Multicenter Feasibility of Multiple-Breath Washout in Preschool Children with Cystic Fibrosis and Other Lung Diseases

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Online Supplement

Supplementary Methods

Study population

In all participating children with cystic fibrosis (CF), the diagnosis was based on a positive family history, positive CF newborn screening, or clinical symptoms characteristic of CF and confirmed by sweat chloride concentrations > 60 mmol/mL and/or cystic fibrosis transmembrane conductance regulator *(CFTR)* mutation analysis (Supplementary Table E1) according to established diagnostic criteria as previously described [1, 2]. Multiple-breath washout (MBW) measurements were performed as part of diagnostic work up in children with CF and those with non-CF pulmonary disease (history of recurrent wheeze (n=9), primary ciliary dyskinesia (PCD) (n=3), status post esophageal atresia with tracheo-esophageal fistula (n=1)). Children with lung disease were excluded from the study if they had a respiratory infection within 2 weeks prior to investigation. *Pseudomonas aeruginosa* status was determined from microbiological results of throat swabs and/or sputum samples at the time of investigation and the medical history. In addition, a control group of children without evidence of lung disease based on a detailed symptom and history questionnaire, review of the medical records and physical examination by a pediatric pulmonologist, was prospectively recruited from children of co-workers of the different study sites. Children were excluded from this control group when they had cardiac, respiratory, or neuromuscular disease or a history of prematurity, respiratory hospitalization, wheezing, chronic cough, asthma, use of anti-asthmatic drugs, or respiratory infection within 3 weeks prior to investigation. Reference values of healthy children in Germany were used to derive z-scores for weight, height and BMI [3, 4].

Multiple-breath washout test

Nitrogen (N_2)-MBW investigations in preschool children were established at the Airway Research Center North (ARCN), the Biomedical Research in Endstage and Obstructive Lung Disease (BREATH) and the Translational Lung Research Center Heidelberg (TLRC) before the start of this study and got established at the Universities of Giessen and Marburg Lung

Center (UGMLC) and the Charité – Universitätsmedizin Berlin as part of this study [5, 6]. Children were awake and sitting upright during MBW testing performed with a commercially available mainstream ultrasonic flowmeter (Exhalyzer D, Eco Medics, Duernten, Switzerland) with N_2 as tracer gas as previously described [6-8]. Dead space reducers (DSR) were used according to child's body size and manufacturer´s recommendations (DSR set 1 <15 kg, DSR set 2 ≥15 to ≤35 kg, and DSR set 3 >35 kg body weight) with all children breathing evenly through a hard mouthpiece while wearing a nose clip. The mouthpiece is the identical one used in older subjects at our centers. Spiroware 3.2.1 (client 21679/ server 21680; EcoMedics, Duernten, Switzerland) was used for data collection and analysis. A minimum of five consecutive regular breaths was collected before starting the washout by switching from inspiration of room air to 100% oxygen (O_2). The measurement was finished once N_2 was below 2.5% of the N_2 starting concentration in five consecutive breaths. Every investigation aimed at a minimum of two acceptable measurements to be rated successful. All washout curves were saved, but only technically acceptable washout curves that met previously defined quality criteria were used to derive the LCI from a minimum of two measurements [9-11]. Repeated investigation of the same patient at different time points was allowed. One MBW investigation could consist of numerous measurements with a minimum of one measurement while at least two successful measurements were needed for a successful investigation.

Technical requirements, training and quality control

Pre-study tech-check

Inclusion in the study was possible for partner sites with an Exhalyzer D (Eco Medics, Duernten, Switzerland) with equipment available. The CF centers at the five DZL sites and one associated partner site were invited to submit feasibility questionnaires for the evaluation of technical specifications of MBW hardware and software versions installed. Specifications were reviewed within four weeks and five sites were selected as study sites that had an Exhalyzer D (Eco Medics, Duernten, Switzerland) with equipment available. Following this

selection process, a datasheet containing the standardized MBW protocol and the data acquisition and analysis software version installed at the coordinating center in Heidelberg (see above) were distributed and the study team at each site was instructed to install this software version on their MBW PC.

Pre-study training session

A dedicated training session on performance of MBW according to the study protocol and on systematic washout interpretation was held at the coordinating site prior to the start of the study. Members of all participating sites attended such a teaching session. A supervised MBW investigation was carried out by the trainees to ensure standardized execution and quality.

