The Use of Lung Clearance Index to Assess Cystic Fibrosis–Related Lung Disease

Outline

- **The natural history of lung disease in CF and the use of FEV_1 vs LCI to assess pulmonary dysfunction**
  - The physiological underpinnings of lung disease in CF
  - The strengths and limitations of spirometry and multiple breath washout techniques to assess pulmonary dysfunction, and methods for determining LCI

- **LCI as an outcome measure in CF**
  - The use of LCI to discriminate between CF and health, to detect early lung disease and to track early disease progression
  - Correlation between LCI and data obtained with multiple imaging modalities
  - Relation between LCI, inflammatory markers, and pulmonary exacerbations

- **LCI as an outcome measure in CF clinical trials**
  - Clinical trial data from dornase alpha and hypertonic saline trials
  - Evaluating the clinical significance of LCI results

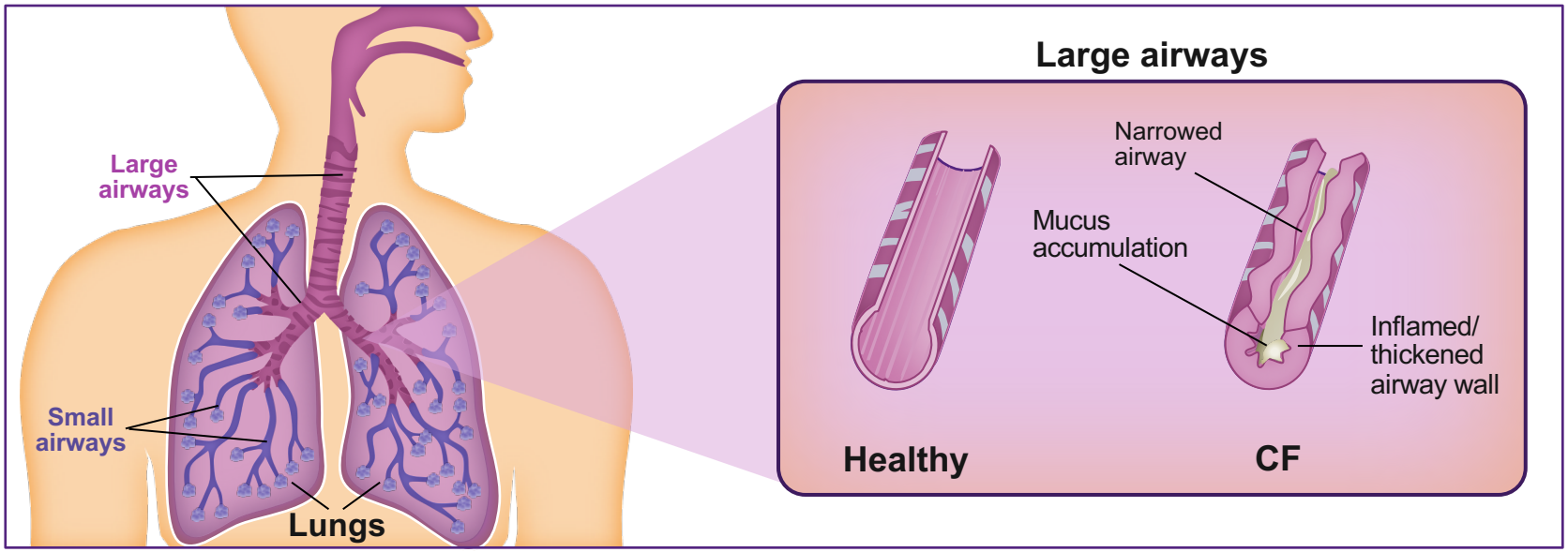
CF, cystic fibrosis; FEV_1, forced expiratory volume in 1 second; LCI, lung clearance index.

Background

FEV_1, LCI, and the Physiology of Early Lung Disease in CF

FEV_1: The Cornerstone of Pulmonary Function Testing

- FEV_1 is the volume of air forcefully expelled from the lungs in the first second
- It is the most widely used method for clinical monitoring of CF lung function
- FEV_1 provides a measure of total airway resistance
- It correlates best with airway wall thickness and mucus plugging, both features of larger airway obstruction



Horsley A, Siddiqui S. *Respirology*. 2015;20(1):33-45.

FEV_1: The Cornerstone of Pulmonary Function Testing (cont)

Strengths

- Widely used in the clinic and in clinical trials[1,2]
- When expressed as percent predicted, FEV_1 is the most useful objective measure of pulmonary status[3]
- Validated prognostic indicator of lung disease progression and survival time in CF[2,4]
- Recognized as a primary outcome by the FDA and EMA[5]

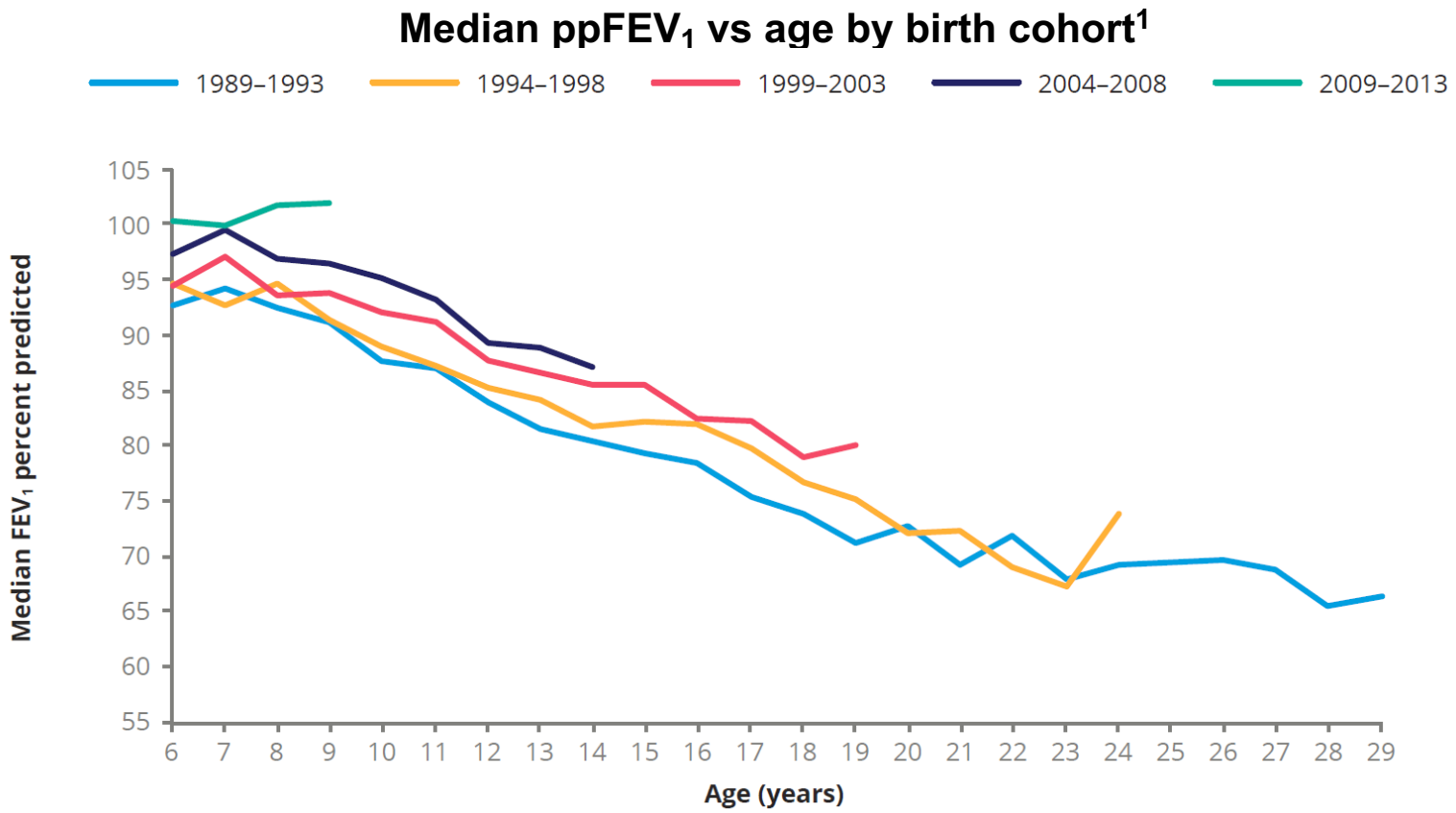
Limitations

- Not sensitive to small airway damage that occurs earlier in CF lung disease[6,7]
- Not sensitive to mild lung disease[6,7]
- FEV_1 values often do not correlate with more detailed assessments of pulmonary disease severity such as CT imaging[8,9]
- Spirometry can be difficult for young children to perform[10]
- Spirometry measurements are dependent on patient cooperation and effort[10]

CT, computed tomography; FDA, Food and Drug Administration; EMA, European Medicines Agency.

1. De Benedictis FM, et al. *Eur J Clin Pharmacol.* 2010;67(Suppl 1):49-59. 2. Liou TG, et al. *Am J Epidemiol.* 2001;153(4):345-352. 3. Yankaskas JR, et al. *Chest.* 2004;125(1 Suppl):1S-39S. 4. Corey M. *Proc Am Thorac Soc.* 2007;4(4):334-337. 5. Kent L, et al. *J Cyst Fibros.* 2014;13(2):123-138. 6. Gustafsson PM, et al. *Thorax.* 2008;63(2):129-134. 7. Horsley A, Siddiqui S. *Respirology*. 2015;20(1):33-45. 8. Brody AS, et al. *J Pediatr.* 2004;145(1):32-38. 9. de Jong PA, et al. *Eur Respir J.* 2004;23(1):93-97. 10. Aurora P, et al. *Am J Respir Crit Care Med.* 2004;169(10):1152-1159.

