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Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis

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Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. P.M. Gustafsson, P. Aurora, A. Lindblad. ©ERS Journals Ltd 2003.

ABSTRACT: Many children with cystic fibrosis (CF), receiving modern, aggressive CF care, have normal spirometry results. This study aimed to see if homogeneity of ventilation distribution is impaired early in the course of CF lung disease, and if ventilation inhomogeneity is a more frequent finding than abnormal spirometry in children benefiting from modern CF care.

The study compared spirometry findings to two indices of ventilation inhomogeneity (mixing ratio (MR) and lung clearance index (LCI)) from multiple-breath inert gas washout in 43 children with CF, aged 3–18 yrs, and 28 healthy children.

In total, 10/43 CF subjects (23%) had reduced forced expiratory volume in one second (FEV_1) and 14/34 (41%) showed abnormal maximum expiratory flow at 25% of forced vital capacity (MEF_{25}). In contrast, MR was abnormal in 31/43 (72%) and LCI in 27/43 (63%). MR was abnormal in 22/33 CF subjects with normal FEV_1, *versus* 0/28 controls ($p<0.001$), and abnormal MR was found in 10/20 CF subjects with normal MEF_{25}, *versus* 0/22 controls ($p<0.001$). Nine of the 10 CF subjects with reduced FEV_1 and 12/14 with abnormal MEF_{25} showed abnormal MR.

Inert gas washout discloses airway dysfunction in the majority of children with cystic fibrosis with normal lung function judged by spirometry. These findings suggest that multiple-breath inert gas washout is of greater value than spirometry in detecting early cystic fibrosis lung disease.

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The care of children with cystic fibrosis (CF) has changed in the last 2 decades, with increasing emphasis on the detection and aggressive treatment of early CF lung disease [1]. One of the practical consequences of the shift to earlier treatment has been the increasing difficulty of interpreting lung function results obtained from young CF subjects. Parameters derived from maximal expiratory flow-volume loops (spirometry), such as the forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and maximum expiratory flow at 25% of FVC (MEF_{25}), have traditionally been used to monitor lung function in CF patients. Whilst FEV_1 and FVC are still recognised to be good predictors of prognosis in subjects with moderate-to-severe CF lung disease [2–4], they are now of less value in monitoring children with CF. The majority (82%) of children aged 6–18 yrs attending the CF centre in Gothenburg, Sweden, have FEV_1 results that are within the normal range. A recent report from the University Children's Hospital in Vienna, Austria, describes how the predicted FEV_1 of a 6-yr-old child attending their CF clinic has risen from 75 to 108% predicted over the last decade [5]. There is clearly a need for alternative, more sensitive measures of lung function that can be used to monitor paediatric CF subjects.

The rationale for this study is the long-standing belief that CF lung disease commences in the peripheral airways [6, 7]. Whilst spirometry parameters are known to be insensitive to peripheral airways disease [8, 9], inhomogeneity of ventilation distribution, which can be assessed by multiple-breath washout (MBW) of inert tracer gases, is known to be sensitive to peripheral airways obstruction [10–13]. The aim of this study was to see if homogeneity of ventilation distribution is impaired early in the course of CF lung disease, and if ventilation inhomogeneity is a more frequent finding than abnormal spirometry in children benefiting from modern CF care.

Material and methods

Study subjects

All paediatric CF patients aged 3–18 yrs attending the regional CF centre in Gothenburg for annual checkup over a 3-yr period were asked to participate in the study. All were in a stable condition with no signs of infective exacerbation at the time of test. A total of 28 healthy subjects (no history of wheeze, chronic cough or sputum production, asthma or allergy) of similar age and sex distribution were recruited as controls. All participants were of Caucasian origin. The study was approved by the Regional Ethics Committee for Human Research and informed oral consent was obtained from the parents and also from the participants themselves when applicable.

Study design

All children performed a minimum of three FVC manoeuvres, and FEV1, maximum expiratory flow at 50% of FVC (MEF50), and MEF25 were recorded. The residual volume (RV) and total lung capacity (TLC) were determined plethysmographically in subjects aged $\geqslant$6 yrs. All subjects then performed a minimum of three washin-washouts of a gas mixture containing 4% sulphur hexafluoride (SF_6) and 4% helium (He). Two indices of ventilation inhomogeneity: the mixing ratio (MR); and the lung clearance index (LCI), were calculated. In the primary analysis, subjects were classified according to whether they had normal or abnormal spirometry or RV/TLC ratio, and prevalence of ventilation inhomogeneity was determined for each group. Secondary analyses determined correlation between different measures of lung function and age. The relationship between lung function findings and chronic airway colonisation by typical CF bacterial pathogens, pancreatic function and genotype, were assessed in the CF group. Chronic colonisation was defined as sputum or naso-pharyngeal cultures found to be positive repeatedly over a 6-month period.

