p=0.0023), night awakenings due to asthma and exercise-induced asthma symptoms (both p<0.0001). Patients were similarly distributed among GINA steps, but subjects with SAD used a higher daily dose of ICS (544±336 *versus* 386±267 micrograms of beclomethasone equivalents). Compared to patients without SAD, patients with SAD were less likely treated with extra-fine inhaled therapy (ICS and ICS+LABA) (16.3% *versus* 60.4%) and more frequently treated with anti-leukotriene agents (20.0% *versus* 6.5%).

By standard spirometry, approximately one third of patients had larger airways involvement (as documented by an altered FEV1/FVC ratio<LLN). After the exclusion of these patients, when considered “isolated” SAD (i.e. SAD without central airway involvement, thus with a FEV1/FVC≥LLN, n=178), SAD was present in 31.5% (n=70) according to IOS definition, compared to 10.5% (n=23) according to standard spirometry (FEF₂₅₋₇₅<60). Detailed standard spirometry and IOS measurements of subjects with SAD and without it are summarized in **Table 2**.

229

230 **Correlations between IOS measurements, asthma control and standard spirometry features**

231 Since asthma control categories had different prevalence of SAD, with SAD being more
 232 prevalent in less-controlled asthma, we analyzed and showed that all IOS measurements, namely
 233 R5, X5, R5-R20, Ax, Fres, progressively worsened with the aggravation of asthma control
 234 categories (**Table 3**). SAD was present in virtually all the asthmatic patients with UC, in two third
 235 of those with PC and one third of those with WC.

236 **Supplementary Table 1** shows the coefficients of correlations between all standard
 237 spirometry and IOS indices for this cohort of patients. When looking at the correlations between
 238 FEF₂₅₋₇₅ and R5–R20, the standard spirometry and IOS indices conventionally used to assess
 239 peripheral airways resistance, we found a very weak inverse correlation between FEF₂₅₋₇₅ and R5–
 240 R20 ($r=-0.28$, $p<0.0001$). When patients were stratified for FEV1/FVC $\leq 70\%$, and for or FEV1
 241 $\leq 80\%$, we found no correlations between R5–R20 and FEF₂₅₋₇₅, with the exception of the group
 242 with FEV1/FVC $<70\%$ showing a weak, negative correlation ($r=0.36$, $p<0.001$, *not shown*).

243

244 **Baseline predictors of SAD and classification tree analysis**

245 Using logistic regression analysis, we calculated the probability of having SAD for each
 246 variable resulted statistically significant in **Table 1**, which was thus included in the model.
 247 Univariable and multivariable analysis are shown in **Table 4**. By multivariable analysis, increased
 248 FeNO (OR 2.05, 95% CI 1.14-3.70), female sex (OR 2.27, 95% CI 1.29-4.06), current or former
 249 heavy smoker status (OR 3.06, 95% CI 1.60-6.05), older age (OR 3.08, 95% CI 1.77-5.49), night
 250 awakenings due to asthma (OR 3.34, 95% CI 1.85-6.17), overweight (OR 3.64, 95% CI 1.99-6.85),
 251 and exercise-induced asthma symptoms (EIA) (OR 6.39, 95% CI 3.65-11.45) resulted independent

252 predictors of SAD. Of note, EIA resulted the strongest predictor of SAD, associated with almost
253 6.4-fold higher likelihood of having SAD.

254 The results of classification tree analysis using the binary outcome variable SAD yes/no are
255 presented in **Figure 1**. The first partition produced two subgroups with respective SAD incidences
256 of 87% (presence of EIA) and 34% (absence of EIA). These latter subgroups were further
257 partitioned by the presence of overweight within the EIA group (with an incidence of SAD in the
258 resultant subgroups of 96% and 79%, respectively) and night awakenings due to asthma within the
259 group without EIA (with an incidence of SAD in the resultant subgroups of 69% and 34%,
260 respectively). Additional divisions resulted from the use of extra-fine ICS (with or without
261 additional beta-agonists); at least one emergency room visit due to asthma before the clinical
262 assessment; the smoking status; age > 50 years old; at least one asthma exacerbation despite therapy
263 before the clinical assessment.

