ONLINE DEPOSITORY

A new double-tracer gas single-breath washout to assess early cystic fibrosis lung disease

Florian Singer, Georgette Stern, Cindy Thamrin, Chiara Abbas, Carmen Casaulta, Urs Frey, Philipp Latzin.

Recruitment

The study was performed between November 2009 and December 2010 at the Children's University Hospital of Bern, Switzerland. We recruited 68 children aged between 5 and 16 years attending the outpatient clinic with confirmed cystic fibrosis (CF) diagnosis prior to the CF newborn screening introduced in January 2011. Healthy controls (n = 53) of similar age were recruited from local schools, playgroups, and paediatric surgery outpatient clinics attending for follow-up of stable upper- or lower limb fractures. Exclusion criteria for children with CF were fever, acute worsening of respiratory symptoms, significant drop in spirometry indices, and IV antibiotic treatment. Exclusion criteria for control children included any of the following: (i) doctor diagnosis of asthma, (ii) past history of severe lower respiratory tract infection, and (iii) injuries other than stable upper-limb fractures four weeks prior to the study. Preterm birth before 34 weeks gestation, neuromuscular or bone diseases likely to affect lung function, cardiac diseases, and origin other than Central European were exclusion criteria for both study groups.

Hardware for inert tracer gas washout testing

For double-tracer gas single-breath washout (tidal SBW) and nitrogen multiple-breath washout (MBNW) tests we used an available bias flow setup (Exhalyzer D®, Eco Medics AG, Duernten, Switzerland) as recently described and validated against mass spectrometry and a new lung model, respectively [1;2]. The double-tracer gas mixture (Carbagas, Bern, Switzerland) contained 5% sulfur hexafluoride (SF_6), 26.3% helium (He), 21% oxygen (O_2), and balance nitrogen (N_2). This gas mixture had the same molar mass (MM) as medical-grade air and was thus optimized for simultaneous tracing of He and SF_6 washout by MM. Expired gases were sampled near airway opening via Nafion® tubing. MM was measured by a side-stream ultrasonic flowmeter at 200 Hz. A mainstream ultrasonic flowmeter measured tidal flows, an infrared sensor measured carbon dioxide (CO_2), and a laser sensor measured O_2. The patient interface consisted of a bacterial filter and a snorkel mouthpiece to prevent air leaks near the mouth. Using a dead space reducer and a disposable hygienic insert provided by the manufacturer, this system accorded to current paediatric equipment recommendations [3]. Prior to testing, signal calibration and verification were performed daily. The main-stream ultrasonic flowmeter was calibrated using a 1 L precision syringe. The side-stream ultrasonic flowmeter, and O_2 and CO_2 sensors were calibrated using medical-grade calibration gas and pure O_2 (Carbagas, Bern, Switzerland). Directly before tracer gas washin, the apparatus dead space and the bias flow was flushed with double-tracer gas or 100% O_2 during expiration to prevent re-inspiration of expired or ambient gas. Bias flow at 1 L/s flushed the system completely within mean (SD) 0.17 (0.01) s and was estimated to exceed the children's peak inspiratory flow.

Double-tracer gas single-breath washout measurement

Two research fellows (FS, CA) executed all tests. All investigators adhered to the calibration, measurement, and analysis protocols developed in-house. For tidal SBW testing, children were motivated to breathe regularly while wearing a nose clip and watching television for distraction. At least five normal tidal breaths as monitored by online flow-volume loops and tidal volumes within 10% of each other were required. In the tidal SBW test phase, children inhaled a tidal volume of double-tracer gas from functional residual capacity prior to exhaling back to functional residual capacity. The tidal SBW accorded to measurement quality criteria if there was no evidence of air leaks as monitored by volume and MM signals, and pre-test and test breaths had similar, congruent flow-volume-loops, five tidal pre-test and the test breath volumes were within 10%, and inspiratory peak flow had not exceeded the by-pass flow during double-tracer gas washin. Between tidal SBW tests, children relaxed for a minimum of ten breaths of room air and until MM signals returned to baseline. Prior to tidal SBW analyses, data were anonymised.