Methods

Spirometry: acceptability and analysis. Spirometry was performed using a pneumotachometer (PNT) in a Jaeger Masterscreen body plethysmograph (Erich Jaeger AG, Wurzburg, Germany). The best FVC and FEV1 results were noted. The recording including the highest sum of FEV1 and FVC was used to obtain MEF50 and MEF25. The authors encouraged the participants to expire for at least 3 s and they were required to produce two repeatable FEV1 results (within 5%). However, in some of the youngest subjects (two CF subjects and one control subject) it was difficult to achieve acceptable complete flow/volume curves, preventing the authors from reporting the MEF50 and MEF25 in these subjects. For subjects aged $\geqslant$7 yrs the results from forced expiratory measurements were expressed as % predicted or SD scores using normative data obtained in healthy Swedish children aged 7–18 yrs using a PNT method [14]. For those aged <7 yrs normative FEV1 data obtained in the same laboratory in 93 healthy children aged 4–7 yrs were used [15]. RV and TLC were determined in the body plethysmograph as the mean of three tests and findings were related to Swedish normative data in subjects aged 7–18 yrs [14]. The eight youngest CF patients could not perform RV/TLC determinations. No analyses of MEF50, MEF25 or RV/TLC ratio data were undertaken in the participants aged <7 yrs as normative data were not available and not all subjects could perform these tests.

Multiple-breath sulphur hexafluoride washout. The participants were investigated in the sitting position. During the SF_6 MBW tests the younger subjects (<10 yrs) watched a video while the older subjects watched a tidal volume trace on a computer screen and were instructed to keep breathing regular with a tidal volume (V_T) between 10–15 $mL \cdot kg^{-1}$ body weight (bw). All participants used a noseclip and breathed through a Fleisch no.1 PNT (Metabo SA, Lausanne, Switzerland) *via* a mouthpiece, except for the subjects aged $\leqslant$6 yrs who breathed *via* a transparent face mask connected to the PNT. The mask covered the mouth and the nose and was sealed with silicon putty to prevent leaks. The putty also served to reduce the dead space in the mask. A sampling tube from a mass spectrometer was introduced in the middle of the air stream between the mouthpiece/mask and the PNT through a short connecting piece. The external dead space was ~15 mL for the mouthpiece system and 30 mL for the mask system.

Each test consisted of two phases: a washin phase during which a dry gas mixture containing 4% SF_6, 4% He, 21% oxygen (O_2), and balance nitrogen (N_2) was administered using a bias flow applied on the external opening of the PNT. Washin was continued until the inspiratory and expiratory SF_6 concentrations were stable and equal, plus another 30 s. At this moment the bias flow was stopped during expiration by disconnecting the T-piece and washout was started. The washout phase continued until the end-tidal SF_6 concentration was <0.1% (*i.e.* 1/40th of the starting concentration).

The PNT was connected to a differential pressure transducer (MP 45-14-87; Validyne Corp., CA, USA; $\pm$2 cm H_2O) and the flow signal was demodulated and amplified (CD12 C-2A; Validyne Corp.). Gas concentrations were measured at the mouth using a respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark). 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 vapour conditions. Gas samples and flow signals were aligned in time. The sample flow of the mass spectrometer was 20 $mL \cdot min^{-1}$ and the gas concentration signals were updated at a rate of 33.3 Hz. The mass spectrometer measured the concentrations of all respiratory gases used (SF_6, He, N_2, O_2, and carbon dioxide (CO_2)) as dry gas concentrations. All signals were recorded at 100 Hz by a computer through a 16-channel AD-conversion board (DAS-1602; Keithley Metrabyte, Taunton, MA, USA). The software corrected the flow signal sample-by-sample for changes in dynamic viscosity caused by the variations in gas composition. One of the two inert tracer gases (SF_6) was used for the evaluations presented in this paper. Helium was included for other assessments of ventilation distribution not presented here.

Calculation of functional residual capacity, lung clearance index and mixing ratio. Each parameter was calculated as the average value from three technically acceptable washouts. The functional residual capacity (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) at that breath divided by the FRC. The CEV was corrected for the external dead space in each breath.

Two indices of overall ventilation inhomogeneity are presented: the LCI and the MR. The LCI was calculated as the number of TO needed to lower the end-tidal tracer gas concentration to 1/40th of the starting concentration [16]. The MR was calculated as the ratio between the actual and the ideal number of breaths needed to lower the end-tidal tracer gas concentration to 1/40th of the starting values [16, 17]. The ideal number of breaths was calculated from the ratio between the logarithm for the end-tidal SF_6 at end-washout and the logarithm for the FRC/(FRC+alveolar V_T) ratio. The alveolar V_T was calculated as average V_T during the MBW minus the predicted airway dead space (V_{daw}=bw$\cdot$2 mL^{-1}).

An increase in either LCI or MR indicates increased ventilation inhomogeneity. FRC, LCI, and MR were calculated for each washout, the mean value from the three recordings in each subject was then calculated for each parameter.