Limitations to the Use of FEV_1

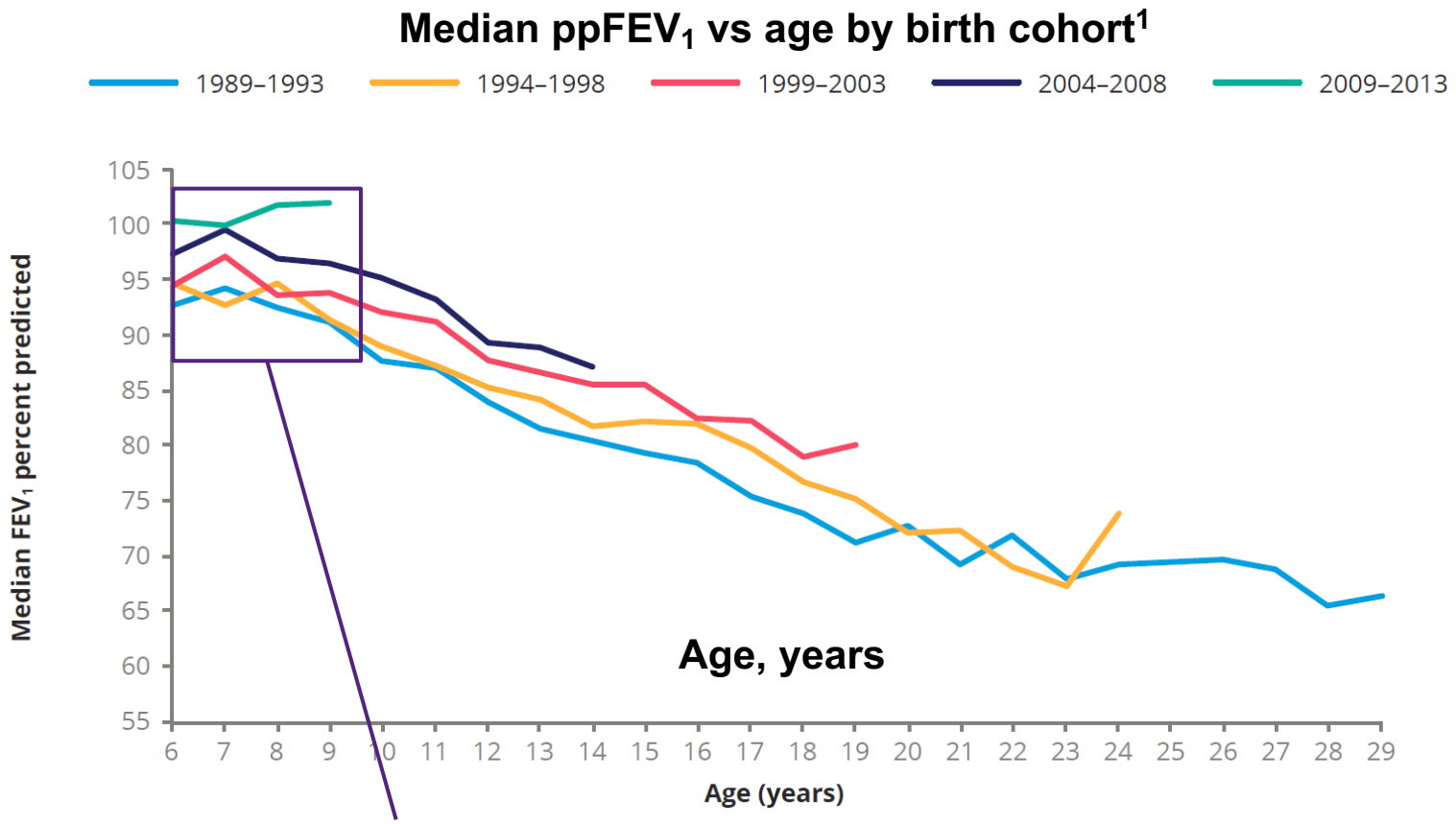


Spirometry is normally only used in patients aged 6 years or older. In infants spirometry often requires sedation[2] and can be difficult to perform in young children[3]

$ppFEV_1$, percent predicted forced expiratory volume in 1 second.

1. Cystic Fibrosis Canada. (2018). The Canadian Cystic Fibrosis Registry 2018 Annual Data Report 2. Linnane BM, et al. *Am J Respir Crit Care Med.* 2008;178(12):1238 1244. 3. Aurora P, et al. *Am J Respir Crit Care Med.* 2004;169:1152-1159.

Limitations to the Use of FEV_1 (cont)



FEV_1 is not sensitive to small airway damage that occurs early in disease[2,3] (small airways contribute ~10% of total airway resistance in healthy adults)[3]

Hence, in early CF, there can be considerable disturbance of large numbers of small airways, with relatively little effect on FEV_1[3]

$ppFEV_1$, percent predicted forced expiratory volume in 1 second.

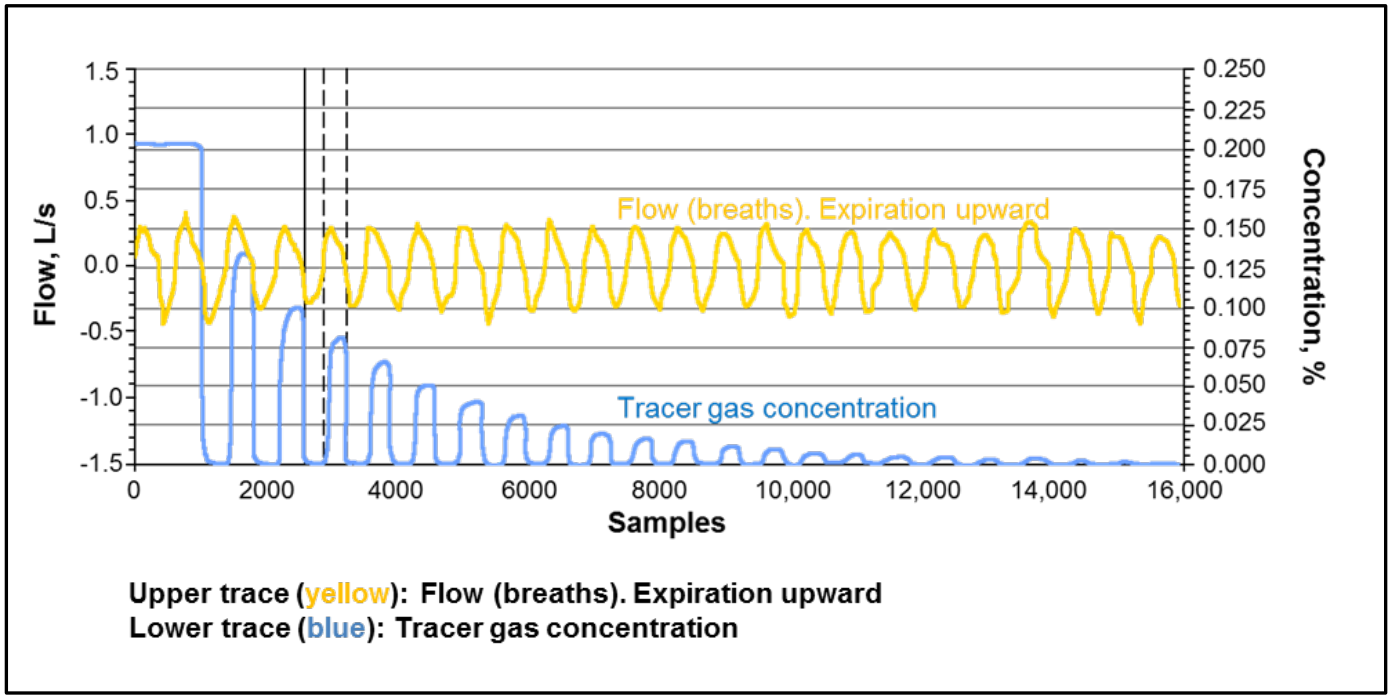
1. Cystic Fibrosis Canada. (2018). The Canadian Cystic Fibrosis Registry 2018 Annual Data Report. 2. Gustafsson PM, et al. *Thorax.* 2008;63(2):129-134. 3. Horsley A et al. *Respirology.* 2015;20(1):33-45.

“The ability to identify early airway dysfunction in these ‘silent years’, when FEV_1 is often within normal range, is of great importance for investigating new therapies in infants and young children and in those with mild disease”

Kent L, et al. *J Cyst Fibros*. 2014;13(2):123-138.

VERTEX

LCI Is a Measurement of Lung Function Capable of Detecting Early Airway Disease in CF



Reprinted from *Respiratory Medicine*, 103(6), Horsley A, Lung clearance index in the assessment of airways disease, 793-799, Copyright 2009, with permission from Elsevier.

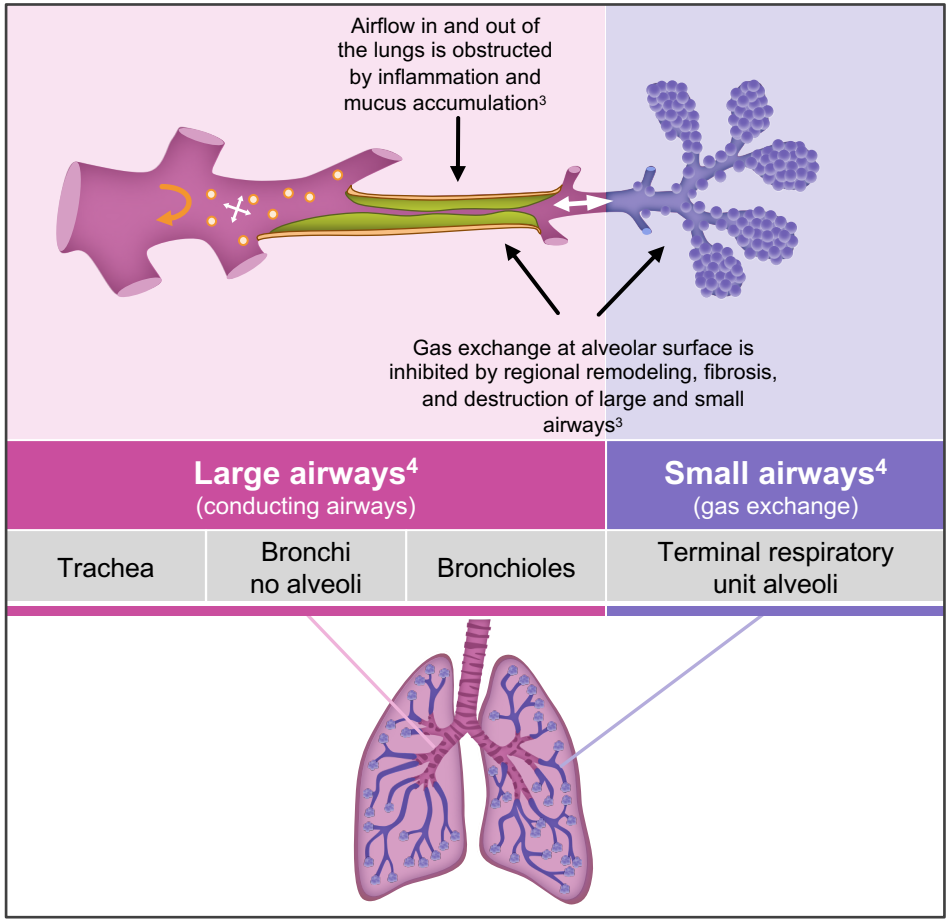
- LCI is a numerical value derived from multiple breath washout (MBW) test data
- LCI represents the number of breaths to reduce the tracer gas to a predefined concentration

LCI, lung clearance index.

