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Utility of single versus multiple breath washout in adult asthma

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Summary

Nitrogen multiple breath washout (N_2 MBW) is a sensitive method to identify peripheral airway involvement in asthma, but is a time-consuming test. The N_2 vital capacity single breath (VC SBW) test offers greater time efficiency, but concordance with N_2 MBW is poorly understood. The prevalence of peripheral airway abnormality was determined by N_2 MBW and N_2 SBW tests in 194 asthmatic subjects aged 18–1 years. N_2 MBW data were related to findings in 400 healthy controls, aged 17–71 years, while N_2 SBW data were compared to findings in 224 healthy controls, aged 15–65 years, to derive equipment-specific reference values. Amongst asthmatic subjects, relationships between N_2 SBW and N_2 MBW outcomes were studied. N_2 SBW relationship with clinical history, spirometry, blood eosinophils and fraction exhaled nitric oxide (FENO) data was also explored. The prevalence of peripheral airway involvement (i.e. abnormal ventilation distribution) determined by N_2 SBW-derived phase III slope (N_2 S_{III}) was 24·7%, compared to 44% determined by N_2 MBW-derived lung clearance index (LCI) ($P<0{\cdot}001$). Predictors of abnormal N_2 S_{III} were older age, smoking history and lower FEV_1. N_2 SBW offers lower sensitivity than N_2 MBW to detect small airway dysfunction in adult asthma, but may be a marker of more severe disease.

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Introduction

The importance of the peripheral (or small) airways in the pathogenesis of asthma and their role as the major determinant of airflow obstruction and disease expression was highlighted in a recent manifesto, published by worldwide asthma/allergy organizations (Braido *et al.*, 2016). The challenge of detecting abnormality in this 'silent lung zone' with spirometry is well recognized (Woolcock *et al.*, 1969; McNamara *et al.*, 1987; Macklem, 1998). A recent literature review estimated that the incidence of peripheral airway involvement, examining data across a range of lung function and imaging techniques was up to 60% in adult asthma (Usmani *et al.*, 2016). Recently, we reported a high prevalence of peripheral airway dysfunction in 42%–47% of adult asthma subjects, recruited from primary care, across multiple breath washout (MBW) indices, using widely available commercial MBW equipment and using equipment-specific reference data generated from a large healthy control group (Kjellberg *et al.*, 2016). Furthermore, we observed that a positive smoking history and/or raised blood eosinophil count was highly predictive of this MBW-detected peripheral airway involvement in asthmatic subjects with reduced FEV_1.

Multiple breath washout offers important insight into the unevenness of gas mixing (ventilation inhomogeneity) within the lung, but is a time-consuming test. Nitrogen vital capacity single breath washout (N_2 VC SBW) also reflects global ventilation inhomogeneity (Robinson *et al.*, 2013) and offers strong feasibility within a shorter testing time in adult subjects. The introduction of the N_2 emission spectrophotometer, that is the fast responding N_2 analyser, in 1946 allowed for continuous recording of N_2 concentration over the course of a single or multiple breaths (Lilly, 1946). WS Fowler described the use of the VC SBW technique to assess ventilation distribution in the late 1940s (Fowler, 1949), based on the steepness of the expirogram-derived phase III slope (S_{III}). It can identify tobacco smokers at risk of developing chronic obstructive pulmonary disease (COPD) (Macklem, 1972) and has been validated against histopathological proven structural peripheral airway abnormalities in the human lung (Cosio *et al.*, 1978), and remains the only ventilation inhomogeneity marker to be validated in this way. Specific to adult asthma, S_{III} steepness

correlates with disease severity as determined by asthma exacerbation frequency (Bourdin *et al.*, 2006). The N_2 VC SBW test remains in use in many laboratories today (Mikamo *et al.*, 2013; Boeck *et al.*, 2016; Di Marco *et al.*, 2017), mainly because of its time-effectiveness, yet the correlation between N_2 MBW and N_2 SBW remains largely unknown, and remains an important gap in current knowledge for clinicians interested in inert gas washout techniques.

