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Clinical characteristics of adult asthma associated with small airway dysfunction

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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 ($S_{cond} \times V_T$ and $S_{acin} \times V_T$; 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% ($S_{cond} \times V_T$) 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.

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].

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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 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 $FENO_{50}$ 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, Hoechberg, 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} x V_T and S_{acin} x 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 Chi^2-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 N_2 MBW variables. ANOVA was subsequently performed using the most closely related clinical characteristics in dichotomized form as categorising (independent) variables and IOS and N_2 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 beta$_2$-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 FEV_1 (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 FEV_1 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 (R^2 0.93; p < 0.001), which linearised using Sqrt (AX) (R^2 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 $S_{cond} \times V_T$ (z) were higher in males vs. females, while R20 (z) was lower. All IOS outcomes except R20, and all MBW variables except $S_{cond} \times V_T$ 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} x V_T (z-score, 2.91 vs. 0.97; p < 0.001) remained different vs. non-smokers, which was also seen with FEV_1/FVC (z-score, −1.48 vs. −0.63; p < 0.05).

FDR and MBW abnormalities were more frequently seen with abnormal FEV_1 (Figs. 1—6). A significant number of subjects had, however, detectable abnormalities missed by FEV_1, while the opposite was much less common. Similar patterns were shown for FEV_1/FVC ratio (data not shown).

FDR was abnormal in 31%, LCI in 44%, $S_{cond} \times V_T$ in 47%, $S_{acin} \times V_T$ 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 $S_{cond} \times V_T$;

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 Chi^2-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.
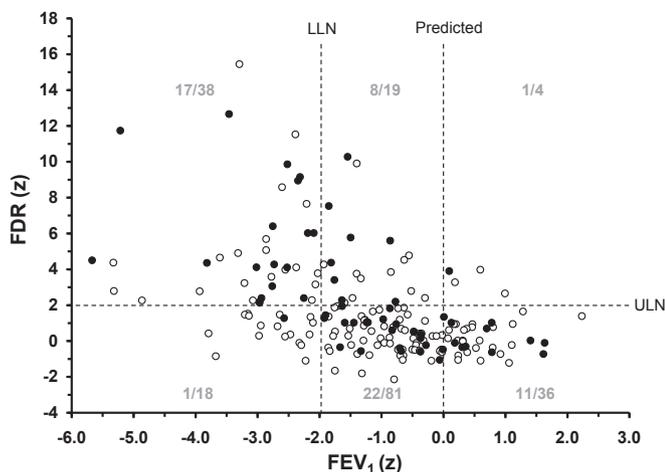

Fig. 1. FDR (z) *vs.* FEV$_1$ (z) in 196 adults subjects with asthma. Open circles denote never-smokers (n = 136) and closed circles denote ever-smokers (n = 60). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for FDR. LLN, lower limit of normal for FEV$_1$. Predicted, predicted value for FEV$_1$.

p < 0.05 *vs.* S$_{acin}$ × 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 N$_2$ 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$_{acin}$ × V$_T$ (p < 0.001), but not S$_{cond}$ × 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).

FEV$_1$ (z), FEV$_1$/FVC (z), and R20 (z) showed close to normal distributions (Figures E17-E19, OLS). In contrast, R5 (z), FDR (z) and z-scores of N$_2$ 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. FEV$_1$ 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$_{cond}$ × 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$_{acin}$ × V$_T$ than with other indices, and not at all with S$_{cond}$ × 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$_{cond}$ × V$_T$ (z) (Table 5). Reduced FEV$_1$ 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$_{acin}$ × V$_T$ (z), while ACT or FENO did not contribute. In addition to reduced FEV$_1$, only ACT<16 was a significant contributor to S$_{cond}$ × 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 N_2 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} 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)
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)***
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} 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)
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)***
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.
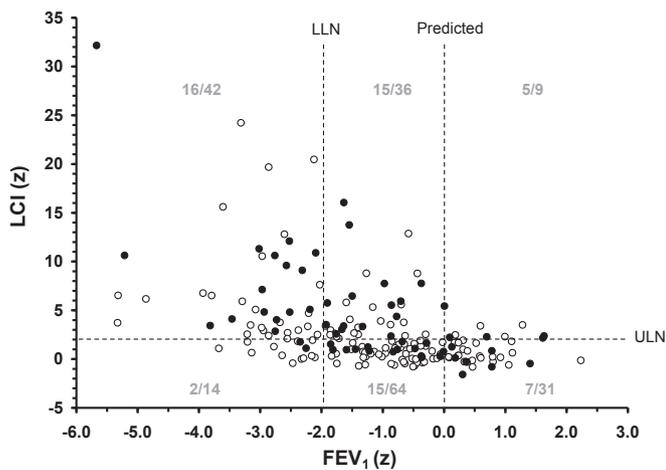