DISCUSSION

In the current study, conducted on physician-diagnosed, community-treated asthma, we observed that subjects with SAD have a less controlled asthma and use a higher mean ICS daily dose compared to patients without SAD. In the multivariable analysis, increased FeNO, female sex, current or former heavy smoker status, older age, night awakenings due to asthma, overweight and EIA symptoms showed to be independently associated with the presence of SAD. Similar variables emerged from the classification tree analysis, providing a complementary approach to data analysis, and supporting -among others- EIA, overweight, night awakenings due to asthma, older age and smoking as the main predictors of SAD.

Our findings showed a worst control of asthma and a higher ICS dose used in patients with SAD compared to those without it, indirectly supporting the implication of SAD in asthma control. These results are consistent with previously published data, in which SAD was associated with a worse clinical expression of asthma [11-14] and the peripheral abnormalities persisted despite a greater daily dose of ICS, suggesting a little effect of current therapies on the airway structural abnormalities, or a poor peripheral delivery of inhaled therapy [32-34]. The sizes of ICS particles may affect the aerodynamic properties of the drugs and its delivery to distal airways, and extra-fine ICSs have significantly higher odds of achieving asthma control at significantly lower prescribed doses than non-extra-fine particles ICS [40-43]. In our study, only 16.3% of patients with SAD were treated with inhaled extra-fine therapy compared to 60.4% of patients without SAD; however, this association with extra-fine therapy was not further confirmed by multivariable analysis. The potential clinical implication on asthma control of receiving inhaled extra-fine therapy compared to standard particles size ICS and ICS/LABA still needs to be precisely defined [44].

Little is known about clinical features associated with SAD in asthmatic patients, and clinical predictors of SAD have never been investigated so far. Our findings allowed the identification of the baseline predictors that may help in distinguishing patients with SAD among

other asthmatic patients. By logistic regression, the independent predictors of SAD include some previously identified asthma risk factors, such as female sex (for late-onset asthma), overweight, smoking and markers of airway Th2-inflammation (FeNO); age higher than 50 years, night awakenings due to asthma and EIA were also found to be independently associated with the risk of SAD. Our results confirm and extend the current scarce and heterogeneous evidence on SAD predictors [16] that, to the best of our knowledge, were never systematically analyzed in a single study. Although a growing body of literature supports the role of IOS compared to other techniques for the assessment of small airways, in clinical routine practice IOS is only rarely used, and even less in community-based asthma management. Since SAD may affect asthma control, its identification by means of surrogates or predictors might be particularly important, especially when IOS cannot be performed.

Similar predictors emerged from the complementary classification tree analysis. Of note, EIA symptoms, overweight and night awakenings due to asthma, that resulted the stronger predictors of SAD from the multivariable analysis, were shown to be key variables in the decision tree analysis as well. This tree-structured method consists of dividing the sample such that, at each stage, the best variable (among those selected) that best partitions the data into homogeneous subgroups is determined. This affords a flexible nonparametric tool for performing exploratory data analysis and constructing simple, interpretable classification schemes, as previously described [45-46]. In our case, the classification tree analysis revealed that the presence of exercise-induced asthma symptoms represents the best baseline feature in terms of increasing the yield of subjects with SAD. The classification tree paradigm was then applied again to the two subgroups (presence *versus* absence of exercise-induced asthma symptoms); the best feature in terms of increasing the yield of asthmatic subjects with SAD and exercise-induced asthma symptoms was found to be overweight, while for asthmatic subjects with SAD and without EIA symptoms resulted the presence of night awakenings due to asthma. The classification tree paradigm was then applied