The recent ERS/ATS inert gas washout consensus statement provided essential guidance to manufacturers on both VC SBW and MBW testing (Robinson *et al.*, 2013) and has led to the emergence of validated commercial equipment suitable for widespread use (e.g. Exhalyzer® D, Eco Medics AG, Dürnten, Switzerland). The N_2 VC SBW test involves a slow maximal inspiration of 100% oxygen (O_2), followed by a slow expiration to residual volume (RV). Each trial takes 1–2 mins to perform. In a busy asthma clinic, the N_2 VC SBW test therefore offers a more feasible tool compared to N_2 MBW test, which typically takes 5–10 mins and requires a greater period between tests for N_2 levels to re-equilibrate within the lung. N_2 VC SBW tests were performed within testing as part of our recent adult asthma cohort assessment. Due to the lack of robust equipment-specific reference data to clearly describe the degree of abnormality detected, we subsequently recruited a large healthy adult control group to describe the degree of abnormality detected.

The primary aim of this study was to assess the prevalence of abnormal ventilation inhomogeneity in an adult asthma population recruited from primary care as determined by the N_2 VC SBW test, in comparison with MBW-derived indices. The secondary aim was to identify clinical characteristics predictive of abnormal N_2 S_{III}.

Methods

Participants

A cohort of 196 (109 females, 56%) subjects with physician-diagnosed asthma aged 18–60 years was recruited from primary care centres across East Skaraborg County in West Sweden. Exclusion criteria were unstable asthma, presence of cardiac disease, hypertension, diabetes or other systemic disease, or current medication affecting response to bronchodilator therapy (e.g. beta-blocker therapy). The study was performed in the Respiratory Research Laboratory, at Skaraborg Hospital Skövde, Sweden, from May 2011 to June 2014 and was approved by the Regional Ethics Committee at the University of Gothenburg, Sweden. All participants gave written consent prior to examination.

A control group of 224 subjects (108 males, 116 females) aged 15–65 years was recruited from the Swedish population register to derive reference equations for N_2 VC SBW S_{III}. Inclusion criteria were no known respiratory disease, no reported history of chronic or recurrent respiratory symptoms and a smoking history of less than five pack-years. Testing occurred across two respiratory function laboratories (Skaraborg Hospital Skövde and University of Gothenburg, Sweden) using identical equipment and testing protocols. The control subjects were tested from March 2011 to December 2016. Ethics approval was granted by the Regional Ethics Committee at the University of Gothenburg, Sweden, and all participants gave written consent prior to examination.

Lung function tests

The N_2 VC SBW test was performed using the Exhalyzer® D (Eco Medics AG). Three technically acceptable recordings were targeted. The test manoeuvre included a full expiration to RV, followed by a slow inhalation of 100% O_2 to total lung capacity (TLC), and subsequently slow exhalation from TLC to RV. The VC breath was measured at a constant slow respiratory flow of approximately 500 ml s^{-1}, facilitated by the use of a resistor. Test acceptability was defined by a coefficient of variation (CV %) of expiratory VC less than 10%. The N_2 S_{III} was measured by linear regression between 25% and 75% of exhaled volume visually avoiding the influence of cardiogenic oscillations, phase II (bronchial phase) and/or IV (start of airway closures).

Multiple breath washout was performed according to the recommendations of the ERS/ATS inert gas washout consensus statement, targeting three technically acceptable trials, and using a free breathing tidal breathing protocol. Outcomes calculated were lung clearance index (LCI), functional residual capacity (FRC) and concentration normalized phase III slope (Sn_{III}) indices S_{cond} and S_{acin} (both corrected for expired tidal volume, i.e. S_{cond} x V_T and S_{acin} x V_T) (Robinson *et al.*, 2013). Data were analysed offline using in-house software based on a commercial software package (TestPoint®, Capital Equipment; Billerica, MA, USA), also used for analyses of previous N_2 MBW data (Kjellberg *et al.*, 2016). This software served as the template for the commercial Spiroware software used with Exhalyzer® D. A healthy cohort of 400 subjects aged 17–71 years, recruited via the Swedish population register generated reference data for N_2 MBW outcomes, for further details see online supplement (OLS) to reference (Kjellberg *et al.*, 2016).

