



Clinical characteristics of adult asthma associated with small airway dysfunction



S. Kjellberg^a, B.K. Houltz^{b,c}, O. Zetterström^a, P.D. Robinson^{d,e}, Per M. Gustafsson^{a,b,*}

^a Department of Pediatrics, Central Hospital, Skövde, Sweden

^b The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

^c Department of Clinical Physiology, The Sahlgrenska University Hospital/East, Gothenburg, Sweden

^d Department of Respiratory Medicine, The Children's Hospital at Westmead, Sydney, New South Wales, Australia

^e Discipline of Paediatrics and Child Health, Sydney Medical School, University of Sydney, Australia

ARTICLE INFO

Article history:

Received 23 December 2015

Received in revised form

8 May 2016

Accepted 30 May 2016

Available online 31 May 2016

Keywords:

Allergy

Eosinophils

Impulse oscillometry

Lung clearance index

Nitrogen

Multiple breath washout

Peripheral

S_{acin}

S_{cond}

Smoking

Specific ventilation ratio

Ventilation distribution

ABSTRACT

Suboptimal asthma control is common despite modern asthma therapy. The degree of peripheral airway involvement remains unclear and poor medication delivery to these regions might be a contributing reason for this failure in obtaining adequate symptom control.

A cohort of 196 adults (median (range) age 44 (18–61) years, 109 females, 54 ex-smokers, six current smokers) with physician-diagnosed asthma were recruited from primary care. Subjects were characterized clinically by interviews, questionnaires, skin prick tests (SPT) and blood eosinophil counts. Lung function was assessed by spirometry, impulse oscillometry (IOS) and nitrogen multiple breath washout (N_2 MBW). IOS assessed peripheral airway resistance (FDR, frequency dependence of resistance). N_2 MBW assessed global ventilation inhomogeneity (LCI, lung clearance index), specific indices of peripheral airway function ($S_{\text{cond}} \times V_T$ and $S_{\text{acin}} \times V_T$; V_T , tidal volume), and inter-regional inhomogeneity (specific ventilation ratio). Never-smoking healthy cohorts of 158 and 400 adult subjects provided local reference values for IOS and N_2 MBW variables, respectively.

Peripheral airway dysfunction was detected in 31% (FDR or specific ventilation ratio) to 47% ($S_{\text{cond}} \times V_T$) of subjects. Risk factors for peripheral airway dysfunction were identified. Among subjects with low FEV_1 and either positive smoking history and/or blood eosinophilia ($>4.0\%$), 63% had abnormality across all peripheral airway outcomes, whilst only one subject was completely normal.

Abnormal peripheral airway function was present in a large proportion of adult asthmatics at baseline. Reduced FEV_1 , a positive smoking history, and/or blood eosinophilia identified “a small airway asthma subtype” that might benefit from peripheral airway targeted therapy.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Despite advances in modern asthma treatment, several studies have shown suboptimal asthma control remains an issue for a significant proportion of asthma sufferers [1–3]. Peripheral airway involvement in asthma pathogenesis has been confirmed by histopathology [4,5], physiology [6,7] and imaging studies [8–10], and yet the role of the peripheral airways in the clinical expression of asthma remains largely unknown and under-appreciated [11]. Targeting treatment to the peripheral airways has been highlighted

as a potential tool to improve asthma outcomes [12]. Such an approach is supported by interventional studies comparing conventional relatively large particle inhalation therapy to ultra-fine particle treatment [13–16], demonstrating beneficial effects of the latter on peripheral airway function, asthma control [17,18], and quality of life [19]. The peripheral airways are conventionally defined as those with an internal diameter less than 2 mm in the adult, corresponding to approximately airway generation 8 and more distally [20]. Due to their large combined cross-sectional area the peripheral airways constitute a low resistance zone [21], and spirometry is therefore predicted not to be sensitive to obstruction of these small airways. In addition, uneven distribution of peripheral obstruction can be masked by increased flow via airways that are not flow restricted [22].

* Corresponding author. Department of Pediatrics, Central Hospital, Skövde, Sweden.

E-mail address: per.gustafsson@vgregion.se (P.M. Gustafsson).

Assessments of peripheral airway function are now available to asthma clinics using modern versions of two old lung function testing methods, the forced oscillation technique (FOT) [23] and nitrogen based multiple breath washout (N_2 MBW) [24]. These tests derive information about peripheral airway mechanics and the non-uniformity of ventilation distribution occurring in the small airways, respectively. Whilst commercial FOT devices like impulse oscillometry (IOS, Jaeger Masterscreen, Care Fusion, Würzburg, Germany) have been commercially available for 20 years [25], it is only recently that standardisation efforts for MBW have led to the development of a validated commercially available N_2 MBW device (Exhalyzer[®] D, Eco Medics AG, Duernten, Switzerland) [26,27]. Advances in MBW data analysis have provided additional insight into the mechanisms determining ventilation inhomogeneity [28,29]. This provides the opportunity not only to better characterize peripheral airway function in adult asthmatic subjects, but also to compare the utility of these two techniques to detect peripheral airway involvement, and to determine potential driving factors.

The primary aim of the present study was to assess the prevalence of peripheral airway dysfunction in an adult asthmatic cohort recruited from primary care centres. The secondary aim was to investigate its relationship to a range of clinical asthma features easily accessible in asthma clinics or general practice.

2. Methods

Asthmatic subjects aged 18–60 years were recruited by contacting primary care centres across Skaraborg County in West Sweden. Inclusion criteria were a physician diagnosis of asthma based on a history of recurrent dyspnoea, wheeze or cough responding to bronchodilator and/or inhaled corticosteroid therapy, and spirometry-proven reversible airway obstruction. Exclusion criteria were unstable asthma at the time of evaluation dictating a need to change therapy, known cardiac disease, hypertension, diabetes or other systemic disease, or current medication potentially affecting the response to bronchodilator therapy, such as a beta-blocker therapy. The study was performed in the Respiratory Research Laboratory, at the Central Hospital, Skövde, Sweden from May 2011 to June 2014, and was approved by the Regional Ethics Committee at the University of Gothenburg, Sweden.

Structured history and physical examination were performed by either of two authors (OZ and PG). Questions focused on onset and course of asthma, concurrent clinical allergies and related conditions, exposures to allergens, a history of ever smoking tobacco regularly, number of pack-years smoked, and medication taken. Due to the variety of medication combinations used across the cohort, an arbitrary score was assigned for different medication combinations (see online supplement, OLS). Questionnaires completed were the ACT (Asthma Control Test) [30] and the ACQ (Asthma Control Questionnaire) [31]. Skin prick tests (SPT) were performed in duplicate on the volar aspects of both forearms, respectively, using positive histamine and negative controls, and a standard panel of pollens, house dust mite, furred pets allergens and four mould allergens (Soluprick SQ; ALK, Copenhagen, Denmark) (Table E7, OLS). A mean weal of ≥ 3 mm was regarded as positive.

Lung function testing of asthmatic subjects was undertaken after withholding all medication for at least 12 h. Assessments started by triplicate measurements of fraction of exhaled nitric oxide at an expiratory flow of 50 mL/s (FENO₅₀), using an online chemiluminescent analyser (Eco Medics AG, Duernten, Switzerland) (See OLS for further details). The mean value of three recordings within 10% was noted. A mean FENO₅₀ value > 25 ppb was defined as abnormal.

IOS was performed using a Jaeger Masterscreen system (Care Fusion, Würzburg, Germany). One minute recordings were performed in the upright sitting position, during relaxed tidal breathing via a mouthpiece and a microbe filter (MicroGard II, bacterial/viral filter, Care Fusion, Hoechst, Germany), with the subject wearing a nose-clip and with the hands supporting the cheeks. At least two technically acceptable recordings were performed per subject. Sequences with artefacts from swallowing or evidence of glottic closures were excluded. Mean values of resistance at 5 Hz and 20 Hz (R5 and R20), frequency dependence of resistance (FDR; R5–R20), area under the reactance curve (AX), and the square root of AX (Sqrt (AX)) were calculated. Previously collected data from 158 healthy never smoker subjects (85 females) aged 18–62 years, recruited via the Swedish population register, were used for calculation of reference values (see OLS for further details). Results are reported as absolute values and z-scores based on findings in the healthy reference cohort.

N_2 MBW was performed using the Exhalyzer[®] D N_2 MBW device (Eco Medics AG, Duernten, Switzerland). Three technically acceptable N_2 MBW tests were performed in all subjects, in accordance with the recently published consensus statement [26]. While the commercial software delivered with Exhalyzer[®] D (Spiroware 3.1) was used for data recording, in-house software written with a commercial software package (TestPoint[®], Capital Equipment, MA, USA) was used for off-line analysis, and for quality control purposes. The basic algorithms in the TestPoint[®] software are applied in Spiroware. The OLS describes the calculations of all indices of ventilation inhomogeneity derived from N_2 MBW, including the lung clearance index (LCI; a global index of ventilation inhomogeneity), specific indices of peripheral airway function derived by Sn_{III} analysis (S_{cond} and S_{acin}) [26] and compartment analysis [28]. In the present paper all Sn_{III} -derived indices were corrected for lung size and breathing pattern by multiplying Sn_{III} by expiratory tidal volume of each subsequent breath, before further calculations [26]. Thus, $S_{cond} \times V_T$ and $S_{acin} \times V_T$ are reported. The presence of abnormal inter-regional ventilation distribution was assessed by “compartment analysis” using recently published algorithms [28]. The OLS provides details on the calculations of the slower and faster lung compartments, their respective specific ventilation (regional alveolar tidal volume/regional lung volume), and the specific ventilation ratio (SV-ratio; specific ventilation of “faster”/“slower” compartment). Results are presented as absolute values and as z-scores. Reference values were generated using data for N_2 MBW variables derived from a healthy never smoker adult cohort of 400 subjects (208 females) aged 17–71 years, recruited via the Swedish population register. Derivation of N_2 MBW indices, including the recent novel indices of slower and faster ventilated lung volumes and their specific ventilation, and details about the healthy reference population, are provided in the OLS.

Spirometry was performed using the Jaeger Masterscreen (Care Fusion, Würzburg, Germany) in accordance with current European Respiratory Society (ERS) and American Thoracic Society (ATS) standards [32]. Spirometry data was expressed as z-scores (“z”) using the Hedenström Swedish reference equations [33,34].

2.1. Statistics

Statistical analysis of the data generated was performed using Statistica 7™ (StatSoft, Tulsa, OK, USA). Z-scores for IOS and N_2 MBW outcomes in the asthma population were calculated as the difference between measured value and predicted value divided by the residual standard deviation (RSD) in the reference population. Upper limit of normal (ULN) was defined as predicted value + 1.96 RSD and lower limit of normal (LLN) as predicted value – 1.96 RSD.

Because several lung function variables did not show normal

distribution non-parametric tests were used, Mann Whitney U-tests for comparisons of outcomes between groups and subgroups and Chi²-test with Yates correction for assessment of differences in proportions. Correlation matrices including lung function variables and clinical features were produced to identify clinical characteristics associated with impaired IOS and N₂ MBW variables. ANOVA was subsequently performed using the most closely related clinical characteristics in dichotomized form as categorising (independent) variables and IOS and N₂ outcomes as the dependent variables. ANOVA results included overall model fit, and F-values and p-levels for the specific association between a peripheral airway outcome and an explanatory clinical characteristic. A two tailed p-value < 0.05 was considered significant.

3. Results

A total of 196 subjects (109 female, 56%) were included, median (range) age 44 (18–61) years. Demographics are summarised in Table 1.

Only 21 subjects (15%) were not on regular inhaled controller therapy (inhaled corticosteroid, ICS or ICS/long-acting beta₂-agonist (LABA) combination therapy or oral montelukast; OLS Table E10). Twenty-four (12%) were on low dose ICS alone, whilst 66 (49%) used high dose ICS alone (budesonide ≥800 mcg/day or fluticasone ≥400 mcg/day), or combined with LABA and/or montelukast. Eight subjects used pressurized metered dose inhalers (pMDIs) for controller medication delivery, and the remainders used dry powder inhalers (DPIs).

Based on ACT, 40% had insufficiently and 20% poorly controlled asthma. Asthma onset occurred most frequently during adult years (20–40 yrs, 29%) followed by preschool years (24%) and adolescence (15%). Seasonal and perennial allergic rhinitis was reported by 74% and 39%, respectively. A pet in the home was reported by 33% (21% cat, 17% dog). Two-thirds had a least one positive SPT, blood eosinophilia (>4.0%) was present in 72 subjects (37%), and 60 (31%) had a smoking history, while only six were current smokers.

Spirometry and FENO50 results are summarised in Table 2. Spirometry variables were in general moderately reduced, with FEV₁ (z) <−3.0 in only 19 subjects (9.6%), and 139 subjects (71%) within the normal range (Fig. 1). No gender difference was seen, while all spirometry variables except FEV₁ and FVC, were significantly lower among ever-smokers (n = 60) vs. never-smokers. Median (range) FENO50 was 17.1 (2.3–189.5) ppb, abnormal in 59 (30%) subjects, and higher in males (p < 0.05).

3.1. Peripheral airway function tests

IOS and MBW results are summarised in Table 3. R5 and R20 were abnormal in 42% and 30%, respectively. FDR (z) was raised in 31% and AX in 33%. AX showed a curvilinear relationship with FDR (R² 0.93; p < 0.001), which linearised using Sqrt (AX) (R² 0.93; p < 0.001). Only 4 subjects (2%) showed discordance when classified as normal/abnormal using Sqrt (AX) and FDR. For these reasons, only FDR was used in subsequent analyses.

FDR (z) and S_{cond} × V_T (z) were higher in males vs. females, while R20 (z) was lower. All IOS outcomes except R20, and all MBW variables except S_{cond} × V_T were significantly more elevated in ever-smokers vs. never-smokers, and the differences persisted for several variables even when subjects with ≥5 pack-years were excluded. When excluding current smokers, FDR (z-score, 1.12 vs. 0.69; p < 0.05), LCI (z-score, 2.32 vs. 1.34; p < 0.01), and S_{acin} × V_T (z-score, 2.91 vs. 0.97; p < 0.001) remained different vs. non-smokers, which was also seen with FEV₁/FVC (z-score, −1.48 vs. −0.63; p < 0.05).

FDR and MBW abnormalities were more frequently seen with abnormal FEV₁ (Figs. 1–6). A significant number of subjects had, however, detectable abnormalities missed by FEV₁, while the opposite was much less common. Similar patterns were shown for FEV₁/FVC ratio (data not shown).

FDR was abnormal in 31%, LCI in 44%, S_{cond} × V_T in 47%, S_{acin} × V_T in 42%, and SV-ratio in 31% of subjects (Fig. 6). MBW variables were more frequently abnormal than FDR (p < 0.01 vs. LCI and S_{cond} × V_T;

Table 1
Demography and clinical features in full asthma cohort, and in females and males, never-smokers and ever-smokers, respectively.

Variable	All	Females	Males	Never-smokers	Ever-smokers
N	196	109	87	136	60
Sex (M/F)	87/109	0/109	87/87	62/74	25/35
Age (yrs)	44 (18; 61)	44 (18; 60)	45 (18; 61)	43 (18; 61)	47 (18; 61)*
Weight (kg)	79 (50; 160)	72 (50; 160)	83 (55; 126)***	79 (50; 160)	79 (50; 120)
Height (cm)	173 (149; 200)	167 (149; 185)	180 (162; 200)***	173 (149; 200)	172 (150; 187)
BMI (kg/m ²)	25.8 (17.7; 55.4)	25.5 (19.3; 55.4)	26.2 (17.7; 36.9)	25.4 (19.3; 55.4)	27.0 (17.7; 37.1)
ACT	20.5 (6; 25)	20.0 (8; 25)	21.0 (6; 25)	20.0 (9; 25)	21.0 (6; 25)
ACT<16	39/196	22/109	17/87	27/136	12/60
ACT<20	77/196	45/109	32/87	55/136	22/60
ACQ	0.83 (0.00; 5.17)	0.83 (0.00; 4.00)	0.83 (0.00; 5.17)	0.83 (0.00; 4.00)	0.67 (0.00; 5.17)
ACQ>1.0	86/190	49/106	37/84	65/131	21/59
B-eosinophils (%)	2.8 (0.0; 26.7)	2.6 (0.0; 16.9)	3.1 (0.0; 26.7)	2.5 (0.0; 26.7)	3.4 (0.0; 13.0)
B-eosinophils >4.0%	72/196	37/109	35/87	48/136	24/60
No. positive SPT	3 (0; 13)	2 (0; 13)	4 (0; 12)*	3 (0; 13)	2 (0; 11)
All SPT negative	65/192	41/107	24/85	45/133	20/59
Age at asthma onset					
Preschool	47/196	26/109	21/87	34/136	13/60
School age	32/196	19/109	13/87	20/136	12/60
Adolescence	30/196	18/109	12/87	23/136	7/60
20–40 yrs	57/196	36/109	21/87	37/136	20/60
> 40 yrs	30/196	10/109	20/87*	22/136	8/60
Allergic rhinitis	146/196	80/109	66/87	103/136	43/60
Pack-years smoking	0.0 (0.0; 30.0)	0.0 (0.0; 25.0)	0.0 (0.0; 30.0)	0.0 (0.0; 0.0)	5.0 (0.5; 30.0)
Ever smokers	60/196	35/109	25/87	0/136	60/60
Current smokers	6/196	4/109	2/87	0/136	6/60

Data displayed as Median and range or proportion of subjects. *p < 0.05, ***p < 0.001, Mann-Whitney U test and Chi²-test for proportions, refer to comparisons between females and males, and between never-smokers and ever-smokers, respectively.

Table 2

Spirometry and FENO50 findings in full asthma cohort, and in females and males, never-smokers and ever-smokers, respectively.

Variable	All	Females	Males	Never-smokers	Ever-smokers
N	196	109	87	136	60
FEV ₁ (L)	3.14 (1.22; 5.35)	2.91 (1.22; 4.78)	3.66 (1.55; 5.35)***	3.16 (1.22; 5.35)	3.02 (1.52; 5.06)
FVC (L)	4.29 (1.76; 7.23)	3.90 (1.76; 6.27)	5.12 (2.60; 7.23)***	4.31 (1.76; 7.23)	4.24 (2.32; 6.52)
FEV ₁ /FVC (%)	73.0 (45.4; 89.8)	74.6 (55.3; 88.0)	71.5 (45.4; 89.8)***	73.7 (55.8; 89.8)	67.3 (45.4; 81.8)***
MEF ₅₀ (L/s)	2.9 (0.9; 7.0)	2.8 (0.9; 5.1)	3.0 (0.9; 7.0)	3.0 (0.9; 7.0)	2.4 (0.9; 6.8)**
MEF ₂₅ (L/s)	0.9 (0.2; 3.6)	0.9 (0.2; 2.4)	0.9 (0.2; 3.6)	1.0 (0.3; 3.6)	0.7 (0.2; 2.0)***
FEV ₁ (% pred)	87.6 (39.3; 123.8)	89.7 (48.1; 120.8)	86.6 (39.3; 123.8)	89.0 (44.5; 123.8)	83.0 (39.3; 120.8)
FVC (% pred)	95.1 (48.7; 128.9)	95.7 (61.5; 128.9)	93.4 (48.7; 127.5)	93.4 (51.4; 128.9)	96.6 (48.7; 116.3)
FEV ₁ /FVC (% pred)	93.0 (55.0; 115.9)	93.8 (65.8; 115.9)	92.1 (55.0; 115.9)	94.5 (65.8; 115.9)	87.6 (55.0; 111.0)**
MEF ₅₀ (% pred)	61.7 (17.5; 153.5)	61.5 (21.4; 112.4)	62.0 (17.5; 153.5)	63.7 (21.4; 153.5)	55.1 (17.5; 132.8)**
MEF ₂₅ (% pred)	53.9 (14.4; 139.7)	56.1 (17.4; 139.7)	52.2 (14.4; 137.7)	57.4 (17.4; 139.7)	46.0 (14.4; 136.3)**
FEV ₁ (z)	−1.10 (−5.67; 2.23)	−0.86 (−3.93; 1.63)	−1.22 (−5.67; 2.23)	−0.98 (−5.33; 2.23)	−1.39 (−5.67; 1.63)
FEV ₁ /FVC (z)	−0.85 (−6.16; 1.96)	−0.72 (−4.23; 1.76)	−0.92 (−6.16; 1.96)	−0.63 (−5.12; 3.15)	−0.32 (−4.59; 2.04)**
MEF ₅₀ (z)	−1.61 (−3.85; 2.01)	−1.66 (−3.85; 0.49)	−1.53 (−3.68; 2.01)	−0.63 (−4.23; 1.96)	−1.48 (−6.16; 1.17)**
MEF ₂₅ (z)	−1.51 (−4.55; 1.90)	−1.36 (−4.55; 1.40)	−1.69 (−4.50; 1.90)*	−1.52 (−3.73; 2.01)	−2.03 (−3.85; 1.47)*
FENO50 (ppb)	17.1 (2.3; 189.5)	13.7 (2.3; 90.2)	20.0 (3.3; 189.5)*	16.9 (2.3; 189.5)	17.6 (3.3; 97.5)

Data displayed as Median and range. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Mann-Whitney U test, refer to comparison between females and males, and between never-smokers and ever-smokers, respectively.

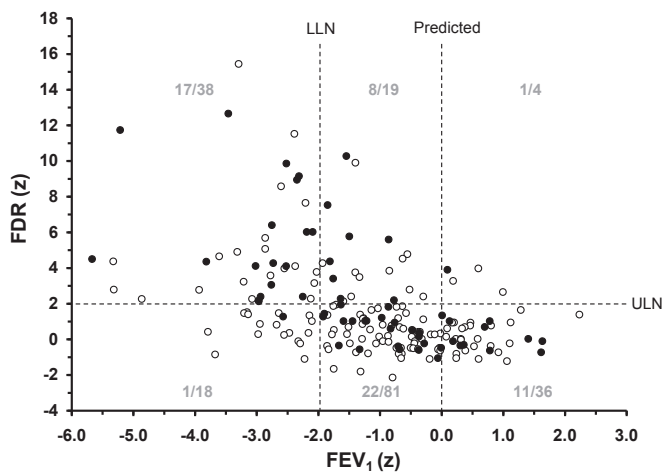


Fig. 1. FDR (z) vs. FEV₁ (z) in 196 adults subjects with asthma. Open circles denote never-smokers ($n = 136$) and closed circles denote ever-smokers ($n = 60$). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for FDR. LLN, lower limit of normal for FEV₁. Predicted, predicted value for FEV₁.

$p < 0.05$ vs. $S_{\text{acin}} \times V_T$). LCI and FDR classification was concordant in 77%, whilst abnormal LCI but normal FDR occurred in 18%. Similar findings were seen for the other N₂ MBW variables vs. FDR (data not shown), indicating that IOS and MBW provide complementary information despite relatively good overall agreement.

Ever-smokers showed significant over-representations of abnormal FDR ($p < 0.05$), LCI ($p < 0.01$) and $S_{\text{acin}} \times V_T$ ($p < 0.001$), but not $S_{\text{cond}} \times V_T$ ($p = 0.30$), and a tendency for SV-ratio ($p = 0.051$) (Figs. 1–5), despite their generally modest tobacco smoke exposure (median 5 pack-years).

FEV₁ (z), FEV₁/FVC (z), and R20 (z) showed close to normal distributions (Figures E17–E19, OLS). In contrast, R5 (z), FDR (z) and z-scores of N₂ MBW variables demonstrated marked skewness to the right, suggesting the presence of a peripheral airway dysfunction asthma subtype (Figures E20–E25, OLS).

3.2. Models of relationships to clinical characteristics

Tables 4A–C summarise the closest relationships identified between FDR or MBW variables and clinical characteristics in the cohort. Complete correlation matrices are provided in the OLS

(Tables E16–E18). ACT and ACQ correlated closely ($R 0.84$, $p < 0.001$), and therefore only ACT is included. FEV₁ was the background variable most strongly related to FDR and MBW outcomes in the asthma cohort as a whole, and regardless of smoking category. Across the whole cohort, and in never-smokers, blood eosinophils showed the second strongest association with peripheral airway outcomes, with the weakest relationship to FDR. Percentage eosinophils did not contribute to any peripheral airway outcome in ever-smokers. ACT showed the weakest correlations with peripheral airway indices in general, but correlated significantly with $S_{\text{cond}} \times V_T$ irrespective of smoking history.

Across the whole cohort, FENO and extent of smoking (pack-years) showed similarly strong relationships to peripheral airway outcomes. Number of pack-years correlated better with FDR and $S_{\text{acin}} \times V_T$ than with other indices, and not at all with $S_{\text{cond}} \times V_T$. Allergic sensitization was not associated with any outcome measures (Tables E16–E18, OLS). Medication score correlated negatively with FENO in the asthma cohort as a whole ($R -0.24$, $p < 0.01$) and among asthmatics who had not smoked ($R -0.26$, $p < 0.01$), while no significant correlation was seen among ever-smokers ($R -0.16$, $n.s.$).

