# Exploring the relevance and extent of small airways dysfunction in asthma: AssessmenT of smalL Airways involvemeNT In aSthma, the ATLANTIS study

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**Link to full study:**

**https://clinicaltrials.gov/ct2/show/NCT02123667?term=NCT02123667&rank=1**

## Summary

### Background

Small airways dysfunction (SAD) is well-recognized in asthma, yet its role in asthma severity and asthma control is unclear.

### Methods

This multinational observational study investigated participants without and with asthma (GINA severity stage 1-5). They underwent spirometry, body plethysmography, impulse oscillometry (IOS), Multiple Breath Nitrogen Washout (MBNW), computed tomography (CT) and questionnaires. Structural equation modeling (SEM) was applied in asthma to assess the contribution of all physiological and CT parameters to SAD. With SEM, we defined a clinical-SAD and CT-SAD score. Asthma subjects were classified in SAD groups using model-based clustering. Asthma severity, control and health care utilization in the past year were compared with the SAD scores and SAD groups.

### Findings

We investigated 773 asthma and 99 control participants (median [interquartiles] age 46 [34, 54] and 41 [29, 52] years, 58% and 57% females, respectively). All physiologic measures contributed to the clinical SAD model with SEM analysis. SAD prevalence was dependent on the measure used and lowest with MBNW Sacin that reflects ventilation heterogeneity in the most peripheral, pre-acinar/acinar airways. IOS and spirometry, reflecting dysfunction of small-to-mid-sized airways, contributed most to the Clinical-SAD score and SAD Groups. Clinical-SAD Group1 (n=452) had "milder" SAD, i.e. comparable MBNW Sacin (ventilation heterogeneity in pre-acinar/acinar airways) values with controls. Group2 (n=312) had more abnormal physiologic SAD measures than Group1, particularly IOS and spirometry, and more severe asthma (asthma control, treatments, exacerbations,

quality of life). Clinical-SAD scores were higher in Group2 (“more severe” SAD) and related to asthma control, severity, and exacerbations. Clinical-SAD and CT-SAD scores did not significantly correlate.

**Interpretation**

SAD has multiple components and physiologic parameters from spirometry, body plethysmography, IOS and MBNW contribute to SAD. SAD is present across all asthma severity and particularly in severe disease. The clinical classification of SAD in two groups, i.e. a “milder” and “more severe” SAD group, by the easy-to-conduct measures IOS and spirometry, is meaningful given its association with GINA asthma severity stages, asthma control, quality of life, and exacerbations.

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## Research in context

### Evidence before this study

Small airways dysfunction (SAD) has been understudied, though it significantly contributes to airway obstruction, a hallmark of asthma. So far, studies on the role of SAD in asthma have been performed in small sample sizes and/or subgroups of asthma. Moreover, these studies investigated only a subset of available potential measurements of SAD and did not include both spirometry, body plethysmography, impulse oscillometry (IOS), Multiple Breath Nitrogen Washout (MBNW), CT scans and questionnaires.

### Added value of this study

This is the largest study to date involving 773 evaluable asthma patients and 99 controls without airway obstruction specifically designed to determine the prevalence and impact of small airways dysfunction SAD in asthma. The study shows that SAD is present in asthma across all stages of severity, with highest prevalence in GINA 5. We were able to define a SAD score from a combination of lung function measurements that reflects the amount of physiological small airways impairment in asthma. The score associated significantly with measures of asthma control, history of exacerbations and disease severity. Model-based clustering delineated two clinical SAD groups that differed in age, duration of asthma, and disease severity. Of interest, values of Sacin, that measures ventilation heterogeneity in pre-acinar/acinar airways, were in the normal range in Group1. The difference between Clinical SAD Group1 and Group2 was particularly clear with clinically available SAD measurements, such as IOS and spirometry, followed by $FEV_1$, while differences were small with CT SAD parameters. In summary, we can cluster asthma patients in two subgroups based on SAD measured with easy-to-conduct, clinically applicable measures.

### Implications of all the available evidence

Small airways dysfunction (SAD) has been understudied in asthma. Our results show the clinical relevance of SAD, which is present across all severity stages of asthma. It is particularly present in severe disease, likely reflecting structural lung changes that are not responsive toe the use of oral corticosteroids and/or high dose inhaled corticosteroids. Moreover, SAD relates to asthma stability, severity, quality of life, exacerbation rates and health care utilization and can be delineated by easy-to-conduct, clinically applicable measures such as IOS and spirometry. Therefore, this aspect of asthma needs further consideration in the management of the disease.

## Introduction

Asthma is a prevalent obstructive airway disease that affects the entire bronchial tree. The small airways, defined by a diameter ≤2 mm and referred to as the "silent zone" of the lungs, contribute to the resistance in the airways of patients with obstructive airways disease[1]. This is of clinical importance since small airways can be inflamed in asthma and hence narrowed[2-4]. Small airway narrowing can also occur due to smooth muscle contraction after inhaling allergic and non-allergic irritants. Moreover, remodeling can affect small airway wall stiffness, thereby changing their distensibility[5].

Small airways dysfunction (SAD) has been postulated to exist at all severities of asthma, whereas some studies suggest that the prevalence increases with asthma severity [6,1]. However, it is still not clear what proportion of asthma patients suffers from SAD, and which tests or combination of tests best defines it. Lack of best practice is due to the fact that published studies investigating the small airways in asthma included only small-sized and/or relatively homogeneous populations regarding asthma severity, or only tested one or a few physiologic SAD measures [6-8]. The ATLANTIS (AssessmenT of smalL Airways involvemeNT In aSthma) study subjected a large asthma cohort to all available, clinically applicable, potential SAD tests, including spirometry, body plethysmography (e.g. residual volume), impulse oscillometry (IOS), and Multiple Breath Nitrogen Washout (MBNW) and CT scan. The physiological tests may reflect abnormalities in different parts of the bronchial tree or different aspects of small airways dysfunction, providing different perspectives on SAD[9,10]. Lung imaging by CT scan can provide additional insight regarding SAD, but the relationship with physiologic measures of SAD in asthma has not been studied extensively and only in small groups.

The ATLANTIS study assessed which (combination of) biomarkers, physiological testing and imaging markers best measures the presence and extent of SAD in asthma. It builds on both a

baseline and 1-year follow-up phase. We assessed SAD through a series of baseline measurements using published criteria defining small airways dysfunction for each test, both for physiological and CT measures. The final result of the model-building process is a score defining to what extent SAD is present in each individual patient, a score that was built from baseline data and validated at follow-up. With this score, its usefulness for prediction of asthma severity, asthma control, quality of life and history of exacerbations was evaluated

Here we present the clinical baseline data of the ATLANTIS study. The main aim is to identify which combination of biomarkers, physiologic testing and imaging approaches best measures the presence and extent of SAD in asthma cross-sectionally and their relationship with asthma severity, control, quality of life and history of exacerbations over time[9]. The study allowed us to develop novel predicted, upper limit of normal (ULN) and lower limit of normal (LLN) values of physiological parameters infrequently studied (e.g. IOS).

## Methods

### Participants

Participants were recruited (2014-2016) from general practitioners, chest physician's databases and by advertisements in 29 centers across 9 countries worldwide. Inclusion criteria were: 1) age 18-65 years; 2) clinical asthma diagnosis $\geq$ 6 months, confirmed by a chest physician according to GINA 2012[11] and supported by objective evidence of any of the following at the baseline visit or in the previous 5 years: a) positive airway hyperresponsiveness to methacholine, ***or*** b) positive reversibility, defined as $\Delta FEV_1 \geq$ 12% and $\geq$ 200 mL over baseline $FEV_1$ within 30 minutes after inhaling 400 µg of salbutamol pMDI with or without a spacer ***or c)*** Peak Expiratory Flow variability (i.e. highest - lowest value over the day/mean value of the two, ×100) >20%, measured during 7 days ***or*** d) documented reversibility after a cycle (e.g. 4 weeks) of maintenance anti-asthma treatment; 3) stable asthma on any previous regular asthma treatment ("rescue" β2-agonists alone included) at a stable dose for $\geq$ 8 weeks before baseline; 4) lifetime smoking $\leq$ 10 pack-years. Main exclusion criteria were a COPD diagnosis confirmed by a chest physician and an asthma exacerbation during 8 weeks before baseline.

Controls were included based on 1) age 18-65 years; 2) no respiratory symptoms compatible with asthma or COPD in the past 2 years; 3) normal spirometry: baseline $FEV_1 \geq$ 80%predicted, $FEV_1$/Forced Vital Capacity (FVC)> LLN (lower limit of normal); 4) normal airways responsiveness: PC20$\geq$ 16 mg/mL, PD20$\geq$ 1.4 mg; 5) lifetime smoking $\leq$ 10 pack-years. Diagnosed upper/lower respiratory tract diseases were exclusion criteria. The Medical Ethics Committee of each center approved the protocol; all patients gave written informed consent.

### Study design and procedures

Participants were followed for 1 year with 6-month clinic and 3-month telephone follow-ups[9]. The clinical and CT tests were performed at 3-day baseline visits. The methods for spirometry,

hyperresponsiveness, MBNW, IOS, body plethysmography, CT, questionnaires, blood tests, and health care utilization are described in the Supplement. Medications during an eight-week period before evaluation were used to assess GINA severity[11]. Potential indices of SAD used, with hypothetical location in the airways between brackets, were: % fall in FVC during hyperresponsiveness testing (% fall FVC, air trapping in peripheral airways), spirometry: Forced Expiratory Flow $(FEF)_{25-75}$ and $FEF_{50}$, both corrected for FVC (conducting small airways), body plethysmography: Residual Volume/Total Lung Capacity (RV/TLC) and Functional Residual Capacity (FRC) %predicted (air trapping due to obstruction in both central and peripheral airways), IOS: R5-R20 (heterogeneity in resistance of small-to-mid-sized airways, AX and X5 (reactance or distensibility of more central, conducting small airways), MBNW: Scond and Sacin (gas exchange measures, assessing convectional ventilation heterogeneity in peripheral conducting (Scond) and pre-acinar/acinar (Sacin) airways). Alveolar NO was not incorporated in this analysis since it was only available in a subset of participants (See Supplement). Indices of "large airways dysfunction", which may also capture small airways abnormalities, were $FEV_1$%predicted, $FEV_1$/FVC, IVC, FeNO, R20, $PC_{20}$, $PD_{20}$ and 3 severity categories of airway hyperresponsiveness (Supplement).