again to the following subgroups, leading to the identification of a group of variables that could be used to improve the efficiency of the screening for SAD in asthmatic patients. The best number of divisions was calculated by the system. Of note, 5 out of 7 predictors identified by multivariable analysis coincided with those emerged from the decision tree analysis (5 out of 8): EIA symptoms, overweight, night awakenings due to asthma, current or former heavy smoker status, and older age. Both the analyses showed that EIA was the most important factor associated with the presence of SAD, followed by overweight and night awakenings due to asthma. Specifically, the decision tree analysis showed that overweight asthmatics with EIA would have a 94% prevalence of SAD, and in those without EIA but with night awakenings due to asthma the prevalence of SAD would be around two-third of patients. Although it would be tempting to speculate that these factors are central to promote SAD development, the nature of our analysis does not allow us to provide clear causal explanations. On the contrary, it might be useful to investigate characteristic phenotypes of asthmatic patients with higher prevalence of SAD, that might be identified according to their clinical symptoms at the first medical evaluation (i.e. the overweight patient with EIA; the patient with night awakenings without EIA, etc.).

Overall, conventional spirometry measurements are usually considered unable to sensitively evaluate small airways [9,10,13,18]. The prospect of detecting lung disease at an early stage has led to widespread measurement of FEF_{25-75} but there is no consensus regarding the reliability of FEF_{25-75} in the assessment of small airways [35-38]. Although we observed good correlations between the parameters of IOS and standard spirometry in our study, FEF_{25-75} , correlated only weakly with $R5-R20$ overall and within the subset with $FEV1/FVC < 70\%$, suggesting that SAD may be assessed by standard spirometry only in most severe cases, and potentially overlooked in all the others. Although comparing the accuracy of IOS to standard spirometry was not the aim of our study, IOS sensitively captured SAD, as demonstrated by the fact that some asthmatic patients of our cohort have a normal spirometry. In detail, IOS was particularly useful to confirm the diagnosis of asthma

in those patients with asthmatic symptoms but with a normal standard spirometry, raising the diagnostic yield for SAD of more than 20%. This fact is not surprising, since physiologically, it has been estimated that obstruction of 75% of the small airways is required before changes can be detected by routine pulmonary function tests [6]. Under normal circumstances, small airways contribute only minimally to airway resistance, and for this reason they are known as the “quiet zone” [9,21]. Small airways are likely the key area that determines the transition from physiologic to pathophysiologic behavior of the bronchial tree in asthma [39].

Our study has some strengths and limitations. First, we defined asthma as diagnosed by physician, with the potential risk of over-diagnosis. However, this is the gold standard used for several real life studies [32, 40-41], and in most of the cases diagnosis was supported by a standard spirometry and/or methacholine challenge. Another limitation is the lack of data on treatment adherence and the cross-sectional design of the study. On the other hand, all the patients have been evaluated by the same pulmonologist expert in asthma using the same approach to disease management for all the patients. IOS, standard spirometry, and FeNO were performed, in accordance with European Respiratory Society guidelines, by the same pulmonary function technologist with the same instruments for all the patients, allowing a high internal comparability and reproducibility of our results. The utility of R5–R20 for the assessment of small airway involvement is not completely accepted [18]. However, we decided to rely on an altered R5-R20 as a marker of SAD because of the growing body of literature that used this index for the same purpose [9,12,13,15,16,32]. Furthermore, in our study R5-R20 appeared to correlate closely with that of X5, AX and Fres, representative markers of peripheral airway abnormalities, supporting R5-R20 as a surrogate of peripheral airway mechanics.

Finally, a limitation of the current study is the underrepresentation of milder (GINA step 2) and severe asthma phenotypes (GINA step 5), in contrast with the overrepresentation of the intermediate phenotype (GINA step 3). Nevertheless, this represents the epidemiology of the community-treated

364 asthma in Italy, and this should be taken into account when data are interpreted. Moreover, our
365 analysis lies on a large number of asthma patients with complete data on disease duration, allergic
366 sensitization and asthma control, which are very rarely reported in real life studies.

367 In conclusion, subjects with SAD had less controlled asthma and used higher mean ICS
368 dosage compared to patients without SAD. The independent predictors of SAD were identified;
369 among others, EIA, overweight, asthma-related night awakenings, older age and smoking resulted
370 strongly associated with SAD by means of two different statistical methods. Overall, these findings
371 may be of help in distinguishing subjects with SAD among asthmatic patients, especially when IOS
372 cannot be performed.