The overall strength of the ANOVA models including dichotomized clinical characteristics as independent variables was similar across the peripheral airway outcomes with the lowest specific R^2 and F-values for $S_{\text{cond}} \times V_T$ (z) (Table 5). Reduced FEV₁ was the strongest contributor to all models while raised FENO (>25 ppb) did not contribute significantly to any peripheral airway index.

A history of tobacco smoking was the second most important contributor to FDR (z), while the contribution of ACT <16 did not quite reach significance, and raised blood eosinophils or FENO did not contribute. Positive smoking history and raised blood eosinophils were both highly significant contributors to raised LCI (z) and $S_{\text{acin}} \times V_T$ (z), while ACT or FENO did not contribute. In addition to reduced FEV₁, only ACT <16 was a significant contributor to $S_{\text{cond}} \times V_T$ (z), while raised blood eosinophils was close to producing a significant contribution ($p = 0.053$). Smoking history, raised blood eosinophils and low ACT, but not FENO, contributed significantly to a raised SV-ratio.

The outcome with respect to abnormal FDR and MBW indices was further explored with respect to the presence or absence of the most significant “clinical risk factors” identified by ANOVA. Results are tabulated in the form of a tick box scheme (Table 6). Median values for the corresponding actual outcomes are given Fig. 7. Median (range) values are given in Table E19 (OLS), and group

Table 3
IOS (Impulse Oscillometry) and N₂ MBW (multiple breath washout) findings in full asthma cohort, and in females and males, never-smokers and ever-smokers, respectively.

Variable	All	Females	Males	Never-smokers	Ever-smokers
N	196	109	87	136	60
R5 (kPa/L/s)	0.47 (0.24; 1.36)	0.50 (0.25; 1.36)	0.43 (0.24; 1.05)**	0.45 (0.24; 1.36)	0.53 (0.25; 1.05)*
R20 (kPa/L/s)	0.37 (0.18; 0.68)	0.41 (0.23; 0.68)	0.33 (0.18; 0.58)**	0.36 (0.18; 0.68)	0.39 (0.18; 0.59)
FDR (kPa/L/s)	0.09 (−0.03; 0.68)	0.08 (−0.03; 0.68)	0.10 (0.02; 0.53)	0.08 (−0.03; 0.68)	0.11 (0.02; 0.53)**
AX (kPa/L)	0.34 (0.03; 7.01)	0.36 (0.08; 7.01)	0.30 (0.03; 6.40)	0.32 (0.07; 7.01)	0.47 (0.03; 6.40)*
Sqrt (AX) (kPa/L) ^{1/2}	0.58 (0.17; 2.65)	0.58 (0.17; 2.65)	0.55 (0.17; 2.53)	0.56 (0.27; 2.65)	0.69 (0.17; 2.53)*
R5 (z)	1.63 (−1.77; 13.30)	1.68 (−1.77; 13.30)	1.48 (−1.18; 10.68)	1.43 (−1.77; 13.30)	2.31 (−0.99; 10.68)*
R20 (z)	1.10 (−1.54; 5.48)	1.45 (−1.28; 5.48)	0.84 (−1.54; 5.27)*	1.02 (−1.54; 5.48)	1.47 (−1.54; 4.85)
FDR (z)	0.94 (−2.14; 15.44)	0.61 (−2.14; 15.44)	1.39 (−0.72; 12.66)**	0.69 (−2.14; 15.44)	1.31 (−1.06; 12.66)**
AX (z)	0.53 (−1.06; 37.23)	0.49 (−1.06; 37.23)	0.58 (−0.94; 34.85)	0.41 (−1.06; 37.23)	1.12 (−0.94; 34.85)*
Sqrt (AX) (z)	0.77 (−1.46; 14.24)	0.68 (−1.42; 14.24)	0.82 (−1.46; 12.91)	0.62 (−1.42; 14.24)	1.29 (−1.46; 12.91)*
LCI	7.77 (6.36; 20.65)	7.72 (6.36; 17.67)	7.85 (6.60; 20.65)*	7.69 (6.36; 17.67)	8.34 (6.38; 20.65)***
S _{cond} × V _T (L)	0.027 (0.003; 0.117)	0.027 (0.007; 0.099)	0.027 (0.003; 0.117)	0.026 (0.003; 0.117)	0.032 (0.007; 0.099)
S _{acin} × V _T (L)	0.097 (0.022; 0.548)	0.084 (0.022; 0.452)	0.107 (0.027; 0.548)**	0.085 (0.022; 0.452)	0.130 (0.024; 0.548)***
SV-ratio	3.57 (2.44; 10.05)	3.60 (2.44; 8.62)	3.57 (2.56; 10.05)	3.49 (2.44; 10.05)	3.83 (2.77; 8.62)**
LCI (z)	1.70 (−1.59; 32.15)	1.57 (−1.59; 24.22)	1.84 (−0.79; 32.15)	1.34 (−0.81; 24.22)	2.71 (−1.59; 32.15)***
S _{cond} × V _T (z)	1.69 (−4.29; 26.05)	1.24 (−1.91; 13.71)	2.15 (−4.29; 26.05)*	1.37 (−4.29; 26.05)	2.11 (−2.87; 18.04)
S _{acin} × V _T (z)	1.59 (−2.19; 24.11)	1.34 (−2.19; 20.67)	1.92 (−2.11; 24.11)	0.97 (−2.19; 20.67)	2.91 (−2.10; 24.11)***
SV-ratio (z)	0.73 (−1.58; 15.92)	0.74 (−1.58; 12.47)	0.55 (−1.53; 15.92)	0.40 (−1.58; 15.92)	1.24 (−1.15; 12.98)**

Data displayed as Median and range. R5, resistance at 5 Hz. R20, resistance at 20 Hz. FDR, frequency dependence of resistance, (R5–R20). AX, reactance area. Sqrt(AX), square root of AX. LCI, lung clearance index. SV-ratio, specific ventilation ratio. *p < 0.05, **p < 0.01, Mann-Whitney U test, refer to comparisons between females and males, and between never-smokers and ever-smokers, respectively.

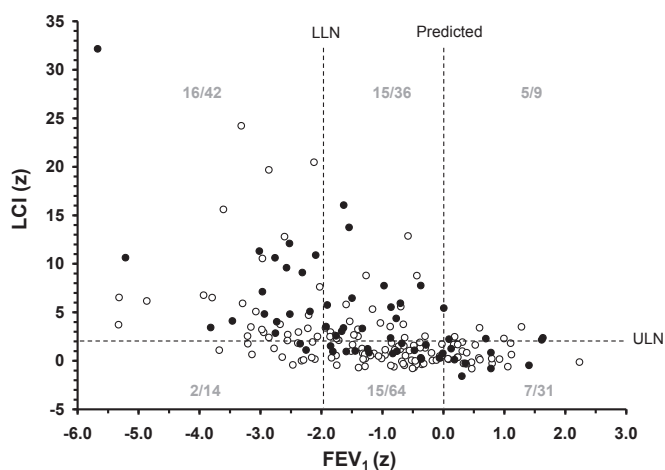


Fig. 2. LCI (z) vs. FEV₁ (z) in 196 adults subjects with asthma. Open circles denote never-smokers (n = 136) and closed circles denote ever-smokers (n = 60). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for LCI. LLN, lower limit of normal for FEV₁. Predicted, predicted value for FEV₁.

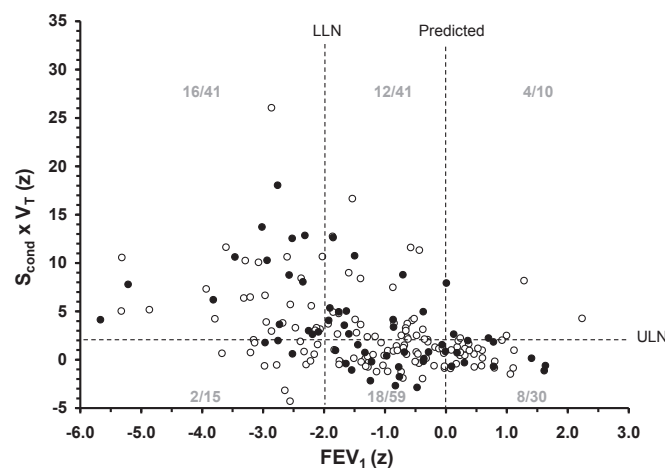


Fig. 3. S_{cond} × V_T (z) vs. FEV₁ (z) in 196 adults subjects with asthma. Open circles denote never-smokers (n = 136) and closed circles denote ever-smokers (n = 60). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for S_{cond} × V_T. LLN, lower limit of normal for FEV₁. Predicted, predicted value for FEV₁.

comparisons for the different outcomes are given in Table E20-26 (OLS). The combination of reduced FEV₁ with a smoking history, and/or raised blood eosinophils indicated a very high risk of presenting with peripheral airway dysfunction and very low chance of having completely normal findings (Table 6). A similar outcome pattern was seen for the combination of reduced FEV₁ and ACT < 16.

Seventy subjects (36%) were absent of the three most important risk factors (reduced FEV₁, a history of smoking and raised blood eosinophils). The prevalence of abnormal peripheral airway function indices among these subjects ranged from 10% (FDR) to 30% (S_{cond} × V_T), and all indices were within normal range in 36/70 subjects (51%). Among the 116 subjects with normal FEV₁ and ACT ≥ 16 the presence of abnormal peripheral airway function indices ranged from 15% (FDR) to 30% (S_{cond} × V_T), while FDR and MBW indices were all normal in 46%.

Among the 56 subjects with reduced FEV₁, 38 had a smoking history, raised blood eosinophils, or both, while the 18 remainders lacked these two features (Table 6). These subgroups differed

significantly (medians) with respect to FDR (z) (4.11 vs. 1.37; p < 0.01), LCI (z) (6.04 vs. 2.52; p < 0.001), S_{cond} × V_T (z) (6.28 vs. 2.55; p < 0.01), S_{acin} × V_T (z) (5.06 vs. 1.37; p < 0.001), SV-ratio (z) (4.03 vs. 0.99; p < 0.001). ACT scores were similar (19.5 vs. 20.0; n.s.), while age differed (51.4 vs. 39.5 years; p < 0.001).

4. Discussion

This is the first report on the prevalence of peripheral airway dysfunction as determined by both IOS and N₂ MBW in an adult asthma cohort recruited from primary care. It provides convincing evidence that peripheral airway dysfunction is a common finding in adult asthma, present in up to half of the overall cohort. IOS and MBW provide complementary information, while MBW seems to be a more sensitive and discriminative method. Simple clinical features did predict the presence of abnormal small airway function. These were reduced FEV₁ in combination with a positive

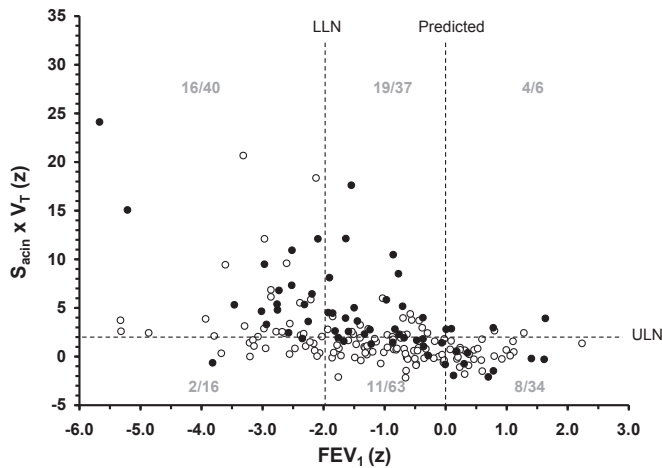


Fig. 4. $S_{\text{acin}} \times V_T (z)$ vs. $FEV_1 (z)$ in 196 adults subjects with asthma. Open circles denote never-smokers ($n = 136$) and closed circles denote ever-smokers ($n = 60$). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for $S_{\text{acin}} \times V_T$. LLN, lower limit of normal for FEV_1 . Predicted, predicted value for FEV_1 .

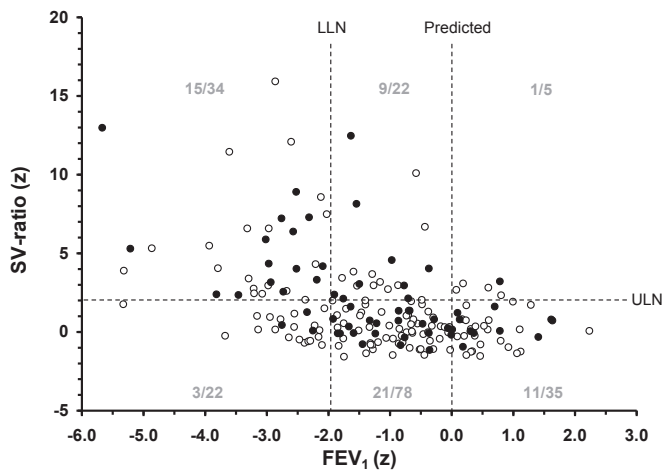


Fig. 5. SV-ratio (z) vs. $FEV_1 (z)$ in 196 adults subjects with asthma. Open circles denote never-smokers ($n = 136$) and closed circles denote ever-smokers ($n = 60$). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for SV-ratio. LLN, lower limit of normal for FEV_1 . Predicted, predicted value for FEV_1 .

smoking history and/or raised blood eosinophils, or reduced FEV_1 in combination with poorly controlled asthma ($ACT < 16$), while reduced FEV_1 in the absence of the additional features did not signal small airway involvement. Our findings show that even a short history of past smoking may leave the lungs with significantly impaired small airway function. The extent of allergic sensitization was not associated with small airway dysfunction. The present findings are novel and could potentially serve as a simple clinical guide to identify a “small airway asthma subtype”.

4.1. Do we need both IOS and N_2 MBW outcomes, plus several different MBW variables?

The study findings suggest added utility when both IOS and N_2 MBW are used and physiological reasons further justify their complementary nature. IOS reflects the mechanics (resistance and compliance) of the airways reached by the pressure waves generated by the IOS device. We would expect that these pressure waves travel preferentially through the more patent (*i.e.* less resistive) of parallel pathways at branch points. Therefore FDR reports peripheral airway resistance of relatively better functioning airways and lung units, while indices from ventilation distribution tests give weight to relatively poorly ventilated lung units. Such units slow the clearing of the inert marker gas, causing it to emerge later in the expiration (*i.e.* increased phase III slope), and delay marker gas clearance of the lung as a whole (*i.e.* higher LCI). Striking differences between FDR and LCI were observed (Figure E26, OLS). As FEV_1 decreased the scatter in difference between LCI (z) and FDR (z) increased, suggesting the presence of different mechanisms driving the reduction in FEV_1 (Figure E27, OLS). A similar pattern was observed with $S_{\text{cond}} \times V_T (z)$ and $S_{\text{acin}} \times V_T (z)$ (Figure E28–E29, OLS). These observations indicate that important aspects of peripheral airway disease may be missed if only one method or index is used. Future studies should focus on better understanding of the reasons behind these differences.

4.2. Risk factors for peripheral airway dysfunction

High and low risk groups for peripheral airway dysfunction were identified. Subjects with low FEV_1 , positive smoking history and/or raised blood eosinophils had abnormality across all peripheral outcomes in two-thirds of subjects and almost no-one had completely normal peripheral outcomes. A combination of low FEV_1 and evidence of uncontrolled asthma ($ACT < 16$) resulted in a

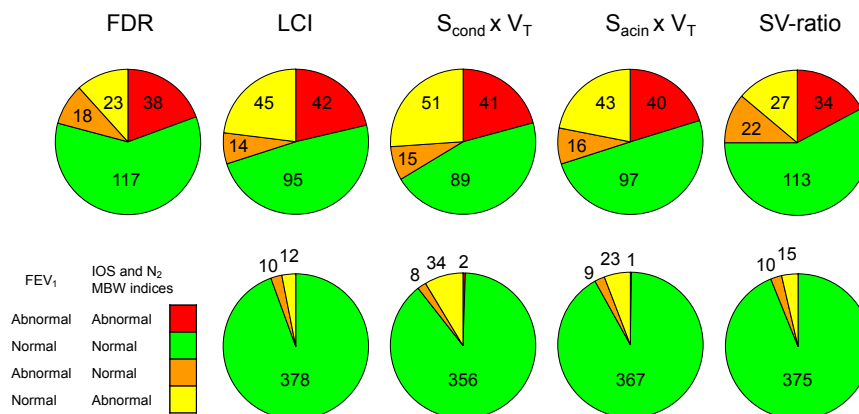


Fig. 6. Number of subjects in asthma cohort (upper panel) and in N_2 MBW healthy control cohort (lower panel) with different outcomes at spirometry, IOS or N_2 MBW. (FDR, frequency dependence of resistance, (R5–R20). LCI, lung clearance index. SV-ratio, specific ventilation ratio).

Table 4A

Correlation matrix for full asthma cohort.

Variable	FDR	LCI	$S_{\text{cond}} \times V_T (z)$	$S_{\text{acin}} \times V_T (z)$	SV-ratio (z)
FEV ₁ (z)	−0.47***	−0.50***	−0.42***	−0.47***	−0.45***
B-eosinophils (%)	0.18*	0.39***	0.39***	0.38***	0.38***
Pack-years smoking	0.25***	0.18**	0.10	0.30***	0.18*
FENO50 (ppb)	0.05	0.28***	0.28***	0.20***	0.30***
ACT	−0.15*	−0.17*	−0.31***	−0.11	−0.20**

R-values and p-levels are given.*p < 0.05, **p < 0.01, ***p < 0.001.

Table 4B

Correlation matrix for never-smokers.

Variable	FDR	LCI	$S_{\text{cond}} \times V_T (z)$	$S_{\text{acin}} \times V_T (z)$	SV-ratio (z)
FEV ₁ (z)	−0.39***	−0.43***	−0.39***	−0.40***	−0.39***
B-eosinophils (%)	0.25***	0.52***	0.34***	0.56***	0.47***
Pack-years smoking	—	—	—	—	—
FENO50 (ppb)	0.11	0.34***	0.41***	0.26***	0.38***
ACT	−0.16	−0.25**	−0.28***	0.25**	−0.26**

R-values and p-levels are given.*p < 0.05, **p < 0.01, ***p < 0.001.

Table 4C

Correlation matrix for ever-smokers.

Variable	FDR	LCI	$S_{\text{cond}} \times V_T (z)$	$S_{\text{acin}} \times V_T (z)$	SV-ratio (z)
FEV ₁ (z)	−0.63***	−0.63***	−0.49***	−0.63***	−0.56***
B-eosinophils (%)	0.04	0.16	0.12	0.13	0.15
Pack-years smoking	0.26*	0.09	0.09	0.20	0.16
FENO50 (ppb)	−0.07	0.24	−0.05	0.19	0.14
ACT	−0.16	−0.08	−0.38***	0.02	−0.11

R-values and p-levels are given.*p < 0.05, ***p < 0.001.

Table 5ANOVA outcomes in the asthma cohort as a whole for z-scores of IOS and N₂ MBW variables using a model including five dichotomized independent variables.

Independent variables	Dependent variables				
	FDR (z)	LCI (z)	$S_{\text{cond}} \times V_T (z)$	$S_{\text{acin}} \times V_T (z)$	SV-ratio (z)
Overall model fit					
Adjusted R ²	0.27	0.27	0.22	0.28	0.28
F-value	15.40	15.76	11.79	15.25	15.21
p-level	<0.001	<0.001	<0.001	<0.001	<0.001
FEV ₁ <LLN					
F-value	52.21	31.38	22.54	30.44	29.08
p-level	<0.001	<0.001	<0.001	<0.001	<0.001
Ever-smoker					
F-value	10.58	8.91	1.71	22.23	5.51
p-level	<0.001	0.003	0.193	<0.001	0.020
B-eos >4.0%					
F-value	2.18	9.39	3.79	9.56	9.01
p-level	0.148	0.002	0.053	0.002	0.003
ACT<16					
F-value	3.71	3.46	12.19	0.45	4.41
p-level	0.056	0.64	0.001	0.502	0.037
FENO50 > 25 ppb					
F-value	1.73	1.30	0.92	0.06	2.13
p-level	0.190	0.256	0.340	0.802	0.146

LLN, lower limit of normal. B-eos, blood-eosinophil count (%).

similar outcome. Normal FEV₁ findings in never-smokers without blood eosinophilia were associated with lower risks (maximum 30%) of having an abnormal outcome for a particular small airway index and increased the chance of having completely normal small airway function to approximately 50%. The skewed distribution of peripheral airway outcomes was in contrast to the pattern observed with both FEV₁ (z), which is recognised to poorly reflect peripheral airway function, and R20 (z), which is a measure of central airway resistance. This further supports the presence of a “small airway

asthma subtype”.

Asthma therapy directed to the peripheral airways might be particularly beneficial in such a population. Our findings need to be demonstrated in other cohorts, however, and interventional studies are required before the validity of this concept is confirmed.

FEV₁ reduction alone, without the presence of additional “risk factors”, did not predict small airway dysfunction. However, there was a significant detrimental effect when combined with either a smoking history and/or raised blood eosinophils (Table 6, Fig. 7).

Table 6

Proportion of subjects in the asthma cohort with abnormal (+) or all negative outcomes in subgroups, defined with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively. ACT, Asthma Control Test.

FEV ₁ ↓	Ever-smoker	B-eos >4.0%	FDR +	LCI +	S _{cond} × V _T +	S _{acin} × V _T +	SV-ratio +	FDR and all N ₂ MBW +	All negative
☒	☒	☒	8/8	8/8	6/8	7/8	8/8	5/8	0/8
☐	☒	☒	3/16	9/16	4/16	10/16	3/16	0/16	4/16
☒	☒	☐	9/10	8/10	10/10	9/10	7/10	6/10	0/10
☐	☒	☐	6/26	11/26	12/26	13/26	7/26	1/26	7/26
☒	☐	☒	15/20	16/20	15/20	18/20	14/20	13/20	1/20
☐	☐	☒	7/28	12/28	14/28	9/28	8/28	1/28	11/28
☒	☐	☐	6/18	10/18	10/18	6/18	5/18	1/18	4/18
☐	☐	☐	7/70	13/70	21/70	11/70	9/70	0/70	36/70
FEV ₁ ↓	Ever-smoker and/or B-eos >4.0%								
☒	☒	☒	32/38	32/38	31/38	34/38	29/38	24/38	1/38
☐	☒	☒	16/70	32/70	30/70	32/70	18/70	2/70	22/70
☒	☐	☐	6/18	10/18	10/18	6/18	5/18	1/18	4/18
☐	☐	☐	7/70	13/70	21/70	11/70	9/70	0/70	36/70
FEV ₁ ↓	ACT <16								
☒	☒		13/15	14/15	14/15	13/15	10/15	9/15	0/15
☐	☒		6/24	10/124	13/24	10/24	8/24	0/24	5/24
☒	☐		25/41	28/41	27/41	27/41	24/41	16/41	5/41
☐	☐		17/116	35/116	38/116	33/116	19/116	2/116	53/116

Small airway dysfunction was related to FENO50, but more strongly to blood eosinophils. These relationships were present because all three were related to the overall severity of disease. Disparity between blood eosinophilia and other measures of airway inflammation, such as sputum eosinophilia are well recognised [35,36]. This supports our hypothesis in this dataset that this differing strength of relationship can be explained by the preferential deposition of ICS in proximal airways affecting FENO50 to a greater degree than blood eosinophils. We were surprised by the lack of association between the number of positive SPT, small airway dysfunction and asthma severity on the whole. In fact, the number of positive SPT did not correlate with any other study finding.

4.3. Smoking is detrimental for peripheral airway function in asthma

There was striking evidence of the detrimental effect of smoking on markers of peripheral airway function, which persisted after excluding subjects with more significant smoking history (≥ 5 pack-years). Smoking has been shown to have a clear detrimental effect on the small airways, using techniques such as MBW [37]. In our

cohort, S_{acin} × V_T was more closely associated than S_{cond} × V_T with a history of tobacco smoking and number of pack-years, suggesting a more peripheral location of airway pathology in subjects who had smoked. Niewoehner and co-workers studied the small airways by histopathology in young smokers who had died suddenly outside hospital. The authors reported that inflammation of the respiratory bronchioles was a universal finding, and proposed that it predated centrilobular emphysema [38]. Before drawing firm conclusions about potentially greater vulnerability in asthmatics, it is necessary to compare the effects of similar tobacco smoke exposure on IOS and MBW indices in atopic asthmatics to that in non-atopic non-asthmatic subjects.