**Computed tomography**

Volumetric whole lung scans were obtained at full inspiration (near total lung capacity) and at end of expiration, near FRC. Scans were analyzed by a single observer (SB) using semi-automated software, Apollo (VIDA Diagnostics, Iowa), with various quality control parameters[12,13]. The supplement describes CT acquisition, quantitative airway morphometry and lung densitometry. SAD parameters used were: ex- and inspiratory Mean Lung Density and their ratio (E/I MLD), ex- and inspiratory lung volume and their ratio (E/I LV), expiratory Voxel Index (VI-856) and inspiratory VI-950 (% of Voxels with CT numbers <-856 and <-950 Hounsfield Units respectively, inspiratory Percentile15, Inspiratory median Lumen area, Wall area (WA) and Total area, these latter three divided by body

surface area (BSA), inspiratory median percentage WA, and inspiratory Pi10 and Po20%WA (hypothetical airway with internal perimeter of 10 mm and outer perimeter of 20 mm respectively).

**Statistical analyses**

Detailed statistical information, including power analysis[14], is provided in the Supplement. The following variables reflecting SAD were used in the clinical SAD analysis: $FEF_{50}$/FVC, $FEF_{25-75}$/FVC, $FEV_1$%predicted, $FEV_1$/FVC, IVC%predicted, % fall FVC at $PC_{20}$ or $PD_{20}$, RV/TLC %predicted, FRC%predicted, R5-R20, X5, AX, Scond, Sacin. For CT SAD analysis, variables were: MLD ratio, Lung Volume ratio, VI-856, Pi10, Po20%WA.

Several steps were performed for clinical SAD and CT SAD SEM analysis separately[15]. A correlation matrix evaluated correlations among observed variables, high correlations indicating presence of underlying latent variables. An exploratory factor analysis for observed variables was performed to identify the underlying SAD factor structure. The final underlying SAD factor structure was tested by specifying a confirmatory factor model. Once the measurement model was set and fit the data properly, it was used to classify each patient into SAD groups, using model-based clustering. The SAD Groups and SAD scores from the clinical SAD and the CT-scan SAD model were compared, evaluating the rate of agreement, using Chi-square and Pearson's correlation tests. The clinical SAD model was additionally tested in the subgroup with a CT scan, by adding the CT scan variables to the model. Full information maximum likelihood (FIML) method was used for dealing with missing data in SEM analysis[16].

Relationships of physiologic SAD variables with asthma severity, control and healthcare utilization were analyzed by Poisson regression. Continuous prediction equations, their lower- and upper limit of normal (LLN and ULN) from the literature[17] and from formulas based on ATLANTIS controls are provided in Supplemental Table 1. Statistical analyses and data processing were performed using

Statistical Analysis Systems (SAS®) Software (release 9.2) and Mplus Version 7.4 on a Windows 7 operating system.

## Results

The main reason for screening failure was not fulfilling inclusion/exclusion criteria (n=99, Figure 1).

## Participants

Baseline characteristics are shown in Table 1, Table 2 (asthma only) and Supplemental Table 2. Gender, age and smoking habits were comparable between asthma and control participants. Asthma participants demonstrated higher BMI, heart rate, blood pressure, blood cell counts, and prevalence of atopy. Hyperresponsiveness was only present in asthma participants. All physiologic parameters were significantly worse in asthma. Asthma participants had lower MLD expiratory values, inspiratory airway lumen, wall, and total area, also when divided by BSA (body surface area) on CT. Asthma participants had a moderately severe health status impairment (Table 2) and lower lung-related quality of life (higher EuroQol-5Dim-5Levels score) than controls, median (Q1;Q3) value of 95.0 (90.0;100.0) versus 80.0 (70.0;90.0).

## Association of physiologic parameters with asthma severity, control and health care utilization

X5, Scond, RV/TLC, R5-R20 and R5 values (Figure 2A) showed the highest positive correlations with GINA severity[15]. GINA severity was also associated, as expected, with lower $FEV_1$, $FEF_{50}$, and $FEV_1$/FVC values. Table 3 shows that GINA5 had the highest SAD prevalence rate for every physiologic variable (measurements >ULN or <LLN). Sacin had the least SAD prevalence rate in all GINA stages, the lowest prevalence being with GINA1 (12%), rather similar, higher prevalences in GINA2-4 (18-19-20%), and highest in GINA5 (41%). This contrasts with other SAD variables, where prevalences either remain constant over the GINA stages (% fall FVC), continuously increase from GINA1-GINA5 (body plethysmography), or increase in steps, e.g. Scond and $FEF_{25-75}$ showed lowest prevalences in GINA1-2, higher in GINA3-4 and highest in GINA5. R5-R20 and AX showed somewhat comparable rates in GINA1-3, higher in GINA4 and highest in GINA5 (Table 3). Sacin

also contrasted with <LLN prevalence distributions in $FEV_1$, i.e. GINA1-GINA5 26%-29%-36%-47%-72%.

A lower Asthma Control Test (ACT) score was particularly associated with higher AX and R5 and lower FVC and $FEV_1$ (Figure 2B).

For exacerbations in the past year, highest positive correlations were with RV/TLC, R5-R20, AX and Sacin and highest negative correlations with $FEV_1$, FVC, IVC, $FEF_{25-75}$, $FEF_{50}$ (Figure 2C). The number of exacerbations was independently predicted by SAD parameters from spirometry, IOS, body plethysmography, hyperresponsiveness severity, female gender and height (Table 4). There was also a negative association with Raw. Independent parameters for unscheduled consultation visits were $FEV_1$, hyperinflation with body plethysmography, hyperresponsiveness severity, and female gender (Table 4).

**Prevalence of LAD and SAD in asthma**

Figure 3 (upper panel) shows the prevalence rates of large and small airways dysfunction, based on LLN and ULN. Sacin had the lowest SAD prevalence (19.2%), % fall FVC the highest (73.1%).

**SAD Model**

Figure 4 shows the final clinical SAD model based on cross-sectional data. It presents both the loadings to the three latent variables, and the goodness of fit values (Supplemental methods), showing good coherence of this model to SAD. IOS parameters R5-R20, AX and X5 loaded to the first latent variable, $FEF_{50}$ and $FEF_{25-75}$ both corrected for FVC, to the second latent variable, while Sacin (MBNW) loaded both to the first and second latent variable. The lung volume parameter RV/TLC %predicted and Scond (MBNW) loaded to the third latent variable. Hyperresponsiveness was only tested at the first visit, hence could not be taken into account in the longitudinal design of the SAD SEM model. Therefore, we also analyzed the clinical SAD model at baseline including hyperresponsiveness, and the % fall FVC loaded on the third latent variable without much change in

goodness of fit values. The baseline model without and with % fall FVC correlated highly (r=0.99; Supplemental Figure 2A). Since the cross-sectional SAD model with and without % fall FVC were almost identical, the model without % fall FVC was tested longitudinally; the same model structure was confirmed at all visits (Supplemental Figure 2B).

**Correlations of clinical SAD score with physiologic and clinical parameters**

A higher SAD score reflects more severe SAD. The highest positive and negative correlations ($r > 0.60$ and $r < -0.60$) of the SAD score existed with physiologic parameters on which the score was based, i.e. IOS parameters AX, R5-R20, and R5 (positively) and X5, spirometric parameters $FEF_{25-75}$ and $FEF_{50}$ (negatively), next being $FEV_1$ %predicted (Figure 5). The highest correlations of non-physiological parameters with the SAD score were duration of asthma, ACQ-6 and number of exacerbations (positively), ACT, Mini AQLQ total and EQ-5D-5L (negatively). Clinical SAD scores increased with higher asthma severity, mean SAD score in GINA1-5 being -0.143, -0.035, -0.048, +0.071 and +0.239 (ANOVA $p < 0.0001$).

**Model-based clustering defined clinical SAD Groups**

Model-based clustering defined two clinical SAD groups, Group1 including 452 patients, Group2 312 patients (Table 3 and Supplemental Table 3 present clinical characteristics). Overall, the 2 clinical SAD Groups were similar regarding age of asthma onset, sex ratio, FeNO, atopy, and smoking habits, while duration of smoking was higher in Group2 (Table 5). Sacin values were comparable between Group1 and the controls, whereas Group2 had significantly higher values than both Group1 and controls. Clinical SAD Group2 was somewhat older, demonstrated higher blood pressure, heart rate and BMI, and a longer asthma duration. Additionally, Group2 had more severe asthma than Group1, according to GINA severity, ACT, ACQ, LABA/ICS use, hyperresponsiveness, blood inflammation (eosinophils), quality of life and health care utilization. All physiologic parameters were worse in

clinical SAD Group2; the two groups were best separated by SAD parameters from IOS followed by spirometry, and additionally $FEV_1$ (Figure 3).

CT scan factors in SAD

CT scans were analyzed in 294 patients (with comparable asthma severity as the non-CT group, Supplemental Table 3). The SEM model provided three factors in CT that contributed to SAD: MLD insp/exp ratio, Lung volume ins/exp ratio and VI-856 (Supplemental Figure 2D). The correlations of the CT SAD score with physiologic and clinical parameters, comparison of CT SAD groups, and additional Clinical SAD analysis in patients who had a CT scan are presented in the Supplement.

**Relationship between Clinical and CT SAD scores**

The Clinical SAD and CT SAD scores showed a significant, weak correlation (r=0.28). There was no significant overlap between the clinical SAD and CT SAD Groups (p=0.103, Supplemental Table 5).

**Discussion**

This large clinical study shows the clinical relevance of small airway dysfunction for asthma, since SAD is present across all severities and particularly in more severe asthma. ATLANTIS was specifically designed to determine the prevalence and impact of SAD in asthma and has performed the most comprehensive evaluation of SAD to date using both physiological and imaging tools. We show that the prevalence of SAD depends on the physiologic measure used, i.e. localization and type of airway narrowing. Of importance, no single variable defines SAD, but IOS, MBNW, lung volumes and spirometry all contribute. For clinical practice, it is important to highlight that SAD associates with GINA severity and –independently- with history of exacerbations over time, particularly when measured by IOS, spirometry and body plethysmography. Moreover, the poorest asthma control was present in the group with the worst clinical SAD score.

Strengths of our study are the large group of asthma patients covering the full severity spectrum and the extensive work-up and quality and experience of the centers. ATLANTIS is a multi-center international study, therefore we feel our results are reliable and applicable to multiple populations. We also included smokers, a factor that by itself may induce some SAD. We felt it important that our study reflects the larger asthma and non-asthma population globally for generalizability, and thus not restricts the impact of our findings. The controls had comparable age, sex ratio and particularly smoking habits as the asthma population, which provided novel LLN and ULN values for physiological parameters infrequently studied, such as IOS and MBNW. We acknowledge that a larger control group might have improved precision of these predicted, LLN and ULN values, and this will be also partially overcome when we add the longitudinal data in the future.