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FIGURE LEGENDS

Figure 1. Classification-tree analysis of predictors of SAD. The first partition is performed by the presence of exercise-induced asthma (EIA) symptoms, that produces two subgroups with respective SAD incidences of 87% and 34%. These latter subgroups are further split according to presence of overweight ($BMI > 25 \text{ kg/m}^2$, for the EIA group) and night awakenings due to asthma (in the group without EIA). The variables that further partition the data are: extra-fine ICS: Extra-fine inhaled corticosteroids (with or without beta-agonists); ER visits: (at least one) emergency room visit due to asthma exacerbation before the baseline visit (indicating a more severe exacerbation compared to “asthma exacerbation”); smoking: current or former smokers (≥ 10 pack/years); age > 50 years old; asthma exacerbation: (at least one) asthma exacerbation despite therapy before the baseline visit.

TABLES

Table 1. Features of the asthmatic patient cohort by the presence of small airway dysfunction

Patient Features	SAD 62% (n=246)	No SAD 38% (n=154)	P values
Demographics, clinical and laboratory features			
Female sex, %(n)	60.6% (149)	45.5% (70)	0.0038
Age, years, mean (SD)	55.4 (17.5)	44.6 (16.8)	<0.0001
BMI (kg/m ²), mean (SD)	26.4 (5.4)	23.4 (3.3)	<0.0001
Current or former smokers (≥10pack/years), %(n)	43.1 (106)	13.6 (21)	<0.0001
Asthma duration, years, median (25%-75% IQR)	15.0 (5.0, 25.0)	11.0 (4.8, 21.0)	0.0290
Presence of Atopy**	58.1% (143)	69.5% (107)	0.0258
Eosinophils, mm ³ , median (25%-75% IQR),	383 (210, 520)	215 (180, 412)	0.0002
FeNO, ppb, median (25%-75% IQR)	32 (16, 53)	20 (12, 38)	0.0002
Standard Spirometry			
FEV1 <80%, %(n)	32.5% (80)	11.7% (18)	<0.0001
FEV1/FVC ratio <70%, %(n)	34.2% (84)	22.7% (35)	0.0181
FEF25-75 <60%, %(n)	49.6% (122)	22.7% (35)	<0.0001
Asthma control			
GINA levels of asthma control			<0.0001
Well Controlled, %(n)	13.8% (34)	59.7% (92)	
Partially Controlled, %(n)	45.5% (112)	37.0% (57)	
Uncontrolled, %(n)	40.7% (100)	3.25% (5)	
Asthma exacerbations, % (n)	53.9% (132)	15.6% (24)	<0.0001
Emergency Room visit, % (n)	18.4% (45)	0% (0)	<0.0001
Hospitalization, % (n)	5.3% (13)	0% (0)	0.0023
Night awakenings due to asthma, % (n)	62.6% (154)	16.9% (26)	<0.0001
Exercise-induced asthma symptoms, % (n)	73.2% (180)	21.4% (33)	<0.0001
Therapy			
GINA steps			0.5232
Step 2	19.9% (49)	22.7% (35)	
Step 3	52.4% (129)	53.9% (83)	
Step 4	23.2% (57)	21.4% (33)	
Step 5	4.5% (11)	2.0% (3)	
ICS, %(n)	100% (246)	100% (154)	1.000
LABA/ICS, %(n)	79.7% (196)	77.3 % (119)	0.6159
Extrafine therapy, %(n)	16.3% (40)	60.4% (93)	<0.0001
ICS Dosage, micrograms, mean (SD)	1088 (672)	772 (552)	<0.0001
LAMA, %(n)	9.3% (23)	5.8% (9)	0.2572
Anti-leukotriene agents, %(n)	20.0% (49)	6.5% (10)	0.0001

Comparisons were analyzed with X² test, Fisher exact tests or Student's t-test for independent samples (2-tailed), where appropriate.