Analysis. The lower limits of normality (LLN) for the spirometry parameters were defined as predicted mean minus 1.96 residual SDs, calculated from published reference data [14, 15]. For males aged $\geq$7 yrs the LLN was 78.4% predicted for FEV1, 66.2% for MEF50, and 60.5% for MEF25 [14]. For females aged $\geq$7 yrs the corresponding figures were 78.8, 69.3 and 62.4% pred [14]. For males aged <7 yrs the LLN for FEV1 was 82.7% pred and for females was 80.5% pred [15]. In contrast to most parameters of lung function, LCI and MR remain constantly healthy throughout childhood [18]. Consequently, the upper limits of normality for the two indices of ventilation distribution in this study were calculated as the group mean from the 28 control subjects plus 1.96 residual SD. These limits were used to classify the CF subjects with respect to normal or abnormal spirometry, and normal or abnormal ventilation distribution. The unpaired t-test was used to compare spirometry and MBW test results between the CF patients and controls, and between subgroups of CF patients. The Chi-squared with Yates correction was used to compare the proportions of subjects with abnormal findings in the two populations. Pearson linear correlation analysis between age and measures of lung function were performed in the two groups separately and the results are presented as r^2 and resulting p-values. A p-value of $\leq$0.05 was regarded as significant.

Results

Altogether, 43 of 70 eligible patients agreed to participate in the study. Demographical data of the patients and controls are given in table 1. Patients and controls did not differ significantly with regards to sex distribution, age, weight or height. Fourteen CF subjects were chronically colonised with *Pseudomonas aeruginosa*, *Burkholderia cepacia* or *Stenotrophomonas maltophilia*.

Lung function results for the two groups are summarised in table 2. Mean FEV1 as % pred was lower in the CF group (89%) compared to the control group (97%), but this difference just failed to reach statistical significance. Among the subjects in whom technically acceptable results could be obtained (34/43 with CF and 26/28 controls), MEF25 % pred was significantly lower among the children with CF than in healthy controls (64 *versus* 86 % pred), whereas the RV/TLC ratios did not differ significantly between the groups. Altogether, 10 of 43 CF subjects (23%) had reduced FEV1 and 14/34 (41%) showed abnormal MEF25. Both indices of ventilation inhomogeneity were significantly elevated in children with CF (p<0.001), with results from the majority of individuals falling above the upper limits of normality, which were calculated to be 1.35 for the MR and 7.17 for the LCI. MR was abnormal in 31/43 (72%) and LCI in 27/43 (63%). MR was abnormal in 22/33 CF subjects with normal FEV1 *versus* 0/28 controls (p<0.01), and abnormal MR was found in 10/20 CF subjects with normal MEF25 *versus* 0/22 controls (p<0.001).

Table 1. – Clinical data of the 43 cystic fibrosis (CF) patients and the 28 healthy controls

	CF patients	Controls
Patients	43	28
Males/females	23/20	14/14
Age yrs	11.5 (3.0–18.2)	11.4 (4.5–18.7)
Weight kg	35.5 (16.5–92.0)	38.3 (20.5–85.5)
Height cm	146 (101–181)	150 (113–189)
Genotype		
ΔF508/ΔF508	24	
ΔF508/other	17	
Other/other	3	
Pancreatic function		
PI	36	
PS	7	
Chronically colonised		
P. aeruginosa	10	
S. maltophilia	2	
B. cepacia	2	

Data are presented as n or median (range). PI: pancreatic insufficiency; PS: pancreatic sufficiency. *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. maltophilia*: *Stenotrophomonas maltophilia*; *B. cepacia*: *Burkholderia cepacia*.

Table 2. – Comparison of lung function tests results from children with cystic fibrosis (CF) and the healthy controls

	CF	Controls	CF-controls
Patients n	43	28	
FEV1 % pred	89.1 (19.8)	96.9 (10.3)	-15.9–0.3[#]
MEF50 % pred[¶]	92.3 (41.2)	107.0 (30.2)	-33.9–4.6
MEF25 % pred[¶]	64.5 (33.5)	86.5 (28.2)	-38.3–-5.7**
RV/TLC[+]	26.5 (10.6)	22.8 (4.3)	-0.7–8.1
MR	1.61 (0.49)	1.19 (0.08)	0.23–0.61***
LCI	8.33 (2.48)	6.33 (0.43)	1.06–2.95***

Data are presented as mean (SD) and 95% confidence interval for the difference between the groups unless otherwise stated. FEV1: forced expiratory volume in one second; % pred: % predicted; MEF50: maximum expiratory flow at 50% of forced vital capacity; MEF25: maximum expiratory flow at 25% of forced vital capacity; RV: residual volume; TLC: total lung capacity; MR: mixing ratio; LCI: lung clearance index. [#]: p=0.06; [¶]: MEF50 and MEF25 results were available for analyses in 34 CF patients and 26 controls; [+]: RV/TLC results were available for analyses in 35 CF patients and 27 controls. **: p<0.01; ***: p<0.001.