We recognize that a quality check of the maneuver to get optimal phase III slope in the MBNW test[18] is key to validity of the measurements, which we have carefully ensured in the present study. The finding of some measurements of ventilation heterogeneity in pre-acinar/acinar airways (Sacin) in the

normal range is not in contrast with the presence of airway dysfunction in Group1, as the body of the available literature on ventilation heterogeneity in adult asthma[19-27] reveals a variable contribution of conducting versus acinar lung regions to treatment response, and consistency in the reversibility towards normal values after exacerbations[19]. Particularly, the persistent derangement of ventilation in conducting airways (Scond) seems more related to airway remodeling, exacerbations, and hyperresponsiveness, whereas the reversible derangement in acinar airway ventilation mainly reflects asthma severity[28]. Accordingly, the worst clinical SAD score was present in the group with the poorest asthma control and higher prevalence in GINA 4 and 5.

Another limitation of the study is that CT scans were not available in all participants, limiting numbers for analyses. However, this allowed us to demonstrate that the clinical SAD model in the full asthma cohort could be replicated in the smaller group with CTs. Future work will expand our analyses by performing parametric response mapping (PRM)[29], a CT voxel-based imaging biomarker tool which uses dynamic image registration between paired inspiratory and expiratory scans to quantify 'functional small airways disease'. A potential limitation is that there was a somewhat higher age in the asthma than control participants, yet this was a small difference (mean age of 46 (34-54) vs 41 (29-52) years respectively) that is likely not of clinical significance, and we adjusted for age in all analyses. We cannot put our clinical SAD score forward as a clinically applicable tool as yet, since this is a cross-sectional study. The score already significantly associates with number of exacerbations, asthma severity and control, and the longitudinal phase of the study will elucidate whether it also predicts future changes in these clinical outcomes. For the same reason we cannot put the “best parameters“ of SAD forward yet, since this also needs prospective data. Additionally, an SADT will be developed to assess SAD by questionnaire[9], which may be easily applicable in the clinic, as MBNW and body plethysmography are not available for all routine settings. Our article did not report on SAD with regard to the underlying pathology[9]. It was not feasible to perform bronchial

and transbronchial biopsies in all participants. However, this will be analyzed in a smaller subset that we will present in the future.

Large and small airways obstruction are important components of asthma pathophysiology[1-3]. Our focus in this study is on the small airways and their specific impact upon asthma symptoms and exacerbations, an area of investigation that has been relatively neglected in our opinion (an overview of relatively small-sized studies is presented in Supplemental Table 7). It would be of interest to analyze in the future data in individuals with Large Airway Dysfunction (LAD) without SAD, or conversely, individuals with SAD and without LAD. Finally, one would like to have a 'gold standard' for SAD, yet our study shows this is not feasible since many physiological parameters contribute to the SAD model. This likely reflects that they represent abnormalities in distinct parts of the bronchial tree and/or contrasting aspects of underlying mechanisms of SAD, thereby providing different information[9].

We were able to define a SAD score that reflects the amount of physiological small airways impairment and is significantly associated with measures of asthma control, exacerbations and severity. We additionally observed two clinical SAD Groups that are comparable in e.g. gender, atopy, FeNO, ICS dose and smoking habits, while Group2 was somewhat older, had a longer asthma duration and more severe asthma according to all parameters tested. Of interest, ventilation heterogeneity in pre-acinar/acinar airways measured as Sacin[20], reflective of dysfunction of the most peripheral small airways, was in the normal range in Group1 only and had a higher prevalence in Group2. The difference between clinical SAD Group1 and Group2 was particularly clear with SAD measurements like IOS and spirometry (Figure 3). Clinical SAD Group 2 represents "more severe" SAD, given particularly the presence of more severe small-to-mid-sized airway obstruction (R5-R20, $FEF_{25-75}$) and less airway distensibility (Ax). In addition

In summary, we can detect asthma subtypes based on presence and extent of SAD measured with easy-to-conduct, clinically applicable tools.

Similarly, with regard to the clinical SAD score, we developed a CT-SAD score. The CT-SAD score significantly associated with GINA severity, but less well than the clinical SAD score. CT SAD Group2 had more severe asthma and the physiologic parameters were significantly different from controls and from Group1. However, the CT SAD Groups had similar levels of small-to-mid-sized airway obstruction (R5-R20) and conducting airway ventilation heterogeneity (Scond), reflective of dysfunction in small-medium size conducting airways, while Group2 had significantly higher air trapping (RV/TLC) and acinar airway ventilation heterogeneity (Sacin) values, reflective of the most peripheral small airways. This suggests that CT scan-derived SAD captures regional differences in mechanisms of airway dysfunction due to air trapping and small airways as a surrogate for peripheral airways impairment[30]. They become apparent in supine position, when airway closure and compliance reduction develop as consequence of severe hyperinflation and expiratory reserve volume reduction[31] in participants with more severe asthma. Notably, we observed a difference in airway distensibility (AX) in participants undergoing CT scan, in comparison to those who did not (see Supplemental Table 3). It is thus understandable that the Clinical SAD score and the CT SAD score were not concordant (r=0.28). Where CT scans (performed in supine position) provide information on SAD particularly by changes driven from increased residual static lung volumes and air trapping[32], the physiologic parameters measured in the sitting position provide information on air trapping (body plethysmography RV/TLC), small airway obstruction (IOS and $FEF_{25-75}$) and heterogeneity of both conducting and acinar airway ventilation (MBNW). This potentially explains why the CT SAD score, in contrast to the clinical SAD score, did not associate with health status or asthma control.

Asthma control is lacking in 50-60% of patients despite guideline-based management[33] and untreated SAD has been proposed as a contributing factor[1]. Drivers of asthma control include treatment

adherence and appropriate use of inhalers, psychological factors and environmental trigger exposures. The current study suggests that asthma control is also determined by the presence of SAD, since ACT was significantly associated with the clinical SAD score and was specifically abnormal in clinical SAD Group2 (most severe SAD). Moreover, a lower ACT score was associated with higher IOS parameters R5 and AX values. These data suggest that asthma control may be partially driven by SAD, but also obstruction in larger airways given its association with $FEV_1$, the gold standard for diagnosis and severity in clinical practice.

Of note, 91% of our asthma population expressed SAD when defined as any abnormal physiologic parameter. Our data imply that they do not all have extensive SAD throughout all airway dimensions, since the prevalence varied with the type of physiologic measure. The lowest prevalence existed with Sacin (19%) and RV/TLC (22%), both reflecting dysfunction of the most peripheral small airways[30]. The highest prevalence was with $FEF_{25-75}$ (68%) and % fall FVC (73%), probably both reflecting obstruction in more small-to-mid-sized airways. Future work will have to elucidate if these different prevalence rates define subtypes of SAD (consistent vs. variable, which level of airway is involved, and what percent of these airways are involved).. We additionally compared our SAD prevalence with literature findings (Supplemental Table 7), yet no study compared all types of physiologic SAD methods. Anderson et al.[6] used R5-R20 >0.03 kPa/L/s as cut-off for abnormality, concluding that abnormal R5-R20 values were present in all severities of asthma, i.e. 65% in British Thoracic Society step2, 64% in step3 and 70% in step4. Our overall prevalence with this cut-off was 70%, while our data extend their findings showing that the prevalence rates of R5-R20 >LLN increase from GINA steps 1-5, being 54%, 65%, 70%, 77%, and 91% respectively. In contrast, the prevalence of Sacin >LLN was lowest in GINA1, almost identical in GINA 2-4 and highest in GINA5, suggesting that mostly peripheral airway dysfunction, and likely structural changes are present in most severe asthma. In summary, our data are comparable with published findings in smaller samples, yet extend these

observations by providing information on all different SAD measurements at the same time in one group of asthma patients across all severities.

Of interest, asthma participants had higher blood pressure than our controls. We did not find literature reporting this observation. Comorbidities are thus not only present in COPD, another obstructive pulmonary disease [34,35], but also occur in asthma patients with an average age of 46 years. The finding is in agreement with previous studies indicating systemic inflammation as one underlying mechanism linking reduced lung function to cardiovascular mortality[36] and a positive association between lower $FEV_1$ and systemic arterial hypertension, while lower ICS doses attenuated the likelihood for hypertension in a population of the same age as ours[37]. Alternatively, hyperinflation could be also considered to have a role via its contribution to changes in intrathoracic pressure that increase left ventricular wall stress, similar to what has been reported in COPD[38].

In conclusion, our data in a large asthma population covering the full spectrum of asthma severity show the complexity of SAD. Notwithstanding this, the clinical classification of Small Airways Dysfunction is meaningful given its association with asthma severity, control and exacerbations. Results show that SAD can be present across all GINA severity stages. Depending on the type of physiologic parameter used, the prevalence rate changes considerably, but is consistently the highest in GINA5. SAD prevalence rates were lowest with Sacin, reflecting pre-acinar/acinar airway abnormalities, and this prevalence was quite comparable over GINA2-4 but again highest in GINA5, suggesting structural abnormalities in severe asthma. In contrast, other physiologic parameters showed either increasing prevalence rates with severity (RV/TLC) or a stepwise increase ($FEF_{25-75}$, R5-R20, AX, X5). Clinical SAD and CT SAD scores did not significantly correlate. SAD derived from the CT scan provides particularly data on air trapping and ventilation impairment in more

peripheral airways, while the physiologic measures show results from both small-medium size conducting airways and peripheral airways. For clinical practice it is important that physiological, easy-to-conduct measures such as IOS and spirometry, delineate two asthma SAD subtypes that differ in exacerbation rates, quality of life, asthma severity and control.