Horsley A. *Respir Med*. 2009;103(6):793-799.

LCI Detects Early Airway Disease in CF

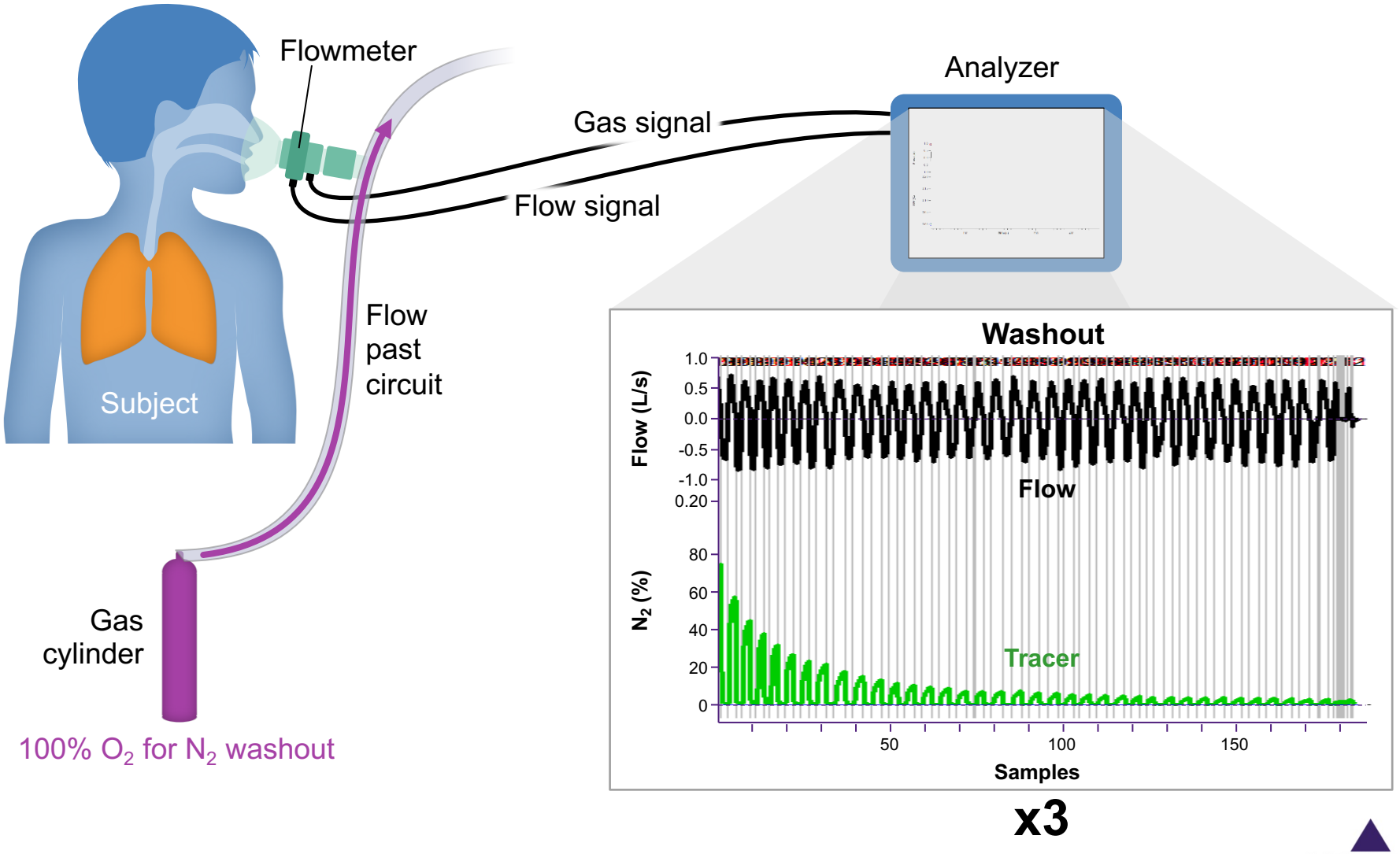
Why more breaths are required to clear tracer gas from CF lungs



- LCI is an indication of ventilation heterogeneity, based on how many breaths it takes to wash a tracer gas out of the lungs during tidal breathing[1]
- LCI is sensitive to damage in both large and small airways[2]
- LCI values increase with lung disease severity[1]
- LCI can detect early lung disease before a noticeable drop in spirometry measurements occurs[1]

1. Horsley A. *Respir Med.* 2009;103(6):793-799. 2. Verbanck S, et al. *J Appl Physiol.* 2012;112(5):782-790. 3. Horsley A, Siddiqui S. *Respirology*. 2015;20(1):33-45. The Human Respiratory System. In: Tu J, et al. *Computational Fluid and Particle Dynamics in the Human Respiratory System.* Springer, 2013; Ch. 2..

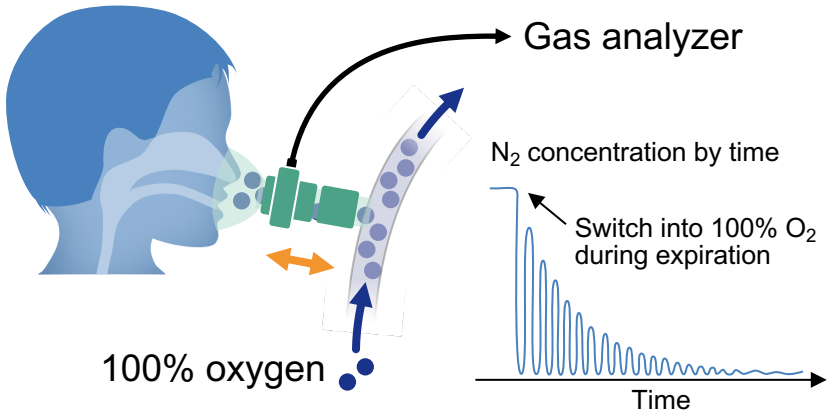
Performing an MBW Test[1,2]



1. Horsley A. *Respir Med.* 2009;103(6):793-799. 2. Subbarao P, et al. *Ann Am Thorac Soc.* 2015;12(6):932-939.

Tracer Gas Selection

Nitrogen (N_2)

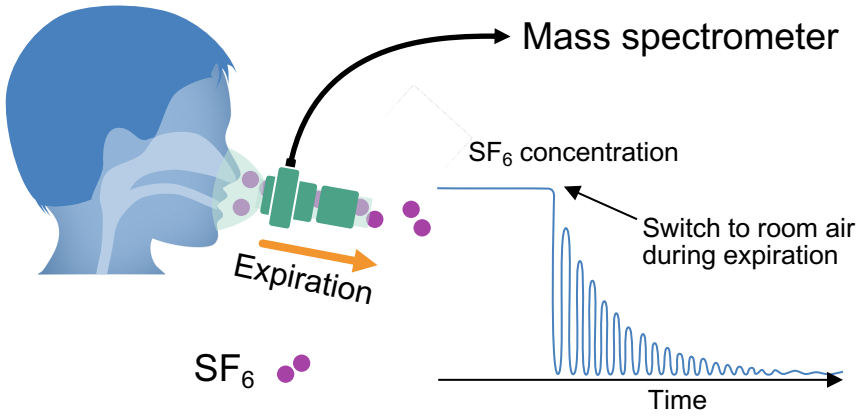


Wash-in
None (N_2 present in the lungs)[1]

Washout
Subjects breathe 100% oxygen (O_2)[1]

Limitation
In infants, O_2 may cause changes in breathing patterns and retinopathy in subjects at risk[2]

Sulfur Hexafluoride (SF_6)



Wash-in
SF_6 until equilibrium is reached at a known gas concentration[1]

Washout
Subjects breathe room air[1]

Limitation
Potent greenhouse gas – access is limited[1]

1. Subbarao P, et al. *Ann Am Thorac Soc.* 2015;12(6):932-939. 2. Schultzke SM, Frey U. *Eur Respir J.* 2013;41(3):500-502.

Strengths and Limitations of LCI

Strengths

- Sensitive to early airway disease before noticeable FEV_1 decline[1,2]
- Requires only passive tidal breathing (vs forceful exhalation)[2]
- Can be performed in infants and young children without the need for sedation and mechanical manipulation[2,3]
- Correlates with HRCT detection of lung structural changes[4]
- Correlates with risk for pulmonary exacerbation in patients with CF[5]
- Worsens with FEV_1 decline in patients with early and moderate lung disease[6]

Limitations

- Few centers have incorporated MBW into routine clinical practice[7]
- Longitudinal data are limited[7]
- Lack of guidelines and normative data to assist with interpreting results[7]
- Limited standardization for staff training, equipment, and analysis software[7]
- Minimum clinically important difference not yet established[8]
- Not practical for patients with advanced lung disease ($ppFEV_1$ <60) due to profound ventilation heterogeneity and extended wash-in and washout periods[8]

HRCT, high-resolution computed tomography.

1. Aurora P, et al. *Thorax*. 2004;59(12):1068-1073. 2. Robinson PD, et al. *Eur Respir J*. 2013;41(3):507-522. 3. Lum S, et al. *Thorax*. 2007;62(4):341-347. 4. Ellemunter H, et al. *Respir Med*. 2010;104(12):1834-1842. 5. Vermeulen F, et al. *Thorax*. 2014;69(1):39-45. 6. Kraemer R, et al. *Am J Respir Crit Care Med*. 2005;171(4):371-378. 7. Subbarao P, et al. *Ann Am Thorac Soc*. 2015;12(6):932-939. 8. Horsley A, Siddiqui S. *Respirology*. 2015;20(1):33-45.