Fig. 2. LCI (z) *vs.* FEV$_1$ (z) in 196 adults subjects with asthma. Open circles denote never-smokers (n = 136) and closed circles denote ever-smokers (n = 60). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for LCI. LLN, lower limit of normal for FEV$_1$. Predicted, predicted value for FEV$_1$.
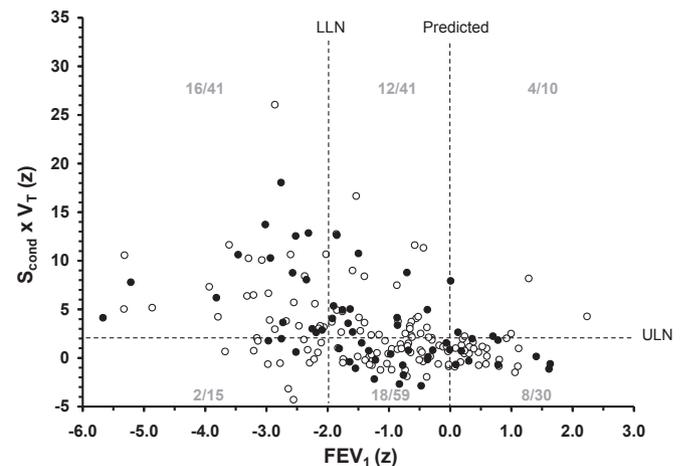

Fig. 3. S_{cond} x V_T (z) *vs.* FEV$_1$ (z) in 196 adults subjects with asthma. Open circles denote never-smokers (n = 136) and closed circles denote ever-smokers (n = 60). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for S_{cond} x V_T. LLN, lower limit of normal for FEV$_1$. Predicted, predicted value for FEV$_1$.

comparisons for the different outcomes are given in Table E20-26 (OLS). The combination of reduced FEV$_1$ 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 FEV$_1$ and ACT<16.

Seventy subjects (36%) were absent of the three most important risk factors (reduced FEV$_1$, 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% ($S_{cond} \times V_T$), and all indices were within normal range in 36/70 subjects (51%). Among the 116 subjects with normal FEV$_1$ and ACT $\geq$ 16 the presence of abnormal peripheral airway function indices ranged from 15% (FDR) to 30% ($S_{cond} \times V_T$), while FDR and MBW indices were all normal in 46%.

Among the 56 subjects with reduced FEV$_1$, 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), $S_{cond} \times V_T$ (z) (6.28 *vs.* 2.55; p < 0.01), $S_{acin} \times V_T$ (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 N_2 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 FEV$_1$ in combination with a positive
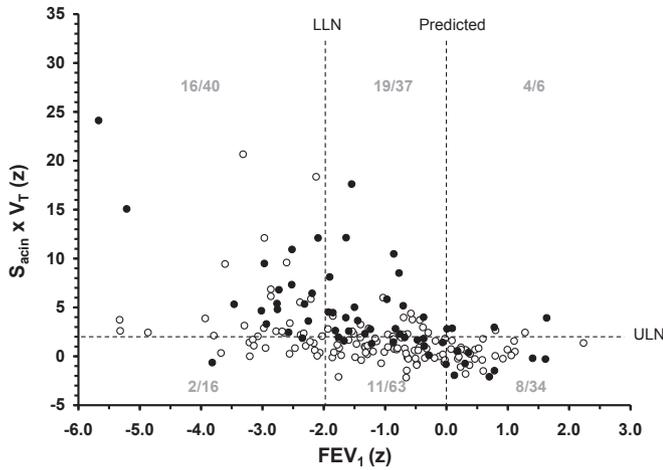
Fig. 4. $S_{acin} \times V_T$ (z) vs. FEV_1 (z) in 196 adults subjects with asthma. Open circles denote never-smokers (n = 136) and closed circles denote ever-smokers (n = 60). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for $S_{acin} \times V_T$. LLN, lower limit of normal for FEV_1. Predicted, predicted value for FEV_1.
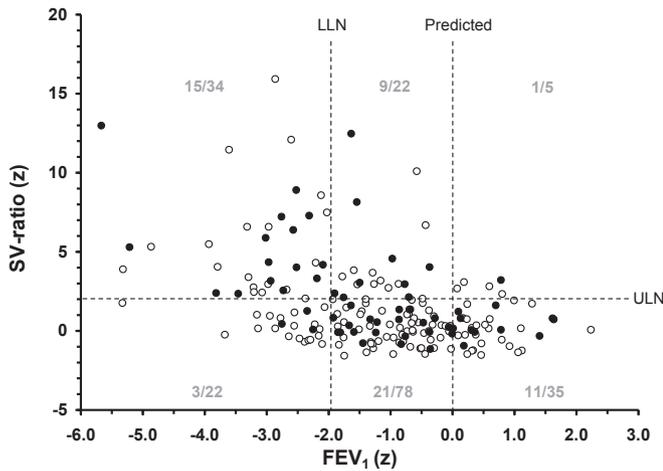