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## Figures and Tables

Figure 1. Flow-diagram of patients and controls without airway obstruction, with reasons for drop out

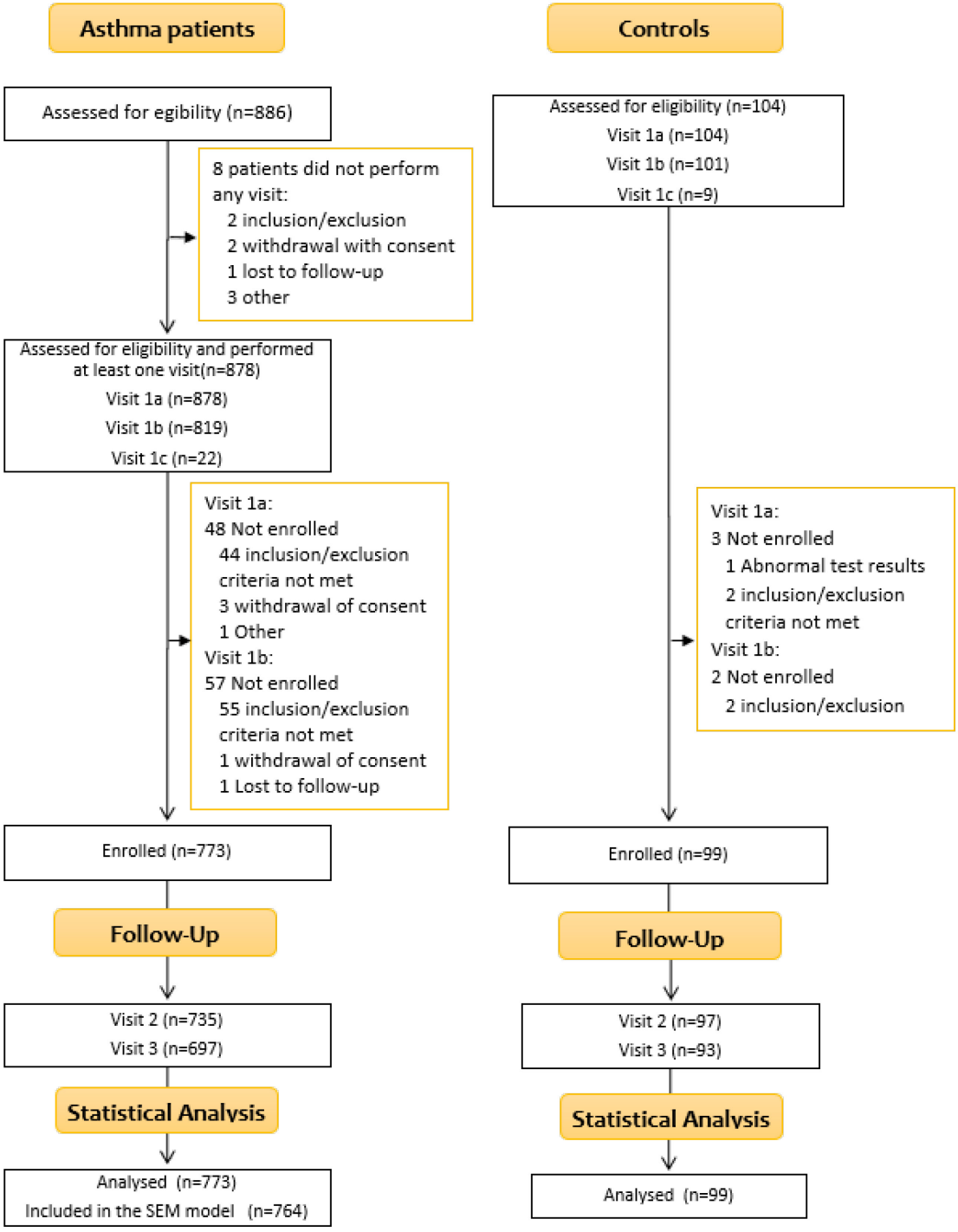
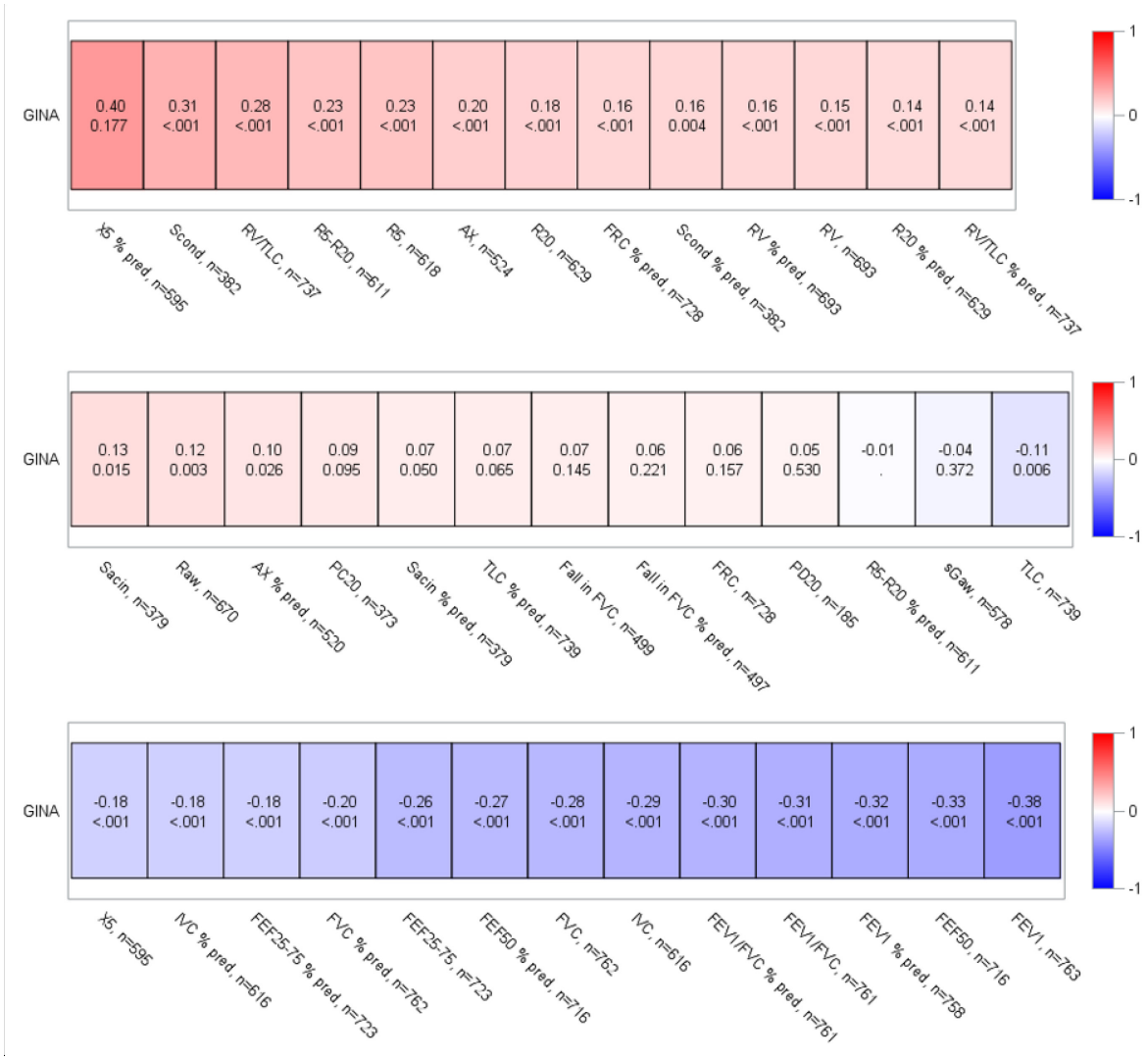
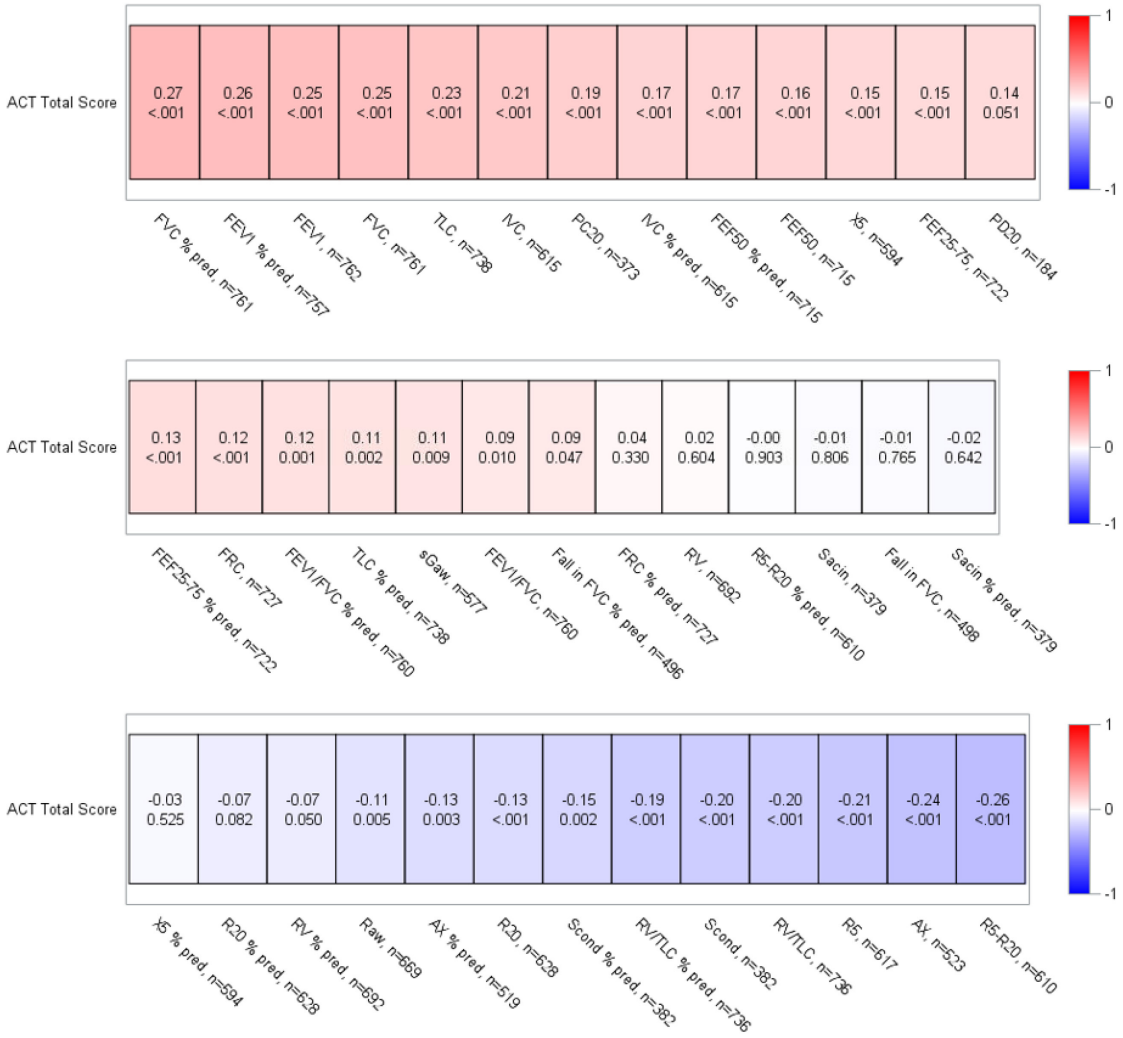
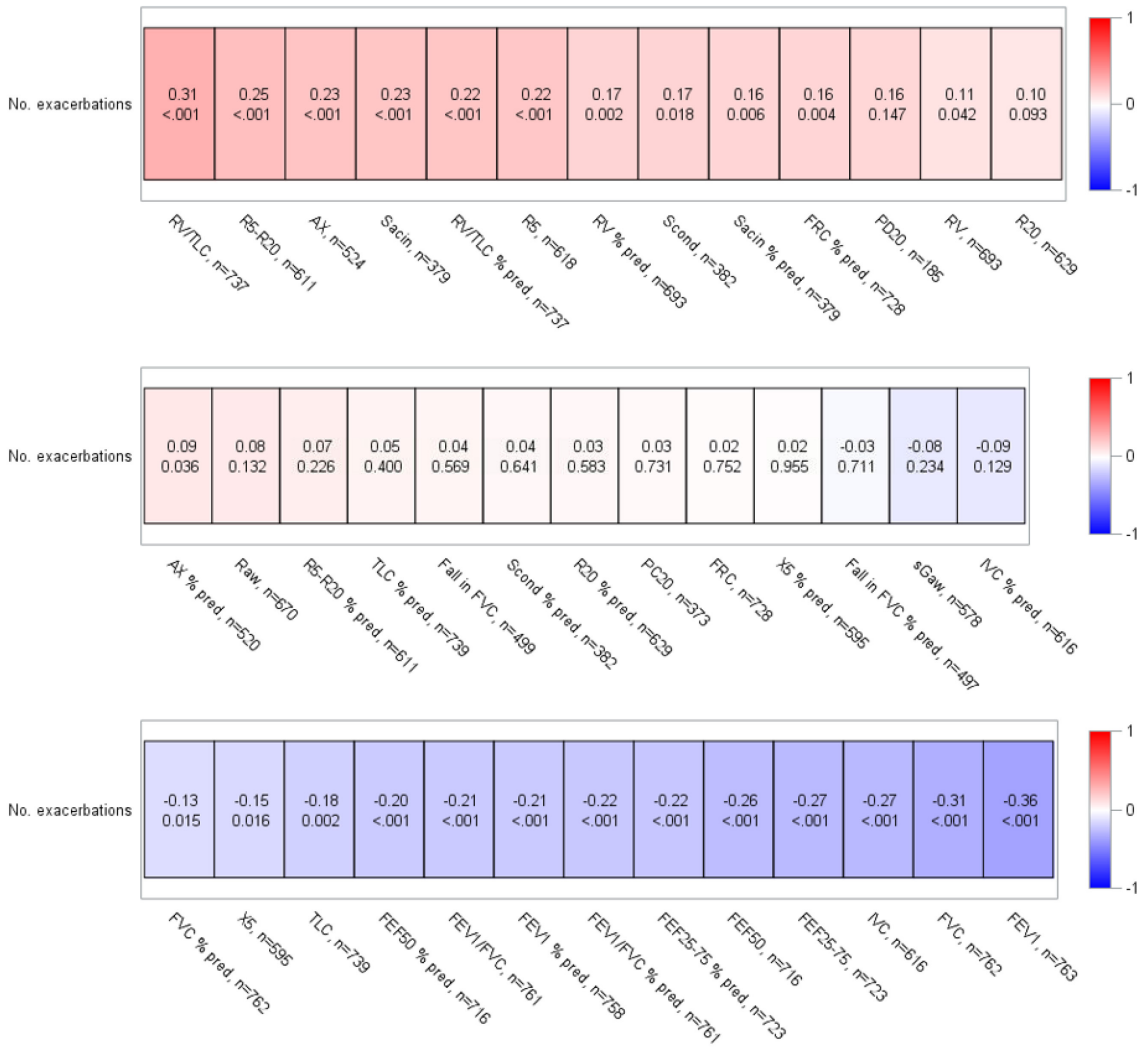


