ONLINE DATA SUPPLEMENT:

Ventilation inhomogeneity in infants with recurrent wheezing

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Running Head: Lung clearance index in wheezy infants

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Methods

Healthy infants: Healthy subjects in a general-population birth cohort, the Canadian Healthy Infant Longitudinal Development (CHILD) study, were recruited to a sub-study of infant lung function.[1] Only healthy asymptomatic children with infant lung function measures were included in this analysis. The following criteria were used to define the healthy infant group:

1. No history of prenatal smoke exposure.
2. No neonatal respiratory issues.
3. No history of prior wheezing.
4. No history of emergency room or hospital visit for respiratory symptoms.
5. Normal chest examination and oxygen saturation at the time of clinical assessment for pulmonary function testing.
6. No viral infection symptoms for a minimum of 6 weeks prior to testing.

Study visits:

Each child was at their baseline respiratory state of health according to their parents and responsible physician and at a minimum of 3 weeks following resolution of an acute exacerbation of respiratory symptoms. A detailed medical history and physical examination including chest auscultation was performed prior to testing. Length and weight were measured using a calibrated stadiometer and digital scale.

Lung function Measurements:

Infants were sedated with chloral hydrate (60-100 mg/kg) and lung function measurements were performed during quiet sleep in the supine position. The sequence of testing was standardized

such that all patients underwent MBW testing prior to measurement of lung volumes by plethysmography and forced expiratory flows by the raised volume rapid thoracoabdominal compression technique (RVRTC) due to known effects of RVRTC maneuvers on MBW measurements[2].

Multiple Breath Washout

MBW tests were performed using an infant facemask (Silkomed, Rendell Baker Masks sizes 2 and 3, Rusch Canada Inc., Benson Medical Industries, Markham, Ontario) sealed with therapeutic putty (Air Putty, Sammons Preston Canada Inc., Mississauga, Ontario). Infants less than 6 months were tested using a size 2 face mask with a 10 mL deadspace while older infants were tested using a size 3 facemask with a 20 mL deadspace. In both case, deadspace was minimized with therapeutic putty. A mass spectrometer (AMIS 2000; Innovision ApS, Odense, Denmark) based set-up and technique was used to perform MBW testing with a sulfur hexafluoride (SF_6)/ Helium (He) gas mixture as previously described.[3] Briefly, participants breathed in a dry gas mixture containing 4% SF_6, 4% He, 21% oxygen (O_2), balance nitrogen (N_2) via an open circuit bias flow system through a mask and an attached pneumotachograph (Hans Rudolph, Shawnee, KS, USA) until equilibrium was reached. Once the end-expiratory inert tracer gas (SF_6) stabilized at 4%, the gas source was removed and the subject breathed room air until concentration of SF6 reached $1/40^{th}$ of the starting concentration. End of test was considered to be the first of three breaths where the end-tidal concentration of SF_6 was below target concentration.[4] Tests were performed in triplicate or until at least two technically acceptable trials had been obtained. LCI is derived from the MBW test, calculated as the cumulative exhaled volume (CEV) needed to reduce the end-tidal concentration of the tracer gas to $1/40^{th}$ of the initial concentration divided by the functional residual capacity (FRC)

$$LCI = \frac{CEV}{FRC}$$

The average of at least two technically acceptable washout tests were used to calculate LCI, FRC, CEV, tidal volume (V_T) and respiratory rate (RR) for each subject.

Plethysmography and RVRTC

Functional residual capacity (FRC_{pleth}) was measured by body plethysmography (nSpire® Infant Pulmonary Lab, Longmont, CO, USA) according to ATS/ERS guidelines[5]. FRC_{pleth} was calculated from the average of 3 acceptable occasions.

Forced expiratory volumes and flows (FVC, $FEV_{0.5}$, FEF_{25-75}, and FEF_{75}) were measured using the RVRTC technique according to ATS/ERS guidelines for raised volume forced expirations in infants.[6] All curves used for analysis had FVC measurements within 10% of the highest FVC. Lung function outcomes are reported as the single best maneuvers with the highest sum of FVC and FEF_{25-75}.

Exhaled Nitric Oxide

Multiple breath online exhaled nitric oxide (FeNO) measurement was performed in infants with wheezing disorders only prior to commencing other pulmonary function measures. Online tidal breathing measurement of FeNO was collected using the Exhalyser D with an ultrasonic flowmeter as previously described[7] and a CLD 88 Exhalyzer chemoluminescent analyzer (Ecomedics AG, Duernton, Switzerland). Recordings were included if > 30 tidal breathing flow-

volume loops (TBFVL) were recorded that were free of sighs, respiratory pauses, leak or irregular volume of breaths. Measures were analyzed according to ATS/ERS recommendations.[8] Mean FeNO and nitric oxide output were calculated using offline analysis software (Wbreath v3.41.5.0 Ndd Medizintechnik, AG, Zurich, Switzerland)

Statistical Analysis

The statistical analysis in current online supplement focused on investigating whether the published reference equation provide a good fit to our data. In addition, the sensitivity analyses were also conducted in the appropriate subgroups to confirm our findings in LCI.

