Ventilation inhomogeneity in children with primary ciliary dyskinesia

Kent Green[1], Frederik F. Buchvald[1], June K. Marthin[1], Birgitte Hanel[1], Per M. Gustafsson[2], Kim G. Nielsen[1].

[1]Danish PCD Center & Pediatric Pulmonary Service, Department of Pediatrics, Copenhagen University Hospital, Denmark. [2]Pediatric Respiratory Physiology Laboratory, Department of Pediatrics, Central Hospital, Skövde, Sweden.

On-Line supplement

Methods

Multiple-breath sulfur hexafluoride washout method.

The studies were done in triplicate in the sitting position. The patients watched a film as distraction or a tidal volume trace on a computer screen and were instructed to keep breathing regular with a tidal volume (VT) between 10–15 ml/kg body weight (bw). They wore a nose clip and breathed through a Fleisch no.1 pneumotachograph (PNT) (Metabo SA, Lausanne, Switzerland) via a mouthpiece, connected to the PNT. A sampling tube from a mass spectrometer was introduced in the middle of the air stream between the mouthpiece and the PNT through a short connecting piece. The post-sample line external dead space was 15 ml. The test consisted of a wash-in phase during which a dry gas mixture containing 4% sulfur hexafluoride (SF_6), 4% helium (He), 21% oxygen (O_2), and balance nitrogen (N_2) was administered using a bias flow applied on the distal port of the PNT. Wash-in was continued until the inspiratory and expiratory SF_6 concentrations were stable and equal, plus another 30 s. At this moment the administration of inert gas mixture was disconnected by taking away the T-piece during an expiration and the washout phase started, and the patient breathed room air. The washout phase continued until the end-tidal SF_6 concentration was below 0.1% over several breaths (i.e. 1/40th of the starting concentration). The PNT was connected to a differential pressure transducer and the flow signal was demodulated and amplified. The PNT was calibrated with separate calibration constants for inspiratory and expiratory flows using a precision syringe. Recorded inspiratory and expiratory flows and volumes were converted to body temperature and ambient pressure, and saturated with water vapor conditions. Gas concentrations (SF_6, He, CO_2 and O_2) were measured at the mouth using a respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark). Sample flow of the mass spectrometer was 20 ml/min and the gas concentration signals were updated at a rate of 33.3 hertz (Hz) and all signals were recorded at 100 Hz by a computer through an 8-channel USB AD-conversion board. The software corrected the flow signal sample-by-sample for changes in dynamic viscosity caused by the variations in gas composition. Gas samples and flow signals were aligned in time using an in-house built device for automated generation of instant gas steps. The same technique is currently used in several other pediatric lung function testing labs worldwide using this set-up and has been produced by one of the co-authors, Dr. Per Gustafsson. One of the two inert tracer gases (SF_6) was used for the assessments presented in this paper. Helium was included for other assessments of ventilation distribution not presented here. Functional residual capacity (FRC) and lung clearance index (LCI) were calculated as the average value from three technically acceptable runs without evidence of gas leaks or other artifacts during washouts. FRC was determined from the cumulative exhaled marker gas (SF_6) concentration divided by the differences in end-tidal gas concentration at the start of the washout and the end-tidal concentration at completion of the washout. The number of lung volume turnovers (TO) at each breath during the washout was calculated as the cumulative expired volume (CEV) corrected for the external dead space (15 ml) up to that breath, divided by the FRC. Only one index of overall ventilation inhomogeneity (the LCI) is given. The LCI was calculated as the number of TO required to lower the end-tidal tracer gas concentration to 1/40th of the starting concentration. An increase in LCI indicates increased global non-uniformity of ventilation distribution. LCI was calculated for each washout, and the mean value of the three recordings in each patient was then calculated.

The concentration normalized slope of phase III (Sn_{III}) for each subsequent breath during MBW was determined to calculate Scond and Sacin. The phase III slope was converted to SnIII by dividing the slope by the mean gas concentration over the slope to allow for gas dilution. The Sn_{III} was further multiplied by tidal volume (VT) giving the Sn_{III} * VT in order to account for inter-individual differences in lung size and breathing pattern.[1] The Sn_{III} * VT was used in all subsequent analyses and is henceforth referred to as Sn_{III} in this paper. For determination of S_{cond} and S_{acin}, Sn_{III} and TO values for each subsequent breath from the three washouts were first averaged. For each breath SnIII was then plotted against the corresponding TO value. S_{cond} was defined as the rate of Sn_{III} increase between TO 1.5 and 6.0. S_{acin} was defined as the first breath Sn_{III} value minus the convection-dependent inhomogeneity contribution to this value (i e Sc_{ond} * TO for the first breath).

<u>Swedish normative data used as reference:</u>

Mean, standard deviation (SD) and upper limit of normality (ULN; mean plus 1.96 RSD) for LCI were 6.33, 0.43 and 7.17, respectively.[2] Reference values for S_{cond} and S_{acin} were also obtained from the same Swedish laboratory and recently reported in a review paper.[3] Mean, SD and ULN for S_{cond} were 0.018, 0.006 and 0.030, and for S_{acin} 0.086, 0.025 and 0.135, respectively.