Figure 2. Monovariate correlations of physiological parameters and GINA severity, ACT score and number of exacerbations

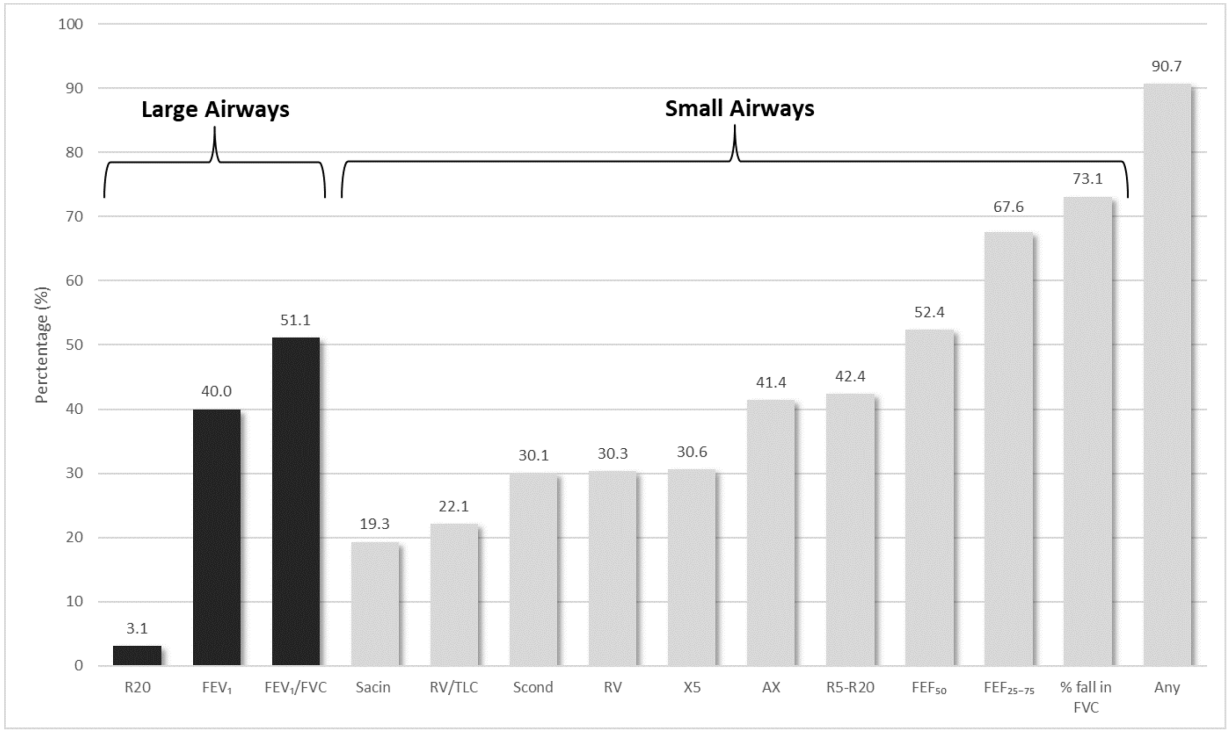


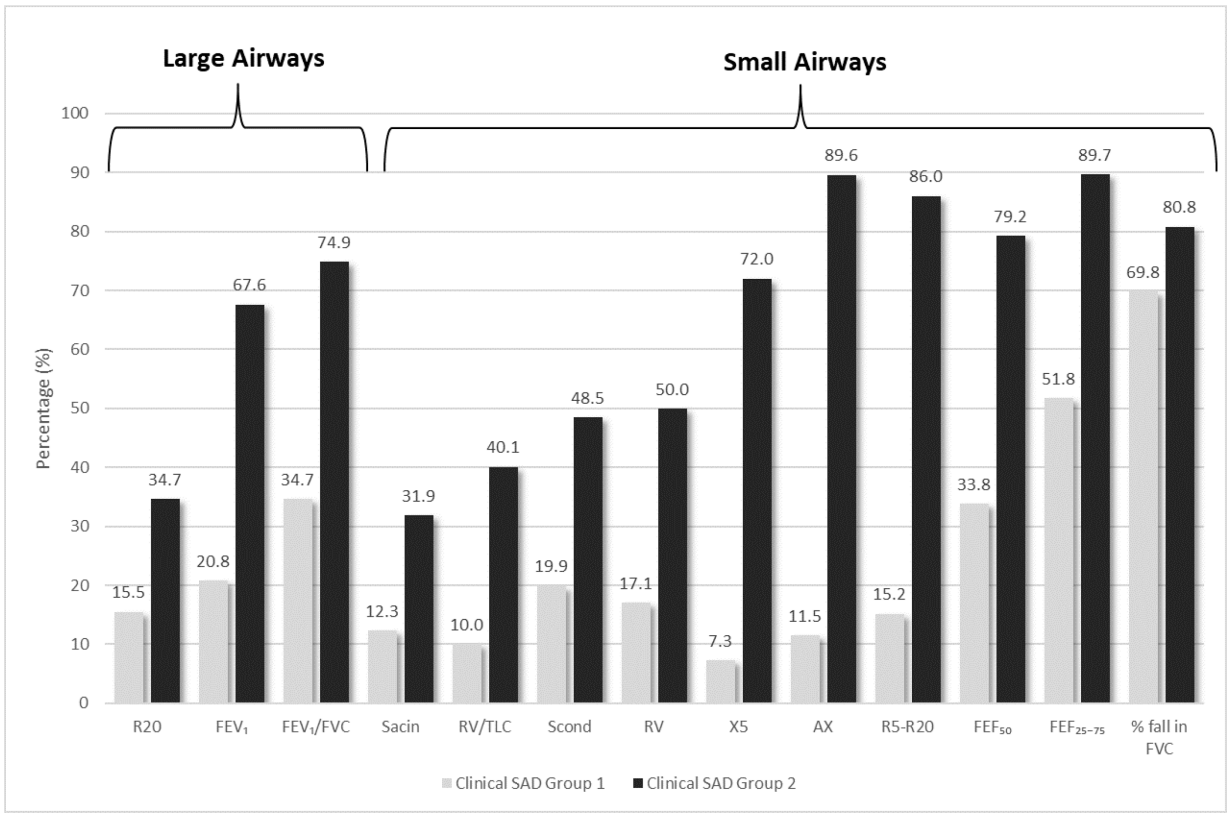

ACT Total Score
0.27 <.001
0.26 <.001
0.25 <.001
0.25 <.001
0.23 <.001
0.21 <.001
0.19 <.001
0.17 <.001
0.17 <.001
0.16 <.001
0.15 <.001
0.15 <.001
0.14 0.051
FVC % pred, n=761
FEV1 % pred, n=757
FEV1, n=762
FVC, n=761
TLC, n=738
IVC, n=615
PC20, n=373
IVC % pred, n=615
FEF50 % pred, n=715
FEF50, n=715
X5, n=594
FEF25-75, n=722
PD20, n=184
1
0
-1
ACT Total Score
0.13 <.001
0.12 <.001
0.12 0.001
0.11 0.002
0.11 0.009
0.09 0.010
0.09 0.047
0.04 0.330
0.02 0.604
-0.00 0.903
-0.01 0.806
-0.01 0.765
-0.02 0.642
FEF25-75 % pred, n=722
FRC, n=727
FEV1/FVC % pred, n=760
TLC % pred, n=738
sGaw, n=577
FEV1/FVC, n=760
Fall in FVC % pred, n=496
FRC % pred, n=727
RV, n=692
R5-R20 % pred, n=610
Sacin, n=379
Fall in FVC, n=498
Sacin % pred, n=379
1
0
-1
ACT Total Score
-0.03 0.525
-0.07 0.082
-0.07 0.050
-0.11 0.005
-0.13 0.003
-0.13 <.001
-0.15 0.002
-0.19 <.001
-0.20 <.001
-0.20 <.001
-0.21 <.001
-0.24 <.001
-0.26 <.001
X5 % pred, n=594
R20 % pred, n=638
RV % pred, n=692
Raw, n=669
AX % pred, n=519
R20, n=638
Scond % pred, n=382
RV/TLC % pred, n=736
Scond, n=382
RV/TLC, n=736
R5, n=617
AX, n=523
R5-R20, n=610
1
0
-1




Legend to Figure 2. Correlations are presented for GINA severity ( top panel), ACT score (middle panel), and Number of exacerbations in the past year (lowest panel). Darkest red is highest positive correlation between parameters. Darkest blue is the lowest negative correlation between parameters. All abbreviations are presented in Table 1.

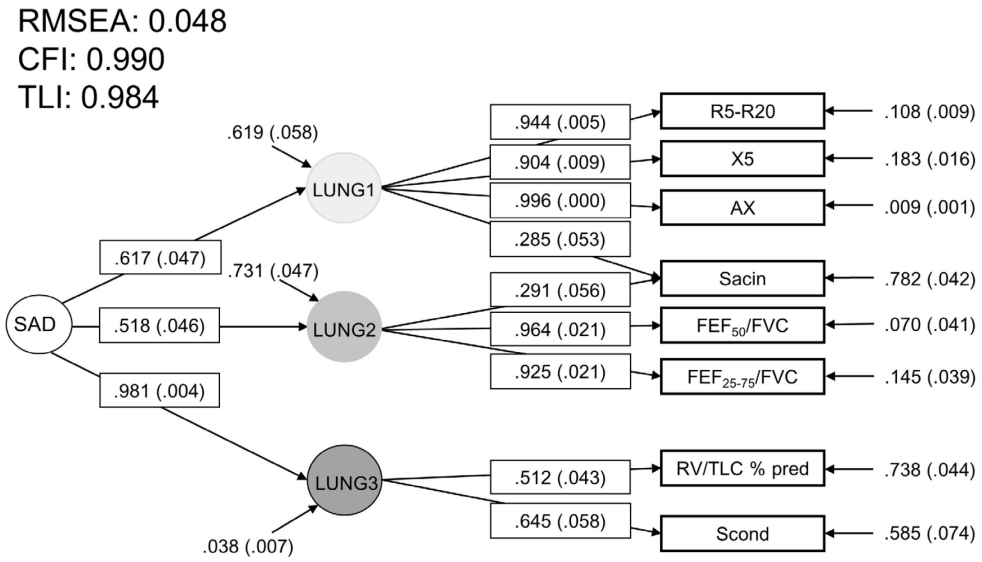
**Figure 3. Prevalence rates of airways dysfunction in the full asthma cohort and in the 2 SAD subgroups**




Legend to Figure 3. Prevalence rates of Large Airways abnormalities, and Small Airways abnormalities in the full cohort of asthma participants (upper Figure), and according to Clinical SAD Group1 and Group2 (lower Figure). Prevalences are based on LLN (Lower Limit of Normal) and ULN (Upper Limit of Normal) values derived from the literature or from ATLANTIS controls without airway disease, noted with*. For abbreviations see Table 1.