FeNO: Fractional exhaled nitric oxide; BMI: Body Mass Index; FEV1: Forced expiratory volume in the first second; FVC: forced vital capacity; FEF25-75: Forced Expiratory Flow Between 25% and 75%; ICS: inhaled corticosteroids; LABA: Long-acting β adrenoceptor agonist; LAMA: Long-acting muscarinic antagonist.

Table 2. Baseline standard spirometry and IOS characteristics of subjects by the presence of small airway dysfunction (defined as R5 – R20>0.07).

Patient Features	SAD	No SAD	<i>p</i> value*
Spirometry values, mean (SD)			
FEV1, (% predicted)	88.9 (19.4)	100.3 (16.6)	<0.0001
FEV1/FVC ratio, (x100)	76.3 (13.3)	80.0 (11.1)	0.0041
FEF25-75, (% predicted)	64.7 (31.6)	77.8 (29.4)	<0.0001
Impulse oscillometry values, median [25%-75% IQR]			
R5 – R20	0.16 [0.12, 0.23]	0.03 [0.018, 0.05]	<0.0001
R5	0.51 [0.43, 0.62]	0.30 [0.25, 0.37]	<0.0001
X5	-0.18 [-0.24, -0.14]	-0.09 [-0.12, -0.07]	<0.0001
Ax	1.69 [1.22, 2.80]	0.29 [0.15, 0.43]	0.0002
F _{Res}	23.46 [20.20, 27.44]	12.06 [9.21, 15.16]	0.0001

Spirometric values were represented as mean (SD) because of their normal distribution, while the values of the impulse oscillometry were represented as median [25%-75% interquartile range], since they were not normally distributed. Variables of the different groups were compared with parametric methods for spirometry values, and with non-parametric methods for impulse oscillometry values.

IQR: interquartile range; FEV1: Forced expiratory volume in the first second; FVC: forced vital capacity; FEF25-75: Forced Expiratory Flow Between 25% and 75%; R5-R20: Resistances at 5 and 20 Hz; X5: Reactance at 5 Hz; Ax: Reactance area; F_{Res}: Resonant frequency in Hz.

Table 3. Impulse oscillometry (R5 – R20, R5, X5, AX, and Fres) measurements in the whole cohort (n=400) divided by GINA control categories.

	UC (n=105)	PC (n=169)	WC (n=126)	P value*
R5	0.603 (0.220)	0.459 (0.175)	0.353 (0.137)	<0.0001
X5	-0.225 (0.116)	-0.169 (0.084)	-0.114 (0.053)	<0.0001
R5-20	0.217 (0.120)	0.126 (0.100)	0.061 (0.059)	<0.0001
Ax	2.656 (1.745)	1.319 (1.042)	0.750 (1.581)	<0.0001
Fres	24.010 (9.930)	18.617 (9.292)	13.807 (7.551)	<0.0001
Prevalence of R5-20>0.07	95.2%	66.3%	27.0%	<0.0001

*One-way ANOVA: cut-off for p value interpretation after Bonferroni correction: **0.017**

UC: uncontrolled asthma; PC: partially controlled asthma; WC: well controlled asthma; R5: Resistance at 5 Hz; R5-R20: Resistances at 5 and 20 Hz; X5: Reactance at 5 Hz; Ax: Reactance area; F_{Res}: Resonant frequency in Hz.

Table 4. Univariate and multivariate analysis of baseline predictors of small airways dysfunction in our asthmatic patient cohort. Multiple binary logistic regressions of all baseline variables associated with small airways dysfunction ($p < 0.05$) in Table 1.