Specifically, if the published reference equations provide a good fit to our healthy control data, the z-score values should have a standard normal distribution with mean 0 and standard deviation of 1. To investigate the fitness of these reference equations to our healthy control data, sign rank test was used to compare the calculated z-scores to zero. Given the age and size difference between the healthy and RW groups, a sensitivity analysis was also conducted by matching the healthy controls and RW based on their age, height, gender and ethnicity. Comparisons of lung function parameters between the two groups were repeated based on the matched data, using two sample t tests or Mann-Whitney U tests where appropriate. Similar analyses were conducted to those with paired measurement of MBW and RVRTC data.

All statistical analyses were based on available data and no missing data were imputed. Statistical significant level was set at 0.05. All analysis was performed using SAS version 9.4 (SAS statistical software, Cary NC).

Results

Relationship between LCI and other lung function measures:

To compare our LCI measures with flow ($FEV_{0.5}$, FEF_{25-75} and FVC) measures, we examined the z-scores for our healthy control population. We found that the published z-scores of LCI, FEF_{25-75} z-score and FRC_{pleth} fit our healthy controls poorly, indicating that our population may differ from the published reference populations (Table E1). Similarly, when matching the two groups by age, height, gender and ethnicity, the LCI remained significantly different (Table E2).

Fewer subjects had paired measures of flow and MBW due to technical issues around sedation and protocol length. For comparison of different lung function measures, we reanalysed the data for the subcohort with paired measures only (59 healthy infants and 24 RW infants). LCI remained higher in the RW group and significantly different compared with healthy controls (Table E3).

Table E1. Published z-scores for pulmonary function in healthy subjects.

Variable	N	Healthy Control (HC) Median (Range)	P value [a]
LCI z-score[1]	113	-0.59 (-2.93, 2.11)	<0.001
$FEV_{0.5}$ z-score[2]	59	-0.05 (-2.08, 2.54)	0.9
FEF_{25-75} z-score[2]	59	0.63 (-1.51, 2.86)	<.0001
$FEV_{0.5}$/FVC z-score[2]	59	0.19 (-1.69, 2.33)	0.1
FVC z-score[2]	59	-0.04 (-2.08 , 1.85)	1.0
FRC_{pleth} z-score[3]	76	-0.72(-2.85 ,1.81)	<0.001

[1] LCI z-score calculated based on Lum et al. 2013 reference equation[9]
[2] Spirometry z-score calculated using Lum et al. 2016 reference equation[10]
[3] FRC_{pleth} z-score calculated using Stock et al. 2001 reference equation[11]
[a] Comparison z-score difference from zero using sign rank test.

Table E2 Comparison of Lung function parameters between healthy and recurrent wheeze for subjects matched by age, height, gender and ethnicity

Variable	N	Recurrent wheeze (RW)†	N	Healthy control (HC)†	Δ (95%CI) RW - HC
LCI z-score	**23**	**0.17 (1.58)**	**23**	**-0.67 (0.81)**	**0.84 (0.09, 1.58)**
FRC_{MBW} z-score	23	0.26 (0.97)	23	0.12 (0.83)	0.14 (-0.40, 0.67)
$FEV_{0.5}$ z-score	15	0.01 (1.35)	14	-0.33 (0.90)	0.34 (-0.54, 1.22)
FVC z-score	15	0.29 (1.35)	14	-0.00 (1.00)	0.29 (-0.62, 1.20)
$FEV_{0.5}$/FVC z-score	15	-0.28 (0.62)	14	-0.15 (0.95)	-0.13 (-0.73, 0.48)
FEF_{25-75} z-score	15	0.14 (0.97)	14	0.34 (0.83)	-0.20 (-0.89, 0.49)
FRC_{pleth} z-score	18	-0.18 (1.46)	16	-0.42 (1.08)	0.24 (-0.67, 1.15)

† Data were presented mean and SD

Δ : Least Square Mean difference between groups derived from linear regressions adjusted for age and height where raw values were used (i.e. for LCI, FeNO and V'NO) ; No adjustment were made for z-scores
CI: confidence interval
Statistically significant results were bolded