Spirometry was performed using a Jaeger Masterscreen system (CareFusion, Würzburg, Germany) in accordance with published ERS and ATS standards (Miller *et al.*, 2005), and expressed in z-scores using Swedish reference equations (Hedenstrom *et al.*, 1985, 1986). All lung function testing of asthmatic subjects was undertaken after withholding asthma medication for at least 12 h. Fraction exhaled nitric oxide (FENO) measurement was performed in triplicate at 50 ml s^{-1} (FENO50) using an online chemiluminescence analyser (Eco Medics AG, Dürnten, Switzerland). FENO50 abnormality was defined as a value >25 ppb.

All asthmatic subjects completed the questionnaire Asthma Control Test (ACT) (Schatz *et al.*, 2006)

Statistics

Statistical analysis was performed using SPSS (IBM SPSS Statistics 24). Predictors of N_2 S_{III} in healthy controls were identified by bivariate correlation analyses. Student's t-test was used to disclose any gender differences in measured N_2 S_{III}. The reference equation for N_2 S_{III} was subsequently identified by multiple linear regression. Z-scores for SBW and MBW outcomes in asthmatics were calculated as the difference between measured value and predicted value divided by residual standard deviation (RSD) in the healthy control group. Upper limit and lower limit of normal (ULN and LLN) were defined as predicted value > +1·96 z-score and < −1·96 z-score, respectively. Parametric and nonparametric data were compared using Student's t-tests and Mann–Whitney U-tests, respectively, whilst between-group comparison of proportions was performed using chi-square tests (with Yates' continuity correction). Spearman rank correlation coefficient (r_s) was used for expression of strength and direction of linear relationships. Hierarchical multiple regression analyses were used to identify the explanation value of a predictor for N_2 S_{III} with the ability to control for another predictor. A two-tailed P-value < 0·05 was considered statistically significant.

Results

Demographic data for both healthy control and asthma cohorts are summarized in Table 1. Spirometry data were rejected for one healthy subject due to technical reasons.

N_2 VC SBW in healthy CONTROLS

Bivariate correlation analysis with N_2 S_{III} as dependent variable and age, height, weight and BMI (body mass index) as independent variables showed a statistically significant correlation between N_2 S_{III} and age (r = 0·38, P<0·001) and between N_2 S_{III} and height (r = −0·31, P<0·001). Student's t-test disclosed a significant difference in N_2 S_{III} between females (mean (SD) 1·11 (0·38)) and males (0·94 (0·35), P<0·001). Consequently, these three anthropometric variables were included in a multiple regression analysis model, to identify predictors for N_2 S_{III}. The multiple regression analysis with N_2 S_{III} as dependent variable and gender, age and height as independent variables gave an overall adjusted R^2 of 0·193 (F = 18·82, P<0·001), with significant contributions of only age (unstandardized beta 0·007, P<0·001) and height (unstandardized beta −0·008, P = 0·033). Age and height were used as independent variables in a new multiple regression model resulting in a model with an adjusted R^2 of 0·194 (F = 27·86, P<0·001) and unstandardized beta for age 0·007 (P<0·001) and unstandardized beta for height −0·010 (P<0·001). From this model, a regression equation predicting N_2 S_{III} was calculated;

$$N_2\ S_{III} = 2{\cdot}489 + 0{\cdot}007 \times \text{age} - 0{\cdot}010 \times \text{height}.$$

The residual standard deviation (RSD) for the healthy control group was 0·3325.

Prevalence of ventilation inhomogeneity determined by N_2 S_{III}

One hundred and ninety-four of 196 (99·0%) asthmatic subjects performed acceptable SBW recordings (defined as at least two technically acceptable tests). Standard deviation (SD) of N_2 S_{III} measurements was < 0·50%/l for these subjects. Forty-eight of 194 subjects (24·7%) had N_2 S_{III} values > +1·96 z-score. The differences between the characteristics of asthmatics with abnormal and normal N_2 S_{III} values are summarized in Table 2: abnormal N_2 S_{III} subjects were significantly older, had higher FENO50 values, greater abnormality across all spirometry and N_2 MBW outcomes, higher rates of smoking and a slightly lower median ACT score. Abnormal N_2 S_{III} was, however, not associated with gender or asthma control (i.e.

Table 1 Demographic and spirometry data of the healthy control population and asthmatic subjects, respectively.