**Figure 4. Cross-Sectional Clinical SEM analyses of small airway function**

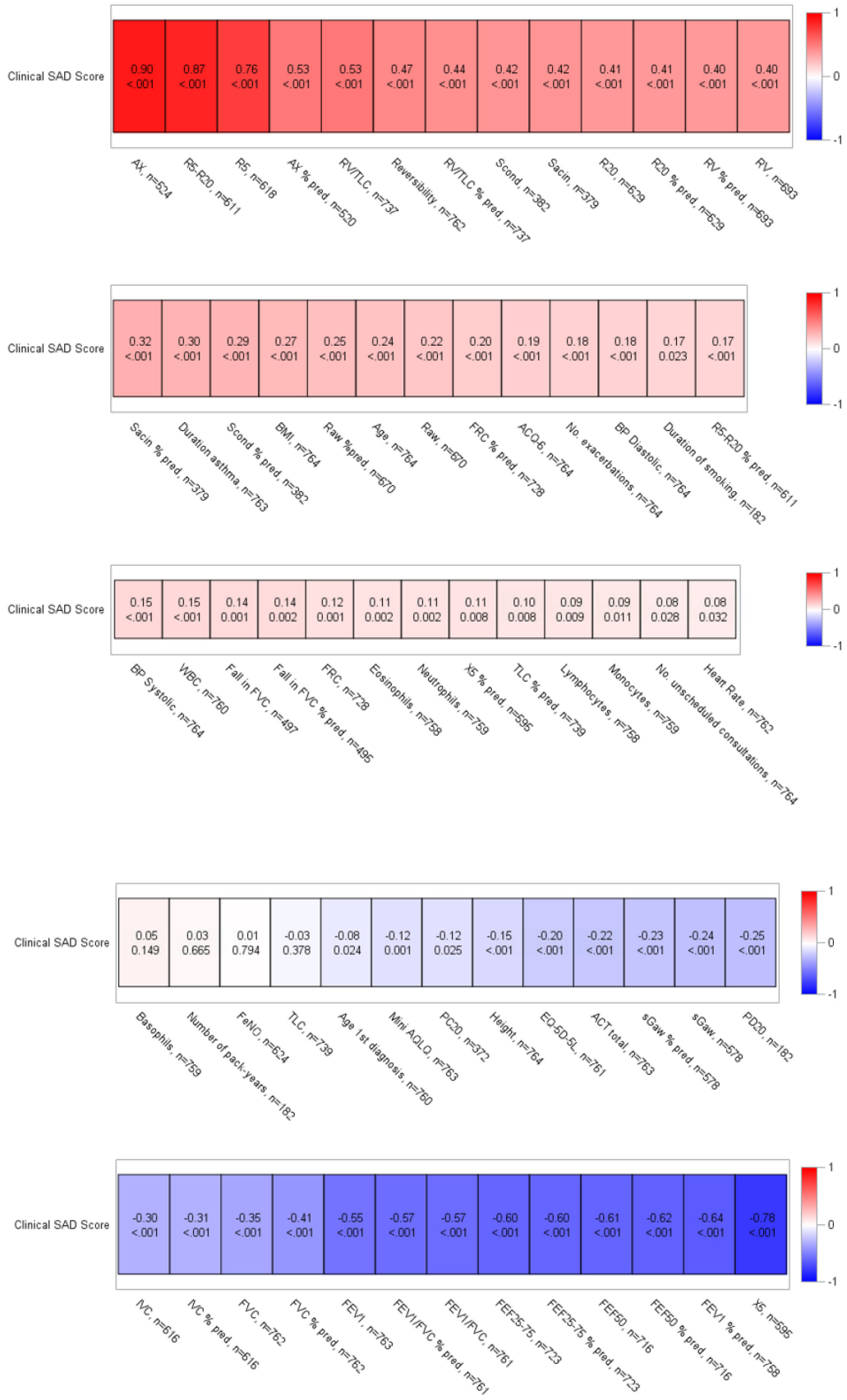



Legend to Figure 4. SAD=Small Airway Dysfunction.

The figure shows the results of Structural Equation Modeling (SEM). The model uses the measured variables presented in squares to define the three latent variables (Lung1, Lung2 and Lung3). The variable SAD is then constructed by a structural model that imputes the relations between these three latent variables (Lung1 loading 0.617, Lung2 loading 0.518 and Lung3 loading 0.981). Thus SEM modeling showed that SAD was built up by three latent variables, represented in circles ( Lung1 loading 0.617, Lung2 loading 0.518 and Lung3 loading 0.981). The measured variables are presented in squares . IOS parameters R5-R20, X5 and AX (reflecting small-to-mid-sized airway obstruction/distensibility) loaded to the first latent variable (Lung1), $FEF_{50}$ and $FEF_{25-75}$ both corrected for FVC (reflecting small-to-mid-sized airway obstruction), to the second latent variable, while MBNW parameter Sacin (reflecting dysfunction in the most peripheral airways) loaded both to the first and second latent variable. The lung volume parameter RV/TLC % predicted (most peripheral airways dysfunction) and MBNW parameter Scond (dysfunction in small-medium size conducting airways) loaded to the third latent variable (Lung3). Please Note that Sacin loaded equally with 0.285 and 0.291 to latent variable Lung1 and Lung2 respectively. Please Note that Sacin loaded equally with 0.285 and 0.291 to latent variable Lung1 and Lung2 respectively. The numbers on the right hand

side represent the variance of the measures, i.e. variance in AX is 0.009, contrasting with the variance in RV/TLC % predicted being 0.738. Goodness of fit of the SEM model was evaluated through the following fit indices: Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), Tucker-Lewis Index (TLI). The closer CFI and TLI are to 1 and the closer RMSEA is to 0 the better is the model fit. The goodness of fit values (Supplemental methods) show there is good coherence of this model to SAD. **Fall in FVC during hyperresponsiveness testing contributed to the model as well, when analyzed in the subgroup of asthmatics who had undergone hyperresponsiveness testing (see also Supplement for model comparison).**

