Supplementary data

Ventilation heterogeneity is a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation

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Appendix 1 – Mathematical derivation of the MBNW indices S_{cond} and S_{acin}

S_{cond} represents the ventilation heterogeneity within the conducting airways, and S_{acin} represents ventilation heterogeneity within the acinar airways. Both indices are derived from the normalized N_2 slope curve, which is computed as follows. In each expiration of a MBNW test, the slope of the alveolar N_2 phase III was determined, and divided by the corresponding mean expired nitrogen concentration to obtain a normalised slope (Sn). The normalised slope of each breath was then plotted against the corresponding lung turnover (cumulative expired volume of that exhalation divided by the FRC). Figure 1 illustrates two such Sn curves, each one resulting from an averaging of Sn values collected from 3 MBW tests; the open symbols represent the average Sn plot of a normal subject and the closed symbols that of an asthma patient. It follows from theoretical considerations (1) that the Sn increase as a function of lung turnover over most of the Sn plot is essentially due to convection-related ventilation heterogeneity, and therefore S_{cond} is calculated as the regression slope between turnovers 1.5 and 6. The offset in the initial part of the Sn versus turnover plot is essentially due to diffusion-related ventilation heterogeneity, and S_{acin} is computed as the Sn value of the first breath minus the small contribution of the rate of Sn rise due to conductive airway ventilation heterogeneity (mean Sn of first breath - S_{cond} * mean lung turnover of 1st breath). For the Sn plots in figure E1, corresponding values are $S_{cond} = 0.02\ L^{-1}$ and $S_{acin} = 0.05\ L^{-1}$ for the normal subject and $S_{cond} = 0.152\ L^{-1}$ and $S_{acin} = 0.172\ L^{-1}$ for the asthma patient.

Figure E1: Example of normalised slopes of an asthmatic (•) versus normal (o) subject.

Appendix 2 – Description of healthy non-asthmatic subjects used in the Receiver Operator Characteristics (ROC) analysis for S_{cond}

17 healthy non-asthmatic subjects were recruited for the purpose of inclusion in the sensitivity and specificity (ROC) analysis undertaken for using S_{cond} as a predictor of AHR. The characteristics of these subjects are summarised in table E1.

Table E1: Baseline characteristics of the healthy non-asthmatic subjects included in the ROC sensitivity and specificity analysis. Values are means and (SD) unless otherwise stated.

	Healthy non-asthmatic subjects (n=17)
M/F	6/11
Age (years) and (range)	26.2 (19-40)
Atopic Y/N	3/14
S_{cond} (L^{-1})	0.019 (0.01)
S_{acin} (L^{-1})	0.094 (0.03)
LCI (CEV/FRC)	7.02 (0.6)
DRR (% fall FEV_1/µmol methacholine+3)*	3.34 (3.06 – 3.65)
FEV_1 (% predicted)	101.6 (12.9)
FEV_1/FVC ratio	0.87 (0.05)

* Geometric mean and 95% CI

Methods

In the healthy non-asthmatic subjects, a baseline MBNW test was performed as described in the methods of the manuscript. Following this, methacholine challenge was performed as described in the manuscript, and for this sensitivity and specificity analysis, response to the challenge was measured by the dose response ratio (DRR), calculated from the final dose as %fall FEV_1/methacholine dose (µmol) + 3. For the sensitivity and specificity analysis reported in the manuscript (n = 57), the results from the 17 healthy non-asthmatic subjects were combined with the 40 asthmatic subjects from the main study (Figure 2 in manuscript).

Results

Figure E2 - Sensitivity and specificity of S_{cond} in predicting the presence or absence of AHR following treatment in the 18 asthmatics from the treatment sub-group

Appendix 3 – Repeatability study of the MBNW indexes S_{cond} and S_{acin}

In a cohort of 10 asthmatic and 11 healthy non-asthmatic subjects, the repeatability of the MBNW indices of S_{cond}, S_{acin} and LCI were assessed. The eligibility criteria for these asthmatic and healthy non-asthmatic subjects were the same as described in the manuscript. The MBNW was performed on two separate days at approximately the same time of day, and the second day's visit was within 8 days of the first visit. The baseline characteristics of these subjects are described below in table E2. In the asthmatic subjects, a methacholine challenge was performed to assess AHR after the MBNW on one of the testing days. The methacholine challenge was performed as per outlined in the methods section of the manuscript.

Table E2: Baseline characteristics of the asthmatic and healthy non-asthmatic subjects included in the repeatability study. Values are means and SD unless otherwise stated

	Asthmatic subjects (n=10)	Healthy non-asthmatic subjects (n=11)	p value#
M/F	4/6	4/7	
Age in years (range)	30.5 (22-42)	37.6 (24-57)	0.1
% predicted FEV_1	85.2 (17.9)	102.6 (14.1)	0.02
AHR present/absent	8/2	-	
S_{cond} (L^{-1})	0.054 (0.03)	0.023 (0.01)	0.001
S_{acin} (L^{-1})	0.134 (0.04)	0.080 (0.02)	0.001
LCI (CEV/FRC)	8.2 (0.9)	6.6 (0.4)	<0.0001

p value is for differences between mean values in asthmatic versus healthy non-asthmatic subjects

Results

The intra-class correlaton coefficients (ICC) for the MBNW indices of S_{cond}, S_{acin} and LCI demonstrate a good repeatability of the indices, and the 95% limits of agreement for the variability of the indices are also reported (table E3).

Table E3: Repeatability and variability of the MBNW parameters S_{cond},S_{acin}, LCI and spirometry in 10 asthmatic and 11 healthy non-asthmatic subjects.

	Asthmatic subjects (n=10)	Healthy non-asthmatic subjects (n=11)
ICC for S_{cond}	0.84	0.80
ICC for S_{acin}	0.95	0.78
ICC for LCI	0.91	0.70
ICC for FEV_1	0.96	0.99
ICC for $FEF_{25-75\%}$	0.93	0.91
95% limits of agreement for S_{cond} (L^{-1})	±0.026	±0.011
95% limits of agreement for S_{acin} (L^{-1})	±0.027	±0.022
95% limits of agreement for LCI (CEV/FRC)	±0.735	±0.718
95% limits of agreement for FEV_1 (L)	±0.369	±0.240
95% limits of agreement for $FEF_{25-75\%}$ (L/s)	±0.662	±0.765

References

1. Crawford, A.B., Makowska, M., Paiva, M., and Engel, L.A. 1985. Convection- and diffusion-dependent ventilation maldistribution in normal subjects. *Journal of Applied Physiology* 59:838-846.