	Healthy controls		Asthmatic subjects	
	Females, *n* = 115	Males, *n* = 108	Females, *n* = 107	Males, *n* = 87
Age (years)	40·2 (17·0)	38·3 (17·9)	41·5 (11·3)	44·0 (10·7)
Height (cm)	167·8 (6·3)	180·9 (6·4) ***	167·3 (7·0)	176·6 (7·1) ***
Weight (kg)	68·2 (11·4)	80·2 (12·8) ***	74·0 (16·9)	86·3 (14·5) ***
BMI (kg cm^{-2})	24·3 (4·1)	24·5 (3·6)	26·4 (5·9)	26·7 (3·9)
FVC z-score	−0·45 (1·58)	−0·23 (1·03)	−0·48 (1·16)	−0·69 (1·52)
FEV_1 z-score	−0·35 (1·37)	−0·08 (0·75)	−1·02 (1·25)	−1·45 (1·60)*
FEV_1/FVC z-score	0·04 (1·36)	0·26 (0·82)	−0·89 (1·15)	−1·09 (1·48)
MEF50 z-score	−0·77 (0·94)	−0·07 0·92) ***	−1·74 (0·99)	−1·39 (1·15)*
MEF25 z-score	−0·52 (1·70)	−0·29 (0·86)	−1·32 (0·97)	−1·55 (0·94)

BMI, body mass index; FVC, forced expiratory volume; FEV_1, forced expiratory volume in 1 s; MEF_{50}, maximal expiratory flow at 50% of expired volume; MEF_{25}, maximal expiratory flow when 25% of FVC remains. Data displayed as mean (SD), Student's t-test was used for comparison between males and females.
*P<0·05, ***P<0·001.

higher rates of poorly or insufficiently controlled asthma on ACT). Median values of percentage blood eosinophils were higher in those with abnormal N_2 S_{III}, but rates of blood eosinophilia (> 4·0%) were not higher among subjects with abnormal N_2 S_{III}.

The proportion of subjects with abnormal N_2 S_{III} values was higher in those with abnormal vs. normal FEV_1: 35/56 (62·5%) versus 13/138 (9·4%), P<0·001, respectively. A significant correlation between N_2 S_{III} and FEV_1 z-scores was found ($r_s = -0·59$, P<0·001). The same pattern was also found between N_2 S_{III} based on FEV_1/FVC abnormality: 22/44 (50·0%) versus 26/150 (17·3%), P<0·001, and $r_s = -0·41$, P<0·001).

Relationship between MBW indices and N_2 S_{III}

All N_2 MBW indices were abnormal in a greater proportion of asthmatics compared to N_2 S_{III} (Table 3). These findings indicate that MBW had the best sensitivity for detecting peripheral airway involvement in adult asthma subjects. The strongest correlation with N_2 S_{III} (z-score) of MBW indices was found with S_{acin} x V_T (z-score, $r_s = 0·60$, P<0·001), followed by LCI (z-score, $r_s = 0·49$, P<0·001), Figures 1–2. Correlation with S_{cond} x V_T was statistically significant but weaker (z-score, $r_s = 0·28$, P<0·001, data not shown). Figure 3 shows the presence/absence of abnormal N_2 S_{III} in relation to presence/absence of abnormal N_2 MBW indices. N_2 S_{III} was normal in approximately half of the subjects with pathological LCI, S_{acin} or S_{cond} values, and abnormal in only 8%–14% of subjects with normal N_2 MBW indices.

Predictors of raised N_2 S_{III}

To identify predictors for N_2 S_{III}, a bivariate correlation matrix was constructed with N_2 S_{III} (z-score) as dependent variable and background data potentially affecting N_2 S_{III}, as independent variables (i.e. gender, weight, BMI, FENO50, smoking history, blood eosinophil counts, ACT score and spirometry results). The first matrix included all asthmatic subjects whilst the other included only asthmatic subjects with an abnormal N_2 S_{III}. Across the entire asthma cohort, the strongest predictors of abnormal N_2 S_{III} were ACT score ($r_s = -0·228$, P<0·01), blood eosinophil counts ($r_s = 0·203$, P<0·01) smoking pack-years ($r_s = 0·211$, P<0·01) and spirometry (FEV_1 (z-score, $r_s = -0·586$, P<0·01), FVC (z-score, $r_s = -0·417$, P<0·01), FEV_1/FVC (z-score $r_s = -0·411$, P<0·01)). By contrast, in those asthmatic subjects with an abnormal N_2 S_{III}, the strongest predictor was smoking history (pack-years, $r_s = 0·406$, ever smoker, $r_s = 0·401$, both P<0·01). A hierarchical multiple regression was performed to assess the ability

Table 2 Characteristics of asthmatic subjects with normal and abnormal N_2 S_{III} values.