Fig. 5. SV-ratio (z) vs. FEV_1 (z) in 196 adults subjects with asthma. Open circles denote never-smokers (n = 136) and closed circles denote ever-smokers (n = 60). Proportions of ever-smokers in each defined rectangle are given. ULN, upper limit of normal for SV-ratio. LLN, lower limit of normal for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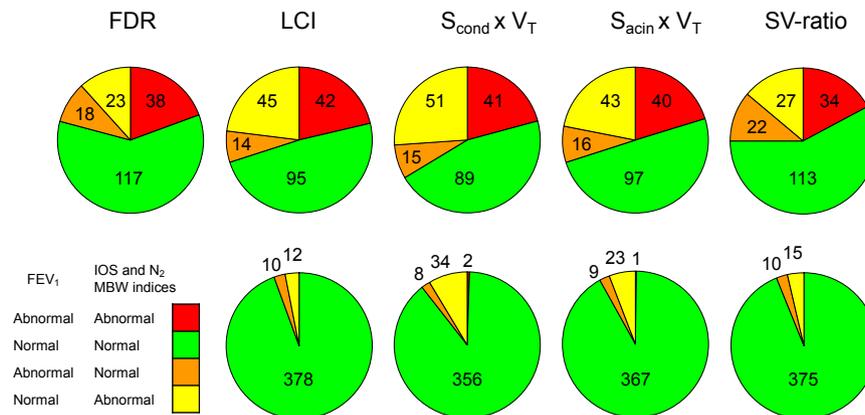

Fig. 6. Number of subjects in asthma cohort (upper panel) and in N_2 MBW healthy control cohort (lower panel) with different outcomes at spirometry, IOS or N_2 MBW. (FDR, frequency dependence of resistance, (R5-R20). LCI. lung clearance index. SV-ratio, specific ventilation ratio).

Table 4A
Correlation matrix for full asthma cohort.

Variable	FDR	LCI	$S_{cond} \times V_T$ (z)	$S_{acin} \times V_T$ (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_{cond} x V_T (z)	S_{acin} x 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_{cond} x V_T (z)	S_{acin} x 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 5
ANOVA outcomes in the asthma cohort as a whole for z-scores of IOS and N_2 MBW variables using a model including five dichotomized independent variables.

Independent variables	Dependent variables				
	FDR (z)	LCI (z)	S_{cond} x V_T (z)	S_{acin} x 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 FEV_1 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 FEV_1 (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.

FEV_1 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.

$FEV_1\downarrow$	Ever-smoker	B-eos >4.0%	FDR +	LCI +	S_{cond} x V_T +	S_{acin} x 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

$FEV_1\downarrow$	Ever-smoker and/or B-eos >4.0%		FDR +	LCI +	S_{cond} x V_T +	S_{acin} x V_T +	SV-ratio +	FDR and all N_2 MBW +	All negative
✓	✓		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

