Early detection of cystic fibrosis lung disease: multiple breath washout vs. raised volume tests

On-line supplement

Sooky Lum, Per Gustafsson, Henrik Ljungberg, Georg Hülskamp, Andrew Bush, Siobhán B. Carr, Rosemary Castle, Ah-fong Hoo, John Price, Sarath Ranganathan, John Stroobant, Angie Wade, Colin Wallis, Hilary Wyatt and Janet Stocks on behalf of the London Cystic Fibrosis Collaboration.

MATERIALS AND METHODS

Infants and young children diagnosed with CF by a positive sweat test and/or positive genotype for CF mutations[1] under the age of two years were recruited to the London CF Collaboration from five specialist CF centers in London (Royal Brompton Hospital, Great Ormond Street Hospital, King's College Hospital, Royal London Hospital and University Hospital Lewisham) between September 2001 and December 2002.

MEASUREMENT OF LUNG FUNCTION

There are currently no international standards for using the MBW in infants but the equipment and procedure for performing MBW in pre-school children in our laboratory has been described in detail previously.[2] While the procedure for performing MBW in infants was identical to that used in pre-school children, the equipment used differed in terms of smaller pneumotachometer and connectors. Residual deadspace of the mask (by water displacement) was estimated to be 50% of measured volume, i.e. 7.5 or 10 mL for Rendell-Baker mask size 1 or 2, respectively.[3]. Dead-space of the other components was also measured by water displacement and the system dead-space was separated into two components:

- The pre-capillary dead-space was defined as the dead-space between the infant's lips and the capillary inlet (i.e. dead-space of the mask).
- The post-capillary dead-space was defined as the dead-space between the capillary inlet and the end of the expiratory port of the pneumotachometer, and was measured as 5 mL.

In the current study, a Rendell-Baker Soucek facemask was applied to the infant's face using therapeutic putty to form an airtight seal. The same facemask was used for both MBW and RVRTC tests. Flow was measured by a Fleisch No. 0 pneumotachometer, and gas concentrations were measured by a respiratory mass spectrometer (AMIS 2000; Innovision

A/S, Odense, Denmark). In brief, each test consisted of two phases. During the wash-in phase the infant inspired a dry air mixture containing 4% sulphur hexafluoride (SF_6), 4% helium, 21% oxygen, and balance nitrogen. Helium was included to allow further analyses of ventilation distribution, results of which are not presented here. The SF_6 was the marker gas used for calculating functional residual capacity (FRC) and the lung clearance index (LCI) reported in this study. Wash-in was undertaken using a simple bias flow system and continued until the inspiratory and expiratory SF_6 concentrations were stable and equal to within 0.1%, for a minimum of 5 breaths. The washout phase using room air commenced when the bias flow was disconnected during expiration. This washout phase continued until the end tidal SF_6 concentration was consistently below 0.1% (i.e. $1/40^{th}$ of starting concentration).

FRC was determined from the cumulative volume of exhaled marker gas (SF_6) divided by the difference in end-tidal SF_6 concentration at the start of the washout and end-tidal SF_6 concentration at completion of the washout. LCI is defined as the number of lung turnovers (i.e. number of FRCs) required to clear the lungs to $1/40^{th}$ of the starting concentration of the tracer gas. The lung clearance index (LCI) was calculated by dividing the cumulative expired volume by the FRC, as described previously.[4,5] For this calculation, both the FRC and cumulative expired gas volume were corrected for post-capillary dead-space, but not for pre-capillary dead-space, as the latter correction would have involved assumptions about gas mixing within the facemask apparatus. The mean LCI from three (minimum two) technically acceptable washouts was calculated, and is presented here. Mean FRC was reported from the same washouts after further correction for pre-capillary dead-space.