**Figure 5. Correlations of the Clinical SAD score of asthma participants with all parameters measured**



Legend to Figure 5. For abbreviations see Table 1

**Table 1: Baseline clinical, physiologic and CT characteristics of asthma participants and controls without airway disease**

| Parameter | Asthma | Controls | P - value |
|---|---|---|---|
| | n=773 | n=99 | |
| *Clinical characteristics* | | | |
| Age, years | 46 (34 ; 54) | 41 (29 ; 52) | 0.007 |
| Gender, female N (%) | 450 (58) | 56 (57) | 0.754 |
| Heart rate, bpm | 71 (65 ; 78) | 68 (61 ; 75) | 0.004 |
| BP syst, mmHg | 123 (114 ; 131) | 120 (110 ; 130) | 0.009 |
| BP diast, mmHg | 80 (70 ; 84) | 75 (68 ; 83) | 0.055 |
| BMI, $kg/m^2$ | 26 (23 ; 30) | 24 (21 ; 27) | <0.001 |
| Atopy (Phadiatop), N (%) | 454 (81) | 39 (46) | <0.001 |
| FeNO, ppb | 25 (16 ; 38) | 18 (11 ; 26) | <0.001 |
| Ex-smoker, N (%) | 156 (20) | 19 (19) | 0.393 |
| Current Smoker, N (%) | 27 (4) | 1(1) | |
| Eosinophils, $10^9/L$ | 0.2 (0.1 ; 0.4) | 0.1 (0.1 ; 0.2) | <0.001 |
| Neutrophils, $10^9/L$ | 3.7 (3.0 ; 4.7) | 3.3 (2.7 ; 4.4) | 0.010 |
| $PC_{20}$, mg/mL | 1.25 (0.4 ; 4.2) | 15.23 (16.0 ; 16.0) | <0.001 |
| $PD_{20}$, mg | 0.11 (0.0 ; 0.6) | 1.86 (2.0 ; 2.0) | <0.001 |
| Moderate-severe hyperresponsiveness, N (%) | 271 (48.4) | 0 (0.0) | <0.001 |
| Fall in FVC, % | 17 (12 ; 22) | 4 (1 ; 8) | <0.001 |
| *Lung Physiology characteristics (%predicted)* | | | |
| $FEV_1$, %predicted | 82.7 (69.9 ; 93.8) | 100.4 (91.6 ; 107.3) | <0.001 |
| Change $FEV_1$, %predicted | 7.6 (4.1 ; 12.7) | | |
| $FEV_1$/FVC, %predicted | 85.8 (76.5 ; 93.9) | 98.2 (93.8 ; 102.7) | <0.001 |
| IVC, %predicted | 99.0 (18.21) | 109.7 (15.28) | <0.001 |
| $FEF_{50}$, %predicted | 62.0 (43.2 ; 84.1) | 102.0 (84.8 ; 117.3) | <0.001 |

| | | | |
|---|---|---|---|
| $FEF_{25-75}$, %predicted | 56.6 (37.6 ; 75.6) | 90.7 (75.6 ; 108.1) | <0.001 |
| RV, %predicted | 117.1 (98.4 ; 138.9) | 95.6 (87.0 ; 115.7) | <0.001 |
| TLC, %predicted | 104.9 (95.9 ; 115.5) | 104.8 (96.7 ; 112.5) | 0.616 |
| RV/TLC, %predicted | 106.1 (91.6 ; 125.8) | 92.5 (80.6 ; 109.6) | <0.001 |
| FRC, %predicted | 108.7 (93.4 ; 126.7) | 107.6 (91.9 ; 121.4) | 0.419 |
| Raw, %predicted | 143.0 (91.4 ; 231.1) | 77.6 (62.9 ; 99.5) | <0.001 |
| sGaw, %predicted | 60.5 (42.5 ; 94.7) | 85.0 (61.3 ; 124.6) | <0.001 |
| R20, %predicted | 114.6 (97.4 ; 134.9) | 96.5 (84.7 ; 110.2) | <0.001 |
| R5-R20, %predicted | 278.6 (91.2 ; 640.9) | 69.5 (0.0 ; 161.7) | <0.001 |
| X5, %predicted | 130.4 (94.4 ; 184.7) | 94.6 (77.6 ; 119.7) | <0.001 |
| AX, %predicted | 209.3 (95.0 ; 510.0) | 66.1 (49.9 ; 108.0) | <0.001 |
| Scond*VT, %predicted | 180.5 (100.7 ; 305.3) | 95.6 (44.8 ; 149.6) | <0.001 |
| Sacin*VT, %predicted | 107.2 (76.7 ; 154.8) | 94.1 (61.6 ; 129.8) | 0.014 |
| *CT Scan characteristics* | | | |
| MLD Inspiratory, HU | -837.93 (-856.95 ; -811.97) | -839.89 (-853.81 ; -812.76) | 0.651 |
| MLD Ratio E/I | 0.83 (0.77 ; 0.88) | 0.80 (0.73 ; 0.87) | 0.081 |
| VI-856 | 7.82 (2.5; 19.5) | 7.83 (1.5; 15.5) | 0.347 |
| Lung Volume Ratio | 0.50 (0.43 ; 0.60) | 0.47 (0.38 ; 0.56) | 0.156 |
| Percentile 15 Inspiratory | -921 (-935;-904) | -929 (-940;-899) | 0.463 |
| Median LA/BSA Inspiratory | 10.4 (2.93) | 11.4 (2.83) | 0.027 |
| Median LA Inspiratory | 19.0 (15.7 ; 23.3) | 21.3 (18.5 ; 25.6) | 0.013 |
| Pi10 Inspiratory | 7.21 (6.59 ; 7.77) | 6.70 (6.28 ; 7.84) | 0.073 |
| Po20 %WA Inspiratory | 7.41 (6.67 ; 8.50) | 7.33 (6.42 ; 9.02) | 0.732 |

Legend to Table 1: All parameters are presented as Mean (standard deviation), Median (Quartile1 - Quartile 3), or N (%) as appropriate. BP= Blood Pressure, Syst=Systolic, BMI= Body Mass Index, FeNO=Fraction of exhaled Nitric Oxide, WBC=White Blood Cell, RV= Residual Volume, FRC=Functional Residual Capacity, PC=Provocative Concentration, PD=Provocative Dose, $PC_{20}$ and $PD_{20}$ = the provocative concentration and dose, respectively, that cause a 20% fall in $FEV_1$ from baseline $FEV_1$ during methacholine challenge, Fall in FVC, % fall in FVC at $PC_{20}$ or $PD_{20}$; $FEV_1$=Forced Expiratory Volume in the 1st second, FVC= Forced Vital Capacity, $FEF_{50}$=Forced Expiratory Flow at 50% of FVC, IVC=Inspiratory Vital Capacity, $FEF_{25-75}$= Forced Expiratory Flow at 25%-75% of FVC,RV= Residual Volume, TLC=Total Lung Capacity, FRC= Functional residual Capacity, Raw- airway resistance, sGaw= specific airway conductance, R5-R20= Peripheral Airway Resistance, X5= Resistance at 5 Hz, AX= Area of Reactance, Scond*VT= ventilation inhomogeneity in the conductive zone of the lungs, Sacin*VT= Ventilation inhomogeneity of the acinar zone of the lungs, CT= Computed tomography, MLD Ratio E/I= Mean Lung Density Expiratory to Inspiratory ratio, E=Expiratory, I=Inspiratory, LA= Lumen Area ($mm^2$), BSA= Body Surface Area ($m^2$), VI-856= Voxel index at -856 Hounsfield Units, .

**Table 2 Characteristics of asthma participants**

| ***Parameter*** | |
|---|---|
| GINA 1, N (%) | 135 (17.5) |
| GINA 2, N (%) | 85 (11.0) |
| GINA 3, N (%) | 207 (26.8) |
| GINA 4, N (%) | 300 (38.8) |
| GINA 5, N (%) | 46 (6.0) |
| *Medication use* | |
| SABA, N (%) | 671 (86.8) |
| Short acting anticholinergics, N (%) | 9 (1.2) |
| LABA, N (%) | 86 (11.1) |
| ICS, uncombined N (%), | 183 (23.9) |
| Extra-fine ICS, N (%) | 58 (7.5) |
| Non-extra-fine ICS, N (%) | 127 (16.4) |
| ICS mean daily dose ( BDP equivalent), µg | 669 (446) |
| ICS/LABA, N (%) | 460 (59.5) |
| ICS/LABA mean daily dose (BDP-equivalent), µg | 882 (634) |
| Extra-fine ICS/LABA, N (%) | 124 (16.0) |
| Non-extra-fine ICS/LABA, N (%) | 336 (43.5) |
| Oral corticosteroids, N (%) | 22 (2.8) |
| Oral corticosteroids mean daily dose, mg | 7.5 (5.0 ; 20.0) |
| Montelukast, N (%) | 144 (18.6) |
| LAMA, N (%) | 29 (3.8) |
| Biologics, N (%) | 32 (4.1) |
| Duration of disease, years | 16.7 (5.6 ; 29.3) |
| Age 1st diagnosis <18 years, % | 39 |
| Unscheduled consultations past 12 months, N | 0.3 (1.4) |
| Exacerbations past 12 months, N | 0.2 (0.6) |
| >1 exacerbation past 12 months, % | 14 |

| | |
|---|---|
| ACT, total score | 21.0 (18.0 ; 24.0) |
| ACT < 15, % | 13 |
| ACQ-6, total score | 0.8 (0.3;1.5) |
| ACQ-6 > 1.25, % | 33 |
| EQ-5D-5L, VAS score | 80.0 (70.0 ; 90.0) |
| Mini AQLQ, total score | 5.6 (4.7 ; 6.3) |

Legend to Table 2. Data are presented as N (%) or Median (Q1 to Q3 ranges) as appropriate. ACT=Asthma Control Questionnaire, ACQ-6= Asthma Control Questionnaire-6, EQ-5D-5L= Standardized measure of health status descriptive system, Mini AQLQ= Mini Asthma Quality of Life Questionnaire. Number of exacerbations and unscheduled consultations are based on the past 12 months. The daily dose of ICS (inhaled corticosteroids) is expressed in BDP equivalents, µg/day

**Table 3. Prevalence rates (%) of abnormal SAD parameters (>ULN or <LLN) according to GINA stages**

| Parameter, % | GINA 1 | GINA 2 | GINA 3 | GINA 4 | GINA 5 |
|---|---|---|---|---|---|
| $FEF_{25-75}$ | 41.4 | 43.0 | 50.5 | 54.5 | 80.4 |
| $FEF_{50}$ | 37.3 | 49.4 | 54.1 | 55.3 | 75.0 |
| % fall FVC | 71.7 | 67.9 | 75.2 | 72.7 | 84.2 |
| RV/TLC | 14.0 | 16.3 | 19.3 | 28.1 | 31.1 |
| FRC | 16.2 | 23.4 | 19.1 | 24.5 | 27.3 |
| R5-R20 | 29.9 | 40.0 | 36.5 | 50.5 | 70.6 |
| AX | 32.4 | 34.4 | 35.4 | 49.2 | 67.7 |
| X5 | 22.8 | 31.8 | 28.5 | 33.2 | 53.1 |
| Scond | 20.5 | 20.0 | 30.0 | 33.3 | 63.6 |
| Sacin | 12.3 | 17.8 | 18.5 | 20.5 | 40.9 |

Legend to Table 3. for abbreviations see Table 1. GINA severity was based on past treatment used. Note that the highest prevalence of SAD is always in GINA5, the lowest prevalence across all GINA stages is with Sacin.