LCI as an Outcome Measure in CF

Discriminating between disease and health
Detecting early lung disease
Tracking early disease progression
Correlations with lung imaging modalities
Correlations with local and systemic inflammatory markers
Correlation with risk for pulmonary exacerbations

LCI as an Outcome Measure in CF

Discriminating between disease and health
Detecting early lung disease
Tracking early disease progression

VERTEX

LCI Discriminates Between CF and Healthy Controls

Author	CF, Non-CF (n)	Subjects	LCI Results[1]
Hoo	71, 54	Infants	*P*=0.002
Lum	39, 21	Infants	*P*<0.001
Belessis	47, 25	Infants and children	*P*<0.001
Belessis	30, 25	Infants and children	*P*<0.001
Aurora	48, 45	Preschool	*P*<0.001
Singer	73, 50	Children	*P*<0.001
Amin	17, 28	Children	*P*<0.001
Keen	45, 35	Children	*P*<0.001
Aurora	22, 33	Children	*P*<0.001
Aurora	30, 30	Children	*P*<0.001
Owens	56, 52	Children	*P*<0.001
Gustafsson	43, 28	Children (<18 y)	*P*<0.001
Pittman	5, 10	Children	*P*=NS
Fuchs	68, 38	Children	*P*<0.001
Horsley	18, 29	Children	*P*=0.022
Bakker	15, 15	Children	*P*<0.001
Fuchs	26, 22	Children (<18 y)	*P*<0.001
Fuchs	10, 8	Children (<10 y)	*P*=0.009
Fuchs	139, 102	Children and adults	*P*<0.001
Gustafsson	18, 25	Adults	*P*<0.001
Verbanck	25, 25	Adults	*P*<0.001
Horsley	22, 17	Adults	*P*<0.0001
Horsley	33, 48	Adults	*P*<0.001

- The European Cystic Fibrosis Society Clinical Trial Network Standardization Committee undertook a review of LCI as a clinical endpoint[1]
- They found that, overall, 22 out of 23 studies demonstrated the ability of LCI to discriminate between individuals with CF and healthy, non-CF subjects[1]
- The 23rd study did not reach statistical significance due to suspended enrollment with a low number of participants after experiencing complications associated with the MBW device[2]

1. Kent L, et al. *J Cyst Fibros.* 2014;13(2):123-138. 2. Pittman JE, et al. *Pediatr Pulmonol.* 2012;47(12):1242-1250.

VERTEX

LCI Discriminates Between CF and Healthy Controls (cont)

Mean LCI values of patients with CF across multiple studies

Age Range	LCI
11-44 years	15[1]
>10 years	14.6[2]
8-17 years	14.0[1]
5-19 years	13.7[1]
17-49 years	13.1[3]
9-20 years	12.8[1]
7-18 years	12.4[1]
12-56 years	11.9[1]
6-16 years	11.53[4]
8-18 years	10.1[5]
8-17 years	9.9[1]
41.4 (22.0) weeks	8.4[6]

- Common range in healthy individuals is from 5 to 9
  - Normal values relatively consistent from infancy to adulthood[8]
- Higher values indicate worse lung function[9]
  - Patients with CF have a wide range of LCI depending on severity of disease

ULN LCI values of controls across multiple studies

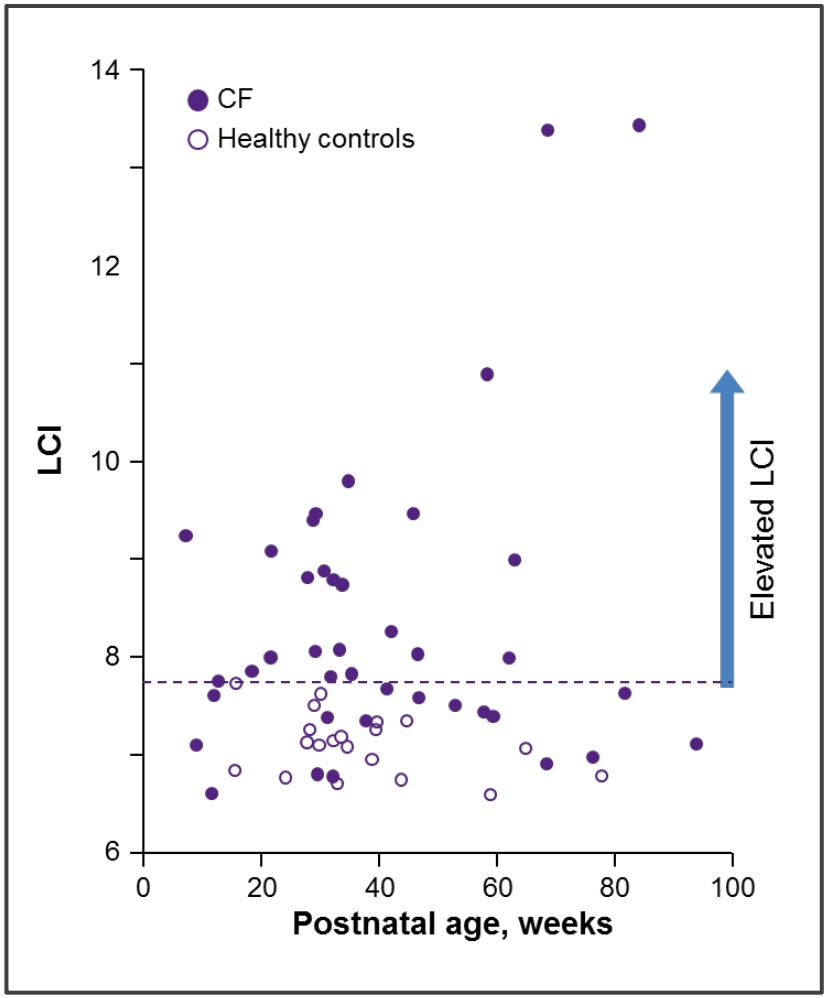
LCI	Age Range
8.2	4.5 (1.4) weeks[7]
8.0	5.1 (0.8) weeks[7]
7.8	19-58 years[3]
7.7	2-6 years[7]
7.5	5-16 years[7]
7.3	6-16 years[7]
7.1	4-18 years[7]
7.1	6-16 years[3]
7.0	5-20 years[7]

ULN, upper limit of normal.

1. Sonneveld N, et al. *ERJ Express.* 2015;46(4):1055-1064. 2. Horsley AR, et al. *Thorax.* 2013;68(6):532-539. 3. Horsley AR, et al. *Thorax.* 2008;63(2):135-140. 4. Aurora P, et al. *Thorax.* 2004;59(12):1068-1073. 5. Robinson PD, et al. *Pediatr Pulmonol.* 2009;44(8):733-742. 6. Lum S, et al. *Thorax.* 2007;62(4):341-347. 7. Fuchs SI, et al. *Paediatr Respir Rev.* 2011;12(4):264-270. 8. Lum, S et al. *Eur Respir J.* 2013;41(6):1371-1377. 9. Horsley A. *Respir Med.* 2009;103(6):793-799.

LCI Detects Early Airway Disease in CF

In Infants



Lum S et al. *Thorax*. 2007;62(4):341-347. Reprinted with permission.

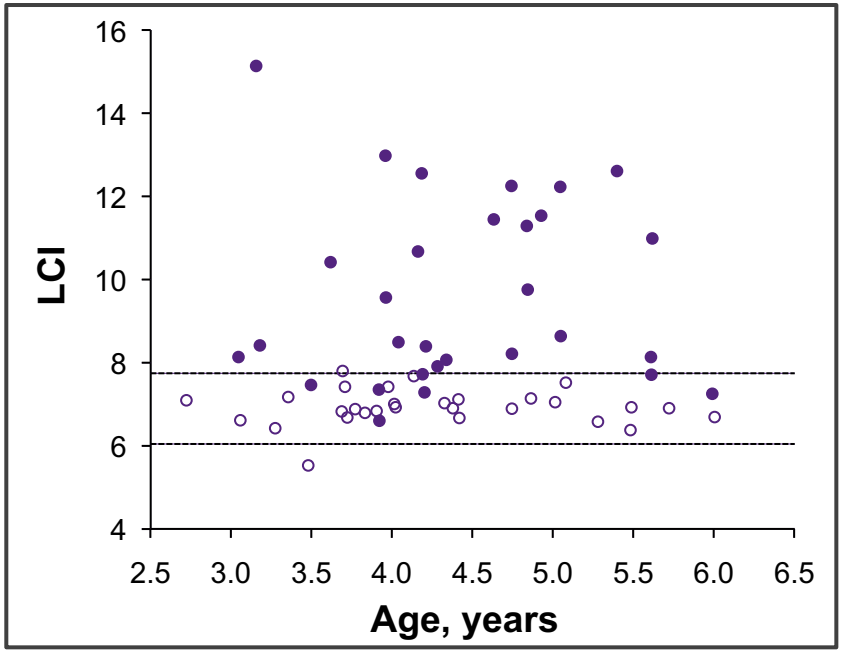
- Measurements obtained in 39 infants with CF and 21 healthy controls
- MBW performed during quiet sleep, before forced expiratory maneuvers
- LCI was significantly increased in CF vs healthy controls

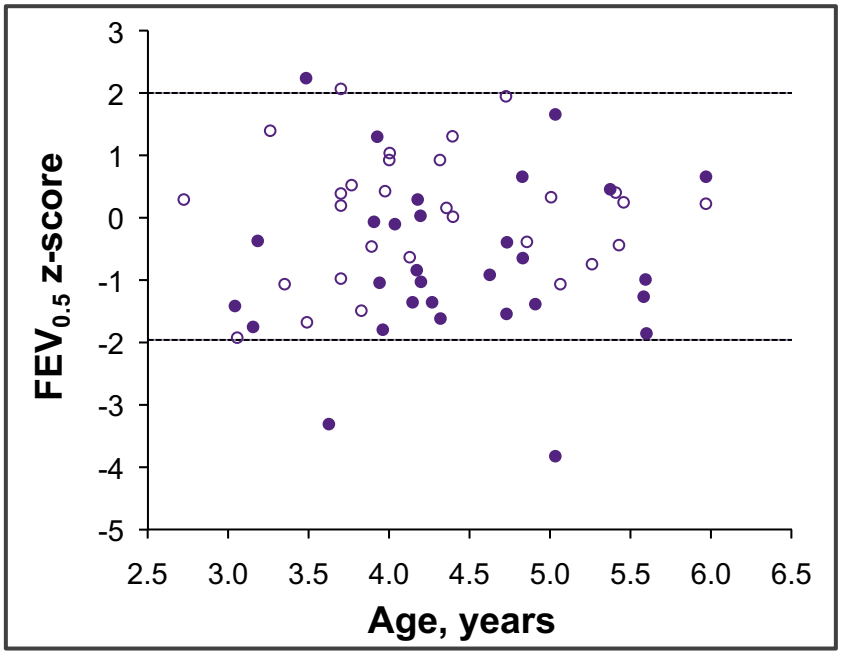
Measure	CF (n=39)	Healthy Controls (n=21)
Age, weeks, mean (SD)	41.4 (22.0)	37.0 (15.1)
LCI, mean (SD)	8.4 (1.5)	7.2 (0.3)
LCI >7.8[a] n (%)	22 (56.4)	0

[a]7.8 was set as the ULN LCI based on the mean LCI in healthy controls of 7.2 (0.3).
Lum S, et al. *Thorax*. 2007;62(4):341-347.