Measurements of airway function at raised lung volume were performed as described previously.[6] Measurements were performed in accordance with recent recommendations,[7] using a prototype version of Jaeger software developed for Jaeger Masterscreen (version 4.54), and used in conjunction with manual inflation using Neopuff Infant Resuscitaire (Fisher & Paykel Healthcare, Auckland, New Zealand) as previously described.[8,9] Briefly, the respiratory muscles were relaxed by administering three to five lung inflations to a pressure of 30 cm H_2O before inflating the jacket to force expiration from raised lung volume. This maneuver was repeated until a minimum of three acceptable and reproducible flow-volume (F-V) curves was obtained. Parameters calculated from the raised volume technique, including forced expiratory volume in 0.5 seconds ($FEV_{0.5}$), forced vital capacity (FVC), forced expiratory flow at 75% of expired forced vital capacity (FEF_{75}) and forced expiratory flow between 25-75% FVC (FEF_{25-75}) were reported from the "best" raised volume curve. The latter was defined as the technically acceptable forced expiratory F-V curve with the highest sum of FVC and $FEV_{0.5}$.[7]

DATA ANALYSIS

Within-test repeatability for all parameters was expressed as the coefficient of variation (CV%) i.e. 100 * [SD / mean]. CV_{LCI} was 4.4% (2.8%) in children with CF, and 3.8% (1.8%) in healthy controls (mean [95% CI] difference: 0.9%, [-0.8, 1.9]). Within-subject variability for parameters derived from the RVRTC technique was similar in both groups and to that reported previously.[10] Results for LCI, $FEV_{0.5}$, FEF_{75}, FEF_{25-75} and FVC and their associated CVs were compared for the CF and control groups.

RESULTS

Details of CF subjects according to specific genotype are summarized in Table E1.

Table E1 CF subjects classified according to their specific genotype

Genotype	n (%)
ΔF508 / ΔF508	24 (62%)
ΔF508 / other*	12 (31%)
1161delC / 1161delC	1 (3%)
Other / unknown second mutation	2 (5%)

* Of these 12 children, five had unknown second mutations, four had G542X mutation and the remaining one each had 1898+1G-A, 1717-1G-A and R560T mutations.

Fifteen (38%) of the CF cohort presented with meconium ileus, 5 (13%) with antenatal bowel pathology (two of whom also had meconium ileus), 17 (44%) presented with failure to thrive or malabsorption syndromes, nine (23%) were diagnosed after recurrent chest infections (seven of whom also had malabsorption syndrome) and five (13%), with a family history of CF. Fourteen infants required hospital admission for intravenous antibiotic therapy between diagnosis and lung function testing for respiratory exacerbations: six on two occasions, one on four occasions, the rest only once. Thirteen (33%) had had *Pseudomonas aeruginosa* isolated from routine clinical cough swabs on at least one occasion. Eleven (28%) had had no clinical evidence of respiratory disease as ascertained by clinical history prior to testing.

Full details of lung function results are summarized in Table E2

Table E2: Lung function results compared by diagnosis

	Cystic Fibrosis (n = 39)	**Healthy Controls (n = 21)**	**Mean (95% CI) difference***	**p value**
Respiratory rate (min^{-1})	38.2 (9.7)	32.0 (5.2)	6.2 (2.3, 10.0)	0.002
Respiratory rate (Z score)†	0.8 (1.4)	-0.3 (0.9)	1.1 (0.4, 1.8)	0.002
Tidal volume (mL)	70.3 (24.2)	71.3 (14.3)	-1.0 (-12.5, 10.6)	0.865
Minute ventilation ($mL.min^{-1}$)	2524 (543)	2235 (374)	287 (22, 554)	0.035
FRC_{MBW} (mL)	170 (54)	156 (38)	14 (-13, 41)	0.295
FRC_{MBW} (Z-score)‡	-0.27 (1.0)	-1.0 (0.9)	0.7 (0.3, 1.2)	0.004
LCI	8.4 (1.5)	7.2 (0.3)	1.2 (0.7, 1.7)	<0.001
$FEV_{0.5}$ (mL)	226 (76)	293 (59)	-67 (-105, -28)	0.001
$FEV_{0.5}$ (Z-score)	-1.6 (1.4)	0.1 (0.9)	-1.7 (-2.3, -1.1)	<0.001
FEV_1 (mL) †	291 (103)	354 (68)	-62 (-115, -9)	0.022
FVC (mL)	299 (111)	362 (75)	-62 (-117, -8)	0.025
FVC (Z-score)	-1.2 (1.0)	-0.1 (0.8)	-1.1 (-1.6, -0.6)	<0.001
FEF_{75} ($mL.s^{-1}$)	208 (94)	306 (78)	-99 (-147, -51)	<0.001
FEF_{75} (Z-score)	-1.8 (1.5)	-0.3 (0.9)	-1.4 (-2.1, -0.8)	<0.001
FEF_{25-75} ($mL.s^{-1}$)	399 (146)	554 (114)	-155 (-228, -82)	<0.001
FEF_{25-75} (Z-score)	-1.9 (1.5)	-0.4 (1)	-1.5 (-2.2, -0.9)	<0.001
$FEV_{0.5}$/FVC	0.77 (0.1)	0.81 (0.06)	-0.04 (-0.08, 0.0)	0.061
$FEV_{0.5}$/FVC (Z-score)	-0.43 (1.26)	0.27 (0.81)	-0.70 (-1.23, -1.16)	0.011

Results expressed as mean (SD) unless otherwise specified.