**Table 4. Relationship of lung physiology variables with number of exacerbations and unscheduled consultations**

**Number of exacerbations**

| Independent variables included in the final model | Coefficient | P-value type 1 | P-value type 3 |
|---|---|---|---|
| $FEF_{25-75,\ corrected\ for\ FVC}$ | -1.226 | 0.034 | |
| R5-R20, kPa/L/s | 2.894 | 0.010 | |
| Raw, kPa*s/L | -2.286 | 0.014 | |
| RV/TLC, ratio | 2.773 | 0.038 | |
| sGaw, 1/kPa*s | -0.316 | 0.027 | |
| Height, cm | -0.053 | <.001 | |
| $PC_{20}$ and $PD_{20}$ categories – Very mild vs Normal | -1.058 | 0.017 | 0.006 |
| $PC_{20}$ and $PD_{20}$ categories - Mild vs Normal | -1.624 | <.001 | |
| $PC_{20}$ and $PD_{20}$ categories - Moderate-severe vs Normal | -1.212 | 0.004 | |
| Sex - Female vs Male | 0.717 | 0.026 | |

**Number of unscheduled consultations due to worsening symptoms**

| Independent variables included in the final model | Coefficient | P-value type 1 | P-value type 3 |
|---|---|---|---|
| $FEV_1$ , L | 0.647 | <.001 | |
| FRC , L | -0.425 | 0.007 | |
| RV/TLC, ratio | 4.659 | 0.001 | |
| sGaw , 1/kPa*s) | -0.466 | <.001 | |
| $PC_{20}$ and $PD_{20}$ categories – very mild vs normal | -0.999 | 0.004 | 0.023 |
| $PC_{20}$ and $PD_{20}$ categories - mild vs normal | -0.888 | 0.008 | |
| $PC_{20}$ and $PD_{20}$ categories - moderate-severe vs normal | -0.792 | 0.012 | |
| Sex (male/female) - Female vs Male | 0.647 | 0.023 | |

Legend to Table 4. MBNW parameters were not used, since this would restrict the number of asthmatics to be analyzed (see Methods). P-value type 3 assesses the statistical difference in hyperresponsiveness severity stages. For abbreviations see Table 1.

**Table 5. Clinical characteristics of asthma participants in Clinical SAD Group1 and Clinical SAD Group2**

| Parameter | Group1 (n=452) | Group2 (n=312) | P-value |
|---|---|---|---|
| Clinical SAD score | -0.256 (-0.34;-0.16) | 0.284 (0.12;0.56) | <0.001 |
| Age, years | 43 (30;53) | 50 (40;58) | <0.001 |
| Gender, female N (%) | 257 (57) | 186 (60) | 0.448 |
| Heart rate, bpm | 70 (64;77) | 72 (65;80) | 0.023 |
| BP syst, mmHg | 120 (110;130) | 125 (117;135) | <0.001 |
| BP diast, mmHg | 78 (70;82) | 80 (72;87) | <0.001 |
| BMI, $kg/m^2$ | 25 (22;28) | 28 (25;32) | <0.001 |
| Atopy, N (%) | 262 (81) | 187 (79) | 0.531 |
| FeNO, ppb | 24 (16;37) | 25 (16;39) | 0.424 |
| Ex-smoking, N (%) | 90 (20) | 65 (21) | 0.474 |
| Duration smoking, years | 10 (5.1;16.7) | 14 (8.0;20.0) | 0.020 |
| GINA 1/2, N (%) | 157 (35) | 60 (9) | <0.001 |
| GINA 3, N (%) | 135 (30) | 70 (22) | <0.001 |
| GINA 4/5, N (%) | 160 (35) | 182 (58) | <0.001 |
| ICS uncombined, N (%) | 98 (22) | 83 (27) | 0.116 |
| ICS/LABA, N (%) | 254 (56) | 202 (65) | 0.018 |
| ICS dose, BDP equivalence | 603.2 (384.9) | 739.9 (482.5) | 0.079 |
| ICS/LABA dose, BDP equivalence | 818.8 (563.1) | 959.6 (710.8) | 0.078 |
| Oral corticosteroids, N (%) | 8 (1.8) | 14 (4.5) | 0.027 |
| Eosinophils, $10^9/L$ | 0.21 (0.12;0.35) | 0.26 (0.16;0.40) | <0.001 |
| Neutrophils, $10^9/L$ | 3.50(2.88;4.47) | 3.90(3.07;4.91) | <0.001 |
| $FEV_1$, %predicted | 90.2 (80.1 ; 98.4) | 70.1 (58.8 ; 81.8) | <0.001 |
| Change $FEV_1$, %predicted | 6.5 (3.6 ; 9.9) | 10.2 (5.5 ; 14.9) | <0.001 |
| $FEV_1$/FVC, %predicted | 90.1 (83.4 ; 96.6) | 78.3 (70.5 ; 86.0) | <0.001 |
| $FEF_{50}$, %predicted | 75.2 (59.1 ; 94.8) | 44.4 (31.5 ; 59.7) | <0.001 |
| IVC, %predicted | 103.3 (18.0) | 93.1 (17.0) | <0.001 |
| $FEF_{25-75}$, %predicted, N (%) | 66.6 (51.7 ; 86.9) | 37.7 (27.8 ; 52.2) | <0.001 |

| | | | |
|---|---|---|---|
| RV, %predicted | 108.9 (92.7 ; 127.2) | 134.2 (110.9 ; 158.8) | <0.001 |
| TLC, %predicted | 104.3 (95.7 ; 114.0) | 105.9 (95.9 ; 116.9) | 0.239 |
| FRC, %predicted | 107.3 (91.7 ; 123.0) | 111.2 (94.8 ; 129.9) | 0.011 |
| Raw, %predicted | 110.1 (81.4 ; 167.8) | 192.3 (139.6 ; 309.3) | <0.001 |
| sGaw, %predicted | 66.5 (47.4 ; 105.1) | 47.0 (33.9 ; 72.4) | <0.001 |
| R20, %predicted | 107.8 (92.2 ; 125.7) | 126.3 (109.7 ; 147.9) | <0.001 |
| R5-R20, %predicted | 129.6 (29.0 ; 304.0) | 636.3 (378.2 ; 1065.0) | <0.001 |
| X5, %predicted | 109.1 (80.9 ; 140.5) | 199.0 (151.6 ; 254.6) | <0.001 |
| AX, %predicted | 115.3 (65.3 ; 198.3) | 613.6 (384.7 ; 868.3) | <0.001 |
| Scond*VT, %predicted | 144.6 (75.9 ; 239.7) | 245.2 (161.7 ; 392.1) | <0.001 |
| Sacin*VT, %predicted | 93.1 (70.6 ; 127.0) | 140.8 (95.8 ; 190.5) | <0.001 |
| No. unscheduled consultations , N | 0.15 (0.57) | 0.50 (2.08) | 0.001 |
| No. exacerbations, N | 0.16 (0.52) | 0.29 (0.76) | 0.002 |
| >= 1 exacerbation, N (%) | 50 (11.1) | 59 (18.9) | 0.002 |
| Duration of disease, years | 11.6 (4.4 ; 24.5) | 21.5 (9.4 ; 35.0) | <0.001 |
| Age at 1st Diagnosis, years | 25 (10 ; 41) | 22 (7 ; 41) | 0.131 |
| Age at 1st Diagnosis < 18 years, N(%) | 162 (36.2) | 134 (42.9) | 0.059 |
| ACT, total score | 22.0 (19.0 ; 24.0) | 20.0(17.0 ; 23.0) | <0.001 |
| ACT score ≤ 15, N (%) | 40 (8.9) | 60 (19.2) | <0.001 |
| ACQ-6, total mean score | 0.66 (0.2 ; 1.3) | 1.00 (0.5 ; 1.8) | <0.001 |
| ACQ-6 score ≥ 1.25, N (%) | 124 (27.4) | 126 (40.4) | <0.001 |
| EQ-5D-5L, VAS score | 83.0 (75.0 ; 90.0) | 80.0 (70.0 ; 90.0) | <0.001 |
| Mini-AQLQ, total score | 5.7 (4.8;6.4) | 5.5(4.5;6.3) | |
| *CT Scan characteristics* | | | |
| MLD Inspiratory, HU | -844.53(-859.56 ; -815.71) | -831.65(-854.46 ; -808.68) | 0.086 |
| MLD Ratio E/I | 0.82 (0.76 ; 0.87) | 0.84 (0.78 ; 0.90) | 0.007 |
| VI-856 | 6.96 (1.92 ; 18.27) | 9.54 (3.18 ; 21.30) | 0.068 |
| Lung Volume Ratio | 0.49 (0.41 ; 0.56) | 0.51 (0.45 ; 0.62) | 0.008 |
| Percentile 15 Inspiratory | -922.33 (-937.51 ; -906.97) | -917.72 (-930.20 ; -900.38) | 0.054 |
| Median LA/BSA Inspiratory | 10.95 (2.66) | 9.67 (3.08) | <0.001 |

| | | | |
|---|---|---|---|
| Median LA Inspiratory | 20.37 (17.32 ; 23.47) | 17.82 (14.59 ; 22.08) | <0.001 |
| Pi10 Inspiratory | 7.12 (6.54 ; 7.77) | 7.28 (6.59 ; 7.78) | 0.641 |
| Po20 %WA Inspiratory | 7.49 (6.71 ; 8.52) | 7.27 (6.57 ; 8.41) | 0.458 |

Legend to Table 5. Data are presented as N (%), Mean (SD) and Median (interquartile ranges) as appropriate; for abbreviations see Table 1 and Table2.