LCI Detects Early Airway Disease in CF

In Preschool Age Children



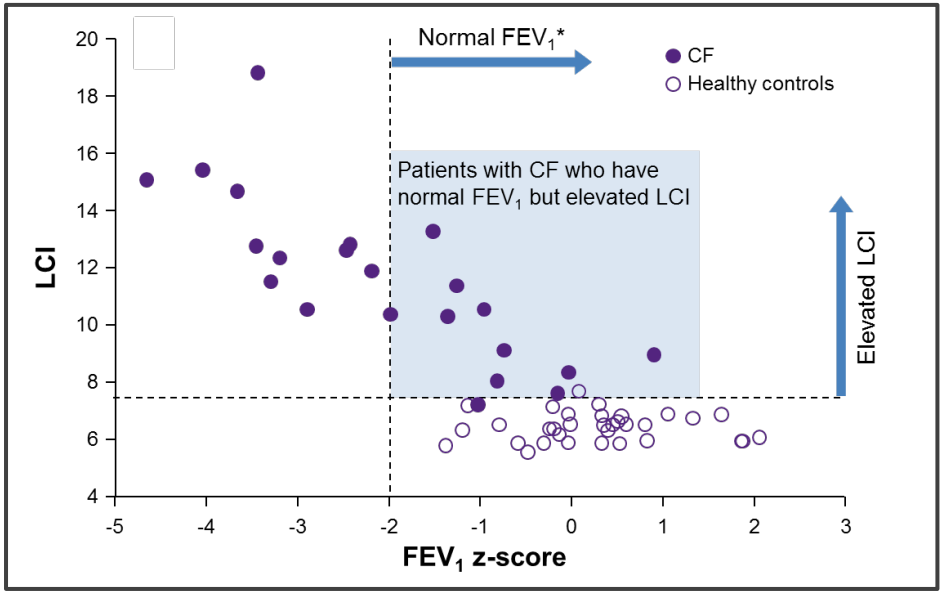


- Measurements obtained in 30 children with CF (aged 2-5 years) and 30 age-matched healthy controls
- Results suggest that LCI is superior to spirometry at detecting abnormal lung function in children aged 2-5 years with CF
  - LCI identified abnormal lung function in a higher proportion of children with CF than spirometry (73% vs 13%)
  - LCI was superior to $FEV_{0.5}$ z-score at identifying children infected with *Pseudomonas aeruginosa*

Horizontal lines show upper and lower limits of normal. $FEV_{0.5}$ z-scores were calculated from reference equations published by Nystad et al, 2002.
Aurora P, et al. *Am J Respir Crit Care Med.* 2005;171:249.

LCI Detects Early Airway Disease in CF

In Children Before Noticeable FEV_1 Decline



Aurora P et al. *Thorax.* 2004;59(12):1068-1073. Reprinted with permission,

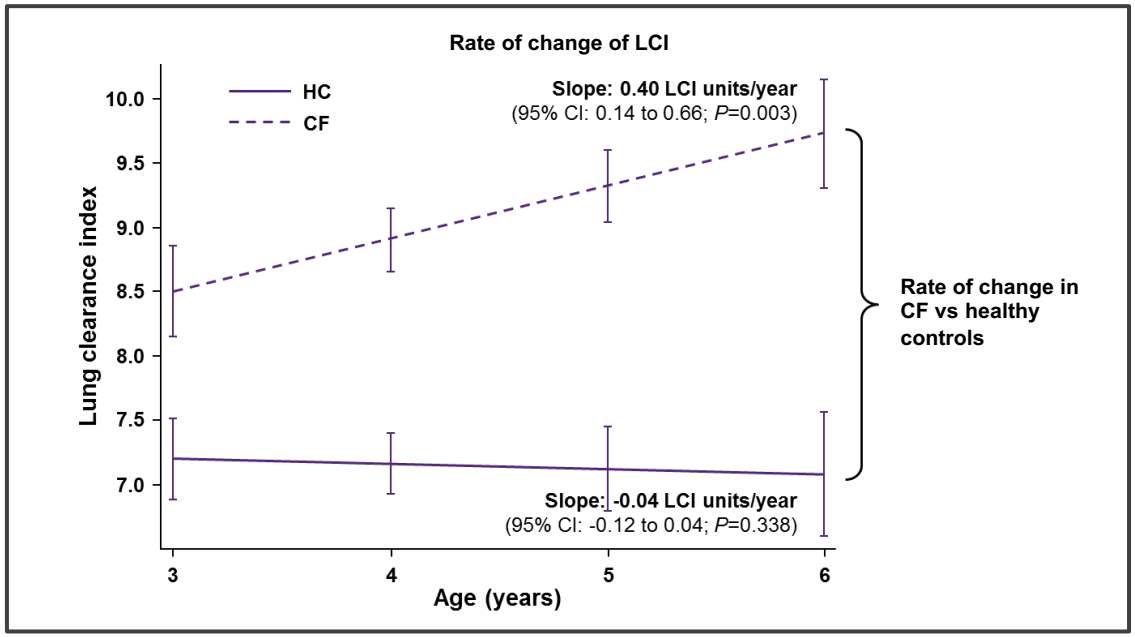
- Measurements obtained in 22 children (aged 6-16 years) with CF and 33 healthy controls
- Results suggest that LCI is superior to spirometry at detecting irregular lung function in children with CF, identifying abnormalities in 95% of children vs the 50% identified with spirometry

*FEV_1 results were converted into standard deviation scores (z-scores) using published reference data from Rosenthal M (*Thorax*, 1993) and Freeman JV (*Arch Dis Child*, 1995), with a z-score of less than -1.96 being categorized as abnormal.

Aurora P, et al. *Thorax.* 2004;59(12):1068-1073.

Utilizing LCI to Track Lung Disease Progression

In Preschool Age Children With CF

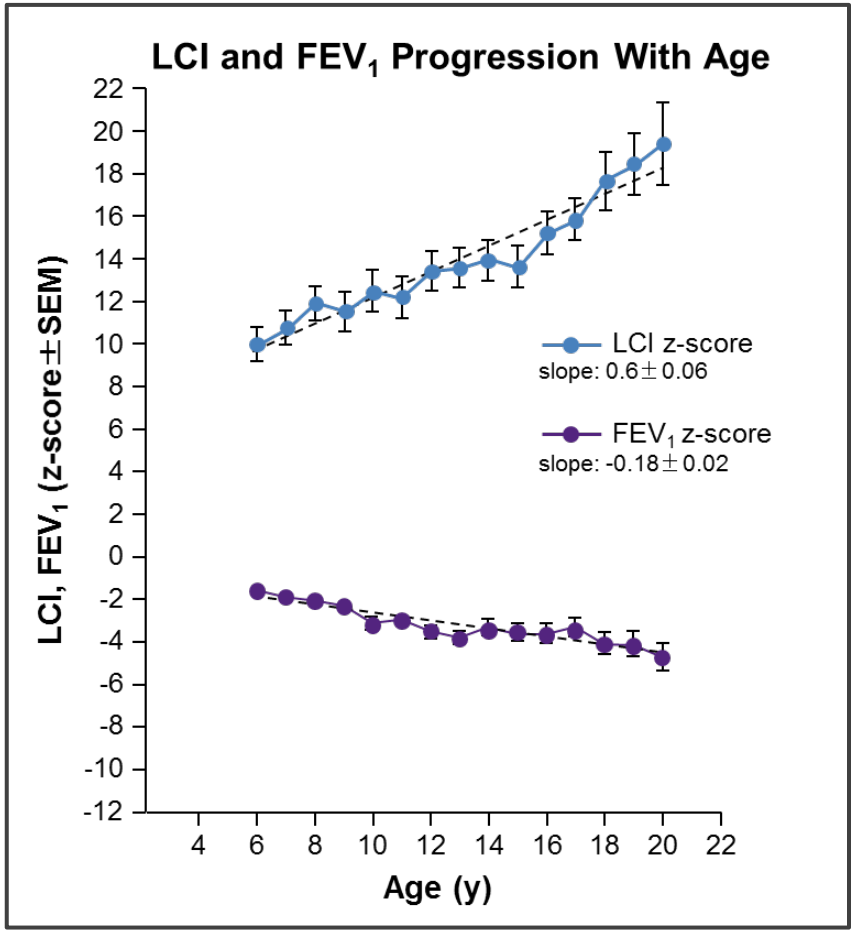


- Data were collected on 156 participants aged 2.5 to 6 years with 800 LCI measurements (78 with CF, 78 age-matched healthy controls)
- The LCI worsened during cough episodes as well as pulmonary exacerbations in children with CF, whereas similar symptoms in healthy children did not impact LCI values
- Results suggest that while both LCI and spirometry discriminated health from disease, only the LCI identified significant deterioration of lung function in CF over time (figure above)

Stanojevic S, et al. *Am J Respir Crit Care Med.* 2017;195(9):1216-1225.