Abbreviation: CI = Confidence Interval; *Difference calculated as CF – control;

† n: CF = 31; HC = 20

FRC_{MBW}: corrected for equipment and mask deadspace.

For interest, lung function results from subjects with CF have been plotted according to mode of presentation, history of respiratory illness, respiratory symptoms at test and sex (Figures E1-3). While no clear pattern was observed with respect to mode of presentation, abnormal lung function results using both MBW and the RVRTC techniques were observed even when subjects had no history of respiratory illness or symptoms at test, whereas, with the exception of a slight elevation of LCI in one child, the three infants diagnosed following a positive family history of CF but with no other modes of presentation had lung function within the normal range (Figure E1). Diminished forced flows and volumes in the presence of a normal LCI were only found in those with recent cough. Due to the small size of these subgroups, results presented here should be interpreted with caution, but could be of potential interest for future hypothesis generation and further investigation.

Figure Legends

Figure E1: Relationship between LCI and a) $FEV_{0.5}$ Z-score, b) FVC Z-score and c) FEF_{25-75} Z-score according to mode of presentation for Cystic Fibrosis.

Legend: The dashed horizontal line represents the upper range of normality for LCI in healthy infants.[2] The dashed vertical line represents the lower 95% limit of normality (i.e. only 2.5% of healthy controls have values below this level) for $FEV_{0.5}$ Z-score, FVC Z-score and FEF_{25-75} Z-score. Any results to the left of this line are unusually low. Z-scores are calculated from published reference equations [11]. Infants in the Right lower quadrant had normal results from both tests. This included all but one of the healthy controls who had an unusually low FEF_{25-75}. Those in the Left upper quadrant had abnormal results from both tests. Those in the Right upper quadrant had abnormally high LCI but normal forced expiratory parameters while those in the lower Left quadrant had LCI within normal limits but diminished FEV or FEF parameters.

Abbreviations: MI= Meconium Ileus; AN bowel = Antenatal bowel pathology; FTT = Failure to Thrive or mal-absorption syndrome; RI = Recurrent respiratory Illness; FH = Family history.

Figure E2: Relationship between LCI and a) $FEV_{0.5}$ Z-score, b) FVC Z-score and c) FEF_{25-75} Z-score according to presence or absence of history of respiratory illness and/or presence of *Pseudomonas aeruginosa* infection on cough swab on at least one occasion. Legend: Explanations for reference lines as for Figure E1.

Abbreviations: CF = Cystic Fibrosis; RI = Respiratory Illness; PA = *Pseudomonas aeruginosa*

Figure E3: Relationship between LCI and a) $FEV_{0.5}$ Z-score, b) FVC Z-score and c)b FEF_{25-75} Z-score according to current symptoms.

Legend: Explanations for reference lines as for Figure E1.

Of the 15 (38%) infants with CF had no current symptoms at time of test, 7 had LCI above the upper limit of normality, 2 had reduced FVC and FEF_{75} (not shown), 3 had diminished $FEV_{0.5}$ while 4 had diminished FEF_{25-75}. While many infants with cough had abnormalities of both LCI and FEFV parameters, the combination of a normal LCI but abnormal FEF_{25-75} was only observed in those with current cough.