Single-breath washout analysis and quality control

The primary outcome was the mean phase III slope ($SIII_{DTG}$) of three technically acceptable tidal SBW. We used available software (WBreath® 3.28, ndd Medical Technologies, Zurich, Switzerland) to record signals and to correct volumes for body temperature and water saturated (BTPS) conditions. Analyses were done using customized software (Matlab® R2006a, The Mathworks Inc., Natick, MA, USA) as described previously. MM, CO_2, and volume signals were aligned in time accounting for different signal rise times. To extract the double-tracer gas signal from MM we subtracted the naturally exhaled CO_2 fraction from the MM signal [1]. The corrected MM and CO_2 expirograms were plotted against expired volume (Figure 2). Two independent investigators (FS, CA) fitted a linear regression model to the tidal phase III of MM between 65% and 95% of expired volume. If required, manual adjustments of volume limits were applied to exclude adjacent tidal phases. Quality criteria were presence of both MM and CO_2 phase III over at least 30% of expired volume. This breath size was regarded sufficient for adequate $SIII_{DTG}$ calculation as a sufficient amount of MM samples measured at 200 Hz was available. This high sampling frequency allowed for a predefined numerical quality criterion of the linear regression, *i.e.* $SIII_{DTG}$ fitting R-squared (R^2) of 0.20 or greater. For sensitivity analyses we normalized $SIII_{DTG}$ by multiplying $SIII_{DTG}$ with the tidal volume of the corresponding breath ($SIII_{DTG.VT}$) according to previous reports [4].

Nitrogen multiple-breath washout measurement and analysis

In the pre-test phase, children established at least five regular tidal breaths as monitored by online flow-volume loops and tidal volumes within 10% of each other. An electronically triggered three-way valve switched to 100% O_2 from a cylinder (Carbagas, Bern, Switzerland). In this device, N_2 is measured indirectly based on *Dalton*'s law of partial pressures: 1 - O_2 - CO_2 [2]. During MBNW measurements, breathing pattern and tightness of the system were monitored via N_2 and volume signals. Air leaks, hyperventilation or sighs led to rejection of the measurement. After complete MBNW (three breaths below $1/40^{th}$ of the starting N_2 concentration), children relaxed during natural N_2 washin for the duration of the previous MBNW plus one extra minute [5]. Prior to the subsequent MBNW, expired N_2 had to return to baseline. For analysis, gas signals were synchronized to the flow signal using the re-inspired post-capillary dead space to produce a step response in CO_2 and O_2 [1;2]. Tidal flows and derived volumes were converted to BTPS conditions. MBNW trials with FRC measurements within 10% were accepted [6;7]. We calculated lung clearance index (LCI) by dividing cumulative expired volume by functional residual capacity [8]. Secondary outcomes were indices of conductive (Scond) and *acinar* (Sacin) ventilation inhomogeneity. Scond is the slope fitted to the evolution of multiple-breath N_2 phase III slopes across the range of 1.5 - 6 lung turnovers. Sacin is the N_2 phase III slope of the first breath from MBNW minus Scond times the lung turnover of the first breath [9]. Because these indices derive from the breathing effort needed to clear lung-resident N_2 to $1/40^{th}$ of the starting N_2 concentration, their values increase with increasing ventilation inhomogeneity. For data sampling and analysis available software was used (WBreath® 3.28, ndd Medical Technologies, Zurich, Switzerland; Spiroware® 3.1, Eco Medics AG; Duernten, Switzerland).

Additional Results

Study population

Thirty-nine (64%) children with CF were delta F508 homozygous. In children with CF, infection status according to *Lee* et al. [10] was: 14 (23%) children never had *Pseudomonas aeruginosa* infection, 21 (34%) were currently free, 17 (28%) were intermittently infected, and 9 (15%) were chronically infected. Thirty-six months prior to the study, 28 (46%) children with CF did not receive IV antibiotic treatment, 30 (49%) children with CF had one or two IV antibiotic treatments, and three (5%) children had more than two IV antibiotic treatments. Comparable to previous MBNW data in children with CF, LCI was positively correlated with airways obstruction (OLS Figure 1).

Phase III slope sensitivity analysis

The predefined quality criterion R^2 of $SIII_{DTG}$ fitting (0.20) was clearly exceeded by the measured mean (SD) fitting R^2 0.64 (0.23).

Tidal breathing patterns slightly differed between children with CF and healthy children. Comparing CF and healthy children, mean (SD) tidal volume was 0.50 (0.20) L and 0.71 (0.19) L, $p < 0.001$, mean tidal expiratory flow was 0.29 (0.09) L/s and 0.35 (0.14) L/s, $p = 0.010$, respectively. However, tidal volumes normalized by body weight were similar in children with CF and controls, 15.4 (5.5) and 16.7 (6.8) mL/kg, $p = 0.261$.