Figure 1 a–d shows results from spirometry and MBW plotted against age according to clinical status. There was no significant correlation between % pred FEV1 and age within either the control or the CF group (fig. 1 a). However, in the CF group all subjects aged <10 yrs had an FEV1 within the normal range, and FEV1 % pred was significantly lower in children aged >10 yrs when compared with those aged <10 yrs (83.0 *versus* 97.6 % pred; p=0.015). There was no significant correlation between any of the other lung function parameters and age in either the CF or control groups (fig. 1 b–d). While % pred MEF25 was also lower among CF subjects aged >10 yrs (mean (SD) 62.0 (29.8) %) than in younger children (83.3 (37.8) %), this did not reach statistical significance (p=0.086; fig. 1 b), and no significant differences according to age group were observed for any of the other parameters, including indices of ventilation inhomogeneity.

There was a close correlation between LCI and MR both among children with CF (r^2=0.95, p<0.001) and in the healthy controls (r^2=0.51, p<0.001). The following descriptions (and figures) are therefore limited to MR results as identical patterns were observed with respect to the LCI. There was a significant correlation between MR and % pred FEV1 both in the CF group (r^2=0.40; p<0.001), and in the controls (r^2=0.30; p=0.002; fig. 2a). The MR was significantly higher in the 10 CF patients who had an abnormally low FEV1, than in the 33 who had an FEV1 within the normal range (2.02 *versus* 1.43; p<0.001). The MR was significantly associated with MEF25 % pred, both in the CF group (r^2=0.40; p<0.001), and in the control group (r^2=0.21; p=0.010; fig. 2b). The MR was significantly higher in the 14 CF patients with an abnormally low MEF25 than in the 20 with values within the normal range (1.99 *versus* 1.34; p<0.001). Altogether, nine of the 10 CF subjects with reduced FEV1 and 12/14 with abnormal MEF25 showed abnormal MR.