Table E3: Group characteristics of CF cohorts*

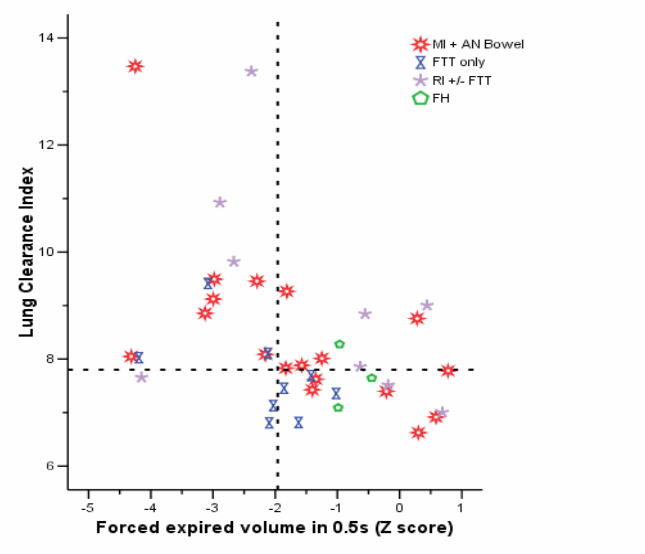
	Current cohort: Lum et al		Ranganathan et al†	
	Cystic Fibrosis	Healthy Controls	Cystic Fibrosis	Healthy Infants
N (% boys)	39 (31%)	21 (43%)	47 (40%)	187 (51%)
N (% white)	36 (92%)	20 (95%)	46 (98%)	186 (99%)
N (%) maternal smoking	12 (31%)	6 (29%)	13 (28%)	75 (40%)
Gestational age (w)	39.0 (2.1)	39.7 (1.2)	39.0 (2.0)	39.8 (1.5)
Birth weight (kg)	3.0 (0.6)	3.3 (0.4)	3.1 (0.6)	3.3 (0.5)
Birthweight Z-score	-0.5 (1.1)	-0.3 (0.8)	-0.2 (1.2)	-0.2 (0.8)
Test age (w)	41.4 (22.0)	37.0 (15.1)	29.1 (18.2)	17.3 (19.7)
Test weight (kg)	8.1 (1.8)	8.5 (1.2)	6.7 (2.0)	6.1 (2.3)
Test weight Z-score	-0.7 (1.1)	0.0 (0.7)	-1.4 (1.5)	-0.1 (0.9)
Test length (cm)	70.6 (7.2)	72.2 (4.9)	65.4 (7.3)	61.9 (8.9)
Test length Z-score	0.08 (1)	1.1 (0.8)	-0.5 (1.4)	0.4 (0.9)

*Data presented as means (SD) unless otherwise stated.
† Ranganathan et al [12]