$FEV_1\downarrow$	ACT<16		FDR +	LCI +	S_{cond} x V_T +	S_{acin} x V_T +	SV-ratio +	FDR and all N_2 MBW +	All negative
✓	✓		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} x V_T was more closely associated than S_{cond} x 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
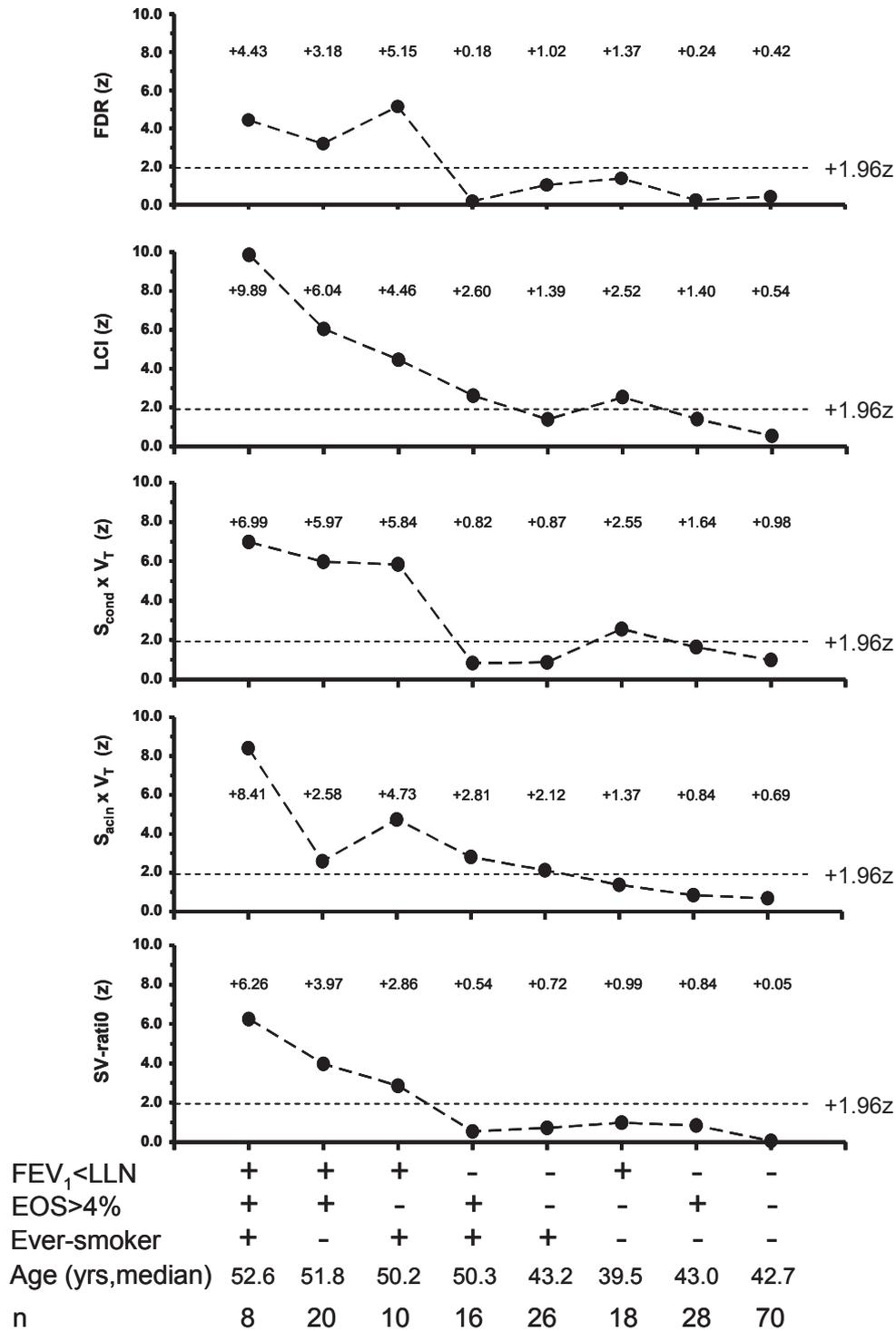
Fig. 7. Peripheral airway function outcomes given as z-scores of medians (filled symbols and numerically). Subgroups defined with respect to presence/absence of reduced FEV_1, EOS >4.0%, and smoking history, respectively, are indicated below plots. EOS>4%, blood eosinophil counts >4.0%. LLN, lower limit 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 N_2 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 N_2 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 FEV_1 would remain abnormal with an FEV_1/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 FEV_1, 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.

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Conflict of interest

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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.

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