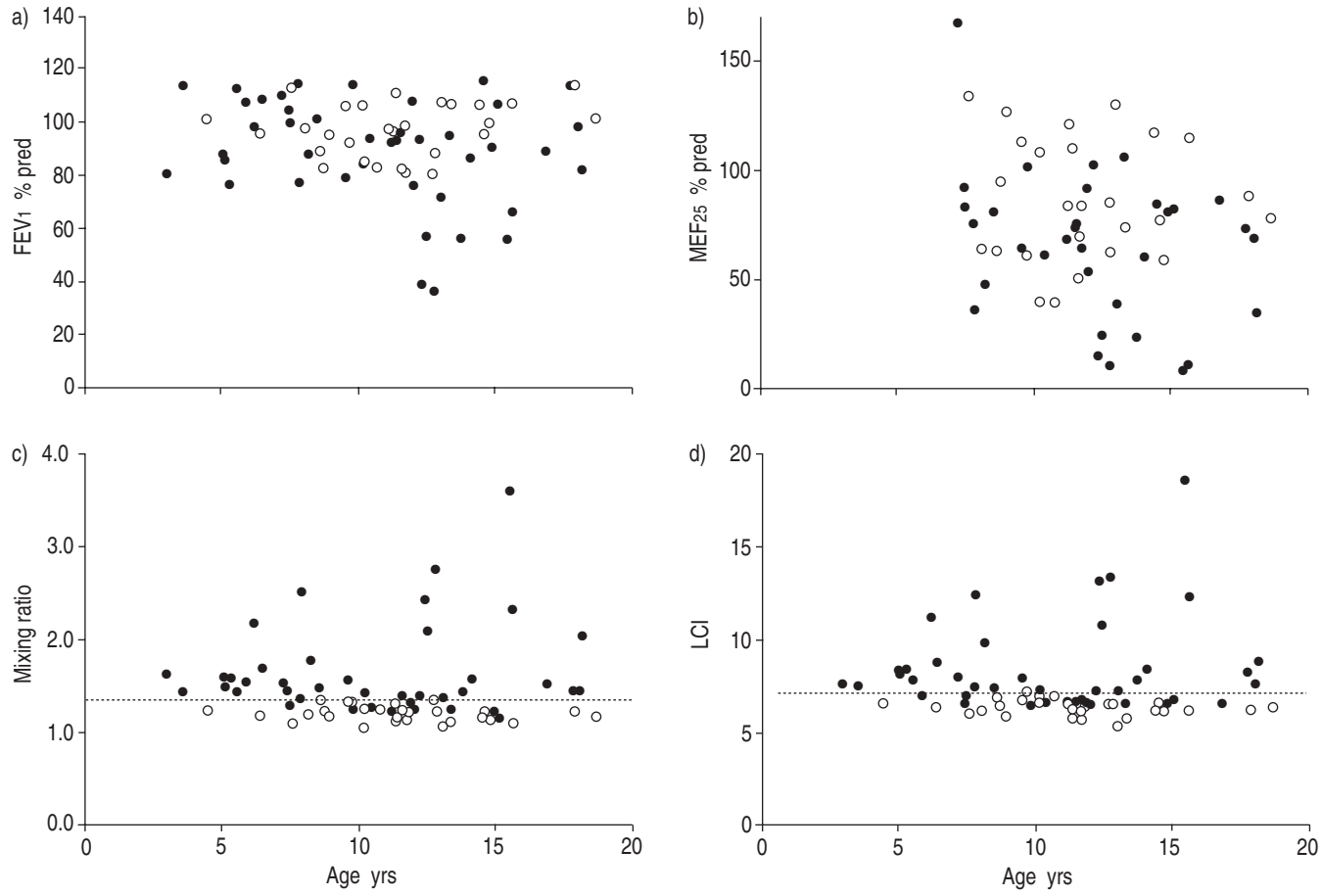

Fig. 1. – a) Forced expiratory volume in one second (FEV_1) % predicted *versus* age in 43 patients with cystic fibrosis (CF) (●) and 28 healthy controls (○). b) Maximum expiratory flow at 25% of forced vital capacity (MEF_{25}) % pred *versus* age in 34 patients with CF (●) and 26 healthy controls (○). c) Mixing ratio from multiple-breath washout (MBW) *versus* age in 43 patients with CF (●) and 28 healthy controls (○). d) Lung clearance index (LCI) from MBW *versus* age in 43 patients with CF (●) and 28 healthy controls (○). The dashed horizontal lines in figures c and d denote the upper limits of normality for mixing ratio (1.35) and LCI (7,17), respectively.

The subgroup of CF subjects with chronic bacterial airway colonisation (n=14) were significantly older than those that were noncolonised (13.7 *versus* 9.3 yrs; p<0.001), and had significantly worse lung function results from all tests (table 3). None of the lung function variables differed among CF patients with respect to pancreatic function or genotype (data not presented). Whereas both spirometric and RV/TLC results were virtually identical in the noncolonised CF subjects and controls, LCI and MR were both significantly higher (p<0.001) in this CF subgroup (table 4). The noncolonised CF subjects were on average aged 2 yrs younger than the controls (9.3 *versus* 11.5 yrs; p=0.029).

The number of CF and control subjects with MR or LCI findings above or below the upper limits of normal in relation to classification using the more conventional measures of lung function is summarised in table 5. It can be seen that, whereas almost all CF subjects with abnormal spirometry or RV/TLC values had abnormal MR and LCI results, the indices of ventilation inhomogeneity were also abnormally elevated in 50–67% of the children with CF who had normal spirometry or RV/TLC findings. The proportion of patients with an abnormal MR despite normal FEV_1 was highest in the CF subgroup <10 yrs (14/16 patients; 88%) and among those who were not chronically colonised (16/25 patients; 64%). There was close agreement between the numbers of CF subjects classified as abnormal or normal according to either MR or LCI.

As can be seen from table 5, 22 of 33 CF subjects with normal FEV_1 had a pathological MR, but this pattern was not observed in any of the healthy controls (p<0.001). Similarly, an abnormal MR was found in 50% of the CF subjects who had a normal MEF_{25} and in 50% of those with a normal RV/TLC ratio, but in none of the controls for either test (p<0.001 for both). Data from table 5 also indicate that an abnormal MR was found in 9/10 CF subjects with reduced FEV_1, in 12/14 with reduced MEF_{25}, and in all 11 with pathological RV/TLC ratios.

Discussion

Summary

In this cross-sectional study spirometry and RV/TLC findings were compared to indices of ventilation inhomogeneity in a cohort of 43 children with CF and in a matched cohort of 28 control children. All control children had normal FEV_1 and, by definition, normal gas mixing indices. Ventilation inhomogeneity was detected in one-half to two-thirds of CF subjects with normal spirometry or RV/TLC results, and in almost all CF children aged <10 yrs who had normal FEV_1. Abnormal gas mixing was found in virtually all CF patients with abnormal spirometry findings, abnormal RV/TLC ratios, or chronic bacterial airway colonisation, as well