4.4. Strengths and weaknesses of the study

In a recent large population based study of subjects aged 16–75 years living in West Sweden, including Skaraborg County, physician diagnosed asthma was reported by 8.3% (female/male ratio 1.23) [39]. Clinical investigation in a subgroup of 744 subjects with current asthma showed similar allergy prevalence, smoking histories, spirometric lung function impairment and medication patterns as

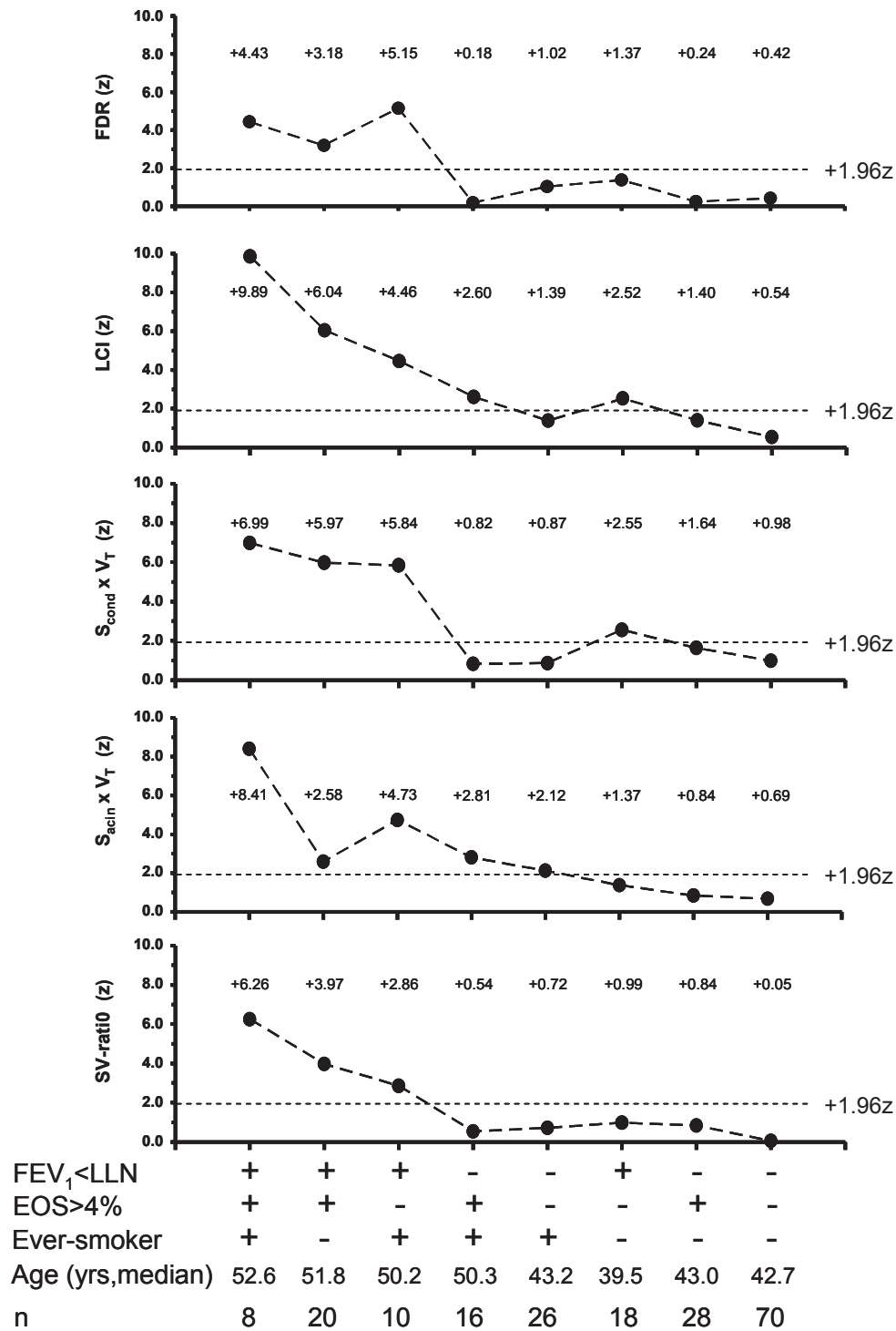


Fig. 7. Peripheral airway function outcomes given as z-scores of medians (filled symbols and numerically). Subgroups defined with respect to presence/absence of reduced FEV₁, EOS >4.0%, and smoking history, respectively, are indicated below plots. EOS>4%, blood eosinophil counts >4.0%. LLN, lower limit of normal.

in our study [40]. We therefore believe that our asthma cohort is representative for an adult asthma population managed in primary care.

Normative values for IOS and N₂ MBW were derived from our own large reference populations of healthy never-smokers, tested by our own staff using the same study protocols and equipment as used for the asthma cohort, and all data were analyzed by two of the authors (SK and PG). The N₂ MBW reference population was

twice as large as the largest published reference population data (n = 180) to date [41]. The approach used for variables such as S_{cond} x V_T involved exclusion of outliers (see expanded discussion in OLS), and the high prevalence of S_{cond} x V_T in our adult asthma cohort must be interpreted in light of this. Future normative data studies will need to collect more detailed exposure data to try to explain the cause of high values among outliers. Prospective follow-up of our outlier subjects would clarify subsequent development of

asthma or COPD. Our IOS reference population included only 158 subjects aged 18–63 years and was weighted towards the younger age range. However, our reference data showed strong agreement with the recently published reference equations of Schulz et al. ($n = 397$ adults, aged 45–91 years, including 162 between 45 and 64 years) [42], the largest adult normative study published to date. Application of Schulz's reference equations on the subset of 92 asthmatic subjects aged 45–61 years in our cohort led to no significant change to our data (data not shown).

The diagnosis of asthma in our study (physician diagnosed asthma) did not require physiological evidence (e.g. bronchodilator response, bronchial reactivity or significant peak flow variability over time). This was done for logistical reasons and because we wanted our cohort to constitute a representative sample of an asthma population managed in primary care. Subjects within our cohort in whom FEV₁ would remain abnormal with an FEV₁/FVC ratio <70%, after bronchodilation might have been classified as suffering from COPD or ACOS (Asthma COPD Overlap Syndrome) [43], which in turn would warrant exclusion from the study, if a purist approach was taken. Several features of our cohort strongly support the asthma diagnosis, however: symptoms started before age 20 years in 56%, and after age 40 in only 15%, high rate of allergic sensitization, allergic rhinitis, blood eosinophilia, plus raised FENO₅₀ in many despite ongoing ICS therapy.

4.5. Summary and conclusions

In our cohort of adult subjects with physician diagnosed asthma, recruited from primary care, between one-third and half of subjects showed evidence of peripheral airway involvement. Reduced FEV₁, in combination with past or current tobacco smoking, and/or raised blood eosinophils identified “a small airway asthma subtype”, a finding that needs to be validated in other asthma cohorts. It remains to be seen if therapeutic targeting of peripheral airways, can lead to significant benefits in either the degree of peripheral airway dysfunction or improvements in other asthma related outcomes, particularly in this asthma subtype. Finally, our findings suggest that tobacco smoke exposure has detrimental effects on peripheral airway function in subjects with asthma and that even a modest smoking history is not forgotten by the asthmatic lung.

Funding source

This study was supported by grants from the Västra Götaland Research Council, Gothenburg, Sweden (grant nos: VGFOUREG-233551, VGFOUREG-308431, VGFOUREG-390031); The Research Fund at Skaraborg Hospital (grant nos: VGSKAS-91151, VGSKAS-162881, VGSKAS-228231, VGSKAS-308701), Skövde, Sweden; The Research Fund at R&D Centre, Skaraborg Primary Care, Skövde, Sweden (grant nos: VGFOUSKB-81971, VGFOUSKB-227601); The Swedish Heart-Lung Foundation, Stockholm, Sweden (Project number: 20110575), and the SAKS Research Foundation, Uppsala, Sweden.

Financial disclosure

The authors have no financial relationships relevant to this article to disclose.

Conflict of interest

The authors have no conflict of interest relevant to this article to disclose.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.rmed.2016.05.028>.

References

- [1] E.D. Bateman, H.A. Boushey, J. Bousquet, W.W. Busse, T.J. Clark, R.A. Pauwels, et al., Can guideline-defined asthma control be achieved? The gaining optimal asthma control study, *Am. J. Respir. Crit. Care Med.* 170 (8) (2004) 836–844.
- [2] K.F. Rabe, M. Adachi, C.K. Lai, J.B. Soriano, P.A. Vermeire, K.B. Weiss, et al., Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys, *J. Allergy Clin. Immunol.* 114 (1) (2004) 40–47.
- [3] K.F. Rabe, P.A. Vermeire, J.B. Soriano, W.C. Maier, Clinical management of asthma in 1999: the Asthma Insights and Reality in Europe (AIRE) study, *Eur. Respir. J.* 16 (5) (2000) 802–807.
- [4] Q. Hamid, Y. Song, T.C. Kotsimbos, E. Minshall, T.R. Bai, R.G. Hegele, et al., Inflammation of small airways in asthma, *J. Allergy Clin. Immunol.* 100 (1) (1997) 44–51.
- [5] M. Kraft, R. Djukanovic, S. Wilson, S.T. Holgate, R.J. Martin, Alveolar tissue inflammation in asthma, *Am. J. Respir. Crit. Care Med.* 154 (5) (1996) 1505–1510.
- [6] S.R. Downie, C.M. Salome, S. Verbanck, B. Thompson, N. Berend, G.G. King, Ventilation heterogeneity is a major determinant of airway hyper-responsiveness in asthma, independent of airway inflammation, *Thorax* 62 (8) (2007) 684–689.
- [7] S. Verbanck, D. Schuermans, M. Noppen, A. Van Muylem, M. Paiva, W. Vincken, Evidence of acinar airway involvement in asthma, *Am. J. Respir. Crit. Care Med.* 159 (5 Pt 1) (1999) 1545–1550.
- [8] R.V. Cadman, R.F. Lemanske Jr., M.D. Evans, D.J. Jackson, J.E. Gern, R.L. Sorkness, et al., Pulmonary 3He magnetic resonance imaging of childhood asthma, *J. Allergy Clin. Immunol.* 131 (2) (2013), 369–76 e1–5.
- [9] E.E. de Lange, T.A. Altes, J.T. Patrie, J.D. Gaare, J.J. Knake, J.P. Mugler 3rd, et al., Evaluation of asthma with hyperpolarized helium-3 MRI: correlation with clinical severity and spirometry, *Chest* 130 (4) (2006) 1055–1062.
- [10] T. Ueda, A. Niimi, H. Matsumoto, M. Takemura, T. Hirai, M. Yamaguchi, et al., Role of small airways in asthma: investigation using high-resolution computed tomography, *J. Allergy Clin. Immunol.* 118 (5) (2006) 1019–1025.
- [11] C.S. Farah, G.G. King, N.J. Brown, S.R. Downie, J.A. Kermode, K.M. Hardaker, et al., The role of the small airways in the clinical expression of asthma in adults, *J. Allergy Clin. Immunol.* 129 (2) (2012), 381–7, 7 e1.
- [12] O.S. Usmani, Small-airway disease in asthma: pharmacological considerations, *Curr. Opin. Pulm. Med.* 21 (1) (2015) 55–67.
- [13] J. Cohen, W.R. Douma, N.H. ten Hacken, J.M. Vonk, M. Oudkerk, D.S. Postma, Ciclesonide improves measures of small airway involvement in asthma, *Eur. Respir. J.* 31 (6) (2008) 1213–1220.
- [14] H. Hauber, R. Taha, C. Bergeron, V. Migounov, Q. Hamid, R. Olivenstein, Effects of hydrofluoroalkane and dry powder-formulated corticosteroid on sputum inflammatory markers in asthmatic patients, *Can. Respir. J.* 13 (2) (2006) 73–78.
- [15] M. Hoshino, Comparison of effectiveness in ciclesonide and fluticasone propionate on small airway function in mild asthma, *Allergol. Int.* 59 (1) (2010) 59–66.
- [16] M. Yamaguchi, A. Niimi, T. Ueda, M. Takemura, H. Matsuoka, M. Jinnai, et al., Effect of inhaled corticosteroids on small airways in asthma: investigation using impulse oscillometry, *Pulm. Pharmacol. Ther.* 22 (4) (2009) 326–332.
- [17] G. Colice, R.J. Martin, E. Israel, N. Roche, N. Barnes, A. Burden, et al., Asthma outcomes and costs of therapy with extrafine beclomethasone and fluticasone, *J. Allergy Clin. Immunol.* 132 (1) (2013) 45–54.
- [18] V. Muller, G. Galfy, N. Eszes, G. Losonczy, A. Bizzi, G. Nicolini, et al., Asthma control in patients receiving inhaled corticosteroid and long-acting beta₂-agonist fixed combinations. A real-life study comparing dry powder inhalers and a pressurized metered dose inhaler extrafine formulation, *BMC Pulm. Med.* 11 (2011) 40.
- [19] E.F. Juniper, D.B. Price, P.A. Stampone, J.P. Creemers, S.J. Mol, P. Fireman, Clinically important improvements in asthma-specific quality of life, but no difference in conventional clinical indexes in patients changed from conventional beclomethasone dipropionate to approximately half the dose of extrafine beclomethasone dipropionate, *Chest* 121 (6) (2002) 1824–1832.
- [20] P.T. Macklem, The physiology of small airways, *Am. J. Respir. Crit. Care Med.* 157 (5 Pt 2) (1998) S181–S183.
- [21] P.T. Macklem, J. Mead, Resistance of central and peripheral airways measured by a retrograde catheter, *J. Appl. Physiol.* 22 (3) (1967) 395–401.
- [22] J.J. McNamara, R.G. Castile, G.M. Glass, J.J. Fredberg, Heterogeneous lung emptying during forced expiration, *J. Appl. Physiol.* 63 (4) (1987) 1648–1657.
- [23] A.B. Dubois, A.W. Brody, D.H. Lewis, B.F. Burgess Jr., Oscillation mechanics of lungs and chest in man, *J. Appl. Physiol.* 8 (6) (1956) 587–594.
- [24] R.C. Darling, A. Courmand, D.W. Richards, B. Domanski, Studies on intrapulmonary mixture of gases. V. Forms of inadequate ventilation in normal and emphysematous lungs, analyzed by means of breathing pure oxygen, *J. Clin. Invest.* 23 (1) (1944) 55–67.
- [25] H.J. Smith, M.D. Goldman, Forced oscillation technique and impulse

- oscillometry, *Eur. Respir. Mon.* 31 (2005) 72–105.
- [26] P.D. Robinson, P. Latzin, S. Verbanck, G.L. Hall, A. Horsley, M. Gappa, et al., ERS/ATS Consensus statement for inert gas washout measurement using multiple and single breath tests, *Eur. Respir. J.* 41 (3) (2013) 507–522.
- [27] F. Singer, B. Houtz, P. Latzin, P. Robinson, P. Gustafsson, A realistic validation study of a new nitrogen multiple-breath washout system, *PLoS One* 7 (4) (2012) e36083.
- [28] P.M. Gustafsson, P.D. Robinson, M. Gilljam, A. Lindblad, B.K. Houtz, Slow and fast lung compartments in cystic fibrosis measured by nitrogen multiple-breath washout, *J. Appl. Physiol.* 117 (7) (2014) 720–729.
- [29] S. Verbanck, D. Schuermans, A. Van Muylem, C. Melot, M. Noppen, W. Vincken, M. Paiva, Conductive and acinar lung-zone contributions to ventilation inhomogeneity in COPD, *Am. J. Respir. Crit. Care Med.* 157 (1998) 1573–1577.
- [30] M. Schatz, C.A. Sorkness, J.T. Li, P. Marcus, J.J. Murray, R.A. Nathan, et al., Asthma control test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists, *J. Allergy Clin. Immunol.* 117 (3) (2006) 549–556.
- [31] H. Pinnock, E.F. Juniper, A. Sheikh, Concordance between supervised and postal administration of the mini asthma quality of life questionnaire (Mini-AQLQ) and asthma control questionnaire (ACQ) was very high, *J. Clin. Epidemiol.* 58 (8) (2005) 809–814.
- [32] M.R. Miller, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, et al., Standardisation of spirometry, *Eur. Respir. J.* 26 (2) (2005) 319–338.
- [33] H. Hedenstrom, P. Malmberg, K. Agarwal, Reference values for lung function tests in females. Regression equations with smoking variables, *Bull. Eur. Physiopathol. Respir.* 21 (6) (1985) 551–557.
- [34] H. Hedenstrom, P. Malmberg, H.V. Fridriksson, Reference values for lung function tests in men: regression equations with smoking variables, *Ups. J. Med. Sci.* 91 (3) (1986) 299–310.
- [35] F.N. Schleich, A. Chevrement, V. Paulus, M. Manise, L. Seidel, et al., Importance of concomitant local and systemic eosinophilia in uncontrolled asthma, *Eur. Respir. J.* 44 (1) (2014) 97–108.
- [36] G.A. Westerhof, D.A. Korevaar, M. Amelink, S.B. de Nijs, J.C. de Groot, J. Wang, et al., Biomarkers to identify sputum eosinophilia in different adult asthma phenotypes, *Eur. Respir. J.* 46 (3) (2015) 688–696.
- [37] S. Verbanck, D. Schuermans, M. Meysman, M. Paiva, W. Vincken, Noninvasive assessment of airway alterations in smokers: the small airways revisited, *Am. J. Respir. Crit. Care Med.* 170 (4) (2004) 414–419.
- [38] D.E. Niewoehner, J. Kleinerman, D.B. Rice, Pathologic changes in the peripheral airways of young cigarette smokers, *N. Engl. J. Med.* 291 (15) (1974) 755–758.
- [39] J. Lotvall, L. Ekerljung, E.P. Ronmark, G. Wennergren, A. Linden, E. Ronmark, et al., West Sweden Asthma Study: prevalence trends over the last 18 years argues no recent increase in asthma, *Respir. Res.* 10 (2009) 94.
- [40] R. Mincheva, L. Ekerljung, A. Bjerg, M. Axelsson, T.A. Popov, B. Lundback, et al., Frequent cough in unsatisfactory controlled asthma—results from the population-based West Sweden Asthma study, *Respir. Res.* 15 (2014) 79.
- [41] S. Verbanck, A. Van Muylem, D. Schuermans, I. Bautmans, B. Thompson, W. Vincken, Transfer factor, lung volumes, resistance and ventilation distribution in healthy adults, *Eur. Respir. J.* 47 (2016) 166–176.
- [42] H. Schulz, C. Flexeder, J. Behr, M. Heier, R. Holle, R.M. Huber, et al., Reference values of impulse oscillometric lung function indices in adults of advanced age, *PLoS One* 8 (5) (2013) e63366.
- [43] P.G. Gibson, J.L. Simpson, The overlap syndrome of asthma and COPD: what are its features and how important is it? *Thorax* 64 (8) (2009) 728–735.

ON-LINE SUPPLEMENT (OLS)

1. IOS reference population and in-house reference values

In-house reference values for IOS (impulse oscillometry) were obtained from an ongoing IOS study in a healthy never-smoker population of children and adults aged 7-62 years recruited from the two nearby communities, Skövde and Tibro, via the Swedish population register. From this healthy IOS cohort, which currently comprises 360 subjects, 158 subjects (85 females) aged 18-62 years were selected as our adult IOS reference population. IOS recordings included two or three 60 second episodes and were performed during relaxed tidal breathing via a mouthpiece and a microbe filter (MicroGard II, Care Fusion, Hoechst, Germany) with the patient wearing a nose-clip and with the hands supporting the cheeks. Sequences with artefacts due to swallowing, sighs or cough were excluded. Mean values of resistance at 5 and 20Hz (R5 and R20), frequency dependence of resistance (R5-R20), area of reactance (AX), and the square root of AX (sqrt(AX)) were calculated.

In principle, AX constitutes the absolute value of Δ frequency $\times$ X5 (reactance at 5Hz) / 2. Δ frequency is calculated as the difference between fres (resonant frequency (Hz), the frequency when reactance (X) equals zero) and 5Hz. AX is the integration of all capacitive reactance from 5Hz to fres and can be said to reflect the elastance (i.e. 1/compliance) of the respiratory system to the force of the pressure waves. In the adult asthma setting a greater AX can be said to reflect the degree of peripheral airway obstruction and airway closures. With increasing peripheral airway obstruction in a subject, fres will increase and capacitive reactance (X5) will show an increasingly greater negative value. In our experience the relative change in fres and X5 may vary among asthmatic subjects during increasing obstruction. The late Michael Goldman, viewed AX as a more sensitive and the same time more robust outcome measure than fres or X5 (personal communication). AX is, however, a "boosted" IOS outcome of peripheral airway obstruction as it multiplies two different but highly correlated signals, leading to a curvilinear relationship with other peripheral airway obstruction indices, *e.g.* FDR. Our solution to this problem is to calculate the square root of AX. This procedure can be seen as taking the geometric mean of X5 and fres, and the result (sqrt(AX)) shows an excellent correlation and agreement with FDR such that they seem to reflect the same physiological event or condition in adult asthma. As a result we see AX or (sqrt(AX)) as redundant when FDR is used as an IOS outcome in adult asthma. The advantage of FDR is that resistance is a concept more easily grasped than the geometric mean of reactance and Δ frequency.

All participants performed spirometry in accordance with the ATS/ERS guidelines (1) and spirometry results were related to Swedish normative data.(2, 3) Demography and spirometry findings in the IOS reference population are given in Table E1.

Table E1. Demographic and spirometry data from the impulse oscillometry (IOS) reference population.

	All n= 158			Females n=85			Males n=73		
	Mean	SD	Median (range)	Mean	SD	Median (range)	Mean	SD	Median (range)
Age (years)	37.3	15.4	38.4 (18.1; 63.0)	39.2	15.4	40.8 (18.1; 62.81)	35.0	15.3	32.1 (18.2; 62.3)
Weight (kg)	72.5	11.6	71.7 (49.0; 110.0)	67.1	8.9	65.3 (49.0; 86.0)	78.8	11.3	79.3 (50.8; 110.0)***
Height (cm)	172.0	9.1	171.0 (151.0; 192.0)	166.1	6.7	166.5 (151.0; 187.0)	178.8	6.3	179.0 (163.0; 192.0)***
BMI (kg/cm ²)	24.4	3.2	24.3 (16.3; 33.1)	24.3	2.9	24.2 (18.8; 31.2)	24.7	3.4	25.3 (16.3; 33.1)
FEV ₁ (%pred)	98.4	12.1	99.1 (68.6; 128.6)	97.9	11.4	98.8 (70.5; 120.9)	99.0	12.9	99.1 (68.6; 128.6)
FEV ₁ (z-score)	-0.16	1.10	-0.08 (-3.22; 2.74)	-0.22	0.98	-0.09 (-2.90; 2.00)	-0.09	1.22	-0.08 (-3.22; 2.74)
FVC (%pred)	97.9	11.6	98.8 (63.1; 124.9)	99.1	10.9	98.9 (73.6; 121.3)	96.6	12.4	98.7 (63.1; 124.9)
FVC (z-score)	-0.21	1.09	-0.11 (-3.77; 2.18)	-0.13	1.06	-0.10 (-2.62; 1.86)	-0.31	1.13	-0.11 (-3.77; 2.18)
FEV ₁ /FVC (z-score)	0.18	0.89	0.13 (-2.54; 3.09)	-0.04	0.74	0.02 (-2.54; 1.46)	0.44	0.98	0.52 (-2.52; 3.09)***
MEF ₅₀ (%pred)	93.3	26.6	91.0 (30.1; 208.6)	83.9	21.0	82.4 (30.1; 139.0)	104.3	28.3	104.6 (42.4; 208.6)***
MEF ₅₀ (z-score)	-0.30	1.14	-0.34 (-2.81; 4.86)	-0.71	0.88	-0.71 (-2.81; 1.40)	0.18	1.22	0.20 (-2.41; 4.86)***
MEF ₂₅ (%pred)	85.7	30.0	81.5 (26.5; 259.6)	82.3	30.1	78.5 (26.5; 259.6)	89.8	29.5	85.8 (33.3; 190.0)
MEF ₂₅ (z-score)	-0.55	1.17	-0.69 (-3.52; 4.30)	-0.69	1.08	-0.70 (-3.52; 4.30)	-0.38	1.25	-0.65 (-2.21; 4.08)

BMI, body mass index. FEV₁, forced expiratory volume in 1 second. FVC, forced expiratory volume. MEF₅₀, maximal expiratory flow at 50% of exhaled volume. MEF₂₅, maximal expiratory flow at 75% of exhaled volume. *p<0.05; **p<0.01; ***p<0.001, for males vs. females, Mann-Whitney U-test.

Statistical analyses using multiple regression analysis, including models with age, height and gender as independent variables and the IOS above as dependent variables, showed that gender was the only significant contributor to R5 and R20 (p=0.028 and p=0.004, respectively). For FDR (frequency dependence of resistance; R5-R20) AX and sqrt(AX), height was the only contributor (p=0.001; p<0.001, and p<0.001, respectively), but was closely related to gender. For these reasons reference values applied for the IOS variables in the asthma population take only gender into account (Table E2). IOS indices in the asthma population are presented as absolute values and as z-scores.

Table E2. Impulse oscillometry (IOS) data from the IOS reference population.