Implementation of the MBW protocol at study sites

Trained members of the study teams of each site locally implemented the MBW standard operating procedure including calibration of the MBW machine, performing standardized MBW measurements in preschool children, as well as organizational procedures and management of acquired data such as pseudonymization and transfer of MBW files for central evaluation to the coordinating center. The previously installed MBW software was checked and corrections were made as necessary.

Data management and central assessment

A diagnostically evaluation of MBW tracings with report to the parents was performed by a pediatrician trained in MBW performance and analysis of the respective study site. Pseudonymized MBW files were sent to the coordinating center for central quality control, evaluation and statistical analysis. The central MBW reader (MS) provided feedback to the study sites to improve their experience in interpretation of diagnostic MBW investigations.

References

1. Hirtz S, Gonska T, Seydewitz HH, Thomas J, Greiner P, Kuehr J, Brandis M, Eichler I, Rocha H, Lopes AI, Barreto C, Ramalho A, Amaral MD, Kunzelmann K, Mall M. CFTR Cl- channel function in native human colon correlates with the genotype and phenotype in cystic fibrosis. *Gastroenterology* 2004: 127(4): 1085-1095.
2. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr* 1998: 132(4): 589-595.
3. Brandt I, Reinken L. [The growth rate of healthy children in the first 16 years: Bonn-Dortmund longitudinal developmental study]. *Klin Padiatr* 1988: 200(6): 451-456.
4. Kromeyer-Hauschild K, Wabitsch M, Geller F, Ziegler A, Geiß HC, Hesse V, v. Hippel, Jaeger U, Johnsen D, Kiess W, Korte W, Kunze D, Menner K, Müller M, Niemann-Pilatus A, Remer Th, Schaefer F, Wittchen HU, Zabransky S, Zellner K, J H. Perzentile für den Body Mass Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatschr Kinderh* 2001: 149(8): 807-818.
5. Stahl M, Joachim C, Wielputz MO, Mall MA. Comparison of lung clearance index determined by washout of N2 and SF6 in infants and preschool children with cystic fibrosis. *J Cyst Fibros* 2019: 18(3): 399-406.
6. Stahl M, Wielputz MO, Graeber SY, Joachim C, Sommerburg O, Kauczor HU, Puderbach M, Eichinger M, Mall MA. Comparison of Lung Clearance Index and Magnetic Resonance Imaging for Assessment of Lung Disease in Children with Cystic Fibrosis. *Am J Respir Crit Care Med* 2017: 195(3): 349-359.
7. Stanojevic S, Davis SD, Retsch-Bogart G, Webster H, Davis M, Johnson RC, Jensen R, Pizarro ME, Kane M, Clem CC, Schornick L, Subbarao P, Ratjen FA. Progression of Lung Disease in Preschool Patients with Cystic Fibrosis. *Am J Respir Crit Care Med* 2017: 195(9): 1216-1225.
8. Anagnostopoulou P, Latzin P, Jensen R, Stahl M, Harper A, Yammine S, Usemann J, Foong RE, Spycher B, Hall GL, Singer F, Stanojevic S, Mall M, Ratjen F, Ramsey KA. Normative data for multiple breath washout outcomes in school-aged Caucasian children. *Eur Respir J* 2019.
9. Jensen R, Stanojevic S, Klingel M, Pizarro ME, Hall GL, Ramsey K, Foong R, Saunders C, Robinson PD, Webster H, Hardaker K, Kane M, Ratjen F. A Systematic Approach to Multiple Breath Nitrogen Washout Test Quality. *PLoS One* 2016: 11(6): e0157523.
10. Robinson PD, Latzin P, Ramsey KA, Stanojevic S, Aurora P, Davis SD, Gappa M, Hall GL, Horsley A, Jensen R, Lum S, Milla C, Nielsen KG, Pittman JE, Rosenfeld M, Singer F, Subbarao P, Gustafsson PM, Ratjen F, Pediatrics ATSAo. Preschool

Multiple-Breath Washout Testing. An Official American Thoracic Society Technical Statement. *Am J Respir Crit Care Med* 2018: 197(5): e1-e19.

11. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR, Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. *Eur Respir J* 2013: 41(3): 507-522.

Supplementary Table E1. Summary of *CFTR* genotypes according to pancreatic status of children with CF

Pancreatic insufficient		Pancreatic sufficient	
CFTR Genotype	Number of subjects	*CFTR* Genotype	Number of subjects
F508del/F508del	35	3120+1G>A/D110H	1
F508del/G542X	2	I507del/3849+10kbC->T	1
F508del/1717-1G->A	1		
F508del/2184delA	1		
F508del/CFTRdele2,3	1		
F508del/I507del	1		
F508del/N1303K	1		
F508del/R553X	1		
E92X/R347P	1		

Exocrine pancreatic function was determined by levels of fecal elastase in stool with ≥ 200µg/g stool as cut-off for pancreatic sufficiency.

Supplementary Table E2. Success rates of MBW investigations in awake preschool children

Cohort	Center	Number of performed investigations, n	Number of performed measurements, mean ± SD	Number of successful measurements, mean ± SD	At least 1 successful measurement, n (%)	At least 2 successful measurements, n (%)
All	ARCN	17	4.0 ± 1.0	2.5 ± 0.6	17 (100.0)	16 (94.1)
	BREATH	21	3.7 ± 1.8*	1.9 ± 0.8	19 (90.5)	17 (81.0)
	TLRC	24	3.8 ± 1.8*	2.0 ± 0.8	22 (91.7)	21 (87.5)
	UGMLC	12	6.2 ± 2.3	2.1 ± 1.1	11 (91.7)	9 (75.0)
	Berlin	17	4.5 ± 2.4	2.1 ± 1.4	13 (76.5)	12 (70.6)
Controls	ARCN	3	3.7 ± 0.6	2.3 ± 0.6	3 (100.0)	3 (100.0)
	BREATH	5	5.2 ± 2.8	2.0 ± 0.7	5 (100.0)	4 (80.0)
	TLRC	3	3.0 ± 1.0	2.0 ± 0.0	3 (100.0)	3 (100.0)
	UGMLC	1	7	3	1 (100.0)	1 (100.0)
	Berlin	3	5.0 ± 2.0	3.0 ± 1.0	3 (100.0)	3 (100.0)
CF	ARCN	4	4.8 ± 1.5	2.0 ± 0.8	4 (100.0)	3 (75.0)
	BREATH	16	3.2 ± 1.2*	1.9 ± 0.9	14 (87.5)	13 (81.3)
	TLRC	20	3.9 ± 2.0	2.0 ± 0.9	18 (90.0)	17 (85.0)
	UGMLC	11	6.1 ± 2.3	2.0 ± 1.1	10 (90.9)	8 (72.7)
	Berlin	12	4.6 ± 2.7	1.9 ± 1.4	9 (75.0)	8 (66.7)
Other lung diseases	ARCN	10	3.8 ± 0.8	2.7 ± 0.5	10 (100.0)	10 (100.0)
	TLRC	1	4	3	1 (100.0)	1 (100.0)
	Berlin	2	3.0 ± 1.4	1.5 ± 2.1	1 (50.0)	1 (50.0)

Definition of abbreviations: MBW, multiple-breath washout. SD, standard deviation. CF, cystic fibrosis. ARCN, CF Center at Airway Research Center North. BREATH, CF Center at Biomedical Research in Endstage and Obstructive Lung Disease Hannover. TLRC, CF

Center at Translational Lung Research Center Heidelberg. UGMLC, CF Center at Universities of Giessen and Marburg Lung Center. Berlin, CF Center at Charité - Universitätsmedizin Berlin. *P<0.05 vs. UGMLC.