Variables	Normal N_2 S_{III} (%/l)	Abnormal N_2 S_{III} (%/l)	*P*-value
Number (male,%)	146 (65, 45%)	48 (22, 46%)	n.s.
Age (years)	42·8 (19·4; 61·3)	48·1 (18·3; 60·7)	0·001
N_2 S_{III} (%/l)	1·0 (0·4; 1·7)	2·5 (1·7; 11·2)	<0·001
N_2 S_{III} (z-score)	−0·04 (−1·96; 1·88)	3·93 (1·99; 31·09)	<0·001
FeNO50 (ppb)	16 (3;141)	22 (2; 190)	0·032
FeNO50 > 25 ppb	37/146 (25%)	22/48 (46%)	0·013
FEV_1 (z)	−0·66 (−3·82; 2·23)	−2·52 (−5·67; −0·43)	<0·001
FVC (z)	−0·11 (−3·34; 3·15)	−1·40 (−5·12; 0·85)	<0·001
FEV_1/FVC (z)	−0·54 (−4·23; 1·96)	−1·94 (−6·16; 1·02)	<0·001
MEF_{50} (z)	−1·36 (−3·73; 2·01)	−2·41 (−3·85; −10·2)	<0·001
MEF_{25} (z)	−1·27 (−3·60;1·90)	−1·76 (−4·55; 0·60)	<0·001
LCI	7·56 (6·36; 15·73)	9·29 (6·61; 20·65)	<0·001
S_{cond} x V_T	0·020 (0·003; 0·120)	0·040 (0·010; 0·100)	<0·001
S_{acin} x V_T	0·080 (0·020; 0·440)	0·150 (0·050; 0·550)	<0·001
LCI (z)	1·08 (−1·59; 19·68)	4·75 (−0·70; 32·15)	<0·001
S_{cond} x V_T (z)	1·04 (−4·29; 26·05)	3·77 (−1·25; 18·04)	<0·001
S_{acin} x V_T (z)	0·93 (−2·19; 17·61)	4·56 (−0·40; 24·11)	<0·001
Ever smokers	38/146 (26%)	22/48 (46%)	0·020
Smoking history ≥5 pack-yrs	17/146 (12%)	16/48 (33%)	0·001
ACT score	21 (8; 25)	20 (6; 25)	0·025
ACT score <16	26/146 (17·8%)	12/48 (25·0%)	n.s.
ACT score <20	53/146 (36·3%)	22/48 (45·8%)	n.s.
ACT score ≥20	79/146 (54·1%)	26/48 (54·2%)	n.s.
Blood eosinophils (%)	2·5 (0·0; 12·5)	3·7 (0·91; 26·7)	0·011
Blood eosinophils >4·0%	49/145 (34%)	23/48 (48%)	n.s.

FeNO50, fraction exhaled nitric oxide at an expiratory flow of 50 ml s^{-1}. FEV_1, forced expiratory volume in 1 s. FVC, forced expiratory volume. MEF_{50}, maximal expiratory flow at 50% of expired volume. MEF_{25}, maximal expiratory flow when 25% of FVC remains. LCI, lung clearance index. ACT, asthma control test. z, z-score. Median (range) or proportions are given. *P*-values refer to Mann–Whitney U-tests or chi-square tests.

Table 3 Proportion of lung function abnormality within adult asthma subjects.

Variables	Number	Percentage
S_{cond} x V_T (%)	91	46·9***
LCI	86	44·3***
S_{acin} x V_T (%)	81	41·8***
MEF_{50} (l s^{-1})	79	40·7 **
FEV_1 (l)	56	28·9
MEF_{25} (l s^{-1})	51	26·3
N_2 S_{III} (% N_2/l)	48	24·7
FEV_1/FVC	43	22·2
FVC (L)	23	11·9

Data arranged in descending order of abnormality. LCI, lung clearance index. FEV_1, forced expiratory volume in 1 s. FVC, forced expiratory volume. MEF_{50}, maximal expiratory flow at 50% of expired volume. MEF_{25}, maximal expiratory flow when 25% of FVC remains. Statistical tests refer to comparison with the rate of abnormal N_2 S_{III} results. **P<0·01; *** P<0·001, chi-square test.