	Females n=85					Males n=73				
	Mean	RSD	Median (range)	LLN	ULN	Mean	RSD	Median (range)	LLN	ULN
R5 Hz (kPa/L/s)	0.376	0.074	0.373 (0.207;0.563)	0.231	0.521	0.318	0.069	0.305 (0.210;0.540)***	0.183	0.453
R20 Hz (kPa/L/s)	0.316	0.067	0.320 (0.170;0.473)	0.185	0.447	0.274	0.059	0.263 (0.170;0.515)***	0.158	0.390
FDR (kPa/L/s)	0.059	0.040	0.053 (0.000;0.150)	-	0.137	0.044	0.038	0.035 (0.000;0.175)*	-	0.118
AX (kPa*L)	0.271	0.181	0.235 (0.070;1.247)	-	0.626	0.197	0.178	0.130 (0.033;0.880)***	-	0.546
SqrtAX ((kPa*L) ^{1/2})	0.498	0.151	0.485 (0.265;1.117)	0.202	0.794	0.413	0.164	0.361 (0.183;0.938)***	0.092	0.734

Lower limit of normal, LLN (predicted mean - 1.96 RSD) and upper limit of normal, ULN (predicted mean + 1.96 RSD). RSD, residual standard deviation. R5 Hz, resistance at 5 Hz, R20 Hz, resistance at 20 Hz. FDR, frequency dependence of resistance (R5 Hz-R20 Hz). AX, reactance area. Sqrt, square root. *p<0.05; **p<0.01; ***p<0.001, for males vs. females, Mann-Whitney U-test.

Figures E1 - E5 demonstrate IOS variables plotted against age in the healthy IOS reference population. An over representation of subjects aged 18-20 years and the higher R5 and R20 values in females are demonstrated. No relationships between IOS variables and age were seen.

Figure E1. R5 (resistance at 5 Hz) vs. age in 158 healthy subjects (85 females). Open symbols denote females, and closed symbols males.

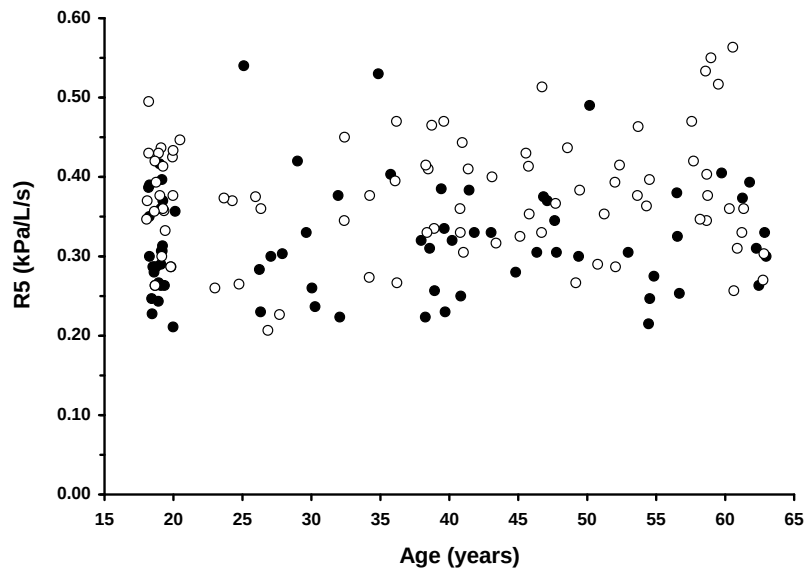


Figure E2. R20 (resistance at 20 Hz) vs. age in 158 healthy subjects (85 females). Open symbols denote females, and closed symbols males.

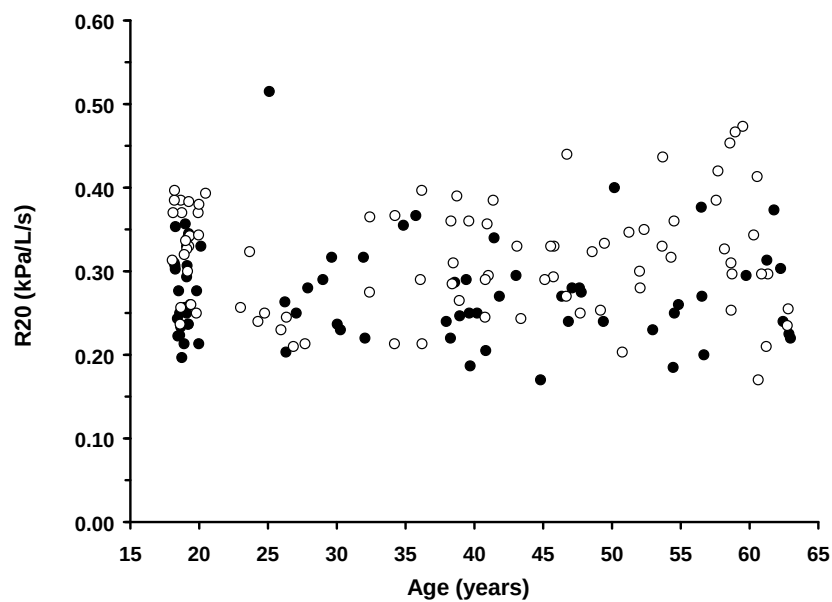


Figure E3. FDR (R5- R20) vs. age in 158 healthy subjects (85 females). Open symbols denote females, and closed symbols males.

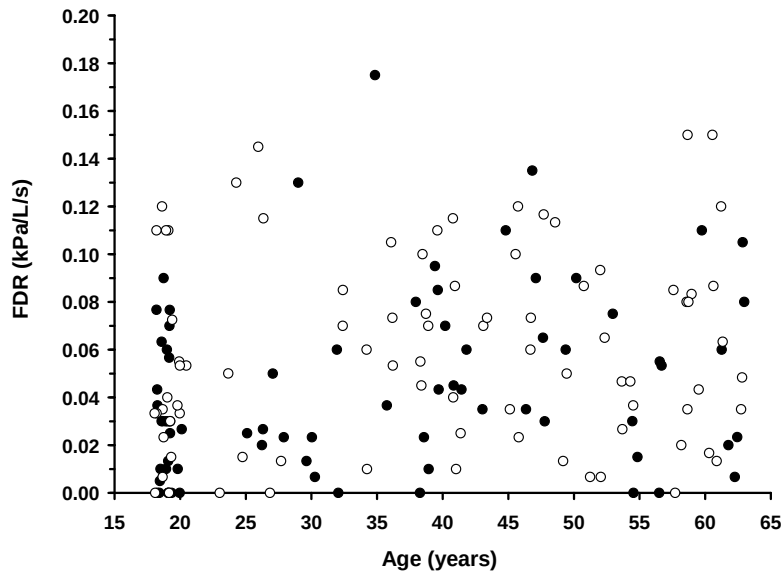


Figure E4. AX (reactance area) vs. age in 158 healthy subjects (85 females). Open symbols denote females, and closed symbols males.

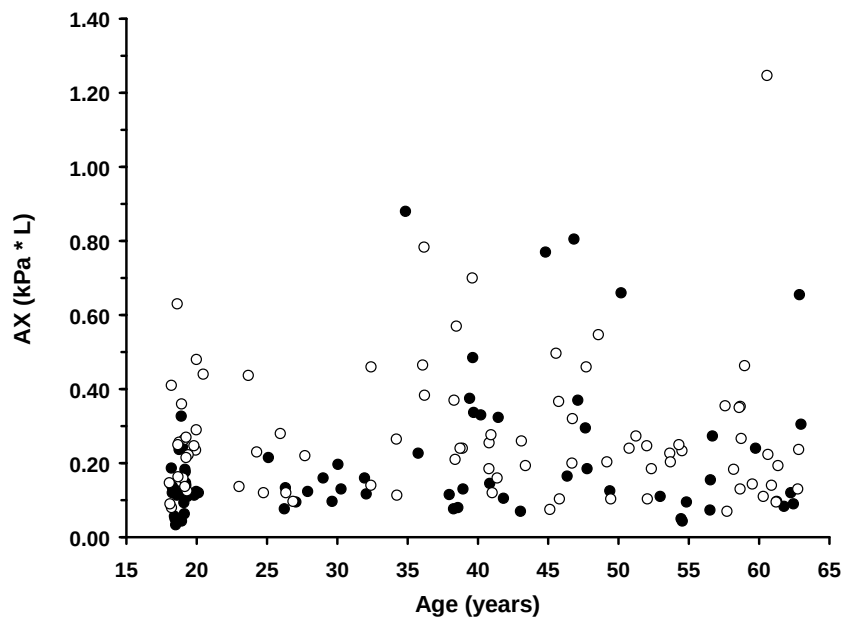
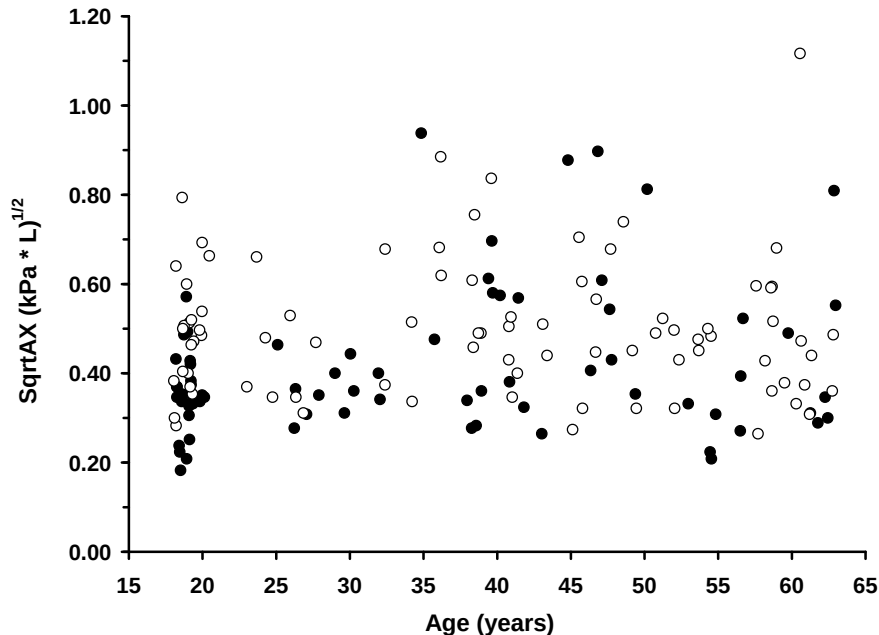


Figure E5. Sqrt(AX) (square root of reactance area) vs. age in 158 healthy subjects (85 females). Open symbols denote females, and closed symbols males.



2. Nitrogen multiple breath washout (N_2 MBW): Methodology, procedures and calculation of indices.

N_2 MBW was performed using an ExhalyzerD® N_2 MBW device (Eco Medics AG, Duernten, Switzerland). This validated device is based on indirect inert gas (N_2) calculation using carbon dioxide (CO_2) and oxygen (O_2) sensors and flow measurement from an ultra-sound flow sensor. While the commercial software delivered with ExhalyzerD® (Spiroware 3.1) was used for data recording, we used our own in-house software, written with a commercial software package (TestPoint®, Capital Equipment, MA, USA), for off-line analysis and for quality control purposes. The basic algorithms used in the custom TestPoint® software are, however, identical to those used in the commercial Spiroware® software, and our software was also used for the previous device validation study.(4) The Exhalyzer D® samples gas and flow signals at 200 Hz.

Prior to each subject test session calibrations of the ultrasonic flow meter, O_2 and CO_2 analysers, and gas and flow signal synchronizations were performed, as suggested by the manufacturer. All test subjects wore a nose clip and breathed via a trimmed silicon mouthpiece (to minimise equipment related dead space volume) attached to a microbe filter (Air Safety Ltd, Morecambe, UK) in line with the recording system. We used the set 3 dead space reducer (Eco Medics AG, Duernten, Switzerland) to reduce main flow sensor dead space volume. The pre-gas-sampling external dead space volume of the system was estimated at 35 mL, and the post-gas-sampling point dead space volume 22 mL. N_2 MBW was performed in the upright sitting position during relaxed tidal breathing. On-line tidal volume monitoring was performed by the operator such that the phase III slope of each breath constituted approximately half the breath. A minimum interval of the preceding washout time

was observed between tests to ensure resting end-tidal N₂ concentration values had returned to normal levels prior to the next test.

TestPoint® off-line data analysis started with a renewed synchronization of the CO₂ and O₂ signals to the flow signal for each recording. The delay time of the gas signals was defined as the time between the moment when the post-gas-sampling dead space volume (22 mL) had been inhaled until the 50% deflection point of the CO₂ and O₂ signals, respectively. The median lag time value for all breaths over the three recordings was applied. After synchronization of CO₂ and O₂ signals, the N₂ concentration was calculated as $N_2\% = 100\% - O_2\% - CO_2\% - Ar\%$, where, Argon (Ar) was assumed to form a fixed portion of the N₂ signal. The analyses of the MBW recordings included visual control of the quality of each breath and correction of the default estimation of phase III slopes (*e.g.* error due to cardiogenic oscillations) when necessary.

The functional residual capacity (FRC) was calculated from the sum of the measured exhaled N₂ volumes contained in all breaths over the washout until 1/40th of the initial end-tidal N₂ concentration (approx. 78%), divided by the difference in initial and final (approx. 1.95%) end-tidal N₂ concentration. Lung volume turnover values (TO) were calculated for each breath over the washout as the cumulative expired volume (CEV) of air at the point of the breath divided by FRC. The CEV of each breath was corrected for total equipment-related dead space volume. LCI was calculated as TO value of the first of three consecutive breaths where end-tidal N₂ concentration had fallen below 1/40th of starting end-tidal N₂ concentration.

The phase III slope of each breath was determined by linear regression from 50 to 95% of the exhaled volume of air. Care was taken to avoid influence of the gas from the bronchial phase (phase II) or the fast rising phase IV, occurring due to airway closures. The concentration normalized phase III slope (Sn_{III}) was calculated by dividing the phase III slope by the mean N₂ concentration over the slope. Prior to S_{cond} and S_{acin} calculations, Sn_{III} values were further normalized by multiplying them by their respective tidal volume (Sn_{III} × V_T), *i.e.* simulating a V_T of 1000 mL, to account for differences in size between subjects and for variations in V_T during the washout. Data from the three MBW recordings were averaged before S_{cond} and S_{acin} calculation. S_{cond} was calculated by linear regression from a plot of Sn_{III} vs. TO as the increase of Sn_{III} from 1.5 to 6 TO. S_{acin} was calculated as the first breath Sn_{III} minus the S_{cond} contribution to Sn_{III}. The Sn_{III} analysis results reported thus constitute S_{cond} × V_T, S_{acin} × V_T and 1st breath Sn_{III} × V_T.

A two-compartment analysis of lung ventilation distribution was undertaken, based on the observation that two different lung compartments are detectable in all lungs, a “faster” ventilated and another, “slower” ventilated compartment. The size (regional FRC) and specific ventilation of these compartments can be subsequently calculated, as well as comparisons of the two contributions to overall ventilation, from the collected MBW data.(5) Calculated indices from compartment analysis included the respective regional resting lung volumes of the faster and more slowly ventilated lung compartments (FRC_{faster} and FRC_{slower}), their effective alveolar tidal volumes (V_{TA,faster} and V_{TA,slower}), specific ventilation of each compartment (V_{TA,faster} / FRC_{faster} and V_{TA,slower} / FRC_{slower}, respectively). In addition, V_{TA,faster} / FRC_{faster} and V_{TA,slower} / FRC_{slower} were normalized by dividing these indices by the V_T/FRC ratio over the washout to account for variations in breathing pattern among subjects, giving the normalized V_{TA,faster} / FRC_{faster} (%) and the V_{TA,slower} / FRC_{slower} (%). Finally, a specific ventilation ratio (SV-ratio) was calculated as the ratio of specific

ventilation of the faster and the slower compartment: $(rV_{TA, \text{faster}} / rFRC_{\text{faster}}) / (rV_{TA, \text{slower}} / rFRC_{\text{slower}})$. The interested reader is directed to the original publication for explanation of the derivation of these indices.

3. N₂ MBW reference population and calculated in-house reference values

In-house reference values for N₂ MBW were obtained from an ongoing study in healthy never-smokers aged 3-86 years. From this population, 400 subjects (208 females) aged 17 - 71 years were selected. They were recruited via the Swedish population register and performed triplets of N₂ MBW, in addition to spirometry in accordance with the ATS/ERS guidelines (1) and FENO₅₀ (fraction exhaled nitric oxide at an expiratory flow of 50 mL/s) recordings in accordance with current ATS/ERS FENO guidelines (39). Demographic data, and spirometry and FENO findings in this healthy control cohort are given in Table E3.

Table E3. Demographic data, spirometry and FENO₅₀ findings in healthy control cohort for N₂ MBW.

Variable	All (n=400)	Females (n=208)	Males (n=192)
Age (yrs)	46.2 (17.1)	47.1 (16.7)	45.3 (17.6)
Height (cm)	173.4 (9.1)	167.3 (6.3)	179.9 (6.8)***
Weight (kg)	74.6 (13.3)	68.7 (11.3)	81.0 (6.8)***
BMI (kg / m ²)	24.8 (3.7)	24.6 (4.1)	25.0 (3.3)
FEV ₁ (z)	0.15 (0.98)	0.10 (0.95) ^a	0.21 (1.02)
FVC (z)	0.01 (1.04)	0.03 (1.10) ^a	-0.01 (0.96)
FEV ₁ /FVC (z)	0.29 (0.92)	0.22 (0.80) ^a	0.36 (1.03)
MEF ₅₀ (z)	-0.25 (1.00)	-0.53 (0.92) ^a	0.04 (0.99) ***
MEF ₂₅ (z)	-0.38 (1.06)	-0.46 (0.97) ^a	-0.30 (1.14) ***
FENO ₅₀ (ppb)	17.5 (9.3)	16.7 (9.6) ^b	18.4 (8.9) ^c

Data displayed as Mean (SD). BMI, body mass index. FEV₁, Forced Expiratory Volume in 1 second. FVC, Forced Vital Capacity. MEF₅₀, Maximum Expiratory Flow at 50% of FVC. MEF₂₅, Maximum Expiratory Flow at 25% of FVC. FENO₅₀, Fraction Exhaled Nitric Oxide at an expiratory flow of 50 mL/s. ppb, parts per billion. z, z-score. a) Valid N=206. b) Valid N=162. c) Valid N=149. *** p<0.001, refers to comparison between males and females, Student's t-test.

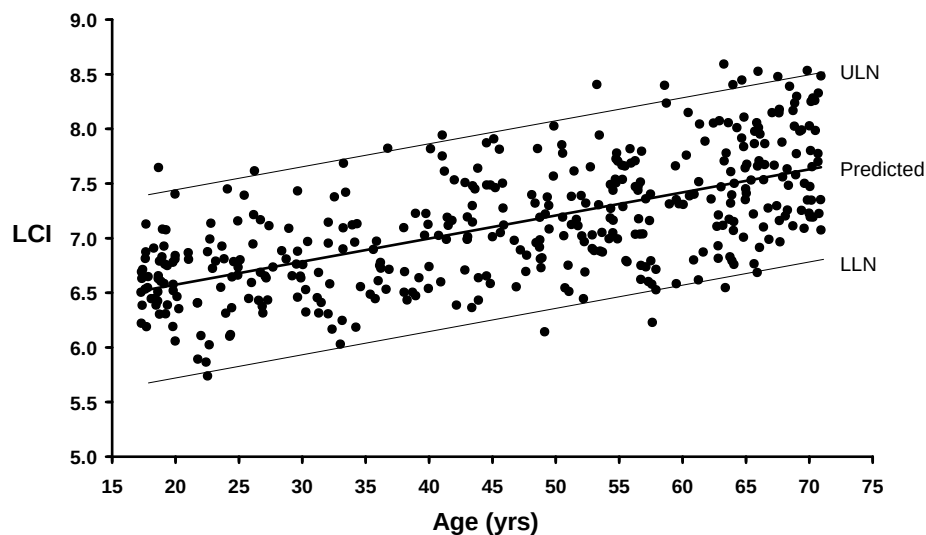
Table E4. N₂ MBW findings in healthy control cohort for N₂ MBW.

Variable	All (n=400)	Females (n=208)	Males (n=192)
LCI	7.13 (0.57)	7.12 (0.59)	7.13 (0.55)
S _{cond} x V _T (L)	0.021 (0.007)	0.022 (0.008)	0.019 (0.005) **
S _{acin} x V _T (L)	0.069 (0.026)	0.066 (0.028)	0.072 (0.024) *
1 st breath S _{nIII} x V _T (L)	0.076 (0.028)	0.074 (0.030)	0.0780 (0.024)
FRC _{slower} / FRC _{full} (%)	64.4 (8.5)	64.2 (8.5)	64.6 (8.6)
V _{TA, slower} / FRC _{slower} (%)	12.7 (4.1)	13.4 (4.3)	11.9 (3.8)
Normalized V _{TA, slower} / FRC _{slower}	0.401 (0.066)	0.401 (0.068)	0.402 (0.065)
V _{TA, faster} / FRC _{faster} (%)	42.3 (15.5)	45.2 (16.3)	39.3 (14.1)
Normalized V _{TA, faster} / FRC _{faster} (%)	1.310 (0.186)	1.315 (0.182)	1.306 (0.184)
SV-ratio	3.33 (0.46)	3.35 (0.47)	3.31 (0.45)
Overall V _T / FRC	31.7 (9.8)	33.8 (10.3)	29.5 (8.6) ***

Data displayed as Mean (SD). N₂, nitrogen. MBW, multiple breath washout. S_{cond}, index of convection dependent ventilation inhomogeneity. V_T, tidal volume. S_{acin}, index of ventilation inhomogeneity arising in the lung periphery, due to interaction between convective and diffuse gas mixing. FRC, functional residual capacity. V_TA, alveolar tidal volume. Normalized V_TA_{slower} / FRC_{slower}, 100* (V_TA_{slower} / FRC_{slower}) / (Overall V_T / FRC)%. SV-ratio, specific ventilation ratio, (V_TA_{faster} / FRC_{faster}) / (V_TA_{slower} / FRC_{slower}). * p<0.05, ** p<0.01, *** p<0.001, refer to comparison between males and females, Student's t-test.

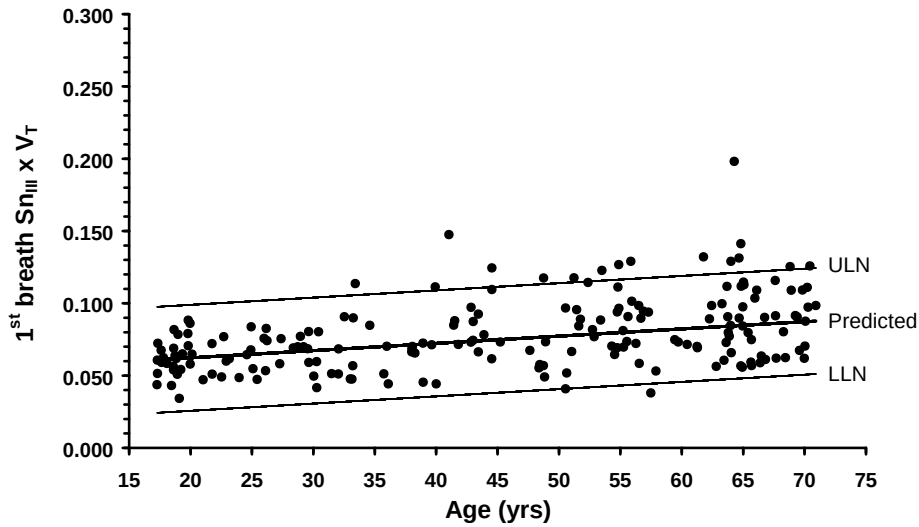
In the healthy control cohort S_{cond} x V_T was significantly greater among females vs. males, while S_{acin} x V_T was greater among the males, and 1st breath Sn_{III} x V_T was numerically greater among the males (Table E4). For this reason separate male and female regression equations were calculated for these indices. The remaining MBW indices did not differ with respect to gender, but females showed significantly higher V_T / FRC. All indices showed significant relationships with age, but were not significantly associated with height or weight. Regression analyses using age as the independent variable were therefore undertaken, and predicted values, residual standard deviations (RSD), and upper limit of normal (ULN; predicted value +1.96 RSD), and lower limit of normal (LLN; predicted value -1.96 RSD) were calculated (Figures E6 - E16). When plotting the Sn_{III} analyses derived indices against age (Figures E6 - E12) it was obvious that there were several outliers, in particular for S_{cond} x V_T among the females. In order to be able to present similar and simple regression equations based on age only, we decided to base the prediction equations for the Sn_{III} derived indices on those male and females subjects demonstrating values below the mean + 1.96 SD for the respective subgroup (Table E 4). The number of subjects included in the calculation of the respective regression equations is given in the legends for the respective figures, which also present the equations. N₂ MBW indices derived in the asthma population are presented as absolute values and as z-scores: (measured value - predicted value) / RSD.

Figure E6. LCI vs. age in healthy control cohort.