Univariable Analysis				Multivariable Analysis		
Variables	OR	95% CI	<i>P value</i>	OR	95% CI	<i>P value</i>
Age, ≥ 50 years old	3.19	2.10, 4.88	<0.0001	3.08	1.77, 5.49	<0.0001
Sex, female	1.84	1.23, 2.78	0.0031	2.27	1.29, 4.06	0.0046
Overweight, BMI $> 25\text{kg/m}^2$	4.07	2.57, 6.54	<0.0001	3.64	1.99, 6.85	<0.0001
Smoking, former or current	4.80	2.89, 8.29	<0.0001	3.06	1.60, 6.05	0.0006
Presence of Atopy	0.61	0.40, 0.93	0.0217	-	-	-
Eosinophil count $> 300\text{ mm}^3$	2.62	1.62, 4.28	<0.0001	-	-	-
FeNO > 25 ppb	2.94	1.93, 4.50	<0.0001	2.05	1.14, 3.70	0.0164
Asthma duration > 15.0 years	1.59	1.05, 2.42	0.0273	-	-	-
FEV1 $< 80\%$	3.64	2.12, 6.54	<0.0001	-	-	-
FEV1/FVC $< 70\%$	1.76	1.12, 2.82	0.0139	-	-	-
FEF25-75 $< 60\%$	3.35	2.12, 5.31	<0.0001	-	-	-
Asthma control (UP and PC versus WP)	9.25	5.75, 15.19	<0.0001	-	-	-
Asthma exacerbations	6.32	3.88, 10.65	<0.0001	-	-	-
Emergency Room visit	6.19	3.59, 27.80	<0.0001	-	-	-
Hospitalization	7.19	4.11, 24.32	0.0003	-	-	-

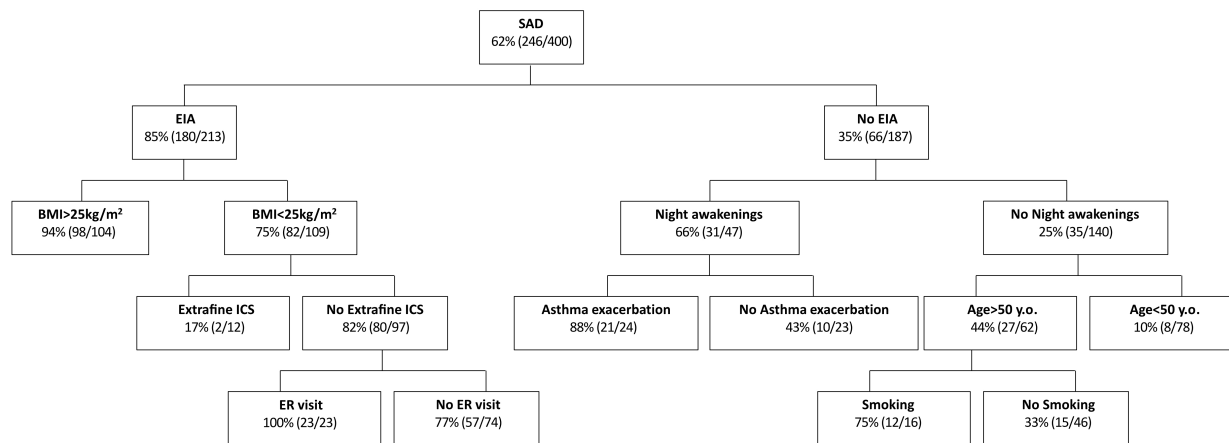
Night awakenings due to asthma	8.24	5.09, 13.74	<0.0001	3.34	1.85, 6.17	<0.0001
Exercise-induced asthma symptoms (EIA)	9.99	6.27, 16.31	<0.0001	6.39	3.65, 11.45	<0.0001
High ICS dosage*	4.45	0.78, 83.63	0.1644	-	-	-
Extra-fine ICS use	0.13	0.08, 0.20	<0.0001	-	-	-
Anti-leukotriene Use	3.60	1.84, 7.75	<0.0001	-	-	-

OR : Odds Ratio; CI : confidence intervals; FeNO: Fractional exhaled nitric oxide; BMI: Body Mass Index.

* >1000 micrograms of Beclometasone equivalent

For the purpose of this analysis, continuous variables associated with small airways disease were converted in binary variable, using an arbitrary values in between the groups of Table 1 (age>50 years old, BMI > 25 kg/m², asthma duration > 15.0 years, eosinophils count > 300 mm³, FeNO >25 ppb, and ICS dosage>1000 micrograms of Beclometasone equivalent). GINA levels of asthma control were dichotomized in uncontrolled *versus* well and partially controlled asthma categories.