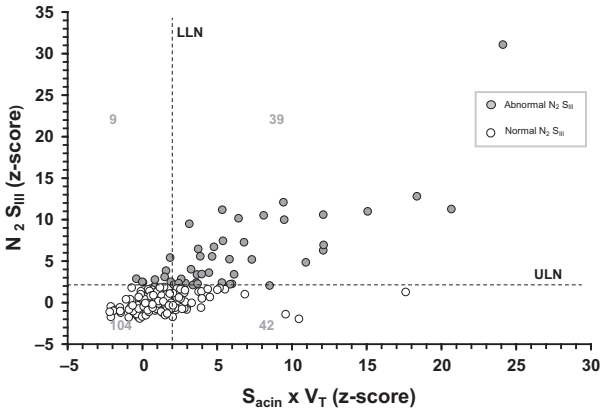

Figure 1 N_2 S_{III} (z-score) versus S_{acin} x V_T (z-score) in 194 asthmatic subjects. A Number of subjects in each quadrant are given. Spearman rho 0·60, P<0·001. ULN, upper limit of normal. LLN, lower limit of normal.

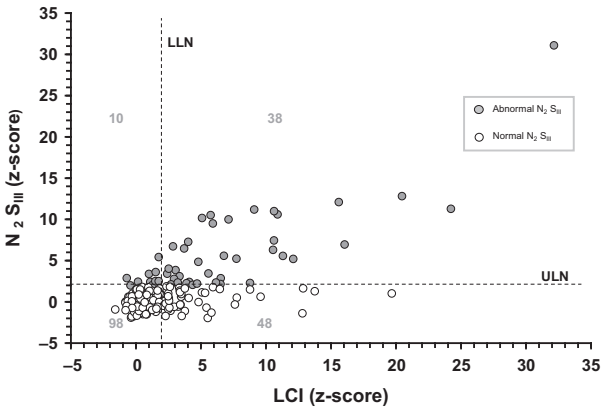

Figure 2 N_2 S_{III} (z-score) versus LCI (z-score) in 194 asthmatic subjects. A Number of subjects in each quadrant are given. Spearman rho 0·49, P<0·001. ULN, upper limit of normal. LLN, lower limit of normal.

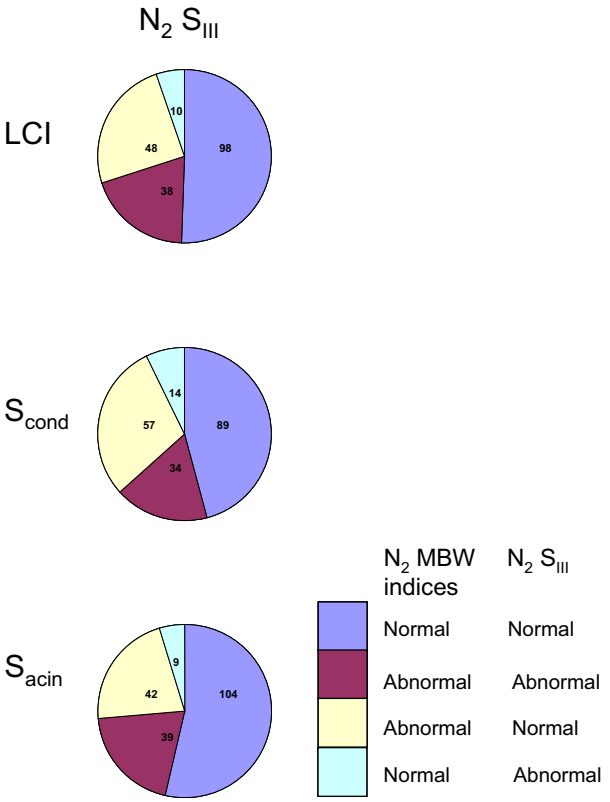

Figure 3 Concordance across N_2 MBW-derived indices and VC SBW S_{III} in asthma subjects.

of the independent variable pack-years smoked to predict N_2 S_{III} (z-score) after controlling for the independent variable (presence versus absence of smoking history). No additional explanation to N_2 S_{III} (z-score) was found in number of pack-years beyond the information given by a positive smoking history.