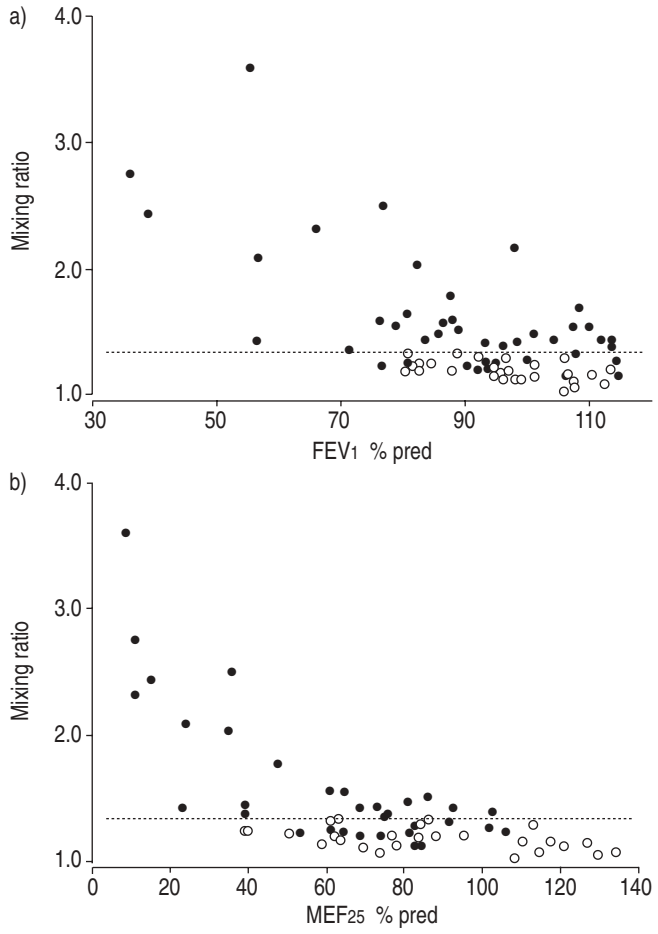

Fig. 2. – a) Mixing ratio (MR) *versus* forced expiratory volume in one second (FEV_1) % predicted in 43 patients with cystic fibrosis (CF) (●) and 28 healthy controls (○). The dashed horizontal line denotes the upper normal limit for MR (1.35). b) MR *versus* maximum expiratory flow at 25% of forced vital capacity (MEF_{25}) % pred in 34 patients with CF (●) and 26 healthy controls (○) aged ⩾7 yrs. The dashed horizontal line denotes the upper normal limit for MR (1.35).

Table 3. – Comparison of age and lung function tests results in the cystic fibrosis (CF) patients with or without chronic bacterial colonisation

	Colonised		Yes-no
	Yes	No	
Patients n	14	29	
Age yrs	13.7 (2.8)	9.3 (3.9)	2.0–6.7***
FEV_1 % pred	74.7 (22.8)	96.1 (13.9)	-32.3–-10.1***
MEF_{50} % pred[#]	67.1 (36.0)	110.0 (36.0)	-68.3–-17.6**
MEF_{25} % pred[#]	43.7 (30.9)	79.1 (27.5)	-55.9–-14.9**
RV/TLC %[¶]	33.0 (14.4)	22.7 (5.4)	3.4–17.1**
MR	1.91 (0.69)	1.47 (0.28)	0.14–0.74**
LCI	9.85 (3.49)	7.60 (1.37)	0.76–3.74**

Data are presented as mean (SD) and 95% confidence interval for the difference between the groups unless otherwise stated. FEV_1: forced expiratory volume in one second; % pred: % predicted; MEF_{50}: maximum expiratory flow at 50% of forced vital capacity; MEF_{25}: maximum expiratory flow at 25% of forced vital capacity; RV: residual volume; TLC: total lung capacity; MR: mixing ratio; LCI: lung clearance index. [#]: MEF_{50} and MEF_{25} results were available for analyses in 20 noncolonised patients; [¶]: RV/TLC results were available for analyses in 22 noncolonised patients and 13 colonised. **: $p<0.01$; ***: $p<0.001$.

Table 4. – Comparison of age and lung function in the children with cystic fibrosis (CF) who did not show chronic colonisation and the healthy control children

	CF (noncolonised)	Controls	CF-controls
Patients n	29	28	
Age yrs	9.3 (3.9)	11.5 (3.2)	-4.0–-0.2*
FEV_1 % pred	96.1 (13.9)	96.9 (10.3)	-7.3–5.7
MEF_{50} % pred[#]	110.0 (36.0)	107.0 (30.2)	16.5–22.6
MEF_{25} % pred[#]	79.1 (27.5)	86.5 (28.2)	-24.1–9.3
RV/TLC[¶]	22.7 (5.4)	22.8 (4.3)	-2.9–2.7
MR	1.47 (0.28)	1.19 (0.08)	0.17–0.39***
LCI	7.6 (1.37)	6.33 (0.43)	0.73–1.81***

Data are presented as mean (SD) and 95% confidence interval for the difference between the groups unless otherwise stated. FEV_1: forced expiratory volume in one second; % pred: % predicted; MEF_{50}: maximum expiratory flow at 50% of forced vital capacity; MEF_{25}: maximum expiratory flow at 25% of forced vital capacity; RV: residual volume; TLC: total lung capacity; MR: mixing ratio; LCI: lung clearance index. [#]: MEF_{50} and MEF_{25} results were available for analyses in 20 CF patients and 26 control subjects; [¶]: RV/TLC results were available for analyses in 22 CF patients and 27 control subjects. *: $p<0.05$; ***: $p<0.001$.

as in two-thirds of those not colonised. The findings indicate that CF lung disease may start early in childhood and long before it becomes evident with the methods currently used for monitoring of lung function.