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Supplementary Table 1. Correlations between impulse oscillometry (R5 – R20, R5, X5, AX, and F_{res}) and standard spirometry (FEV1, FEV1/FVC ratio, FEF₂₅₋₇₅) measures in our cohort of 400 asthmatic patients.

	FEV1, (% predicted)		FEV1/FVC ratio, (x100)		FEF ₂₅₋₇₅ , (% predicted)		R5		X5		R5-R20		Ax	
ALL COHORT (N=400)	r	95% CIs	r	95% CIs	r	95% CIs	r	95% CIs	r	95% CIs	r	95% CIs	r	95% CIs
FEV1, (% predicted)														
FEV1/FVC ratio, (x100)	0.50	(0.43,0.57)												
FEF25-75, (% predicted)	0.80	(0.74,0.82)	0.70	(0.60,0.71)										
R5	-0.32	(-0.39,-0.21)	-0.16	(-0.23,-0.03)	-0.27	(-0.32,-0.14)								
X5	0.44	(0.36, 0.52)	0.18	(0.14,0.32)	0.34	(0.25,0.42)	-0.66	(-0.71,-0.60)						
R5-R20	-0.40	(-0.45,-0.28)	-0.21	(-0.31,-0.12)	-0.28	(-0.36,-0.18)	0.71	(0.66,0.76)	-0.66	(-0.71,-0.60)				
Ax	-0.45	(-0.51,-0.35)	-0.29	(-0.36,-0.17)	-0.38	(-0.41,-0.24)	0.66	(0.60,0.71)	-0.69	(-0.74,0.63)	0.75	(0.71,0.79)		
F _{Res}	-0.36	(-0.42,-0.24)	-0.42	(-0.51,-0.35)	-0.33	(-0.37,-0.19)	0.49	(0.41,0.56)	-0.47	(-0.55,-0.40)	0.61	(0.54,0.66)	0.57	(0.50,0.63)

Spearman's correlation was used for this analysis. The p values of all the correlations in the table were significant (p <0.001).

FEV1: Forced expiratory volume in the first second; FVC: forced vital capacity; FEF25-75: Forced Expiratory Flow Between 25% and 75%; R5-R20: Resistances at 5 and 20 Hz; X5: Reactance at 5 Hz; Ax: Reactance area; F_{Res}: Resonant frequency in Hz. 95% CIs: 95% Confidence Intervals.

#1. Methods: allergy work-up

All the patients withdrew the anti-histamine therapy at least 3 days before the date of skin prick testing, and none received daily glucocorticoid dose ≥ 10 mg at that time. Specific IgE blood testing was performed only in patients that could not reduce the daily dose of glucocorticoids < 10 mg or withdraw the antihistamine, and was considered reliable only if complete as our skin prick test panel. For those patients that were not tested in our clinic, only those that brought the test results at the visit were not re-tested. In particular cases (e.g. patients tested with a panel of aeroallergens not complete as our standardized panel, or patients that was tested > 2 years before, etc.), patients were retested.

#2. Methods: standard spirometry

Patients underwent functional assessment after the withdrawal of short-acting β 2-agonist from at least 12 hours and long-acting β 2-agonist from at least 24 hours. FEV₁, FVC, and forced expiratory flow in the middle half of the patient's exhaled volume (FEF₂₅₋₇₅) were recorded and expressed as a percentage of predicted value (% *predicted*). The FEV₁/FVC value was recorded and expressed as a ratio and as a percentage of predicted value (% *predicted*, LLN).

#3. Methods: impulse oscillometry.

Patients were asked to wear a nose clip and were seated during tidal breathing with their neck slightly extended and their lips sealed tightly around the mouthpiece, while firmly supporting their cheeks with their hands. Assuming the coherence, which is a measure of testing reliability, was acceptable (>0.80 at 10 Hz) and there was no evidence of coughing, swallowing, vocalization, or breath holding, the trial was saved. At least 3 trials were performed, each lasting 30 seconds, and mean values were chosen. Both Rrs and Xrs , which reflect total pulmonary impedance, were measured by the investigator in real time as a function of flow volume and pressure.