Discussion

This is the first study to report the prevalence of ventilation inhomogeneity measured by N_2 S_{III} from N_2 VC SBW in a representative cohort of adult asthmatics recruited from primary care, and the first study to formally compare N_2 VC SBW and MBW findings and sensitivity to detect lung involvement. The findings of this study are unique in the literature and provide significant guidance to interested clinicians about which test should be performed to detect peripheral airway involvement in adult asthmatic subjects. Overall, one-quarter (24·7%) of asthmatics had detectable abnormality on N_2 VC SBW. Abnormal N_2 S_{III} was associated with higher FENO50 levels, eosinophilic inflammation (defined as FENO50 > 25 ppb), and reduced lung function as assessed by spirometry and N_2 MBW indices. Tobacco smoking, in particular a history of five pack-years or more, was closely associated with abnormal N_2 S_{III}. This suggests that raised N_2 S_{III} is a marker of damage or inflammation in the periphery of the airway tree

due to tobacco smoke and/or ongoing eosinophilic inflammation. Our findings are in principal in concordance with the findings in a 38-year follow-up of 595 men (Olofson *et al.*, 2016) in which N_2 S_{III} was identified as a better predictor than FEV_1 of mortality, supporting the concept that N_2 S_{III} is a marker of lung ageing and lung disease severity. The prevalence of ventilation inhomogeneity detected by N_2 VC SBW test was markedly lower than that by N_2 MBW. Normal N_2 MBW indices were associated with non-uniform ventilation measured by the N_2 S_{III} in only 8%–14% of cases, suggesting that this test does not significantly improve detection of small airway dysfunction by N_2 MBW alone. N_2 MBW offered a markedly better assessment of peripheral airway involvement in these asthmatic subjects and should remain the inert gas washout method of choice.

Lower prevalence of abnormal N_2 VC SBW versus N_2 MBW indices

The relatively low prevalence of abnormal N_2 S_{III} does not seem to result from improved reference data, outlined in this manuscript, given the good agreement of our reference equations with selected previously published equations. Figure 4 shows the agreement between our reference equations with previously published equations for men.

Whilst there were variations in the exact methodology used (e.g. the portion of the expired volume S_{III} was measured over), there are several findings of note from these previous publications. Comroe and Fowler (1951) suggested an important influence of age by reporting greater slopes in those above age 50 years. Viegi reported gender to be the only predictor in subjects aged <64 years (Viegi *et al.*, 1988). Buist and Ross (1973) described age and gender to be predictors of N_2 S_{III} and also greater N_2 S_{III} values above the age of 60 years in females. N_2 S_{III} was not only markedly higher above this age, but data were more scattered too. This was mirrored in the findings of Sixt *et al.* (1984) and Roberts *et al.* (1991). Roberts *et al.* also suggested fixed values below age 60 years as an alternative approach to their regression equations. Dosman *et al.* (1981) additionally found weight to be a significant predictor of N_2 S_{III}. The importance of smoking was described by Hedenstrom *et al.* (1985, 1986) who reported the predictors for N_2 S_{III} to include for females age, weight and smoking pack-years and age x pack-years. For males in this cohort, predictors were the same, although height replaced weight.

The lower sensitivity of VC SBW versus MBW can be explained physiologically. Gas mixing in the lungs occurs by two mechanisms: convection and molecular diffusion, predominating within the conducting airways and within the gas exchange zone, respectively. Non-uniform ventilation distribution and gas mixing in the human lung, manifested by a raised S_{III}, occur due to convection-dependent inhomogeneity (cdi), or by interaction between convection and diffusion (i.e. diffusion–convection interaction-dependent inhomogeneity, dcdi) (Crawford *et al.*, 1985). The cdi has two components; gravitational cdi (mainly due to inter-regional differences in expansion among lung units along the pleural pressure gradient), and non-gravitational cdi due to structural differences among closely or widely separated lung units with different mechanical characteristics (compliance and resistance) (Olson & Rodarte, 1984; Wilson *et al.*, 1987). Measured S_{III} differences between VC SBW and MBW can result from different cdi and dcdi contributions. Gravity is predicted to affect N_2 S_{III} more than tidal gas mixing indices. Non-gravitational cdi and dcdi contribute to the S_{III} in VC SBW test too, but to a lesser degree than in MBW due to the greater expansion of the lung and longer breath time, diminishing the influence of differences in time constants between lung units, and increased time for diffusion to overcome concentration differences (Crawford *et al.*, 1986). Furthermore, apical lung regions with higher N_2 concentration will empty early during the expiration, flattering the N_2 S_{III}. Taken together these factors produce a less steep and less discriminative S_{III} with VC