Utilizing LCI to Track Lung Disease Progression

In Preschool Age Children to Adulthood



- Data based on serial lung function measurements performed in 142 children with CF, aged 6 to 20 years
- LCI z-score inversely associated with FEV_1 z-score (figure)
- Results suggest that LCI is more sensitive than spirometry at detecting early disease progression in this population

Kraemer R, et al. *Am J Respir Crit Care Med.* 2005;171(4):371-378.

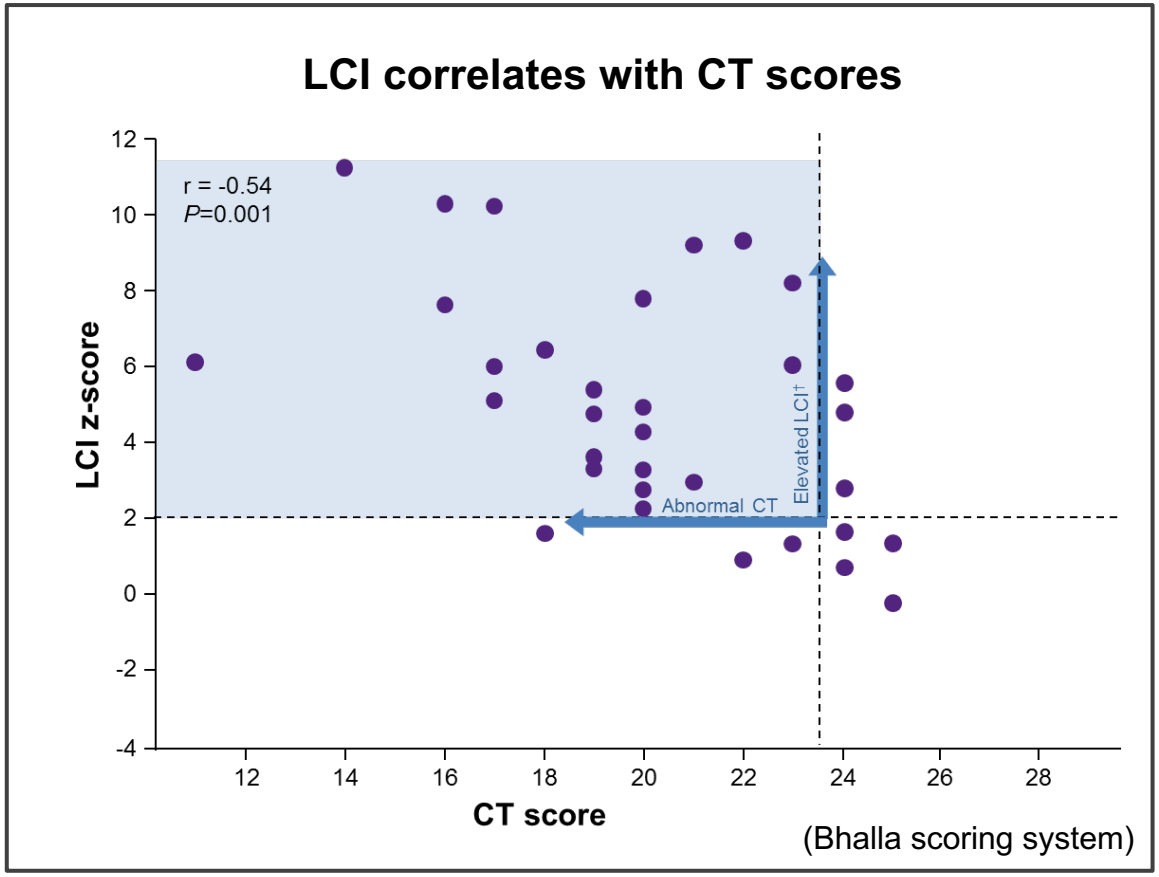
LCI as an Outcome Measure

LCI correlates with lung imaging modalities

LCI Correlates With Lung Imaging Techniques

Computed Chest Tomography

- Thirty-four patients with CF (aged 6-26 years) with ppFEV$_1$ >80 were analyzed by MBW and CT
- Abnormal LCI z-scores and CT scores correlated significantly in 82.3% of subjects
- Structural changes (assessed by CT) were unlikely if LCI was normal (figure)
- Results suggest that diagnostic accuracy of LCI for detecting lung disease in patients with normal ppFEV$_1$ is good based on CT comparison

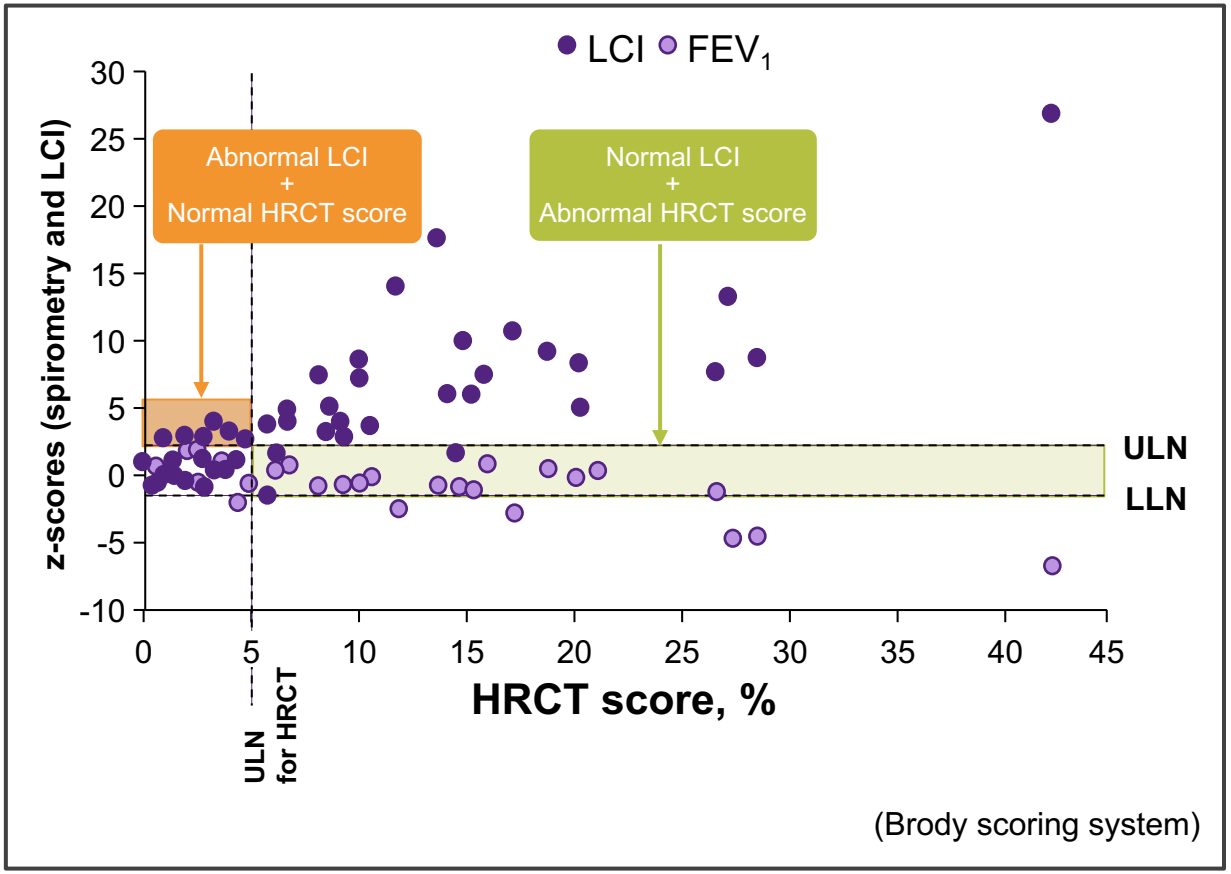


Reprinted from *Respiratory Medicine*, 104(12), Ellemunter H et al, Sensitivity of lung clearance index and chest computed tomography in early CF lung disease, 1834-1842, Copyright 2010, with permission from Elsevier.

LLN for CT score > 23.
ULN for LCI = 2.
Average annual ppFEV$_1$ >80 with at least 4 measurements per year was the cutoff used to screen patients in the center database for eligibility. FEV$_1$ in the study was reported as a z-score.
Ellemunter H, et al. *Respir Med.* 2010;104(12):1834-1842.

LCI Correlates With Lung Imaging Techniques

High Resolution Computed Chest Tomography



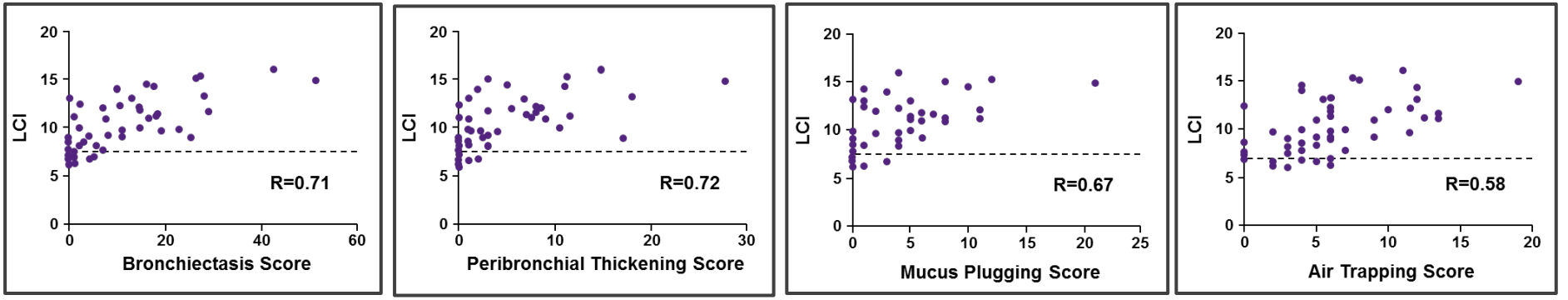
Gustafsson PM et al. *Thorax.* 2008;63(2):129-134. Reprinted with permission.