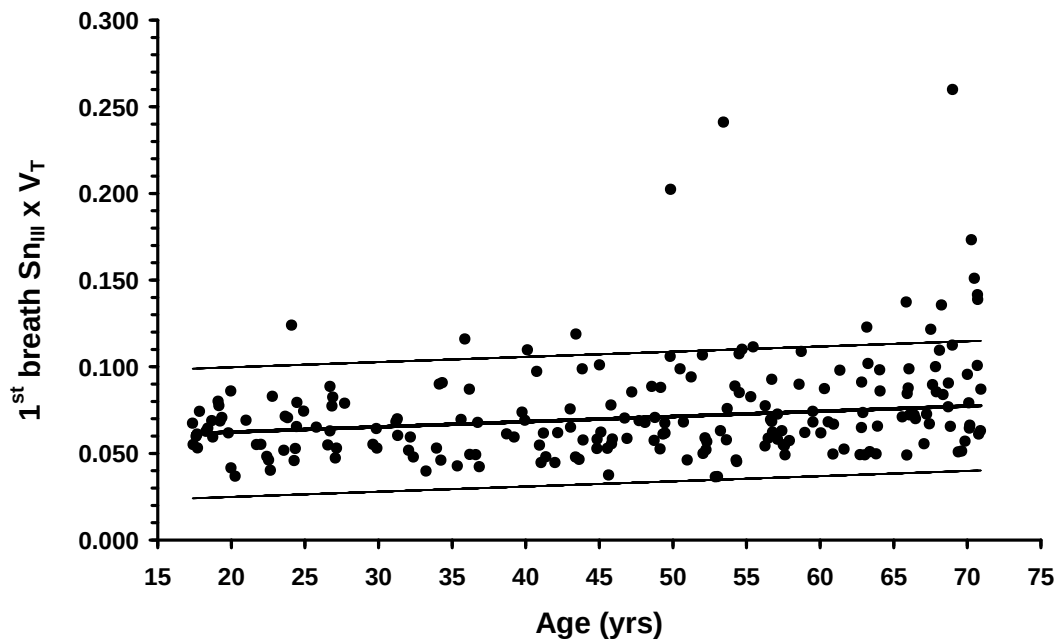
Predicted LCI value = 6.152 + 0.0211 x age; RSD 0.439; R² = 0.4054.
ULN, upper limit of normal. LLN, lower limit of normal.

Figure E7. 1st breath $Sn_{III} \times V_T$ vs. age in full male healthy control cohort.



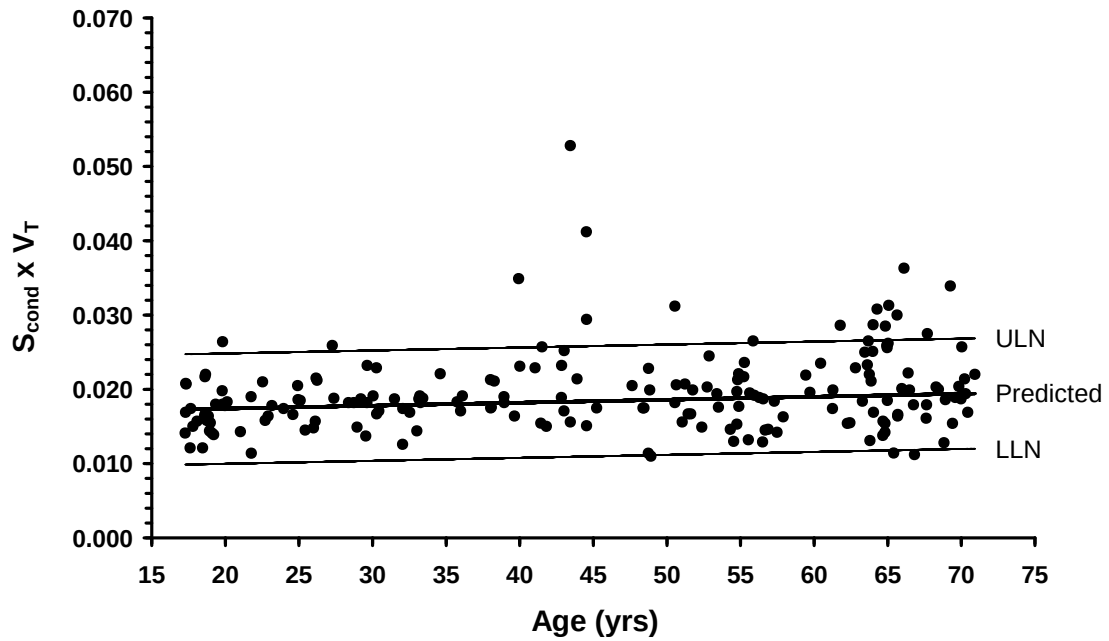
Predicted value = $0.0563 + 0.0003 \times \text{Age}$; RSD 0.01908; $R^2 = 0.1975$. (Regression analysis is based on 184 male subjects with 1st breath $Sn_{III} \times V_T$ less than 0.126; corresponding to mean + 1.96 SD in the full male cohort.) ULN, upper limit of normal. LLN, lower limit of normal.

Figure E8. 1st breath $Sn_{III} \times V_T$ vs. age in full female healthy control cohort.



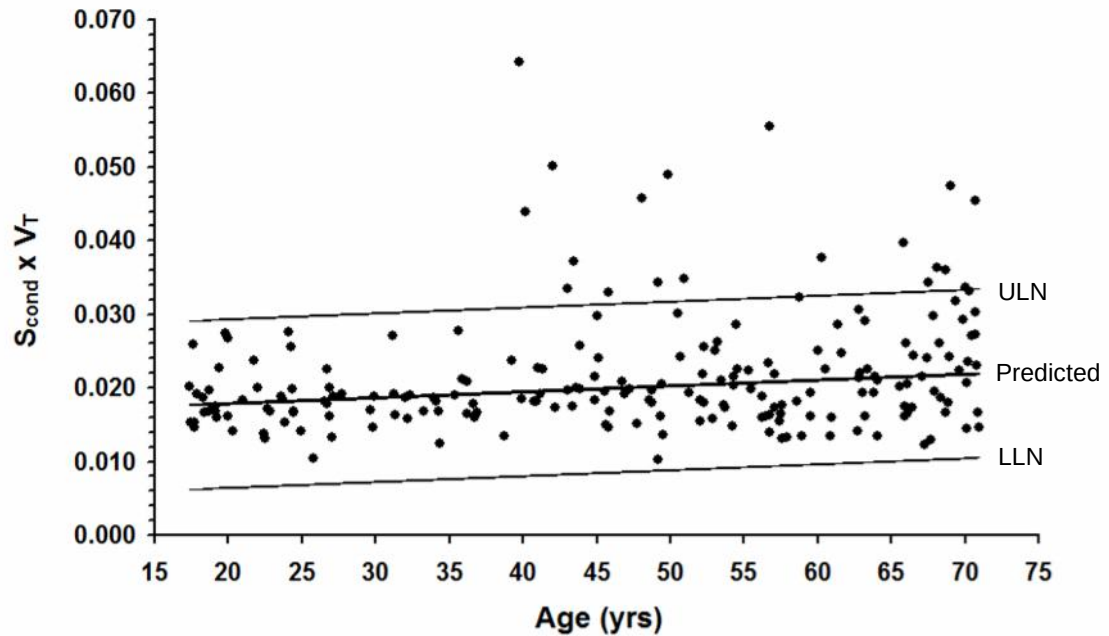
Predicted value = $0.0523 + 0.0005 \times \text{Age}$; RSD 0.01870; $R^2 = 0.0563$. (Regression analysis is based on 199 female subjects with 1st breath $Sn_{III} \times V_T$ less than 0.134; corresponding to mean + 1.96 SD in the full female cohort). ULN, upper limit of normal. LLN, lower limit of normal.

Figure E9. $S_{\text{cond}} \times V_T$ vs. age in full male healthy control cohort.



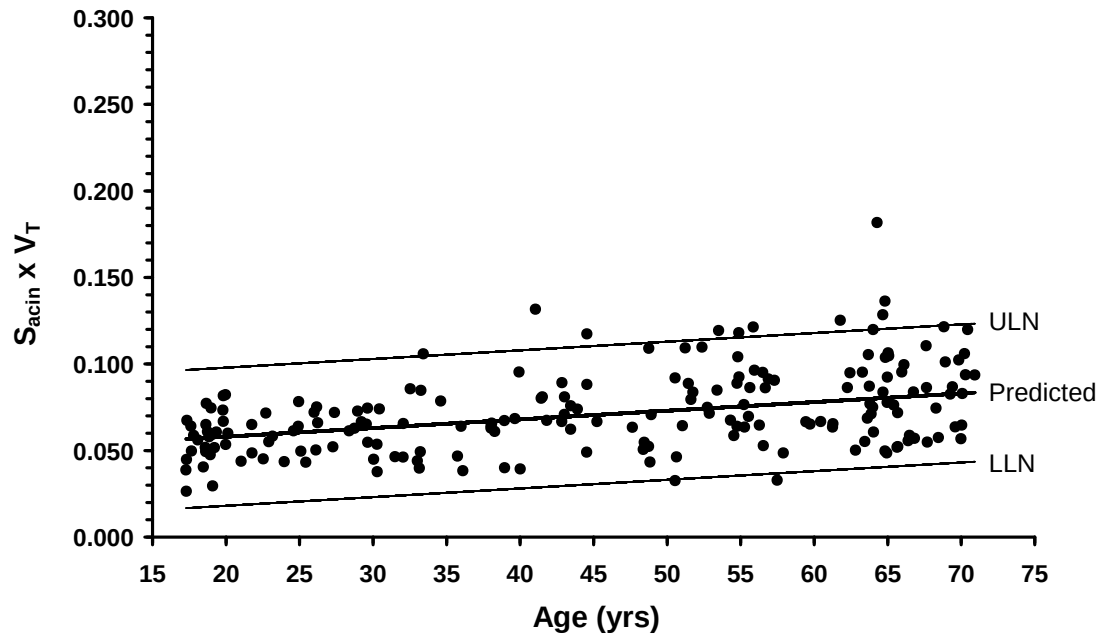
Predicted value = $0.0166 + 0.00004 \times \text{Age}$; RSD 0.003789; $R^2 = 0.0426$. (Regression analysis is based on 183 male subjects with $S_{\text{cond}} \times V_T$ less than 0.030; corresponding to mean + 1.96 SD in the full male cohort). ULN, upper limit of normal. LLN, lower limit of normal.

Figure E10. $S_{\text{cond}} \times V_T$ vs. age in full female healthy control cohort.



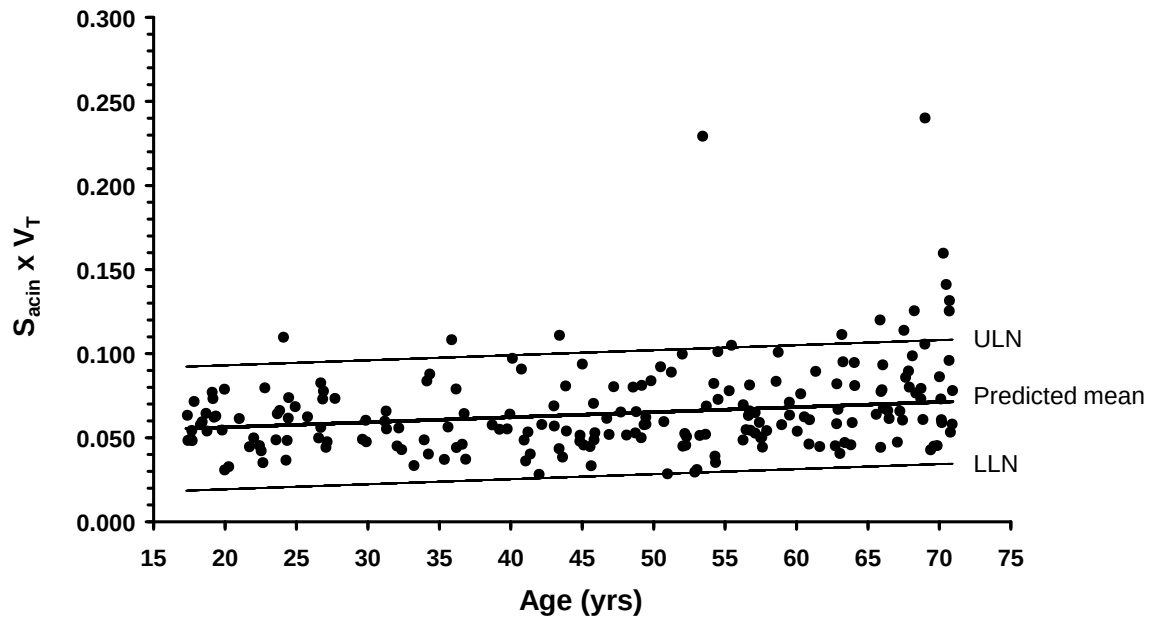
Predicted value = $0.0163 + 0.00008 \times \text{Age}$; RSD 0.005842; $R^2 = 0.0694$. (Regression analysis based on 199 female subjects with $S_{\text{cond}} \times V_T$ less than 0.038; corresponding to mean + 1.96 SD in the full female cohort). ULN, upper limit of normal. LLN, lower limit of normal.

Figure E11. $S_{acin} \times V_T$ vs. age in full male healthy control cohort.



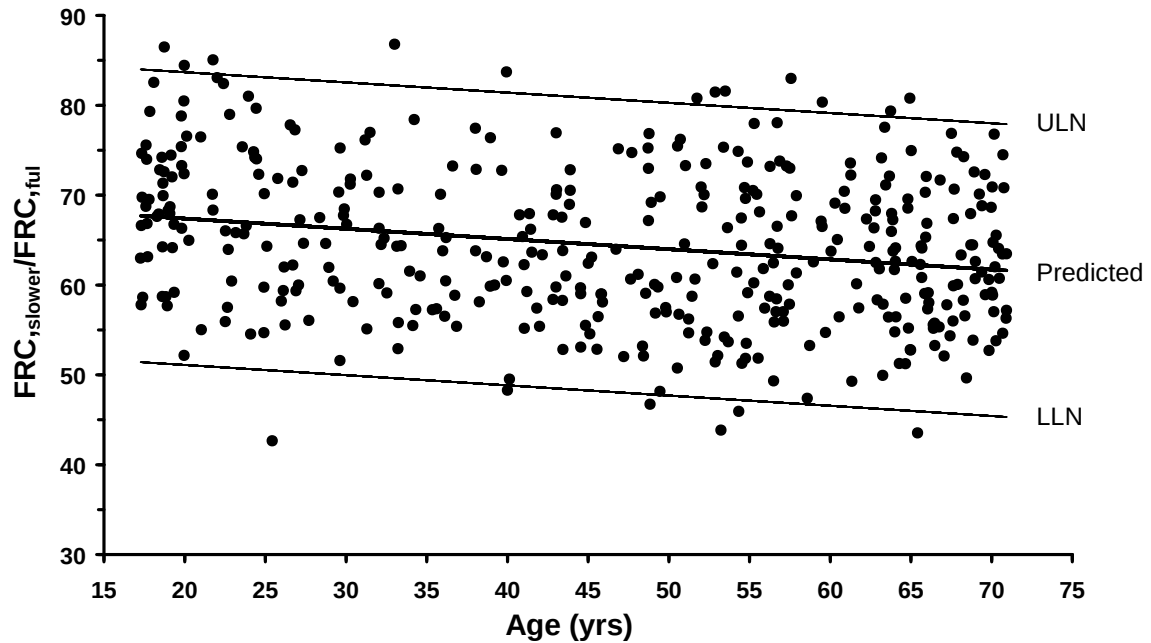
Predicted value = $0.0480 + 0.0005 \times \text{Age}$; RSD 0.02036; $R^2 = 0.1849$. (Regression analysis based on 182 male subjects with $S_{acin} \times V_T$ less than 0.119; corresponding to mean + 1.96 SD in the full male cohort). ULN, upper limit of normal. LLN, lower limit of normal.

Figure E12. $S_{acin} \times V_T$ vs. age in full female healthy control cohort.



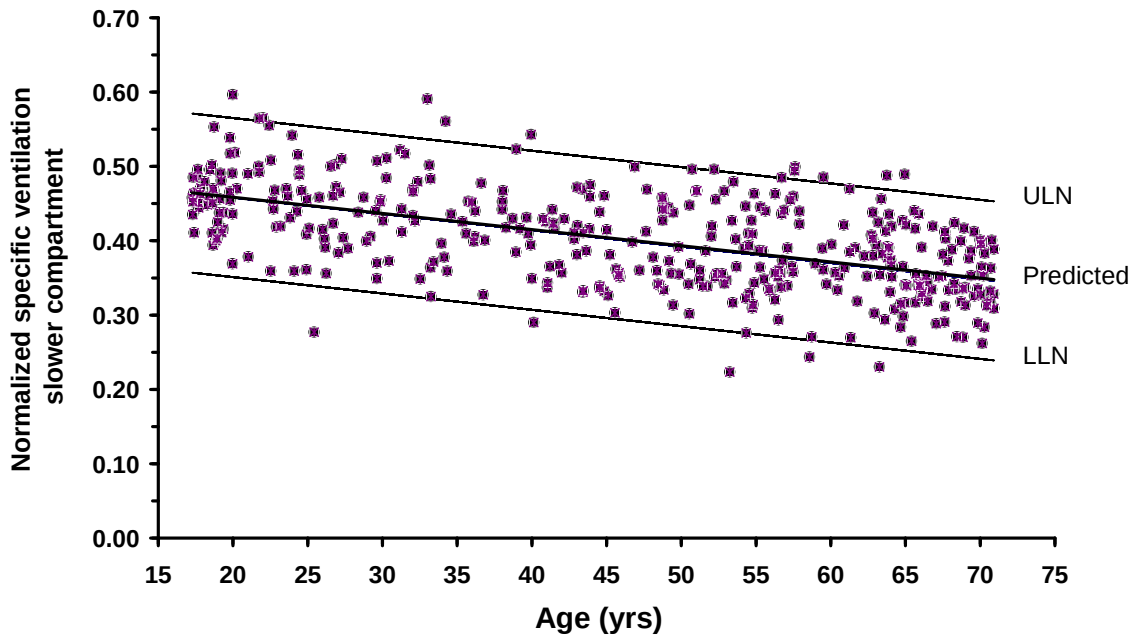
Predicted value = $0.0502 + 0.0003 \times \text{Age}$; RSD 0.01881; $R^2 = 0.0547$. (Regression analysis based on 199 female subjects with $S_{acin} \times V_T$ less than 0.121; corresponding to mean + 1.96 SD in the full female cohort). ULN, upper limit of normal. LLN, lower limit of normal.

Figure E13. $FRC_{\text{slower}} / FRC_{\text{full}}$ (%) vs. age in healthy control cohort.



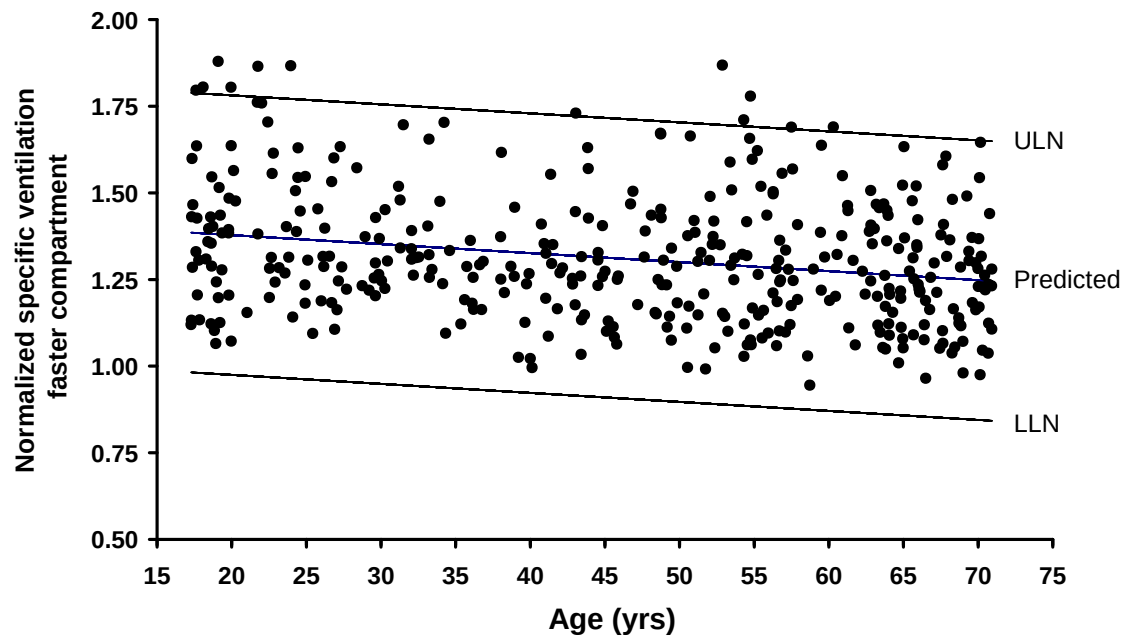
Predicted value = $69.66 - 0.1136 \times \text{Age}$; RSD 8.311; $R^2 = 0.0522$. ULN, upper limit of normal. LLN, lower limit of normal.

Figure E14. Normalized specific ventilation of slower compartment vs. age in healthy control cohort.



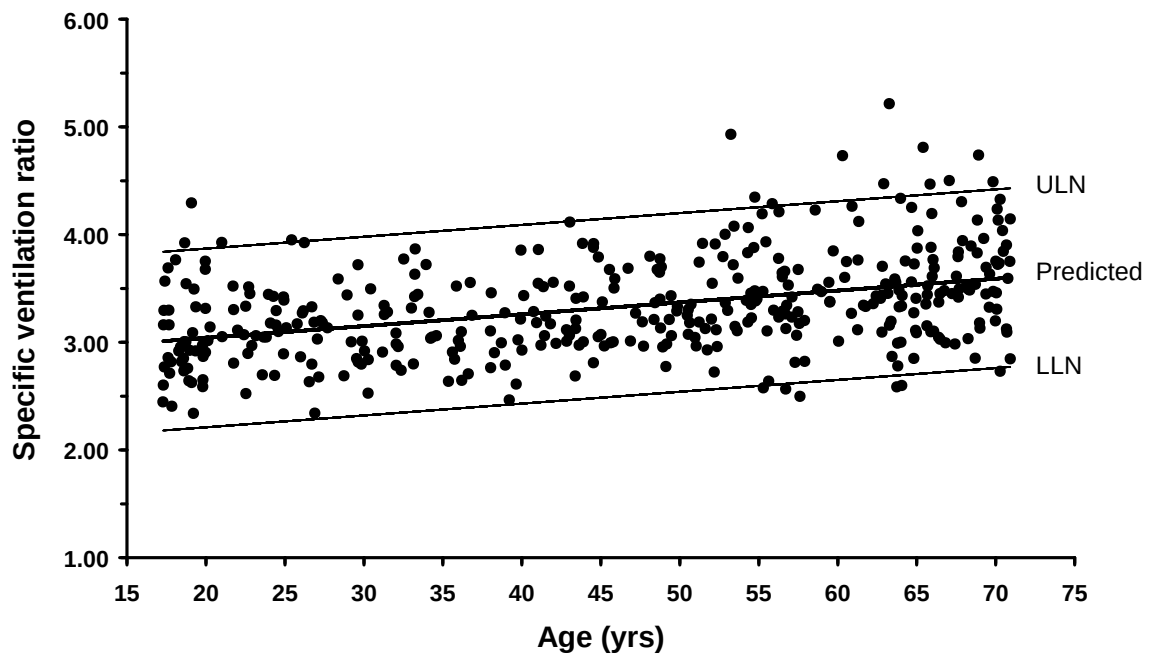
Predicted value = $0.5021 - 0.0022 \times \text{Age}$; RSD 0.0546; $R^2 = 0.173$. ULN, upper limit of normal. LLN, lower limit of normal.

Figure E 15. Normalized specific ventilation of faster compartment vs. age in healthy control cohort.



Predicted value = $1.4301 - 0.0026 \times \text{Age}$; RSD 0.2054; $R^2 = 0.0528$. ULN, upper limit of normal. LLN, lower limit of normal.

Figure E16. Specific ventilation ratio vs. age in healthy control cohort.



Predicted value = $2.820 + 0.011 \times \text{Age}$; RSD 0.423; $R^2 = 0.1671$. ULN, upper limit of normal. LLN, lower limit of normal.

Table E5. ACT and ACQ findings in asthma cohort.

Variable	All (n=196)	Females (n=109)	Males (n=87)
ACT			
6-14	32	18	14
15-18	28	15	13
19	17	12	5
≥20	119	64	55
ACQ (mean values)			
0.0 -0.33	53	25	28
0.34-0.84	51	32	19
1.00-1.33	27	18	9
1.50 -	58	31	27
Missing	7	3	4

ACT, asthma control test. ACQ, asthma control questionnaire.

Table E6. Blood eosinophil findings in asthma cohort.

Variable	All (n=196)	Females (n=109)	Males (n=87)
Blood eosinophil count ($\times 10^9$ /L)			
0.0 - 0.2	125	73	52
0.3 - 0.4	44	24	20
0.5 -	26	11	15
missing	1	1	0
Blood eosinophil count (%)			
≤ 4.0	123	71	52
>4.0; ≤ 6.0	38	24	14
> 6.0	34	13	21
missing	1	1	0

Table E7. Number of positive skin prick tests (SPT) to various specific inhalant allergens among 192 subjects.

Sensitisation	Number of subjects	Percentage of subjects tested
None	65	33.9
Birch pollen	88	45.8
Hazel pollen	56	29.2
Timothy pollen	95	49.5
Mugwort pollen	35	18.2
Cat	90	46.9
Dog dander	88	45.8
Horse dander	59	30.7
D Pteronyssinus (HDM)	41	21.4
D Farinae (HDM)	33	17.2
Alternaria (mould)	17	8.9
Aspergillus (mould)	13	6.8
Penicillium (mould)	9	4.7

Cladosporium (mould)	15	7.8
HDM, house dust mite.		