Supplementary Table E3. Success rates of only the first MBW investigation in this study in awake preschool children

Cohort	Center	Number of performed investigations, n	Number of performed measurements, mean ± SD	Number of successful measurements, mean ± SD	Number of successful investigations, n (%)
All	ARCN	17	4.0 ± 1.0	2.5 ± 0.6	16 (94.1)
	BREATH	18	3.8 ± 1.9*	1.8 ± 0.9	14 (77.8)
	TLRC	21	3.8 ± 1.9*	2.1 ± 0.9	19 (90.5)
	UGMLC	4	7.8 ± 3.0	1.8 ± 1.3	3 (75.0)
	Berlin	14	4.7 ± 2.6	2.1 ± 1.4	10 (71.4)
Controls	ARCN	3	3.7 ± 0.6	2.3 ± 0.6	3 (100.0)
	BREATH	5	5.2 ± 2.8	2.0 ± 0.7	4 (80.0)
	TLRC	3	3.0 ± 1.0	2.0 ± 0.0	3 (100.0)
	UGMLC	1	7	3	1 (100.0)
	Berlin	3	5.0 ± 2.0	3.0 ± 1.0	3 (100.0)
CF	ARCN	4	4.8 ± 1.5	2.0 ± 0.8	3 (75.0)
	BREATH	13	3.3 ± 1.3*	1.8 ± 0.9	10 (76.9)
	TLRC	17	3.9 ± 2.1	2.0 ± 0.9	15 (88.2)
	UGMLC	3	8.0 ± 3.6	1.3 ± 1.2	2 (66.7)
	Berlin	9	5.0 ± 3.0	2.0 ± 1.4	6 (66.7)
Other lung diseases	ARCN	10	3.8 ± 0.8	2.7 ± 0.5	10 (100.0)
	TLRC	1	4	3	1 (100.0)
	Berlin	2	3.0 ± 1.4	1.5 ± 2.1	1 (50.0)

Definition of abbreviations: MBW, multiple-breath washout. SD, standard deviation. CF, cystic fibrosis. ARCN, CF Center at Airway Research Center North. BREATH, CF Center at Biomedical Research in Endstage and Obstructive Lung Disease Hannover. TLRC, CF Center at Translational Lung Research Center Heidelberg. UGMLC, CF Center at

Universities of Giessen and Marburg Lung Center. Berlin, CF Center at Charité - Universitätsmedizin Berlin. *P<0.05 vs. UGMLC.

Supplementary Table E4. Distribution of LCI between study sites in preschool children

Diagnosis		Total	ARCN	BREATH	TLRC	UGMLC	Berlin
Control	Number of children	14	3	4	3	1	3
	LCI, median (range)†	7.3	7.1	7.4	6.7	7.3	7.8
		(6.5–8.3)	(6.8–7.8)	(6.7–7.8)	(6.5–7.5)		(7.2–8.3)
	LCI_{CV}, median (range), %†	2.8	2.6	6.3	2.2	2.0	2.3
		(0.0–10.7)	(2.1–2.9)	(5.4–6.6)	(0.0–10.7)		(2.0–7.1)
	Investigations with abnormal LCI, n (%)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)
CF	Number of children	49	3	13	17	8	8
	LCI, median (range)	8.2	9.7	8.1	8.2	8.3	8.4
		(6.7–15.5)*	(6.9–11.3)	(7.2–9.4)	(6.7–10.8)	(6.8–11.5)	(7.3–15.5)
	LCI_{CV}, median (range), %	4.2	2.1	3.6	4.3	5.0	3.8
		(0.3–17.1)	(1.9–8.9)	(0.3–8.6)	(1.2–8.8)	(2.8–8.5)	(0.7–17.1)
	Investigations with abnormal LCI, n (%)	23 (46.9)*	2 (66.7)	5 (38.5)	7 (41.2)	4 (50.0)	5 (62.5)
Other lung	Number of children	12	10		1		1
diseases	LCI, median (range)†	7.9	7.2		13.9		11.1
		(6.6–13.9)	(6.6–10.7)				
	LCI_{CV}, median (range), %†	2.6	2.6		1.0		5.2
		(0.6–8.4)	(0.6–8.4)				
	Investigations with abnormal LCI, n (%)	6 (50.0)*	4 (40.0)		1 (100.0)		1 (100.0)

Definition of abbreviations: LCI, lung clearance index. CF, cystic fibrosis. CV, coefficient of variation. ARCN, CF Center at Airway Research Center North. BREATH, CF Center at Biomedical Research in Endstage and Obstructive Lung Disease Hannover. TLRC, CF Center at Translational

Lung Research Center Heidelberg. UGMLC, CF Center at Universities of Giessen and Marburg Lung Center. Berlin, CF Center at Charité - Universitätsmedizin Berlin. *P<0.01 vs. control. †In case of a single investigation, the result of this investigation is reported without range.