- Forty-four patients with CF (aged 5-19 years) with ppFEV$_1$ ranging from 44% to 127% were analyzed by MBW, spirometry, and HRCT
- LCI correlated better with HRCT scores (R_s +0.85) than did FEV$_1$ (-0.62)
- LCI is more sensitive than FEV$_1$ for detecting structural lung disease in CF
- A normal LCI almost excludes HRCT abnormalities (figure, green box)
- Authors suggest that in some patients LCI may be even more sensitive than HRCT for detecting lung involvement in CF (figure, orange box)

LLN, lower limit of normal; R_s, Spearman rank correlation coefficient.
The Brody scoring system was used in this study
Gustafsson PM, et al. *Thorax.* 2008;63(2):129-134.

LCI Correlates With Lung Imaging Techniques

High Resolution Computed Chest Tomography (cont)



Owens CM et al. *Thorax.* 2011;66(6):481-488. Reprinted with permission.

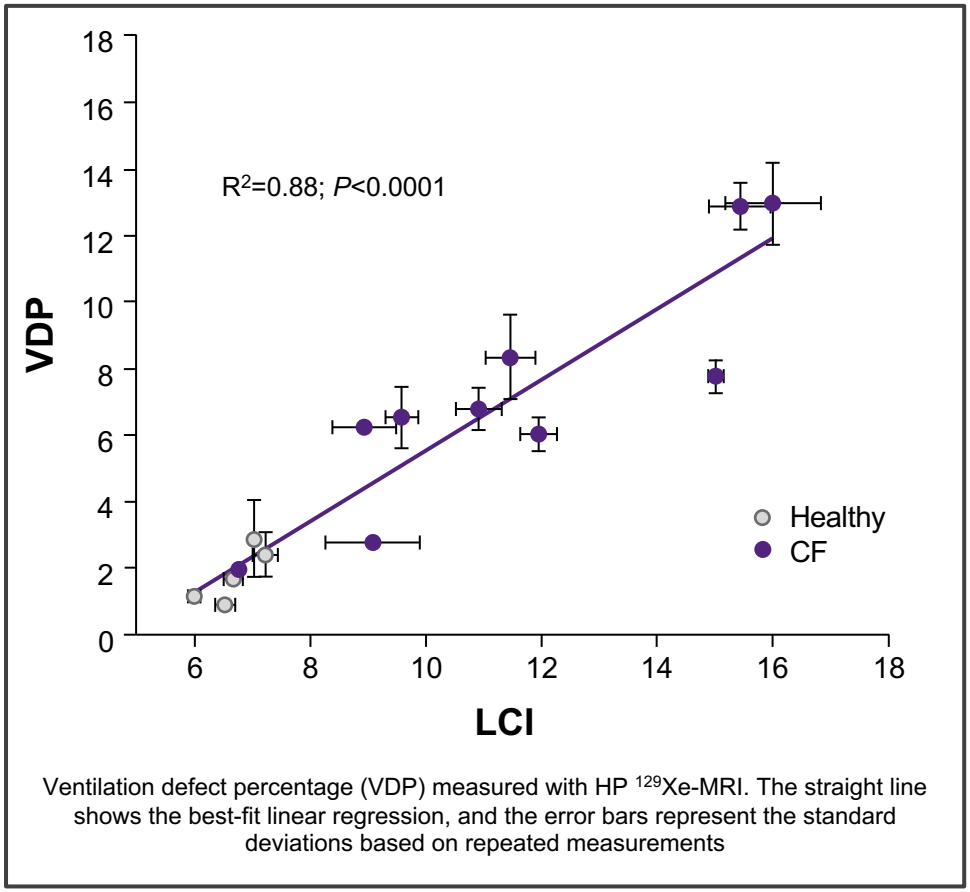
(Brody II Scoring System)

- Data were collected in 60 clinically stable children with CF (mean [SD] age 7.8 [1.3] years) and mean (SD) $ppFEV_1$ of 81.6 (18.3)
- Total CT score correlated more strongly with LCI (Spearman correlation = 0.77) than with spirometry (R = -0.43) or any other marker of lung function
- In addition to the relation with total CT score, LCI was significantly related to all individual elements except the parenchyma score (figure, parenchyma score data not shown)
- Results suggest that LCI and HRCT exhibit similar sensitivity to detect CF lung disease in this population

$ppFEV_1$ calculated using the methods of Stanojevic et al, 2010.
Owens CM, et al. *Thorax.* 2011;66(6):481-488.

LCI Correlates With Lung Imaging Techniques

Hyperpolarized ^{129}Xe-MRI



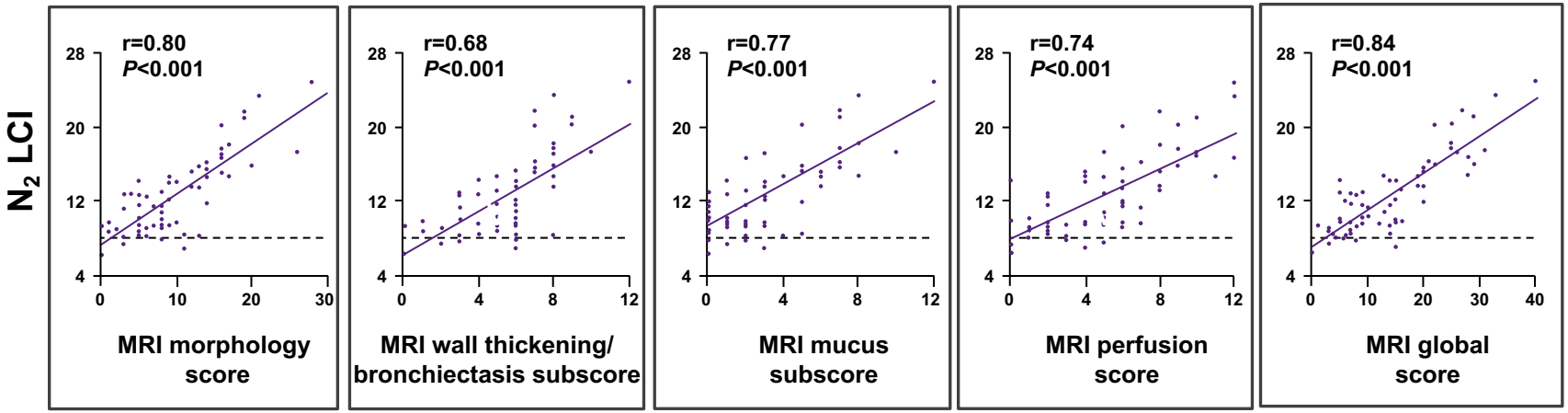
Ventilation defect percentage (VDP) measured with HP ^{129}Xe-MRI. The straight line shows the best-fit linear regression, and the error bars represent the standard deviations based on repeated measurements

- Eighteen participants (aged 8-17 years): 10 clinically stable patients with CF and 8 healthy age-matched volunteers with mean (SD) ppFEV$_1$ at baseline of 84 (14) and 101 (18), respectively
- A significant and strong correlation was observed between VDP and LCI for all subjects (R^2=0.88, *P*<0.0001)

Kanhere N, et al. *Am J Respir Crit Care Med.* 2017 Feb 28. doi: 10.1164/rccm.201611-2228LE.

LCI Correlates With Lung Imaging Techniques

T1 MRI ± Contrast, T2 MRI, and Perfusion MRI



- Sixty-six clinically stable children with CF and mean (SD) age 11.9 (4.4) years and ppFEV$_1$ 83.1 (24.1), plus 25 children with CF and pulmonary exacerbation, age 13.9 (4.5) years and ppFEV$_1$ 65.4 (22.1)
- LCI showed strong correlations with the MRI morphology score (r = 0.80), and subscores for wall thickening/bronchiectasis (r = 0.68), mucus plugging (r = 0.77), abnormal perfusion (r = 0.74), and the MRI global score (r = 0.84)
- MRI provides additional information on the nature and regional distribution of abnormalities that contribute to ventilation inhomogeneity in different disease settings

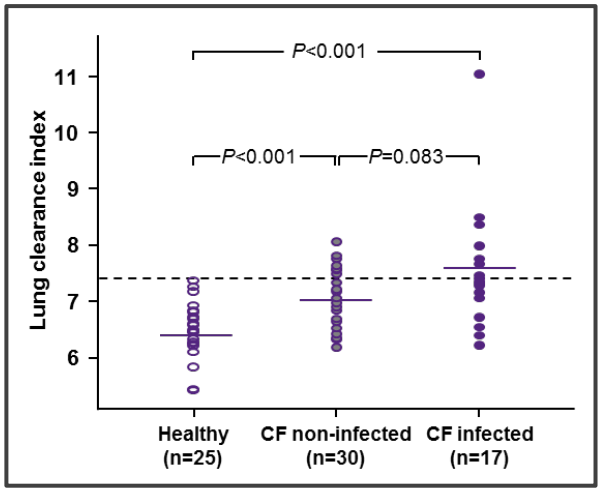
MRI, magnetic resonance imaging.
Source of reference equation for baseline ppFEV$_1$ was not specified.
Stahl M, et al. *Am J Respir Crit Care Med.* 2017;195(3):349-359.