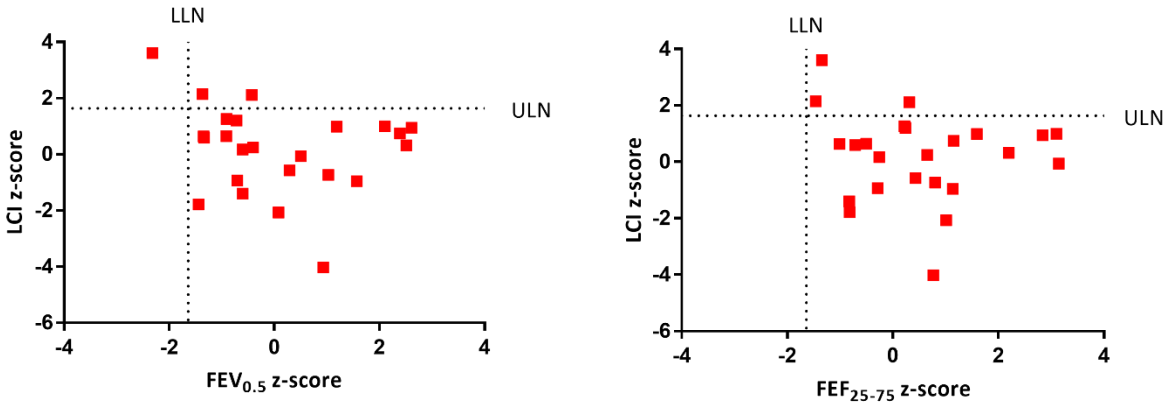
Table E3. Comparison of Lung function parameters between healthy and recurrent wheeze for subjects with both MBW and RVRTC data

Variable	N	Recurrent wheeze (RW)†	N	Healthy control (HC)†	Δ (95%CI) RW - HC
LCI z-score	**24**	**0.17 (1.58)**	**59**	**-0.44 (0.98)**	**0.61 (0.04, 1.18)**
FRC_{MBW} z-score	24	0.03 (1.05)	59	0.21 (0.93)	-0.18 (-0.64, 0.29)
$FEV_{0.5}$ z-score	24	0.09 (1.41)	59	0.10 (1.13)	-0.01 (-0.60, 0.57)
FVC z-score	24	0.01 (1.24)	59	0.05 (0.96)	-0.04 (-0.54, 0.47)
$FEV_{0.5}$/FVC z-score	24	0.07 (0.85)	59	0.14 (0.76)	-0.07 (-0.45, 0.30)
FEF_{25-75} z-score	24	0.52 (1.34)	59	0.73 (1.07)	-0.21 (-0.77, 0.34)
FRC_{pleth} z-score	22	-0.17 (1.51)	55	-0.75 (1.09)	0.58 (-0.03, 1.20)

† Data were presented mean and SD

Δ : Least Square Mean difference between groups derived from linear regressions adjusted for age and height where raw values were used (i.e. for LCI, FeNO and V'NO) ; No adjustment were made for z-scores
CI: confidence interval
Statistically significant results were bolded

Figure E1 Lung function tests for RW subjects in whom both LCI and forced expiratory flow data were available. (LCI z-score were calculated using the reference equation from Lum et.al. 2013, and RVRTC z-score were calculated using Lum et.al. 2016).



REFERENCES:

1. Subbarao P, Anand SS, Becker AB, Befus AD, Brauer M, Brook JR, Denburg JA, HayGlass KT, Kobor MS, Kollmann TR, et al. The Canadian Healthy Infant Longitudinal Development (CHILD) Study: examining developmental origins of allergy and asthma. Thorax 2015;70(10):998-1000.
2. Subbarao P, Lu Z, Kowalik K, Brown M, Balkovec S, Gustafsson P, Lou W, Ratjen F. Changes in multiple breath washout measures after raised volume rapid thoracoabdominal compression maneuvers in infants. Pediatr Pulmonol 2016;51(2):183-188.
3. Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. European Respiratory Journal 2003;22(6):972-979.
4. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HGM, Aurora P, Fuchs SI, et al. Consensus statement for inert gas washout measurement using multiple- and singlebreath tests. Eur Respir J 2013;41(3):507-522.
5. Morris MG, Gustafsson P, Tepper R, Gappa M, Stocks J. The bias flow nitrogen washout technique for measuring the functional residual capacity in infants. ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. Eur Respir J 2001;17(3):529-536.
6. ATS/ERS statement: raised volume forced expirations in infants: guidelines for current practice. Am J Respir Crit Care Med 2005;172(11):1463-1471.
7. Latzin P, Kuehni CE, Baldwin DN, Roiha HL, Casaulta C, Frey U. Elevated exhaled nitric oxide in newborns of atopic mothers precedes respiratory symptoms. Am J Respir Crit Care Med 2006;174(12):1292-1298.
8. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 1999;160(6):2104-2117.
9. Lum S, Stocks J, Stanojevic S, Wade A, Robinson P, Gustafsson P, Brown M, Aurora P, Subbarao P, Hoo A-f, et al. Age and height dependence of lung clearance index and functional residual capacity. Eur Respir J 2012:-.
10. Lum S, Bountziouka V, Wade A, Hoo AF, Kirkby J, Moreno-Galdo A, de Mir I, Sardon-Prado O, Corcuera-Elosegui P, Mattes J, et al. New reference ranges for interpreting forced expiratory manoeuvres in infants and implications for clinical interpretation: a multicentre collaboration. Thorax 2016;71(3):276-283.
11. Stocks J, Godfrey S, Beardsmore C, Bar-Yishay E, Castile R, Society EATFoSfIRFTERSAT. Plethysmographic measurements of lung volume and airway resistance. ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. European Respiratory Society/ American Thoracic Society. Eur Respir J 2001;17(2):302-312.