Accounting the comparison of $SIII_{DTG}$ between children with CF and controls for tidal volume (L) and flow (L/s) in a multivariable linear regression model revealed a mean difference (95% CI) of 1.5 (0.7; 2.3) $SIII_{DTG}$ z-scores, $p < 0.001$.

$SIII_{DTG}$ variability, *i.e.* intra-test coefficient of variation (CV%) of $SIII_{DTG}$, was not associated with the primary outcome $SIII_{DTG}$, age or tidal breathing pattern (Table 1).

Phase II area under the curve analysis

We additionally analyzed area under the SBW curve (AUC) during tidal phase II. To compare groups we applied a two-sample t-test accounting for unequal variances. Mean (SD) AUC in controls and children with CF was 80.6 (38.9) and 51.1 (47.1) mg.L/mol ($p = 0.002$), respectively. There was a weak correlation between AUC and LCI, FEV_1, and Scond but not with Sacin: Pearson correlation coefficients are -0.27 ($p = 0.013$), 0.25 ($p = 0.056$), -0.26 ($p = 0.018$), and -0.12 ($p = 0.310$), respectively.

References

(1) Singer F, Stern G, Thamrin C, Fuchs O, Riedel T, Gustafsson P, Frey U, Latzin P. Tidal volume single breath washout of two tracer gases - a practical and promising lung function test. *PLoS ONE* 2011; 6: e17588.

(2) Singer F, Houltz B, Robinson PD, Latzin P, Gustafsson P. A Realistic Validation Study of a New Nitrogen Multiple-Breath Washout System. *PLoS ONE* 2012; 7: e36083.

(3) Frey U, Stocks J, Coates A, Sly P, Bates J. Specifications for equipment used for infant pulmonary function testing. ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. European Respiratory Society/ American Thoracic Society. *Eur Respir J* 2000; 16: 731-740.

(4) Aurora P, Kozlowska W, Stocks J. Gas mixing efficiency from birth to adulthood measured by multiple-breath washout. *Respir Physiol Neurobiol.* 2005; 145: 125-139.

(5) Kraemer R, Blum A, Schibler A, Ammann RA, Gallati S. Ventilation inhomogeneities in relation to standard lung function in patients with cystic fibrosis. *Am J Respir Crit Care Med* 2005; 171: 371-378.

(6) Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007; 175: 1304-1345.

(7) Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson D, Macintyre N, McKay R, Miller MR, Navajas D, Pellegrino R, Viegi G. Standardisation of the measurement of lung volumes. *Eur Respir J* 2005; 26: 511-522.

(8) Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. *Eur Respir J* 2003; 22: 972-979.

(9) Verbanck S, Schuermans D, Vincken W. Inflammation and airway function in the lung periphery of patients with stable asthma. *J Allergy Clin Immunol* 2010; 125: 611-616.

(10) Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. *J Cyst Fibros* 2003; 2: 29-34.

(11) Stanojevic S, Wade A, Cole TJ, Lum S, Custovic A, Silverman M, Hall GL, Welsh L, Kirkby J, Nystad W, Badier M, Davis S, Turner S, Piccioni P, Vilozni D, Eigen H, Vlachos-Mayer H, Zheng J, Tomalak W, Jones M, Hankinson JL, Stocks J. Spirometry centile charts for young Caucasian children: the Asthma UK Collaborative Initiative. *Am J Respir Crit Care Med* 2009; 180: 547-552.

Table 1. Phase III slope variability and potential confounders.

	Cystic Fibrosis	Healthy Controls
	Pearson correlation coefficients (p-value)	
$SIII_{DTG}$	0.33 (0.073)	-0.42 (0.260)
Age (years)	0.22 (0.236)	0.25 (0.459)
Tidal volume (L)	0.29 (0.108)	-0.53 (0.094)
Mena tidal expiratory flow (L/s)	0.26 (0.157)	-0.34 (0.310)

Non-correlation between intra-test coefficient of variation (CV%) of single-breath washout derived phase III slope ($SIII_{DTG}$) and respective variables.

Figure legend

Online depository figure 1. Association of lung clearance index with airways obstruction. Lung clearance index (LCI) derived from nitrogen multiple-breath washout was highly correlated with forced expiratory volume in one second (FEV_1) in 61 children with CF, *Pearson* $r = -0.57$, $p < 0.001$. Z-scores for LCI and FEV_1 were calculated from the controls and published reference data [11], respectively. Dashed lines reflect limits of abnormal lung function (2 z-scores).