Table E8. Number of positive skin prick tests to various inhalant allergen groups among 192 tested subjects.

Sensitisation	Number of subjects	Percentage of subjects tested
None	65	33.9
Pollens	115	59.9
Furred pets	102	53.1
House dust mites (HDM)	43	22.4
Moulds	28	14.6
Pollens only	17	8.9
Furred pets only	4	2.1
House dust mites only	2	1.0
Moulds only	1	0.5
Pollens and furred pets	45	23.4
Pollens and furred pets and house dust mites	23	12.0
Pollens and furred pets and moulds	17	8.9
Pollens, furred pets, house dust mites and moulds	8	4.2
Furred pets and house dust mites	4	2.1
Pollens and house dust mites	4	2.1
Pollens and moulds	1	0.5
Furred pets, house dust mites and moulds	1	0.5

Table E9. Total number of positive skin prick tests (SPT) among 192 tested subjects.

Sensitisation	Number of subjects	Percentage of subjects tested
None	65	33.9
1	14	7.3
2	10	5.2
3	14	7.3
4	18	9.4
5	19	9.9
6	20	10.4
7	9	4.7
8	11	5.7
9	4	2.1
10	3	1.6
11	3	1.6
12	1	0.5
13	1	0.5

Table E10. Asthma medication and medication scores among 196 asthmatic subjects.

Medication type	Score	Number
No regular or intermittent controller therapy	0	21
Intermittent controller therapy	0	11
MLK only	1	2
Low dose ICS only	1	24
High dose ICS only	2	20
Low dose ICS + MLK	2	1
Low dose Combo	2	34
High dose combo or high dose ICS + MLK	3	66
Low dose combo + MLK	3	7
High dose combo + MLK	4	10

MLK, montelukast. ICS, inhaled corticosteroid. Combo, ICS/long-acting beta₂ agonist combination therapy. "Low dose" corresponds to a maximum daily dose of 400 mcg budesonide or beclomethasone or 200 mcg fluticasone.

Table E11. Number of subjects with different ages at asthma onset separated with respect to different course of disease.

Age at onset and course	All (n=196)	Females (n=109)	Males (n=87)
Preschool age	47	26	21
Persistent	27	18	9
Intermittent or relapsing	20	8	12
School age	32	19	13
Persistent	17	8	9
Intermittent or relapsing	15	11	4
Adolescence	30	18	12
Persistent	21	14	7
Intermittent or relapsing	9	4	5
Young adult life (age 20-40 yrs)	56	35	21
Persistent	28	16	12
Intermittent or relapsing	28	19	9
Middle age (after age 40 yrs)	31	11	20
Persistent	19	6	13
Intermittent or relapsing	12	5	7

Table E12. Allergy rhinitis and polyps in asthma cohort.

Variable	All (n=196)	Females (n=109)	Males (n=87)
Have you had seasonal allergic rhinitis?			
Yes	145	80	65
No	51	29	22
Have you had allergic rhinitis all year round?			
Yes	77	46	31
No	119	63	56
Have you had or do you have a blocked nose ?			
Yes	161	90	71
No	35	19	16
Have you had or do you have nasal polyps?			
Yes	42	20	22
No	150	86	64
Suspected	3	2	1
Missing data	1	1	0

Table E13. Pets at home.

Variable	All (n=196)	Females (n=109)	Males (n=87)
Do you keep any furred pet(s) in your home?			
No	124	67	57
Yes	71	42	29
Cat (s) only	27	15	12
Dog (s) only	23	13	10
Cat and dog	11	7	4
Guinea pig (s) only	6	5	1
Hamster	1	0	1
Cat and Guinea pig	1	1	0
Cat and rabbit	2	1	1
Missing data	1	0	1
Any cat	41	24	17
Any dog	34	20	14

Peripheral airway function in adult asthma - OLS

Table E14. Demography, asthma control questionnaires, blood eosinophil, allergic sensitisation, medication scores, asthma course, FENO50 and smoking history in full asthma cohort and subgroups.

Variable	All	Females	Males	Never smokers	Ever smokers	Previous smokers	Current smokers	Onset age 20-40 yrs	Onset after age 40 yrs
N	196	109	87	136	60	54	6	57	30
Sex (M/F)	87/109	0/109	87/87	62/74	25/35	23/31	2/6	21/36	20/10*
Age (yrs,md,range)	44 (18; 61)	44 (18; 60)	45 (18; 61)	43 (18; 61)	47 (18; 61)*	46 (19; 61)	47 (18; 54)	45 (23; 60)	53 (40; 61)***
Weight (kg)	79 (50; 160)	72 (50; 160)	83 (55; 126)***	79 (50; 160)	79 (50; 120)	79 (50; 108)	80 (55; 120)	79 (56; 120)	88 (59; 125)*
Height (cm)	173 (149; 200)	167 (149; 185)	180 (162; 200)***	173 (149; 200)	172 (150; 187)	172 (150; 187)	176 (161; 180)	170 (149; 187)	178 (160; 195)
BMI (kg/m ²)	25.8 (17.7; 55.4)	25.5 (19.3; 55.4)	26.2 (17.7; 36.9)	25.4 (19.3; 55.4)	27.0 (17.7; 37.1)	26.9 (20.1; 34.7)	28.2 (17.7; 37.1)	26.0 (20.1; 40.7)	28.2 (19.9; 36.9)
ACT	20.5 (6; 25)	20.0 (8; 25)	21.0 (6; 25)	20.0 (9; 25)	21.0 (6; 25)	22.0 (6; 25)	19.5 (14; 20)	21 (8; 25)	20 (7; 25)
Proportion with ACT<16	39/196	22/109	17/87	27/136	12/60	11/54	1/6	11/57	8/30
Proportion with ACT<20	77/196	45/109	32/87	55/136	22/60	19/54	3/6	20/57	14/30
ACQ	0.83 (0.00; 5.17)	0.83 (0.00; 4.00)	0.83 (0.00; 5.17)	0.83 (0.00; 4.00)	0.67 (0.00; 5.17)	0.50 (0.00; 5.17)	1.33 (0.00; 1.83)	0.67 (0.00; 3.83)	1.08 (0.00; 4.17)
# with ACQ>1.0	86/190	49/106	37/84	65/131	21/59	17/53	4/6	20/57	15/30
B-eosinophils (%)	2.8 (0.0; 26.7)	2.6 (0.0; 16.9)	3.1 (0.0; 26.7)	2.5 (0.0; 26.7)	3.4 (0.0; 13.0)	3.2 (0.0; 13.0)	3.9 (2.9; 5.8)	2.6 (0.0; 26.7)	2.9 (0.0; 10.0)
B-eosinophils >4.0%	72/196	37/109	35/87	48/136	24/60	21/54	3/6	22/57	9/30
Number of positive SPT	3 (0; 13)	2 (0; 13)	4 (0; 12)*	3 (0; 13)	2 (0; 11)	2.5 (0; 11)	0 (0; 5)	1 (0; 8)	0 (0; 8)
Proportion with all SPT negative	65/192	41/107	24/85	45/133	20/59	16/54	4/5	25/57	18/30
Proportion with pollen sensitisation	115/192	60/107	55/85	81/133	34/159	33/54	1/5	28/57	10/29
Proportion with furred pet sensitisation	102/192	51/107	51/85	74/113	28/59	27/54	1/5	25/57	7/29*
Proportion with HDM sensitisation	42/192	18/107	24/85	28/113	14/59	13/54	1/5	7/57	3/29
Proportion with mould sensitisation	28/192	15/107	13/85	23/133	5/59	5/54	0/5	3/57	1/29
Medication score	2 (0; 4)	2 (0; 4)	2 (0; 4)	2 (0; 4)	2 (0; 4)	2 (0; 4)	2 (1; 4)	2 (0; 4)	2 (0; 4)
Proportion with medication score>2	83/196	46/109	37/87	56/136	27/60	25/54	2/6	22/57	10/30
Proportion with preschool asthma onset	47/196	26/109	21/87	34/136	13/60	13/54	0/6	-	-
Proportion with school age asthma onset	32/196	19/109	13/87	20/136	12/60	9/54	3/6	-	-
Proportion with adolescence asthma onset	30/196	18/109	12/87	23/136	7/60	6/54	1/6	-	-
Proportion with asthma onset at age 20-40 yrs	57/196	36/109	21/87	37/136	20/60	19/54	1/6	-	-
Proportion with asthma onset after age 40 yrs	30/196	10/109	20/87*	22/136	8/60	7/54	1/6	-	-
Proportion with intermittent/relapsing asthma	84/196	47/109	37/87	52/136	32/60	31/54	1/6	29/57	11/30
Proportion with persistent asthma	112/196	62/109	50/87	84/136	28/60	23/54	5/6	28/57	19/30
Proportion reporting allergic rhinitis	146/196	80/109	66/87	103/136	43/60	40/54	3/6	38/57	14/30
Proportion with reporting nasal polyposis	42/196	20/109	22/87	29/136	13/60	12/54	1/5	16/57	9/30
Proportion with resident cat or dog	65/196	37/109	28/87	42/136	23/60	20/54	3/6	20/57	14/30
Proportion with resident cat or dog + sensitized relevant allergen	24/196	13/109	11/85	14/133	10/59	9/54	1/6	6/57	4/29
FENO50 (ppb)	17.1 (2.3; 189.5)	13.7 (2.3; 90.2)	20.0 (3.3; 189.5)*	16.9 (2.3; 189.5)	17.6 3.3; 97.5)	18.4 (3.3; 97.5)	14.3 (4.6; 62.6)	17.0 (5.5; 90.2)	19.8 (4.6; 141.4)
Proportion with FENO50>25 ppb	59/196	25/109	34/87*	42/136	17/60	16/54	1/6	19/57	13/30
Number of pack-years of smoking	0.0 (0.0; 30.0)	0.0 (0.0; 25.0)	0.0 (0.0; 30.0)	0.0 (0.0; 0.0)	5.0 (0.5; 30.0)***	5.0 (0.5; 30.0)	9.5 (2.0; 20.0)	0.0 (0.0; 25.0)	0.0 (0.0; 30.0)
Proportion ever smokers	60/196	35/109	25/87	0/136	60/60***	54/54	6/6	20/57	8/30
Proportion current smokers	6/196	4/109	2/87	0/136	6/60**	0/54	6/6***	1/57	1/30

ACT, asthma control test. ACQ, asthma control questionnaire. SPT, skin prick test. HDM, house dust mite. FENO50, fraction exhaled nitric oxide at an expiratory flow of 50 mL/s. ppb, parts per billion.

*p<0.05, ** p<0.01, *** p<0.001, Mann-Whitney U-test.

Peripheral airway function in adult asthma - OLS

Table E15. Lung function in full asthma cohort and subgroups. Median and range are given.

Variable	All	Females	Males	Never smokers	Ever smokers	Previous smokers	Current smokers	Onset age 20-40 yrs	Onset after age 40 yrs
N	196	109	87	136	60	54	6	57	40
FEV1 (L)	3.14 (1.22; 5.35)	2.91 (1.22; 4.78)	3.66 (1.55; 5.35)	3.16 (1.22; 5.35)	3.02 (1.52; 5.06)	3.08 (1.52; 5.06)	2.42 (1.82; 3.19)	3.03 (1.22; 5.06)	3.06 (2.01; 4.43)
FVC (L)	4.29 (1.76; 7.23)	3.90 (1.76; 6.27)	5.12 (2.60; 7.23)	4.31 (1.76; 7.23)	4.24 (2.32; 6.52)	4.29 (2.32; 6.52)	3.64 (2.92; 4.77)	4.10 (1.76; 6.75)	4.40 (2.69; 5.98)
FEV1/FVC (%)	73.0 (45.4; 89.8)	74.6 (55.3; 88.0)	71.5 (45.4; 89.8)	73.7 (55.8; 89.8)	67.3 (45.4; 81.8)***	69.4 (48.3; 81.8)	66.3 (45.4; 79.6)	73.7 (56.2; 84.7)	71.7 (50.9; 86.0)
MEF50 (L/s)	2.9 (0.9; 7.0)	2.8 (0.9; 5.1)	3.0 (0.9; 7.0)	3.0 (0.9; 7.0)	2.4 (0.9; 6.8)**	2.5 (0.9; 6.8)	1.9 (0.9; 3.4)	2.8 (0.9; 6.8)	2.5 (1.2; 6.6)
MEF25 (L/s)	0.9 (0.2; 3.6)	0.9 (0.2; 2.4)	0.9 (0.2; 3.6)	1.0 (0.3; 3.6)	0.7 (0.2; 2.0)***	0.8 (0.2; 2.0)	0.5 (0.4; 0.9)	0.9 (0.3; 2.0)	0.8 (0.2; 1.9)**
FEV1 (% pred)	87.6 (39.3; 123.8)	89.7 (48.1; 120.8)	86.6 (39.3; 123.8)	89.0 (44.5; 123.8)	83.0 (39.3; 120.8)	86.8 (39.3; 120.8)	67.6 (41.1; 80.2)*	88.8 (39.3; 110.2)	84.0 (49.0; 117.8)
FVC (% pred)	95.1 (48.7; 128.9)	95.7 (61.5; 128.9)	93.4 (48.7; 127.5)	93.4 (51.4; 128.9)	96.6 (48.7; 116.3)	98.5 (48.7; 116.3)	77.9 (74.4; 89.5)***	95.7 (48.7; 115.5)	88.4 (51.4; 109.9)
FEV1/FVC (% pred)	93.0 (55.0; 115.9)	93.8 (65.8; 115.9)	92.1 (55.0; 115.9)	94.5 (65.8; 115.9)	87.6 (55.0; 111.0)**	87.6 (65.2; 111.0)	84.7 (55.0; 104.8)	93.5 (69.5; 113.6)	96.3 (70.4; 115.9)
MEF50 (% pred)	61.7 (17.5; 153.5)	61.5 (21.4; 112.4)	62.0 (17.5; 153.5)	63.7 (21.4; 153.5)	55.1 (17.5; 132.8)**	56.8 (22.9; 132.8)	32.7 (17.5; 70.4)	63.9 (21.4; 138.2)	57.5 (23.0; 153.5)
MEF25 (% pred)	53.9 (14.4; 139.7)	56.1 (17.4; 139.7)	52.2 (14.4; 137.7)	57.4 (17.4; 139.7)	46.0 (14.4; 136.3)**	48.1 (14.4; 136.3)	29.0 (15.5; 63.6)	55.0 (14.4; 118.2)	49.8 (18.4; 135.2)
FEV1 (z)	-1.10 (-5.67; 2.23)	-0.86 (-3.93; 1.63)	-1.22 (-5.67; 2.23)	-0.98 (-5.33; 2.23)	-1.39 (-5.67; 1.63)	-1.10 (-5.21; 1.63)	-2.55 (-5.67; -1.63)*	-1.01 (-5.21; 0.80)	-1.33 (-5.33; 1.41)
FVC (z)				-0.62 (-5.12; 3.15)	-0.32 (-4.59; 2.04)	-0.14 (-4.49; 2.04)	-2.16 (-2.85; -0.91) ***	-0.42 (-4.49; 1.47)	-1.05 (-5.12; 0.85)
FEV1/FVC (z)	-0.85 (-6.16; 1.96)	-0.72 (-4.23; 1.76)	-0.92 (-6.16; 1.96)	-0.63 (-4.23; 1.96)	-1.48 (-6.16; 1.17) **	-1.48 (-4.26; 1.17)	-1.77 (-6.16; 0.53)	-0.84 (-4.47; 1.68)	-0.43 (-3.54; 1.96)
MEF50 (z)	-1.61 (-3.85; 2.01)	-1.66 (-3.85; 0.49)	-1.53 (-3.68; 2.01)	-1.52 (-3.73; 2.01)	-2.03 (-3.85; 1.47) *	-1.98 (-3.72; 1.47)	-3.03 (-3.85; -1.32)*	-1.47 (-3.85; 1.63)	-1.72 (-3.72; 2.01)
MEF25 (z)	-1.51 (-4.55; 1.90)	-1.36 (-4.55; 1.40)	-1.69 (-4.50; 1.90)	-1.40 (-4.55; 1.90)	-1.65 (-4.50; 0.86)	-1.62 (-3.24; 0.86)	-2.12 (-4.50; -1.00)	-1.39 (-3.19; 0.47)	-1.41 (-2.43; 0.98)
R5 (kPa/L/s)	0.47 (0.24; 1.36)	0.50 (0.25; 1.36)	0.43 (0.24; 1.05)**	0.45 (0.24; 1.36)	0.53 (0.25; 1.05) *	0.52 (0.25; 1.05)	0.62 (0.50; 1.00)	0.51 (0.28; 1.36)	0.47 (0.25; 0.96)
R20 (kPa/L/s)	0.37 (0.18; 0.68)	0.41 (0.23; 0.68)	0.33 (0.18; 0.58)**	0.36 (0.18; 0.68)	0.39 (0.18; 0.59)	0.39 (0.18; 0.59)	0.45 (0.33; 0.54)	0.39 (0.22; 0.68)	0.34 (0.23; 0.54)
FDR (kPa/L/s)	0.09 (-0.03; 0.68)	0.08 (-0.03; 0.68)	0.10 (0.02; 0.53)	0.08 (-0.03; 0.68)	0.11 (0.02; 0.53) **	0.10 (0.02; 0.53)	0.19 (0.11; 0.45)	0.09 (-0.01; 0.68)	0.11 (0.02; 0.45)
AX (kPa/L)	0.34 (0.03; 7.01)	0.36 (0.03; 7.01)	0.30 (0.03; 6.40)	0.32 (0.07; 7.01)	0.47 (0.03; 6.40) *	0.41 (0.03; 6.40)	1.25 (0.34; 4.21)	0.34 (0.03; 6.40)	0.43 (0.09; 7.01)
Sqrt(AX) (kPa/L) ^{1/2}	0.58 (0.17; 2.65)	0.58 (0.17; 2.65)	0.55 (0.17; 2.53)	0.56 (0.27; 2.65)	0.69 (0.17; 2.53) *	0.64 (0.17; 2.53)	1.11 (0.58; 2.05)	0.58 (0.17; 2.53)	0.65 (0.29; 2.65)
RSI (z)	1.63 (-1.77; 13.30)	1.68 (-1.77; 13.30)	1.48 (-1.18; 10.68)	1.43 (-1.77; 13.30)	2.31 (-0.99; 10.68) *	2.26 (-0.99; 10.68)	4.09 (1.68; 8.39)	2.13 (-1.23; 13.30)	1.64 (-1.36; 7.89)
R20 (z)	1.10 (-1.54; 5.48)	1.45 (-1.28; 5.48)	0.84 (-1.54; 5.27)*	1.02 (-1.54; 5.48)	1.47 (-1.54; 4.85)	1.47 (-1.45; 4.85)	2.00 (0.76; 4.28)	1.45 (-1.00; 5.48)	0.88 (-1.21; 4.28)
FDR (z)	0.94 (-2.14; 15.44)	0.61 (-2.14; 15.44)	1.39 (-0.72; 12.66)**	0.69 (-2.14; 15.44)	1.31 (-1.06; 12.66)**	1.12 (-1.06; 12.66)	3.88 (1.27; 9.86)	0.78 (-1.81; 15.44)	1.56 (-0.81; 9.90)
AX (z)	0.53 (-1.06; 37.23)	0.49 (-1.06; 37.23)	0.58 (-0.94; 34.85)	0.41 (-1.06; 37.23)	1.12 (-0.94; 34.85) *	0.91 (-0.94; 34.85)	5.44 (0.38; 21.78)	0.38 (-0.98; 34.85)	1.29 (-0.74; 37.23)
Sqrt(AX) (z)	0.77 (-1.46; 14.24)	0.68 (-1.42; 14.24)	0.82 (-1.46; 12.91)	0.62 (-1.42; 14.24)	1.29 (-1.46; 12.91) *	1.10 (-1.46; 12.91)	4.06 (0.56; 10.30)	0.56 (-1.46; 13.49)	1.43 (-0.85; 14.24)
LCI	7.77 (6.36; 20.65)	7.72 (6.36; 17.67)	7.85 (6.60; 20.65)*	7.69 (6.36; 17.67)	8.34 (6.38; 20.65) ***	8.19 (6.38; 13.48)	10.33 (8.15; 20.65)*	7.80 (6.38; 16.10)	7.87 (7.03; 12.04)
S _{cond} x V _T (L)	0.027 (0.003; 0.117)	0.027 (0.007; 0.099)	0.027 (0.003; 0.117)	0.026 (0.003; 0.117)	0.032 (0.007; 0.099)	0.031 (0.007; 0.099)	0.043 (0.024; 0.071)	0.028 (0.007; 0.117)	0.027 (0.013; 0.087)
S _{acin} x V _T (L)	0.097 (0.022; 0.548)	0.084 (0.022; 0.452)	0.107 (0.027; 0.548)**	0.085 (0.022; 0.452)	0.130 (0.024; 0.548) ***	0.130 (0.024; 0.437)	0.192 (0.045; 0.548)	0.104 (0.024; 0.409)	0.100 (0.039; 0.195)
1 st breath Sn _{max} x V _T (L)	0.104 (0.035; 0.552)	0.094 (0.035; 0.465)	0.123 (0.044; 0.552)**	0.094 (0.035; 0.465)	0.137 (0.040; 0.552) ***	0.137 (0.040; 0.443)	0.209 (0.069; 0.552)	0.117 (0.038; 0.420)	0.105 (0.050; 0.219)
FRC _{slower} / FRC _{full} (%)	57.8 (39.2; 89.2)	58.8 (39.8; 89.2)	57.4 (39.2; 86.5)	59.5 (39.2; 89.2)	52.8 (42.0; 86.5)	53.5 (42.0; 86.5)	48.0 (45.2; 70.0)	55.6 (39.2; 89.2)	57.4 (42.7; 76.8)
V _A _{slower} / FRC _{slower} (%)	10.9 (1.16; 30.4)	11.7 (2.71; 30.4)	10.2 (1.16; 24.6)	11.40 (2.7; 30.4)	9.45 (1.2; 24.6) **	9.45 (3.90; 24.60)	10.12 (1.16; 14.64)	10.94 (3.20; 30.38)	11.70 (4.72; 22.29)
Normalized V _A _{slower} / FRC _{slower}	0.34 (0.08; 0.55)	0.35 (0.11; 0.53)	0.34 (0.08; 0.55)	0.36 (0.08; 0.55)	0.30 (0.09; 0.52) ***	0.30 (0.14; 0.52)	0.22 (0.09; 0.31)	0.34 (0.08; 0.53)	0.32 (0.17; 0.46)
V _A _{faster} / FRC _{faster} (%)	40.2 (9.83; 106.9)	41.6 (16.41; 106.9)	36.9 (9.83; 101.7)*	40.4 (16.4; 106.9)	35.58 (9.83; 91.60)	35.5 (20.4; 91.60)	48.5 (9.8; 67.7)	40.79 (19.02; 105.95)	42.70 (25.31; 91.17)
Normalized V _A _{faster} / FRC _{faster} (%)	1.16 (0.62; 2.26)	1.16 (0.72; 2.26)	1.16 (0.62; 2.04) *	1.18 (0.72; 2.26)	1.13 (0.62; 2.04) **	1.11 (0.62; 2.04)	1.19 (0.74; 1.26)	1.15 (0.80; 2.26)	1.18 (1.03; 1.72)
Specific ventilation ratio	3.57 (2.44; 10.05)	3.60 (2.44; 8.62)	3.57 (2.56; 10.05)	3.49 (2.44; 10.05)	3.83 (2.77; 8.62) **	3.70 (2.77; 7.00)	5.55 (4.09; 8.62)**	3.69 (2.65; 10.05)	3.95 (2.78; 6.52)
LCI (z)	1.70 (-1.59; 32.15)	1.57 (-1.59; 24.22)	1.84 (-0.79; 32.15)	1.34 (-0.81; 24.22)	2.71 (-1.59; 32.15) ***	2.32 (-1.59; 13.75)	7.20 (2.57; 32.15)*	1.89 (-1.59; 20.47)	1.63 (-0.71; 10.62)
S _{cond} x V _T (z)	1.69 (-4.29; 26.05)	1.24 (-1.91; 13.71)	2.15 (-4.29; 26.05)*	1.37 (-4.29; 26.05)	2.11 (-2.87; 18.04)	1.90 (-2.87; 18.04)	4.99 (0.61; 8.76)	1.84 (-1.96; 26.05)	1.68 (-1.25; 18.04)
S _{acin} x V _T (z)	1.59 (-2.19; 24.11)	1.34 (-2.19; 20.67)	1.92 (-2.11; 24.11)	0.97 (-2.19; 20.67)	2.91 (-2.10; 24.11) ***	2.91 (-2.10; 17.61)	6.69 (-0.65; 24.11)	2.03 (-2.11; 18.36)	1.46 (-1.45; 6.80)
1 st breath Sn _{max} x V _T (z)	1.74 (-1.94; 25.71)	1.29 (-1.94; 20.96)	2.79 (-1.33; 25.71)**	1.28 (-1.94; 20.96)	3.36 (-1.86; 25.71)***	3.36 (-1.86; 19.31)	7.14 (0.27; 25.71)	2.13 (-1.86; 18.46)	1.71 (-1.51; 7.63)
FRC _{slower} / FRC _{full} (z)	-0.83 (-3.08; 2.95)	-0.80 (-3.08; 2.95)	-0.84 (-3.05; 2.55)	-0.72 (-3.08; 2.95)	-0.84 (-3.05; 2.55)	-1.25 (-2.72; 2.55)	-2.04 (-2.29; 0.29)	-1.15 (-3.05; 2.95)	-0.84 (-2.60; 1.69)
Normalized V _A _{slower} / FRC _{slower} (z)	-1.22 (-6.85; 2.21)	-1.14 (-5.34; 2.21)	-1.32 (-6.85; 2.05)	-0.85 (-5.94; 2.21)	-1.96 (-6.85; 2.02) ***	-1.85 (-4.90; 2.02)	-3.20 (-6.85; -1.88)*	-1.35 (-5.94; 2.21)	-1.27 (-3.76; 1.63)
Normalized V _A _{faster} / FRC _{faster} (z)	-0.78 (-3.38; 4.57)	-0.72 (-2.94; 4.57)	-0.79 (-3.38; 3.53)	-0.65 (-2.94; 4.57)	-0.96 (-3.38; 3.53) *	-0.98 (-3.38; 3.53)	-0.63 (-3.10; -0.21)	-0.78 (-2.49; 4.57)	-0.63 (-1.34; 2.13)
Specific ventilation ratio (z)	0.73 (-1.58; 15.92)	0.74 (-1.58; 12.47)	0.55 (-1.53; 15.92)	0.40 (-1.58; 15.92)	1.24 (-1.15; 12.98) **	0.80 (-1.15; 8.90)	5.20 (2.11; 12.98)**	0.80 (-1.46; 15.92)	1.28 (-1.53; 7.22)

Statistical comparisons refer to differences between males and females, ever smokers and never smokers, current smokers and previous smokers, subjects with asthma onset after age 40 years and adults with onset at age 20-40 yrs, respectively. *p<0.05, ** p<0.01, *** p<0.001, Mann-Whitney U-test.