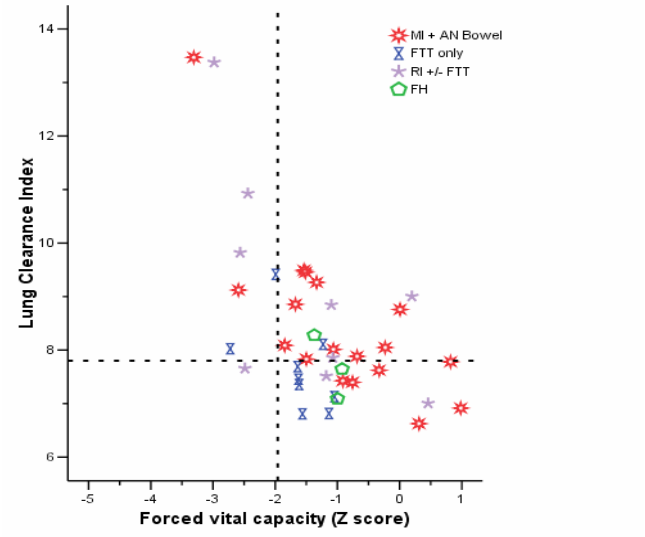
LCI in infants with CF_OLS

Figure E1

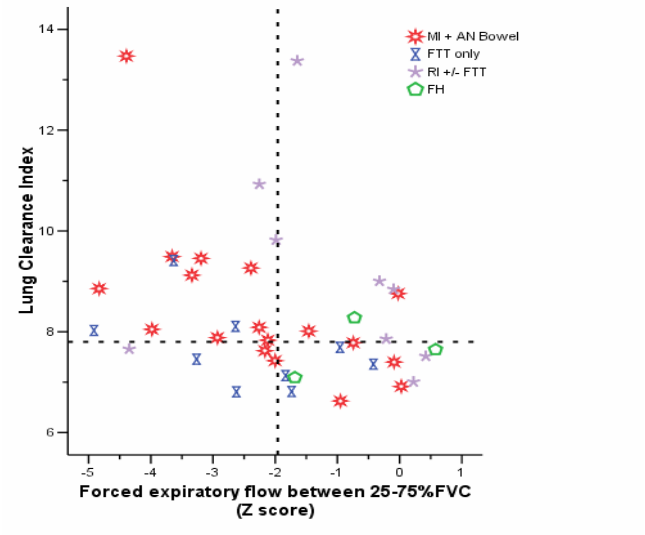
a



b



c



LCI in infants with CF_OLS

Figure E2

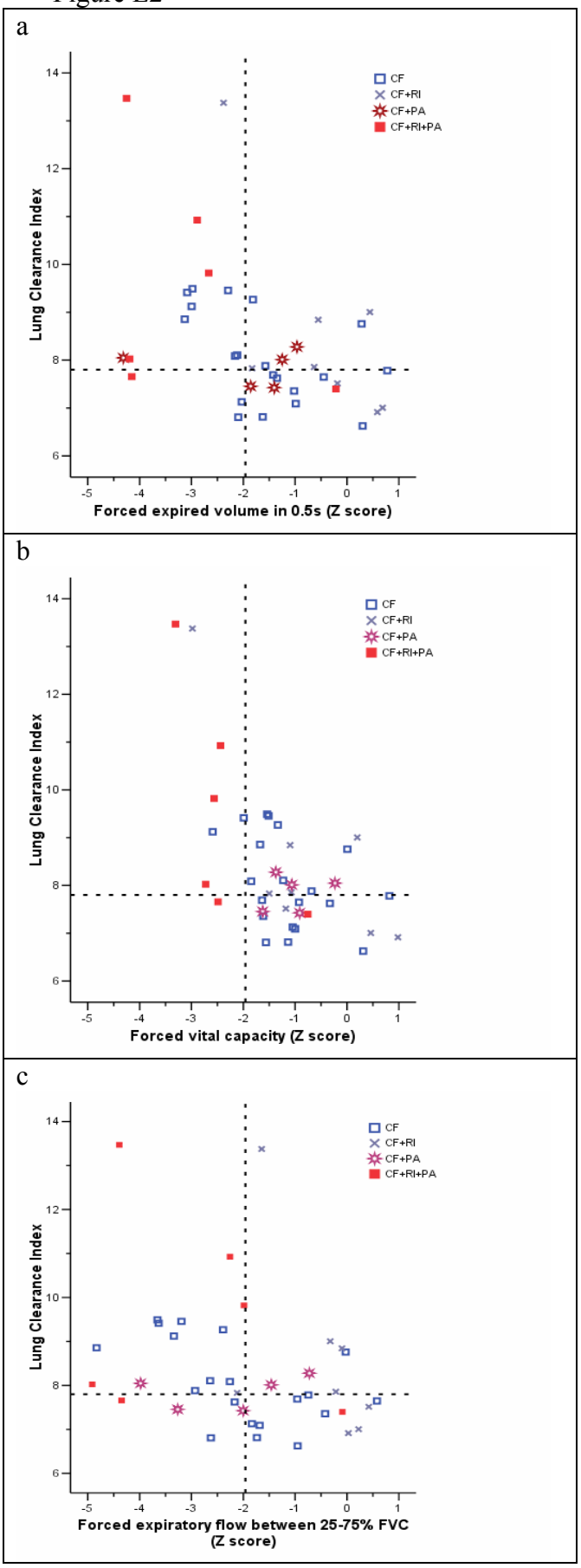
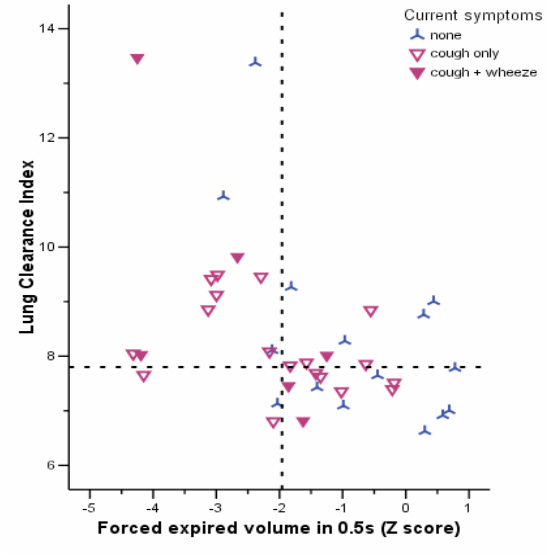
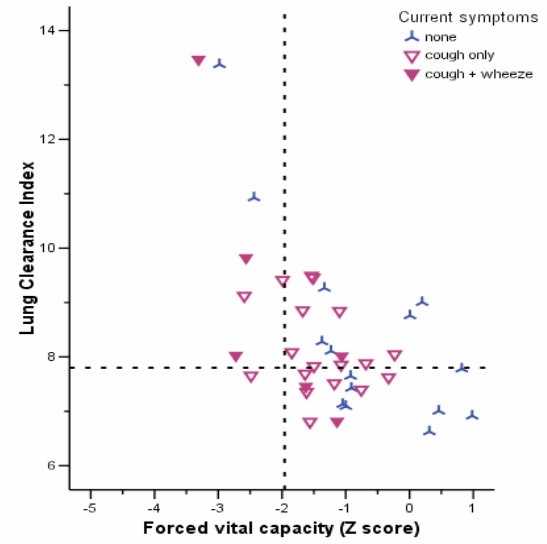