Additional results and discussion

Seventy per cent of the patients (19/27) showed abnormal FEF_{25-75} and 37% (10/27) abnormal FEV_1/FVC ratio. LCI showed a weak but statistically significant correlation with FEF_{25-75} (R^2=0.22; p=0.01) and FEV_1/FVC ratio (R^2=0.38; p<0.001). Among the patients with normal FEF_{25-75}, the MBW variables LCI, S_{cond} and S_{acin} were abnormal in 77% (10/13), 91% (10/11) and 73% (8/11), respectively. When relating Sn_{III} indices to spirometry variables, S_{acin} correlated weakly with both FEV_1/FVC ratio (R^2=0.20; p=0.03) and FEF_{25-75} (R^2=0.19; p=0.04), while S_{cond} did not show correlation with any of the spirometry parameters. See text and Figs. E2 and E3 in online supplement for association between MBW indices and FEF_{25-75} and FEV_1/FVC ratio, respectively. By including spirometry results performed for two patients on a different date than the MBW, correlation between S_{cond} and FEV_1/FVC ratio changed from being statistically significant to insignificant (R^2=0.15; 0.07). Among the patients with normal FEF_{25-75}, LCI, S_{cond} and S_{acin} were abnormal in 9/13 (69%), 8/9 (89%) and 7/9 (78%), respectively (see Fig. E1).

Among the patients with normal FEV_1/FVC ratio, LCI, S_{cond} and S_{acin} were abnormal in 14/17 (82%), 13/14 (93%) and 10/14 (71%), respectively (see Fig. E2).

LCI and S_{acin} both correlated to spirometry findings of airway obstruction (FEF_{25-75} and FEV_1/FVC ratio). In our study, several patients had abnormal FEF_{25-75}. This has previously shown to correlate poorly with peripheral airway abnormalities[4] and should consequently only be interpreted in case of normal FVC since mid-expiratory flow depends on FVC. In addition, FEF_{25-75} is highly variable in healthy patients,[4, 5] and abnormal values should, accordingly, be interpreted with caution.

Figure Legends

Figure E1.

S_{cond} *versus* S_{acin}, z-scores, in 23 patients with primary ciliary dyskinesia (PCD). The dashed lines denote upper limits of normality (mean plus 1.96 SD).

Figure E2.

a) Lung Clearance Index (LCI) from MBW *versus* forced expiratory flow at 25%-75% of forced vital capacity (FEF_{25-75}), z-scores, in 27 patients with primary ciliary dyskinesia (PCD). b) S_{cond} from MBW *versus* FEF_{25-75}, z-scores, in 23 patients with PCD. c) S_{acin} from MBW *versus* FEF_{25-75}, z-scores, in 23 patients with PCD. The dashed horizontal lines denote the upper limits of normality for LCI, S_{cond} and S_{acin} (mean plus 1.96 SD). The dashed vertical lines denote the lower limits of normality (mean minus 1.96 SD) for FEF_{25-75}.

Figure E3.

a) Lung Clearance Index (LCI) from MBW *versus* ratio between forced expiratory volume in one second and forced vital capacity (FEV_1/FVC ratio), z-scores, in 25 patients with primary ciliary dyskinesia (PCD). b) S_{cond} from MBW *versus* FEV_1/FVC ratio, z-scores, in 23 patients with PCD. c) S_{acin} from MBW *versus* FEV_1/FVC ratio, z-scores, in 23 patients with PCD. The dashed horizontal lines denote the upper limits of normality for LCI, S_{cond} and S_{acin} (mean plus 1.96 SD). The dashed vertical lines denote the lower limits of normality (mean minus 1.96 SD) for FEV_1/FVC ratio.

	LCI	S_{cond}	S_{acin}
S_{cond}	R^2: 0.06 ns	-	-
S_{acin}	**R^2: 0.45** **$p<0.001$**	R^2: 0.02 ns	-
FEV_1	R^2: 0.05 ns	R^2: 0.006 ns	R^2: 0.07 ns
FVC	R^2: 0.02 ns	R^2: 0.08 ns	R^2: 0.00 ns
FEF_{25-75}	**R^2: 0.22** **$p=0.010$**	R^2: 0.01 ns	**R^2: 0.19** **$p=0.040$**
FEV_1/FVC	**R^2: 0.38** **$p<0.001$**	R^2: 0.15 ns	**R^2: 0.20** **$p=0.030$**

Table E1: Summary of correlations between indices of MBW and spirometry parameters. Correlations were assessed using linear regression. Presented as: *R^2; p-value*. Correlations with p-values less than 0.05 are highlighted in bold. LCI: Lung Clearance Index. S_{cond} and S_{acin}: Normalized phase III slope indices (see text for explanation). FEV_1: forced expiratory volume in one second. FVC: forced vital capacity. FEF_{25-75}: forced expiratory flow at 25%-75% of FVC.

	Age	Age at diagnosis
LCI	R^2: 0.00	R^2: 0.09
	ns	ns
S_{cond}	R^2: 0.002	R^2: 0.007
	ns	ns
S_{acin}	R^2: 0.001	R^2: 0.11
	ns	ns

Table E2. Association between MBW indices and age and age at diagnosis.

Correlations were assessed using linear regression. Presented as*: R^2; p-value*. LCI: Lung Clearance Index. S_{cond} and S_{acin}: Normalized phase III slope indices (see text for explanation)

Patient	LCI, absolute value	LCI, z-scores	Scond, z-scores	Sacin, z-scores	FEV_1, z-scores	FVC, z-scores
1	10.25	9.11	6.55	-0.08	-1.21	-0.68
2	8.80	5.73	15.42	3.67	-0.85	0.94
3	14.01	17.85	11.10	13.22	-3.70	-0.68

Table E3: Results of the three patients with less definite diagnosis of PCD.

LCI: Lung Clearance Index. S_{cond} and S_{acin}: Normalized phase III slope indices (see text for explanation). FEV_1: forced expiratory volume in one second. FVC: forced vital capacity.

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