Peripheral airway function in adult asthma - OLS

Table E16. Correlation matrix of lung function variables and other clinical variables recorded for the asthma cohort as a whole.

Variables	Age (years)	FENO50 (ppb)	FEV ₁ (Z)	FEV ₁ /FVC (Z)	MEF ₅₀ (Z)	R5-R20 z	LCI (z)	S _{cond} x V _T (z)	S _{acin} x V _T (z)	SV-ratio (z)	ACT-score	ACQ (mean score)	B-eosinophils (%)	Medication score	# positive SPT	# pack-years
Age (years)	1.00	0.22**	-0.08	0.06	0.01	0.27***	0.07	0.09	0.12	0.10	0.04	-0.03	0.26***	-0.03	-0.10	0.25***
FENO50 (ppb)		1.00	-0.31***	-0.16*	-0.17*	0.05	0.28***	0.28***	0.20**	0.30***	-0.11	0.09	0.50**	-0.24**	0.07	0.04
FEV ₁ (Z)			1.00	0.57***	0.74***	-0.47***	-0.50***	-0.42***	-0.47***	-0.45***	0.19**	-0.20**	-0.31***	-0.01	-0.08	-0.13
FEV ₁ /FVC (Z)				1.00	0.78***	-0.34***	-0.50***	-0.33***	-0.49***	-0.40***	0.09	-0.04	-0.27***	0.05	-0.11	-0.23**
MEF ₅₀ (Z)					1.00	-0.44***	-0.43***	-0.33***	-0.45***	-0.39***	0.12	-0.12	-0.29***	0.03	-0.04	-0.21**
R5-R20 z						1.00	0.42***	0.41***	0.54***	0.42***	-0.15*	0.23**	0.18*	0.08	0.01	0.25***
LCI (z)							1.00	0.55***	0.84***	0.88***	-0.17*	0.15	0.39***	0.13	0.01	0.18*
S _{cond} x V _T (z)								1.00	0.29***	0.63***	-0.31***	0.32***	0.27**	0.06	0.04	0.10
S _{acin} x V _T (z)									1.00	0.67***	-0.11	0.11	0.38***	0.16*	0.04	0.30***
SV-ratio (z)										1.00	-0.20**	0.16*	0.38***	0.07	-0.01	0.18*
ACT-score											1.00	-0.84***	-0.20**	-0.04	0.07	-0.08
ACQ (mean score)												1.00	0.18*	0.07	-0.06	0.06
B-eosinophils (%)													1.00	-0.04	0.13	0.12
Medication score														1.00	0.02	0.08
# positive SPT															1.00	-0.13
# pack-years																1.00

Data displayed as R-values. *p<0.05, ** p<0.01, *** p<0.001.

Table E17. Correlation matrix of lung function variables and other clinical variables recorded for the never smokers in the asthma cohort.

Variables	Age (years)	FENO50 (ppb)	FEV ₁ (Z)	FEV ₁ /FVC (Z)	MEF ₅₀ (Z)	R5-R20 z	LCI (z)	S _{cond} x V _T (z)	S _{acin} x V _T (z)	SV-ratio (z)	ACT-score	ACQ (mean score)	B-eosinophils (%)	Medication score	# positive SPT
Age (years)	1.00	0.23*	-0.13	0.07	0.01	0.25***	0.12	0.15	0.09	0.11	0.01	-0.01	0.24**	-0.04	-0.11
FENO50 (ppb)		1.00	-0.37***	-0.15	-0.22*	0.11	0.34***	0.41***	0.26**	0.38***	-0.15	0.15	0.48***	-0.26**	0.08
FEV ₁ (Z)			1.00	0.47***	0.73***	-0.39***	-0.43***	-0.39***	-0.40***	-0.39***	0.20*	-0.23**	-0.37***	0.01	-0.11
FEV ₁ /FVC (Z)				1.00	0.78***	-0.25*	-0.39***	-0.26**	-0.41***	-0.33***	0.12	-0.09	-0.31***	0.11	-0.18*
MEF ₅₀ (Z)					1.00	-0.37***	-0.37***	-0.28**	-0.37***	-0.35***	0.17	-0.20**	-0.35***	0.07	-0.10
R5-R20 z						1.00	0.38***	0.42***	0.44***	0.39***	-0.16	0.28**	0.25***	0.02	0.01
LCI (z)							1.00	0.63***	0.84***	0.87***	-0.25**	0.26**	0.52***	0.02	0.07
S _{cond} x V _T (z)								1.00	0.37***	0.72***	-0.28**	0.27**	0.34***	-0.05	-0.02
S _{acin} x V _T (z)									1.00	0.63***	-0.25**	0.28**	0.56***	0.11	0.11
SV-ratio (z)										1.00	-0.26**	0.22*	0.47***	-0.05	0.04
ACT-score											1.00	-0.79***	-0.20*	0.06	0.08
ACQ (mean score)												1.00	0.17	0.01	-0.09
B-eosinophils (%)													1.00	-0.07	0.14
Medication score														1.00	0.12
# positive SPT															1.00

Data displayed as R-values. *p<0.05, ** p<0.01, *** p<0.001.

Peripheral airway function in adult asthma - OLS

Table E18. Correlation matrix of lung function variables and other clinical variables recorded for the ever smokers (previous and current smokers) in the asthma cohort.

Variables	Age (years)	FENO50 (ppb)	FEV ₁ (Z)	FEV ₁ /FVC (Z)	MEF ₅₀ (Z)	R5-R20 z	LCI (z)	S _{cond} x V _T (z)	S _{acin} x V _T (z)	SV-ratio (z)	ACT-score	ACQ (mean score)	B-eosinophils (%)	Medication score	# positive SPT	# pack-years
Age (years)	1.00	0.26	0.04	0.14	0.09	0.24	-0.08	-0.04	0.05	0.01	0.09	-0.02	0.30*	-0.07	-0.02	0.36**
FENO50 (ppb)		1.00	-0.15	-0.24	-0.09	-0.07	0.24	-0.05	0.19	0.14	-0.00	-0.08	0.57***	-0.16	0.00	0.22
FEV ₁ (Z)			1.00	0.78***	0.80***	-0.63***	-0.63***	-0.49***	-0.63***	-0.56***	0.19	-0.15	-0.17	-0.05	-0.04	-0.27*
FEV ₁ /FVC (Z)				1.00	0.78***	-0.39**	-0.60***	-0.42**	-0.52***	-0.45***	0.06	-0.01	-0.16	0.01	-0.07	-0.19
MEF ₅₀ (Z)					1.00	-0.53***	-0.49***	-0.41**	-0.53***	-0.42***	0.05	-0.02	-0.09	-0.00	0.04	-0.25
R5-R20 z						1.00	0.41**	0.38**	0.60***	0.42***	-0.16	0.21	0.04	0.14	0.07	0.26*
LCI (z)							1.00	0.42**	0.83***	0.91***	-0.08	0.04	0.16	0.29*	-0.03	0.09
S _{cond} x V _T (z)								1.00	0.17	0.46***	-0.38**	0.43***	0.12	0.27*	0.09	0.09
S _{acin} x V _T (z)									1.00	0.72***	0.02	-0.03	0.13	0.18	0.03	0.20
SV-ratio (z)										1.00	-0.11	0.10	0.15	0.27	-0.05	0.16
ACT-score											1.00	-0.92***	-0.21	-0.28*	0.08	-0.23
ACQ (mean score)												1.00	0.22	0.23	-0.03	0.23
B-eosinophils (%)													1.00	0.01	0.13	0.23
Medication score														1.00	-0.24	0.01
# positive SPT															1.00	-0.16
# pack-years																1.00

Data displayed as R-values. *p<0.05, ** p<0.01, *** p<0.001.

Figures E17-25 show the histogram plots of indices collected in this study.

Figure E 17. Distribution of $FEV_1(z)$ observations in the full asthma cohort.

Red curve describes ideal normal distribution

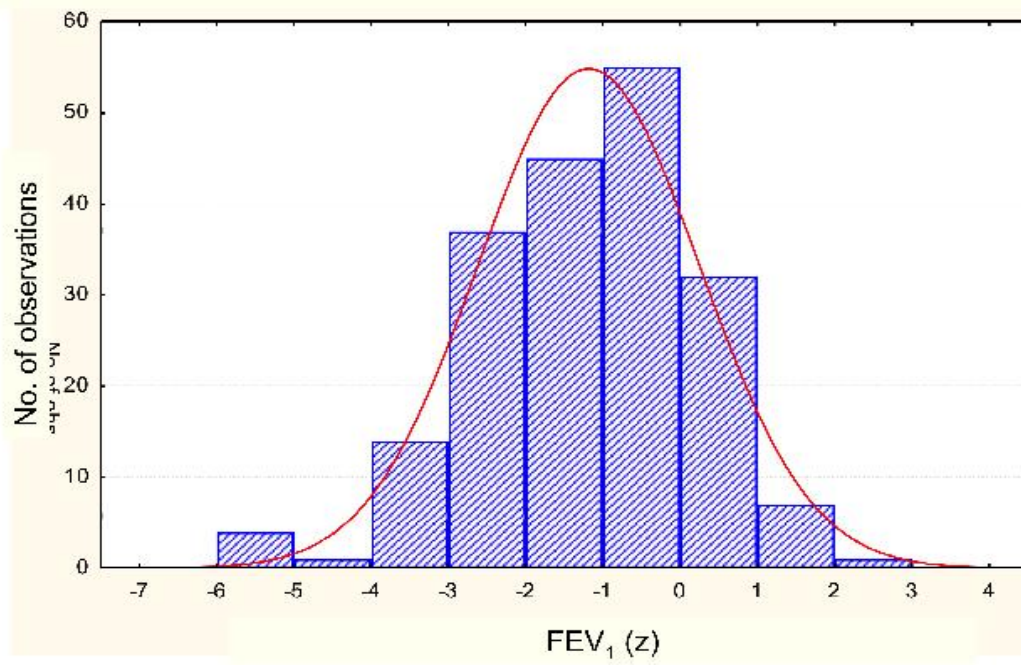


Figure E 18. Distribution of FEV_1 / FVC (z) observations in the full asthma cohort.

Red curve describes ideal normal distribution

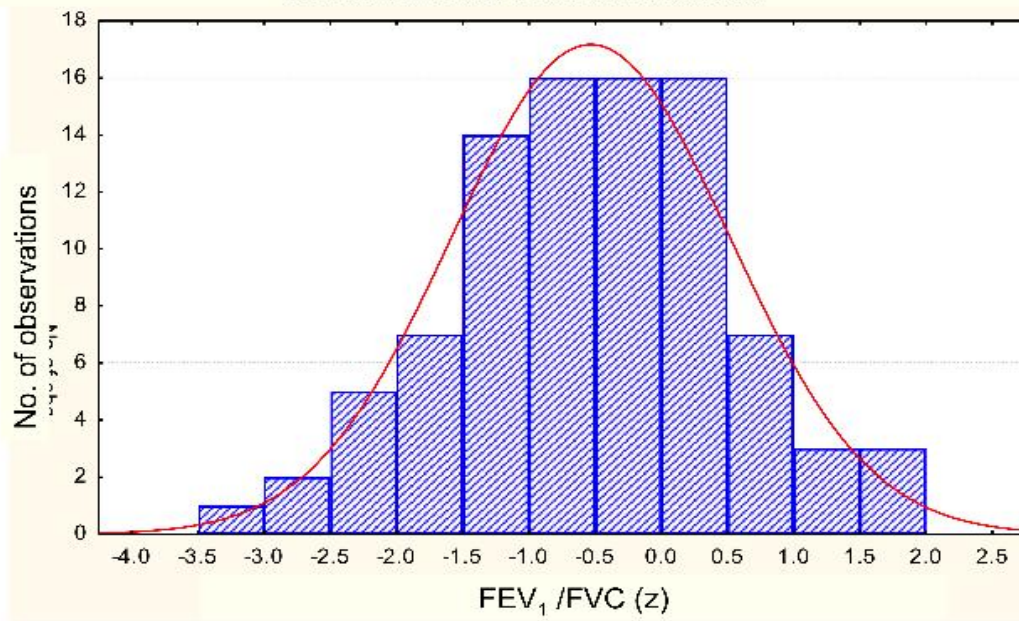


Figure E 19. Distribution of R 20 (z) observations in the full asthma cohort.

Red curve describes ideal normal distribution

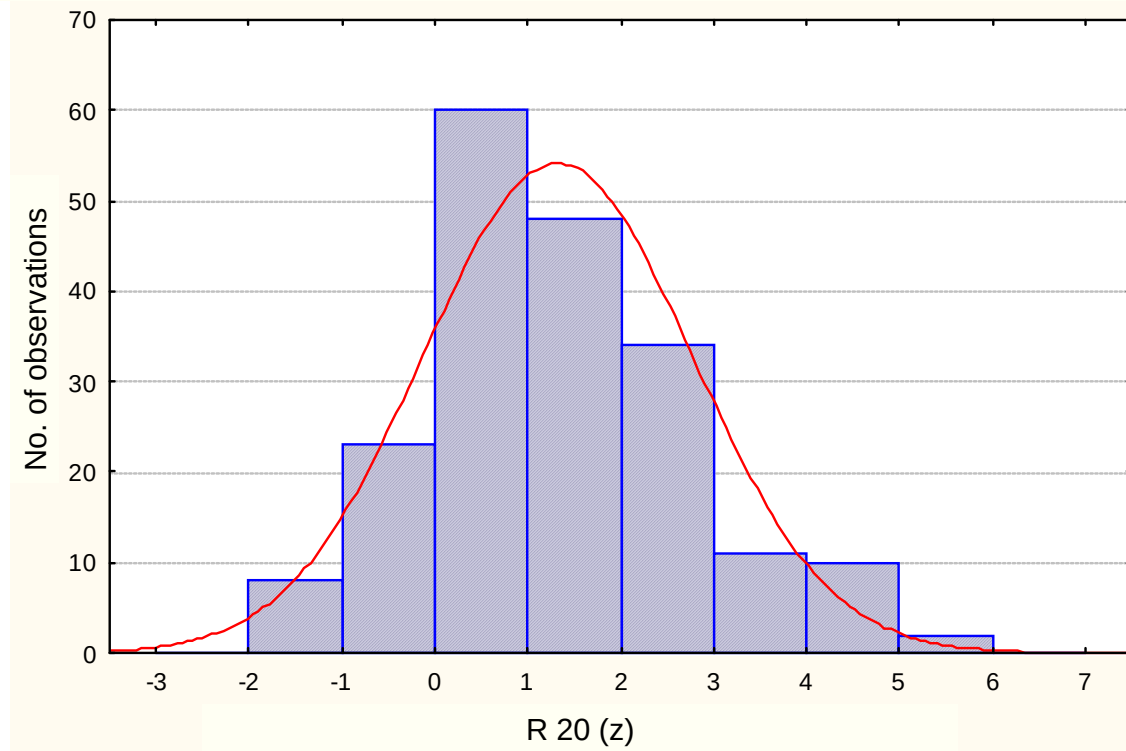


Figure E 20. Distribution of R 5 (z) observations in the full asthma cohort.

Red curve describes ideal normal distribution

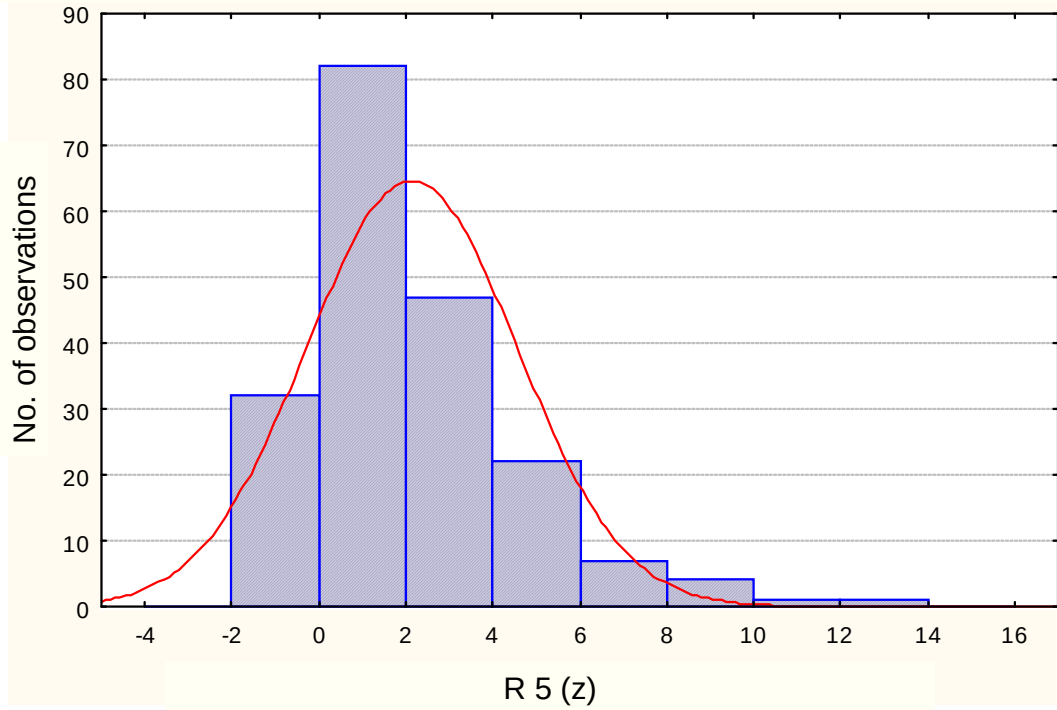


Figure E 21. Distribution of FDR (R 5- R 20) (z) observations in the full asthma cohort.

Red curve describes ideal normal distribution

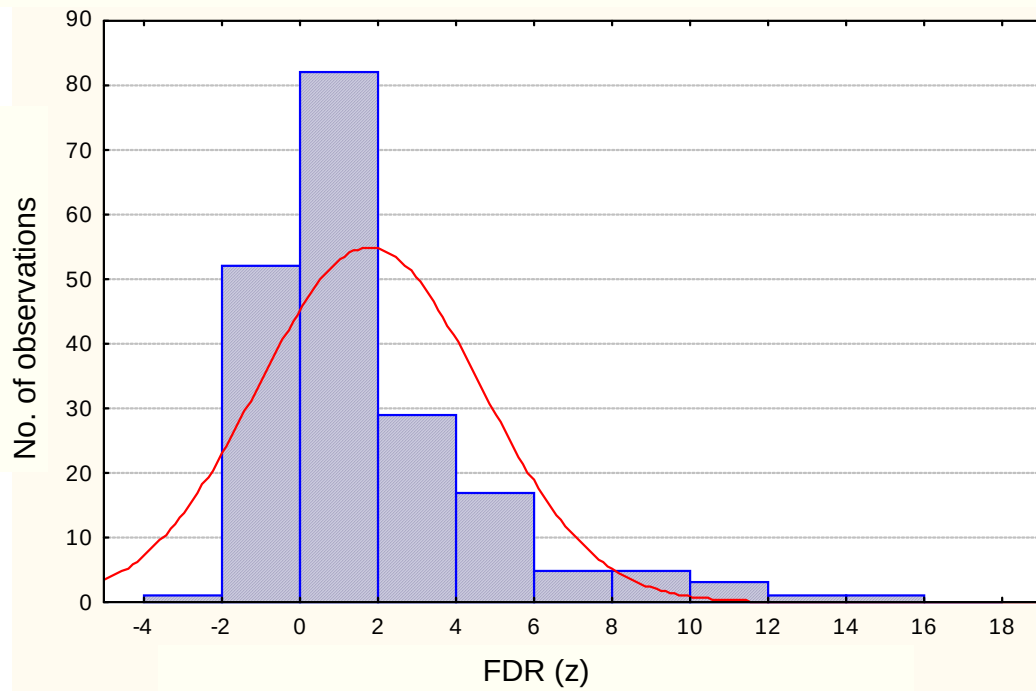


Figure E 22. Distribution of LCI (z) observations in the full asthma cohort.

Red curve describes ideal normal distribution

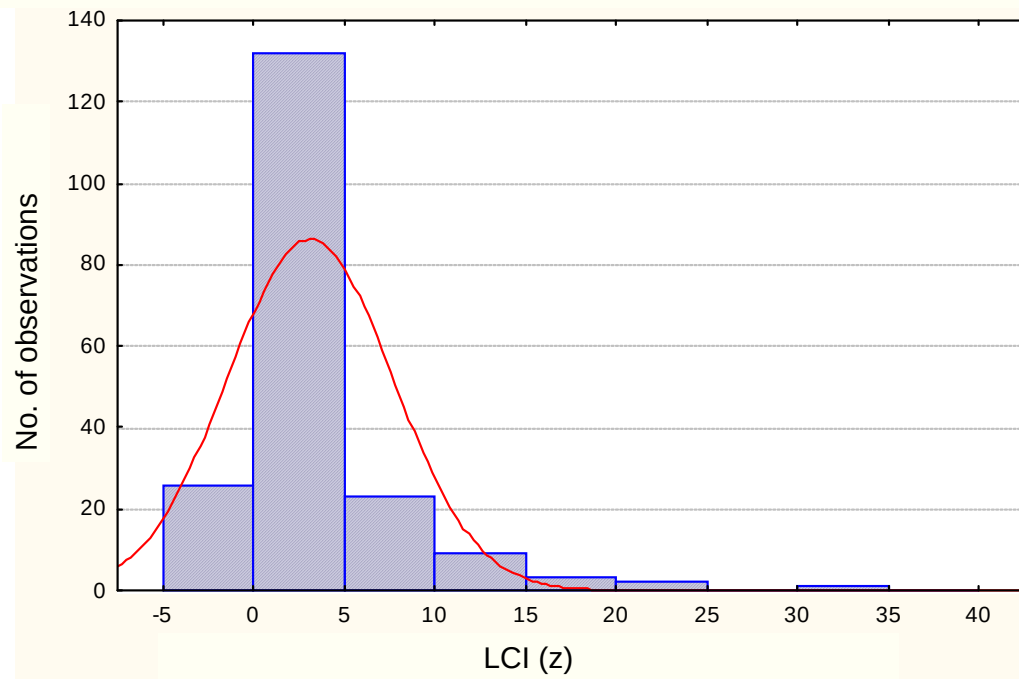


Figure E 23. Distribution of $S_{\text{cond}} \times V_T(z)$ observations in the full asthma cohort.