Figure E3

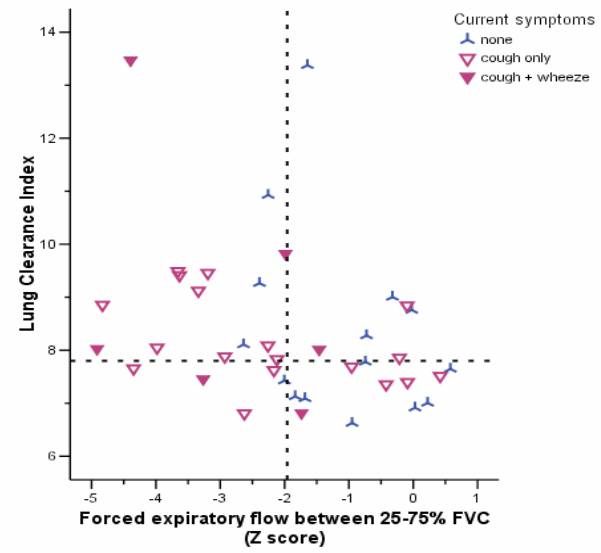
a



b



c



Reference List

1 Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 1998;132:589-595.

2 Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J et al. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. Am J Respir Crit Care Med 2005;171:249-256.

3 Frey U, Stocks J, Coates A, Sly P, Bates J. Standards for infant respiratory function testing: Specifications for equipment used for infant pulmonary function testing. Eur Respir J 2000;16:731-740.

4 Edelman NH, Mittman C, Norris AH, Shock NW. Effects of respiratory pattern on age differences in ventilation uniformity. J Appl Physiol 1968;24:49-53.

5 Larsson A, Jonmarker C, Werner O. Ventilation inhomogeneity during controlled ventilation. Which index should be used? J Appl Physiol 1988;65:2030-2039.

6 Lum S, Hoo AF, Dezateux C, Goetz I, Wade A, DeRooy L et al. The association between birthweight, sex, and airway function in infants of nonsmoking mothers. Am J Respir Crit Care Med 2001;164:2078-2084.

7 ATS_ERS Consensus State, Lum S, Stocks J, Castile R, Davies S, Henschen M et al. Raised volume forced expirations in infants: Recommendations for current practice. Am J Respir Crit Care Med 2005.

8 Lum S, Hoo AF, Stocks J. Influence of jacket tightness and pressure on raised lung volume forced expiratory maneuvers in infants. Pediatr Pulmonol 2002;34:361-368.

9 Lum S, Hulskamp G, Hoo AF, Ljungberg H, Stocks J. Effect of raised lung volume technique on subsequent measures of V'maxFRC in infants. Pediatr Pulmonol 2004;38:146-154.

10 Ranganathan SC, Hoo AF, Lum SY, Goetz I, Castle RA, Stocks J. Exploring the relationship between forced maximal flow at functional residual capacity and parameters of forced expiration from raised lung volume in healthy infants. Pediatr Pulmonol 2002;33:419-428.

11 Jones M, Castile R, Davis S, Kisling J, Filbrun D, Flucke R et al. Forced expiratory flows and volumes in infants. Am J Respir Crit Care Med 2000;161:353-359.

12 Ranganathan SC, Bush A, Dezateux C, Carr SB, Hoo AF, Lum S et al. Relative ability of full and partial forced expiratory maneuvers to identify diminished airway function in infants with cystic fibrosis. Am J Respir Crit Care Med 2002;166:1350-1357.