Previous multiple-breath inert gas washout studies in cystic fibrosis subjects

MBW is not a new technique, and several previous studies, mostly performed during the 1980s and early 1990s, have investigated ventilation distribution in CF subjects using this or related methods. These studies have already shown that indices of ventilation inhomogeneity are raised in CF subjects compared with healthy subjects [19–25], and that ventilation

Table 5. – Number of subjects with abnormal (+) or normal (-) mixing ratios (MR) and lung clearance index (LCI) findings in relation to spirometry and plethysmography results

	CF				Controls			
	MR		LCI		MR		LCI	
	+	-	+	-	+	-	+	-
FEV_1								
+	9	1	9	1	0	0	0	0
-	22	11	18	15	0	28	0	28
MEF_{50}								
+	10	1	10	1	0	2	0	2
-	12	11	9	14	0	24	0	24
MEF_{25}								
+	12	2	12	2	0	4	0	4
-	10	10	7	13	0	22	0	22
RV/TLC								
+	11	0	11	0	0	3	0	3
-	12	12	9	15	0	24	0	24
LCI								
+	27	0			0	0		
-	4	12			0	28		

FEV_1: forced expiratory volume in one second; MEF_{50}: maximum expiratory flow at 50% of forced vital capacity; MEF_{25}: maximum expiratory flow at 25% of forced vital capacity; RV: residual volume; TLC: total lung capacity.

inhomogeneity is correlated to airway resistance [21], FEV1[19], and Schwachman score [22] in CF subjects. Furthermore, there is evidence that ventilation inhomogeneity decreases during treatment of an acute exacerbation in CF subjects [24]. Despite these encouraging early data the MBW technique has not been adopted in clinical practice, as spirometry has been considered to be quicker and easier to perform, and to provide adequate information for clinical care. The impetus for the current study is the recognition that, as CF care becomes more aggressive, the characteristics of the paediatric CF population are changing, and that spirometry may not be sensitive enough to detect the earliest changes of CF lung disease [8]. It is notable that the CF populations reported in most previous studies of MBW had markedly abnormal spirometry results, with mean FEV1 reported as 53% pred [19, 20], 53.1% pred [24], and 68.2% pred [25]. In contrast, the CF population reported here had a mean FEV1 of 89.1% pred.

Methodological aspects

Only 43 of 70 eligible patients were included in the study. The reason why some families declined to participate was the rather long journey to the lung function laboratory. At the time of the study the MBW tests were only performed at the Central Hospital of Skövde, which is situated 160 km from the regional CF center in Gothenburg. The spirometry results from the study group of CF patients who participated in the study are slightly but not significantly lower than the results obtained from paediatric CF patients at the centre as a whole. A recent review of the patient records from the paediatric CF population at this centre showed that the mean (SD) FEV1 was 96 (14) % pred and that 82% of the patients had a normal FEV1 (unpublished data).

Sex distribution and mean age, height and weight were similar for CF and control groups. However, the majority of the controls were aged 7–15 yrs, which was a slightly narrower age distribution than in the CF cohort. MR and LCI results were very similar throughout the age span in the healthy controls, suggesting that the upper limits of normality calculated for these parameters are likely to be valid even at aged <7 and >15 yrs. LJUNGBERG *et al.* [18] supported the view that normal values for LCI and MR are very similar for children aged <7 yrs, as compared with older children. The lack of change of these indices with age in the healthy controls is encouraging.

None of the CF patients in this study were considered chronically colonised with *Staphylococcus aureus* at the time of the test. This probably reflects the frequent use of prophylactic long-term treatment with flucloxacillin at this centre [26].

In this study SF_6 was used as an inert marker gas and a mass spectrometer for measuring the gas concentrations. Previous studies have used the N_2 MBW technique, where resident N_2 is washed out by 100% O_2. The results obtained from SF_6 washout are not expected to differ from those obtained from N_2 washout, but the newer technique does overcome one technical problem. As a gas mixture containing a high O_2 concentration has a higher dynamic viscosity than air, the velocity at which the gas sample is transported through the mass spectrometer capillary will vary significantly with changing O_2 concentration during a N_2 washout. These variations introduce errors in the alignment between the gas sample and the flow signal, which are difficult to correct for when using a mass spectrometer.

A mass spectrometer was used because it enables the concentration of several additional gases, such as He and CO_2, to be recorded simultaneously. Further analyses of recordings including these gases can be performed in the future when suitable software and algorithms are available, offering the potential for more detailed information about lung function. Because SF_6 and He differ in terms of diffusivity, a comparison of their washouts is expected to give additional information. In particular, a comparison of the phase III slope calculated from the plot of expired volume against marker gas concentration may give information about the site of peripheral airway pathology. However, the authors have noted that the phase III slope is affected by age, body size, and V_T. Further methodological work is necessary before data from this study can be interpreted in this way [11, 12].