Red curve describes ideal normal distribution

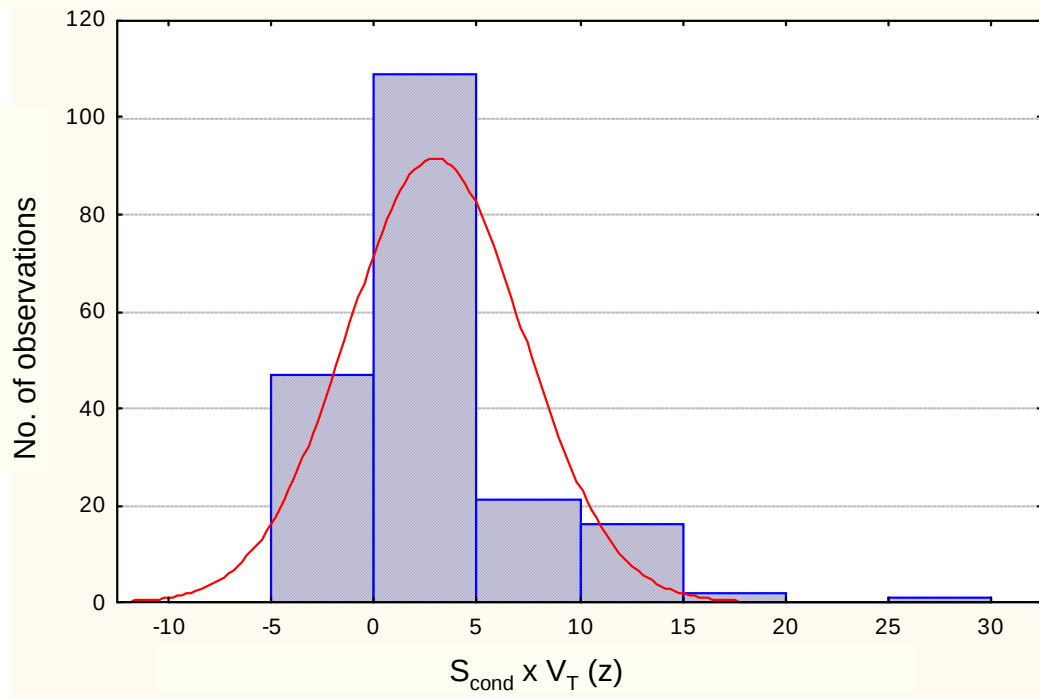


Figure E 24. Distribution of $S_{\text{acin}} \times V_T(z)$ observations in the full asthma cohort.

Red curve describes ideal normal distribution

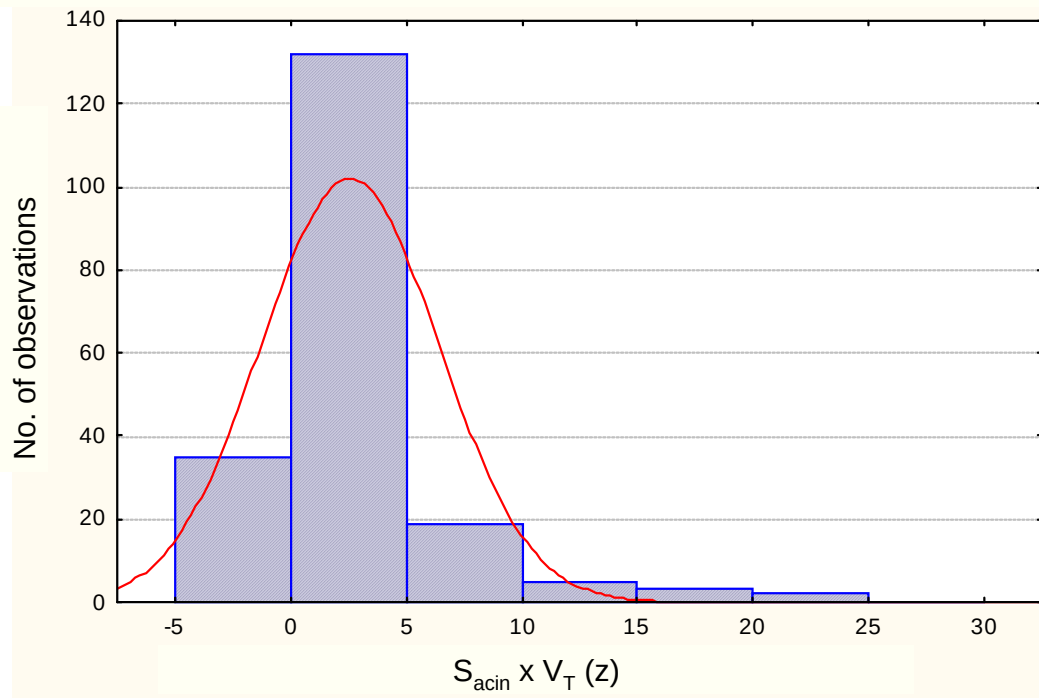


Figure E 25. Distribution of SV-ratio (z) (specific ventilation ratio) observations in the full asthma cohort.

Red curve describes ideal normal distribution

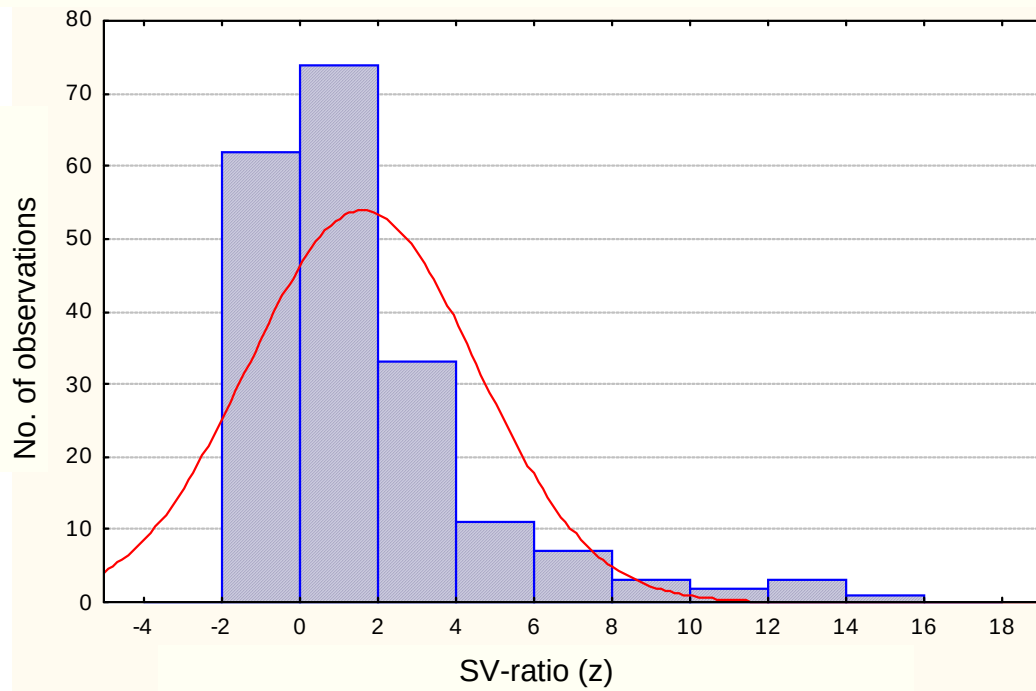
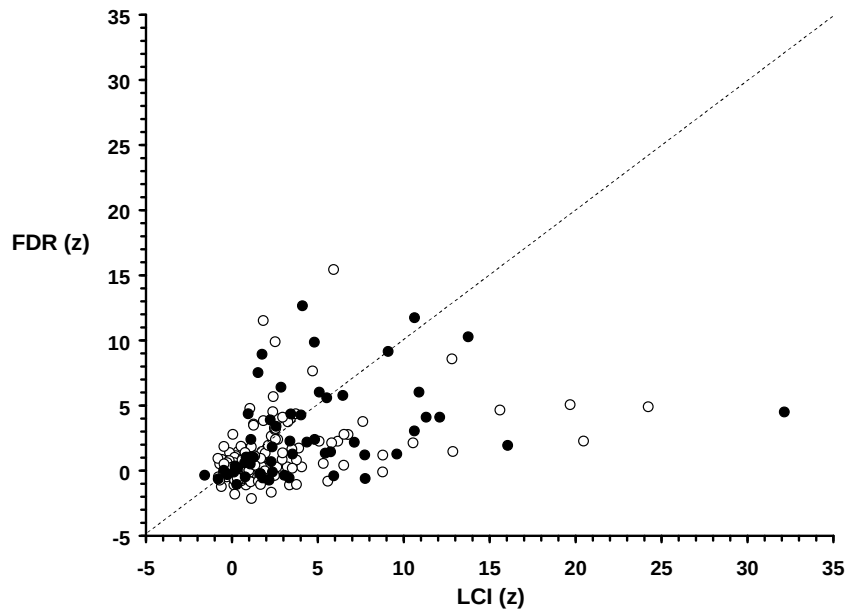
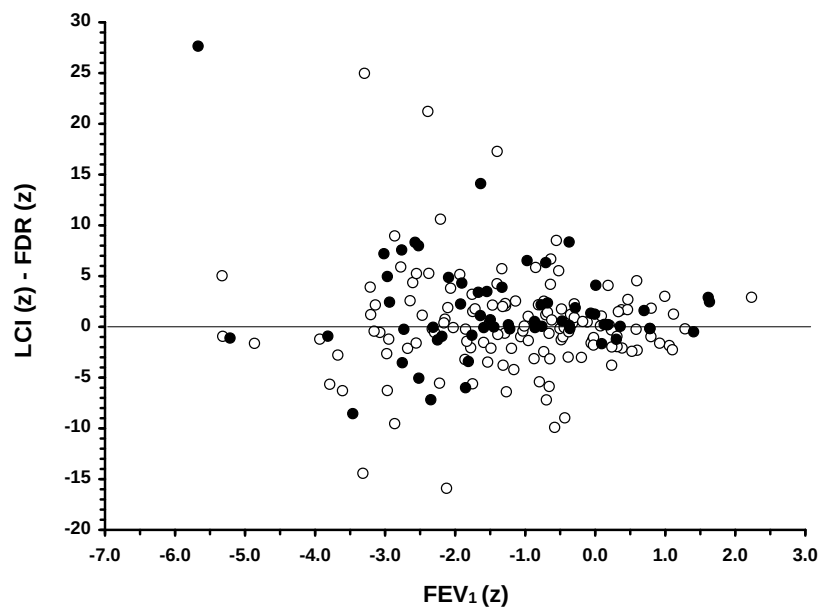


Figure E26. FDR (z) vs. LCI (z) in 136 never smokers and 60 ever smokers from the asthma cohort.



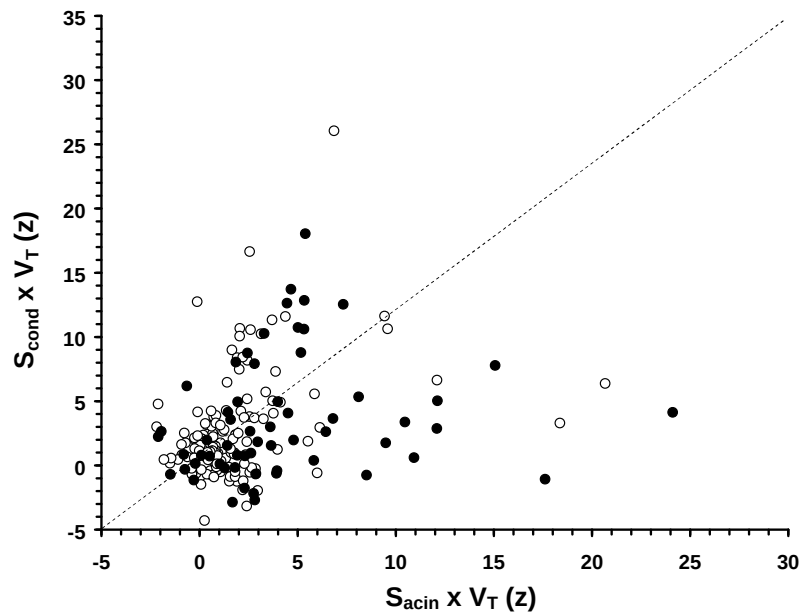
Open circles denote never smokers and closed circles ever smokers. Hatched line represents line of identity.

Figure E27. Figure E 27. LCI (z) - FDR (z) vs. FEV₁ (z) in 136 never smokers and 60 ever smokers from the asthma cohort.



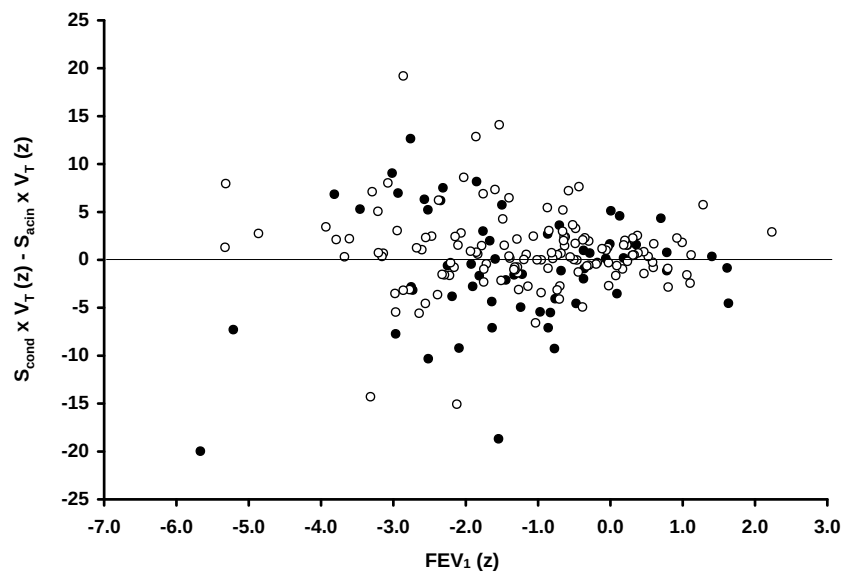
Open circles denote never smokers and closed circles ever smokers.

Figure E28. $S_{\text{cond}} \times V_T(z)$ vs. $S_{\text{acin}} \times V_T(z)$ in 136 never smokers and 60 ever smokers from the asthma cohort.



Open circles denote never smokers and closed circles ever smokers. Hatched line represents line of identity.

Figure E29. $S_{\text{cond}} \times V_T(z) - S_{\text{acin}} \times V_T(z)$ vs. $FEV_1(z)$ in 136 never smokers and 60 ever smokers from the asthma cohort.



Open circles denote never smokers and closed circles ever smokers.

Table E19. Peripheral airway function outcomes given as median (bold) and range with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively.

FEV ₁ ↓	Ever smoker	B-eos >4.0%	n	FDR (z)	LCI (z)	S _{cond} x V _T (z)	S _{acin} x V _T	SV-ratio (z)
☒	☒	☒	8	4.43	9.89	6.99	8.41	6.26
☐	☒	☒	16	2.18; 11.74 0.18	3.43; 32.15 2.60	0.61; 18.04 0.82	-0.65; 24.11 2.81	2.39; 12.98 0.54
☒	☒	☐	10	-1.06; 10.28 5.15	-1.59; 13.75 4.46	-2.70; 7.92 5.84	-0.81; 17.61 4.73	-0.84; 8.14 2.86
☐	☒	☐	26	1.28; 12.66 1.02	1.10; 11.30 1.39	1.97; 13.71 0.87	1.85; 12.09 2.12	0.08; 6.38 0.72
☒	☐	☒	20	-0.63; 7.53 3.18	-0.79; 16.05 6.04	-2.87; 12.63 5.97	-2.10; 12.12 2.58	-1.15; 12.47 3.97
☐	☐	☒	28	1.28; 12.66 0.24	0.00; 24.22 1.40	-4.29; 26.05 1.64	0.25; 20.67 0.84	-0.60; 15.92 0.84
☒	☐	☐	18	-2.14; 4.78 1.37	-0.79; 8.87 2.52	-1.24; 16.65 2.55	-1.50; 5.99 1.37	-1.58; 6.69 0.99
☐	☐	☐	70	-1.10; 11.53 0.42	-0.42; 6.76 0.54	-0.64; 7.31 0.98	0.00; 5.53 0.69	-0.83; 5.49 0.05
				-1.81; 9.90	-0.81; 12.86	-1.96; 11.60	-2.19; 4.37	-1.53; 10.10
FEV ₁ ↓	Ever smoker and/or blood-eos >4.0%							
☒	☒	☒	38	4.11	6.04	6.28	5.06	4.03
☐	☒	☒	70	-0.24; 15.44 0.64	0.00; 32.15 1.65	-4.29; 26.05 0.99	-0.65; 24.11 1.74	-0.60; 15.92 0.74
☒	☐	☐	18	-2.14; 10.28 1.37	-1.59; 16.05 2.52	-2.87; 16.65 2.55	-2.10; 17.61 1.37	-1.58; 12.47 0.99
☐	☐	☐	70	-1.10; 11.53 0.42	-0.42; 6.76 0.54	-0.64; 7.31 0.98	0.00; 5.53 0.69	-0.83; 5.49 0.05
				-1.81; 9.90	-0.81; 12.86	-1.96; 11.60	-2.19; 4.37	-1.53; 10.10
FEV ₁ ↓	ACT<16							
☒	☒		15	4.36	6.52	7.31	4.79	3.89
☐	☒		24	-0.10; 11.74 1.11	0.00; 24.22 1.60	0.83; 18.04 2.22	-0.65; 20.67 1.07	-0.60; 12.09 0.81
☒	☐		41	-0.81; 7.53 2.39	-0.62; 12.86 3.35	-1.91; 16.65 3.30	-1.95; 4.45 2.54	-1.12; 10.10 2.55
☐	☐		116	-1.10; 15.44 0.35	-0.42; 32.15 0.99	-4.29; 26.05 0.95	0.00; 24.11 0.97	-0.83; 15.92 0.31
				-2.14; 10.28	-1.59; 16.05	-2.87; 12.74	-2.19; 17.61	-1.58; 12.47

Ticked boxes denote presence. EOS<4%, blood eosinophil counts >4.0%. FEV₁↓ denotes FEV₁ below LLN, lower limit of normal.

Table E20. Multiple group comparisons of FDR (z) outcome. Groups defined with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively.

			FEV ₁ <LLN	+	+	+	-	-	+	-	-
			EOS>4.0%	+	+	-	+	-	-	+	-
			Ever smoker	+	-	+	+	+	-	-	-
FEV ₁ <LLN	EOS>4.0%	Ever smoker		n=8	n=20	n=10	n=16	n=26	n=18	n=28	n=70
+	+	+		-							
+	+	-		<i>n.s.</i>	-						
+	-	+		<i>n.s.</i>	<i>n.s.</i>	-					
-	+	+		0.004	0.025	0.003	-				
-	-	+		0.050	<i>n.s.</i>	0.041	<i>n.s.</i>	-			
+	-	-		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-		
-	+	-		0.003	0.011	0.002	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-	
-	-	-		0.000	0.000	0.000	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-

P-values for group differences are given. Kruskal-Wallis test: H (7, N= 196) =56.09, p<.0001 for overall differences among group. EOS<4%, blood eosinophil counts >4.0%. LLN, lower limit of normal.

Table E21. Multiple group comparisons of LCI (z) outcome. Groups defined with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively.

			FEV ₁ <LLN	+	+	+	-	-	+	-	-
			EOS>4.0%	+	+	-	+	-	-	+	-
			Ever smoker	+	-	+	+	+	-	-	-
FEV ₁ <LLN	EOS>4.0%	Ever smoker		n=8	n=20	n=10	n=16	n=26	n=18	n=28	n=70
+	+	+		-							
+	+	-		<i>n.s.</i>	-						
+	-	+		<i>n.s.</i>	<i>n.s.</i>	-					
-	+	+		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-				
-	-	+		0.012	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-			
+	-	-		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-		
-	+	-		0.007	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-	
-	-	-		0.000	0.000	0.001	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-

P-values for group differences are given. Kruskal-Wallis test: H (7, N= 196) =57.82, p<0.0001. EOS<4%, blood eosinophil counts >4.0%. LLN, lower limit of normal.

Table E22. Multiple group comparisons of Scond x VT (z) outcome. Groups defined with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively.

			FEV ₁ <LLN	+	+	+	-	-	+	-	-
			EOS>4.0%	+	+	-	+	-	-	+	-
			Ever smoker	+	-	+	+	+	-	-	-
FEV ₁ <LLN	EOS>4.0%	Ever smoker		n=8	n=20	n=10	n=16	n=26	n=18	n=28	n=70
+	+	+		-							
+	+	-		<i>n.s.</i>	-						
+	-	+		<i>n.s.</i>	<i>n.s.</i>	-					
-	+	+		<i>n.s.</i>	<i>n.s.</i>	0.040	-				
-	-	+		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-			
+	-	-		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-		
-	+	-		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-	
-	-	-		0.012	0.003	0.003	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-

P-values for group differences are given. Kruskal-Wallis test: H (7, N= 196) =36.71, p <0.0001. EOS<4%, blood eosinophil counts >4.0%. LLN, lower limit of normal.

Table E23. Multiple group comparisons of Sacin x VT (z) outcome. Groups defined with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively.

			FEV ₁ <LLN	+	+	+	-	-	+	-	-
			EOS>4.0%	+	+	-	+	-	-	+	-
			Ever smoker	+	-	+	+	+	-	-	-
FEV ₁ <LLN	EOS>4.0%	Ever smoker		n=8	n=20	n=10	n=16	n=26	n=18	n=28	n=70
+	+	+		-							
+	+	-		<i>n.s.</i>	-						
+	-	+		<i>n.s.</i>	<i>n.s.</i>	-					
-	+	+		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-				
-	-	+		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-			
+	-	-		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-		
-	+	-		0.006	0.002	0.003	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-	
-	-	-		0.000	0.000	0.000	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-

P-values for group differences are given. Kruskal-Wallis test: H (7, N= 196) =59.81 p <0.0001. EOS<4%, blood eosinophil counts >4.0%. LLN, lower limit of normal.

Table E24. Multiple group comparisons of SV-ratio VT (z) outcome. Groups defined with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively.

			FEV ₁ <LLN	+	+	+	-	-	+	-	-
			EOS>4.0%	+	+	-	+	-	-	+	-
			Ever smoker	+	-	+	+	+	-	-	-
FEV ₁ <LLN	EOS>4.0%	Ever smoker		n=8	n=20	n=10	n=16	n=26	n=18	n=28	n=70
+	+	+		-							
+	+	-		<i>n.s.</i>	-						
+	-	+		<i>n.s.</i>	<i>n.s.</i>	-					
-	+	+		0.027	<i>n.s.</i>	<i>n.s.</i>	-				
-	-	+		0.010	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-			
+	-	-		0.031	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-		
-	+	-		0.017	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-	
-	-	-		0.000	0.000	0.006	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-

P-values for group differences are given. Kruskal-Wallis test: H (7, N= 196) =49.37, p <0.0001. EOS<4%, blood eosinophil counts >4.0%. LLN, lower limit of normal.

Table E25. Multiple group comparisons of ACT score outcome. Groups defined with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively.

			FEV ₁ <LLN	+	+	+	-	-	+	-	-
			EOS>4.0%	+	+	-	+	-	-	+	-
			Ever smoker	+	-	+	+	+	-	-	-
FEV ₁ <LLN	EOS>4.0%	Ever smoker		n=8	n=20	n=10	n=16	n=26	n=18	n=28	n=70
+	+	+		-							
+	+	-		<i>n.s.</i>	-						
+	-	+		<i>n.s.</i>	<i>n.s.</i>	-					
-	+	+		0.027	<i>n.s.</i>	<i>n.s.</i>	-				
-	-	+		0.010	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-			
+	-	-		0.031	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-		
-	+	-		0.017	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-	
-	-	-		0.000	0.000	0.006	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	-

P-values for group differences are given. Kruskal-Wallis test: H (7, N= 196) =49.37, p <0.0001. EOS<4%, blood eosinophil counts >4.0%. LLN, lower limit of normal.

Table E26. Multiple group comparisons of age (years). Groups defined with respect to presence/absence of reduced FEV₁, blood eosinophil count >4.0%, and smoking history, respectively.

			FEV ₁ <LLN	+	+	+	-	-	+	-	-
			EOS>4.0%	+	+	-	+	-	-	+	-
			Ever smoker	+	-	+	+	+	-	-	-
FEV ₁ <LLN	EOS>4.0%	Ever smoker		n=8	n=20	n=10	n=16	n=26	n=18	n=28	n=70
+	+	+	-								
+	+	-	<i>n.s</i>		-						
+	-	+	<i>n.s</i>	<i>n.s</i>		-					
-	+	+	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>		-				
-	-	+	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>		-			
+	-	-	<i>n.s</i>	0.017	<i>n.s</i>	0.035	<i>n.s</i>		-		
-	+	-	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>		-	
-	-	-	<i>n.s</i>	0.021	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	-

P-values for group differences are given. Kruskal-Wallis test: H (7, N= 196) =26.50, p =0.0004 for overall differences among group

References

1. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
2. Hedenstrom H, Malmberg P, Agarwal K. Reference values for lung function tests in females. Regression equations with smoking variables. Bull Eur Physiopathol Respir. 1985;21(6):551-7.
3. Hedenstrom H, Malmberg P, Fridriksson HV. Reference values for lung function tests in men: regression equations with smoking variables. Ups J Med Sci. 1986;91(3):299-310.
4. Singer F, Houlitz B, Latzin P, Robinson P, Gustafsson P. A realistic validation study of a new nitrogen multiple-breath washout system. PLoS One. 2012;7(4):e36083.
5. Gustafsson PM, Robinson P, Gilljam M, Lindblad A, Houlitz BK. Slow and Fast Lung Compartments in Cystic Fibrosis Measured by Nitrogen Multiple-Breath Washout. Journal of applied physiology. 2014.