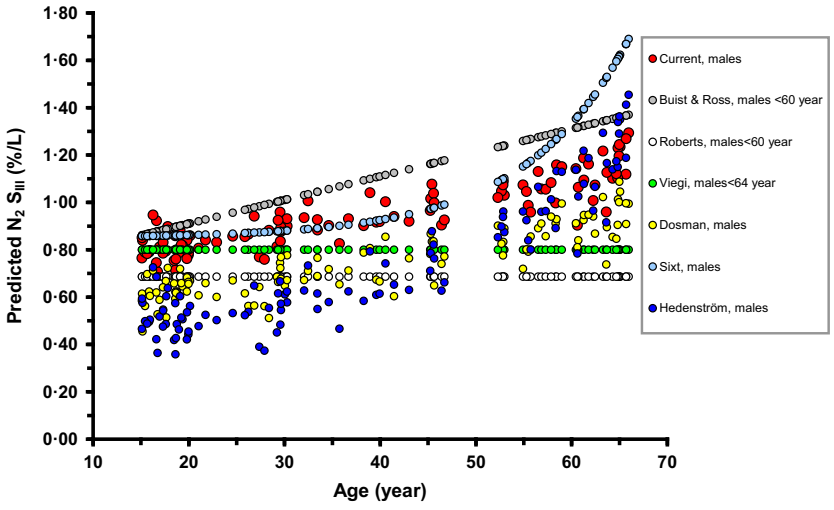

Figure 4 Predicted N_2 S_{III} for males using current and selected previously published reference equations. Age, height and weight from our healthy control group are applied when applicable.

SBW, compared to MBW, resulting in a less sensitive test outcome.

Strengths and weaknesses of the study

This asthma cohort is strongly representative of the adult asthma population managed by primary care, as evidenced by the good agreement of overall demography and clinical findings in our cohort with findings in a recent population-based asthma study of subjects aged 16–75 years living in West Sweden, including Skaraborg County (Lotvall *et al.*, 2009; Mincheva *et al.*, 2014). The study protocol ensured that both N_2 MBW and N_2 VC SBW were performed in the same asthma population, and that results were compared to collected normative values using identical equipment and the same technicians for both VC SBW and MBW (Kjellberg *et al.*, 2016). In this manuscript, our N_2 VC SBW reference equations showed good agreement with previously reported equations. We did not investigate the reversibility of any detected peripheral airway dysfunction in this asthma cohort with bronchodilator inhalation, nor the relative response observed across these gas mixing indices. How much of this abnormality is reversible needs to be addressed in future studies. We cannot relate the findings within this study to imaging or histological data and have assumed that N_2 MBW indices serve as good representations.

Summary and conclusions

In this large cohort, study of asthmatic adults recruited from primary care abnormal ventilation distribution as determined by N_2 VC SBW was detected in approximately 25% of the study population, and in less than half of those with abnormal N_2 MBW indices. Utility of N_2 VC SBW was less than that of N_2 MBW for detection of peripheral airway involvement in adult asthma, and N_2 VC SBW cannot replace MBW. We recommend that MBW is the optimal inert gas washout test for detection of peripheral airway involvement in adult asthma. Abnormal N_2 S_{III} was, however, linked to overall asthma severity as determined by overall lung function impairment and to past smoking history and ongoing eosinophilic airway inflammation, and possibly to disease progression. It may hold useful prognostic information, but further studies are required before recommendations about its clinical utility can be made.

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Conflict of interest

A-C Olin is share holder of PExA AB. The remaining authors have no conflict of interest.

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