Multiple-breath inert gas washout and spirometry

In the present study the authors noticed large differences in LCI and MR between subjects with similar FEV1% pred. The most important finding was that many subjects with normal spirometry results had abnormal MR and LCI and that almost all with abnormal spirometry had abnormal ventilation distribution (table 5). The majority of CF children aged <10 yrs and those without evidence of chronic lower airway bacterial colonisation fell into the first category, with the majority of these children having normal FEV1 and MEF25 results but abnormal MR and LCI results. These findings indicate that MR and LCI are probably both sensitive and specific with respect to CF airway disease.

Older CF subjects, with lower FEV1 and MEF25 results, tended also to have higher MR and LCI results, but this pattern was not consistent (fig. 1c, 1d, 2a, 2b). No significant correlation between age and MR or LCI was found, although a significant relationship was found between % pred FEV1 or MEF25 results and MR results, not only among children with CF, but also in the control group. Some CF subjects with abnormal spirometry results were found to have widely differing ventilation distribution indices. For example, in three CF patients with FEV1 between 55–60 % pred, the MR varied from almost normal (1.4) to 3.0. This observation could be explained by genetic and environmental factors, plus intra-subject variability within the FEV1 data. Given that a starting FEV1 of anywhere between 78–122% pred can be considered normal, then a child with an FEV1 of 60% pred could have lost anything between 22–51% of their starting FEV1. An alternative, physiological, explanation for the incomplete correlation between FEV1 and MBW arises from the observation that MBW is a tidal breathing test, whilst FEV1 is a FVC test. If the airways leading to particularly diseased regions of a subject′s lungs are closed during quiet breathing, then MR and LCI will underestimate ventilation maldistribution. Further studies, comparing results from techniques such as the vital capacity single-breath washout test [27] with MBW indices, are required to address this question.

Significance of abnormal gas mixing

What is the significance of an abnormal MR or LCI in a child with CF? Impaired ventilation distribution can result from asymmetric narrowing of the airway lumen at branch points throughout the airway tree, which in turn may be caused by inflammation, scarring or obstruction by mucus, be secondary to changes in airway tone, or inhomogeneity may result from parenchymal changes in the subtended lung units. From a clinical perspective, the most important consideration

is whether impaired gas mixing represents early changes of CF lung disease, which will subsequently progress, or whether impaired gas mixing is a fixed epiphenomenon, present in most children with CF, but unconnected to future prognosis. An indirect support for inflammation/infection being responsible for the abnormal MBW findings is the fact that patients with chronic colonisation had more pathological MR and LCI results than noncolonised patients. It is well known that children with CF become infected early in life, long before the consequences of that can be detected by conventional lung function tests [28, 29]. The question whether early infection and inflammation are manifested as abnormal gas mixing will only be answered by a combination of longitudinal studies, examining progression of ventilation inhomogeneity and spirometry with age, and intervention studies, examining the effect of treatment upon ventilation inhomogeneity. In the meantime, the observation by the authors of the relationship between high MR and low FEV1 is worth noting, but should not be over interpreted.

There are two further points that suggest that MBW measurements may be of clinical value in CF. First, a number of centres have now reported on the use of MBW in infants [30–32], and the data reported in the current study support previous suggestions that the technique can also be adapted for use in preschoolchildren [22, 23]. The finding that the parameters used for reporting the MBW studies appear to give similar values in healthy children of different age adds to the potential value of this method, as interpretation of forced expiratory parameters can be confounded by the effects of growth and development [33].

Secondly, further adaptation of the technique may provide more detailed evidence as to the site of early CF lung disease, and to the nature of its progression. At present, the site at which ventilation inhomogeneity is being generated in subjects with CF can only be speculated. Spirometry parameters are known to be insensitive to peripheral airways disease and CF disease is known to involve small as well as large airways. The authors suggest that the abnormal ventilation distribution seen in many of the subjects in the current study is the result of changes in the small airways, since these changes were observed in so many subjects with normal spirometry. However, it is also possible that a raised MR can be generated by changes in the large airways, which are not yet detectable by spirometry. In the future, analysis of the progression of the phase III slope through a MBW, and comparison of slopes obtained from gases of differing diffusivities, may allow the site within the airway tree where inhomogeneity is generated to be identified. These more sophisticated interpretations of the MBW are based upon modeling and experimental studies performed in adults, during controlled breathing [11, 12, 34]. Some further methodological work is necessary before these analyses can be applied to measurements obtained during uncontrolled breathing in children.

Conclusions

Abnormal ventilation distribution is found in the majority of children with cystic fibrosis, including young children with normal spirometry measurements. These findings suggest that destructive processes in the airways of children with cystic fibrosis may start early and before they are evident with the methods currently used when monitoring lung function.

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