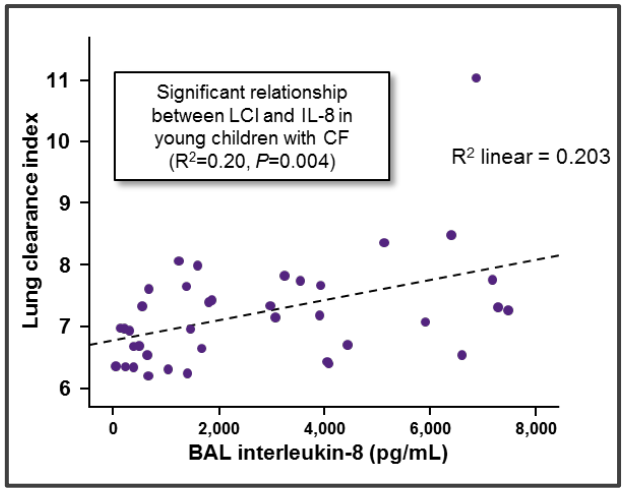
LCI as an Outcome Measure

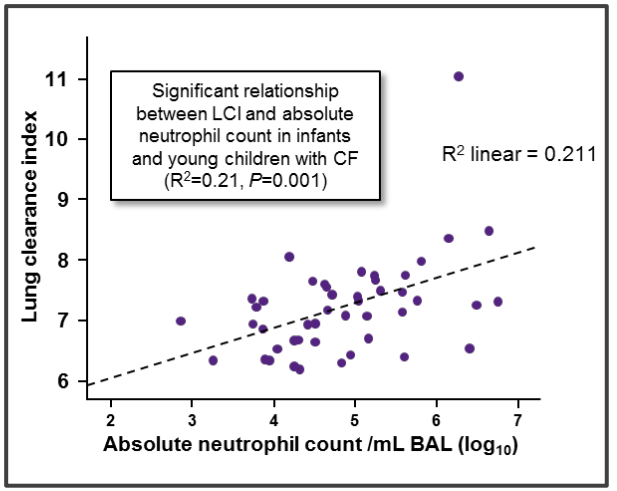
Correlations with local and systemic inflammatory markers
Correlation with risk for pulmonary exacerbations

LCI Correlates With Early CF Inflammation and Infection

Pseudomonas Colonization and Inflammatory Markers in the BAL







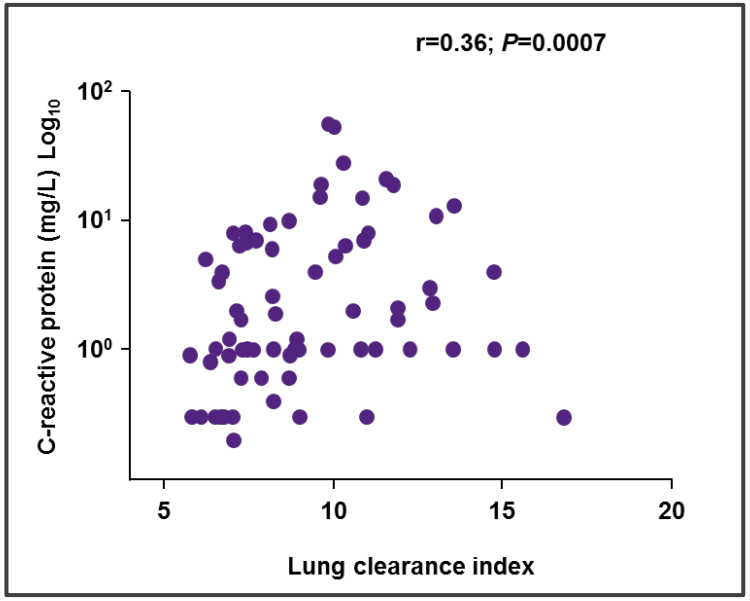
- Forty-seven children with CF (mean [SD] age 1.55 [0.76] years) and 25 healthy children (mean [SD] age 1.26 [0.69] years) had technically acceptable LCI data
- LCI was significantly higher in infants and children with *P. aeruginosa* compared with children with CF without *P. aeruginosa* (*P*=0.083, left figure)
- LCI correlated with BAL IL-8 (R^2=0.20, *P*=0.004) and neutrophil count (R^2=0.21, *P*=0.001)
- An LCI below the ULN had a high negative predictive value (93%) in excluding *Pseudomonas*

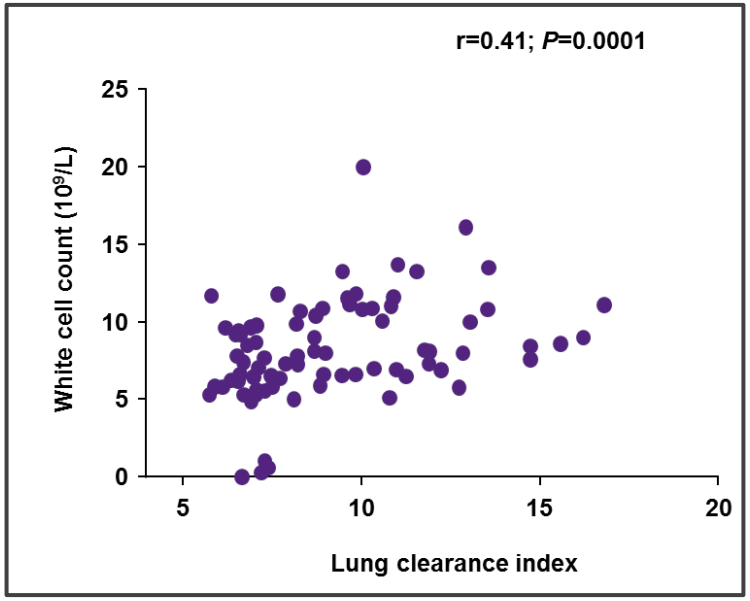
BAL, bronchoalveolar lavage.

Belessis Y, et al. *Am J Respir Crit Care Med.* 2012;185(8):862-873.

LCI Correlates With Early CF Inflammation

Inflammatory Markers in Peripheral Blood





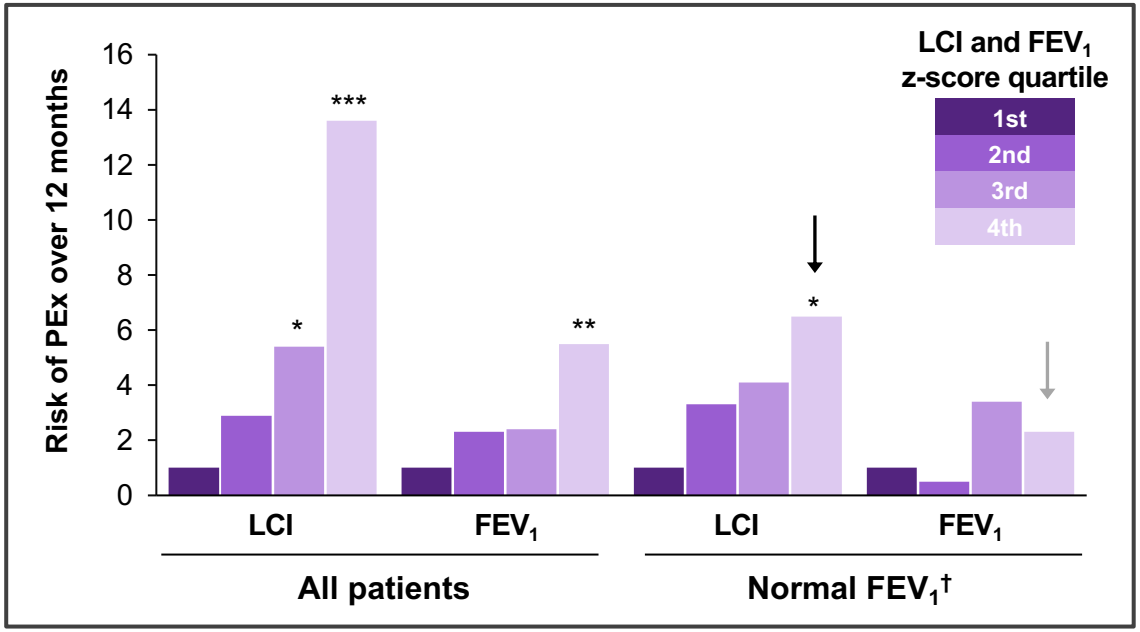
Reprinted with permission from Stuart Elborn, MD, Queen's University Belfast, Belfast, Northern Ireland.

- Data collected from 83 stable patients with CF with mean (SD) age 23.1 (14.6) years and $ppFEV_1$ 77.8 (19.8)
- LCI significantly correlated with CRP and white blood cell counts
- $ppFEV_1$ significantly correlated with CRP and white blood cell counts

CRP, C-reactive protein.
Source of reference equation for baseline $ppFEV_1$ was not specified.
Elborn SJ, et al. *Eur Respir J.* 2014;44(Suppl 58):P1206.

VERTEX

LCI Predicts Risk for Pulmonary Exacerbations

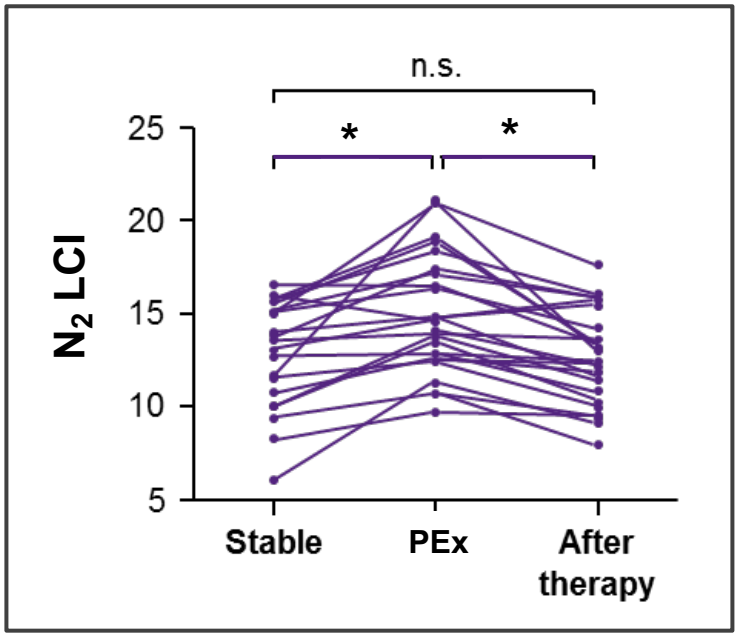


- Sixty-three patients with CF aged 5 to 19 years (median 12.4 years) prospectively followed for 1 year
- Twenty-six patients (41%) experienced 48 PExs
- Baseline LCI z-score (figure, black arrow) was more sensitive than FEV_1 z-score (figure, grey arrow) to predict the occurrence of a PEx over 12 months, especially in the subgroup of patients (n=53) with normal FEV_1 z-score

PEx, pulmonary exacerbation.
*P<0.05; **P<0.01; ***P<0.001; †FEV_1 z-score lower than -2 was considered abnormal.
FEV_1 was expressed as the percent predicted or as z-scores using reference equations from the Global Lung Function Initiative.
Vermeulen F, et al. *Thorax.* 2014;69(1):39-45.

LCI Correlates With Pulmonary Exacerbations

Pulmonary Exacerbations and Post-Treatment Resolution



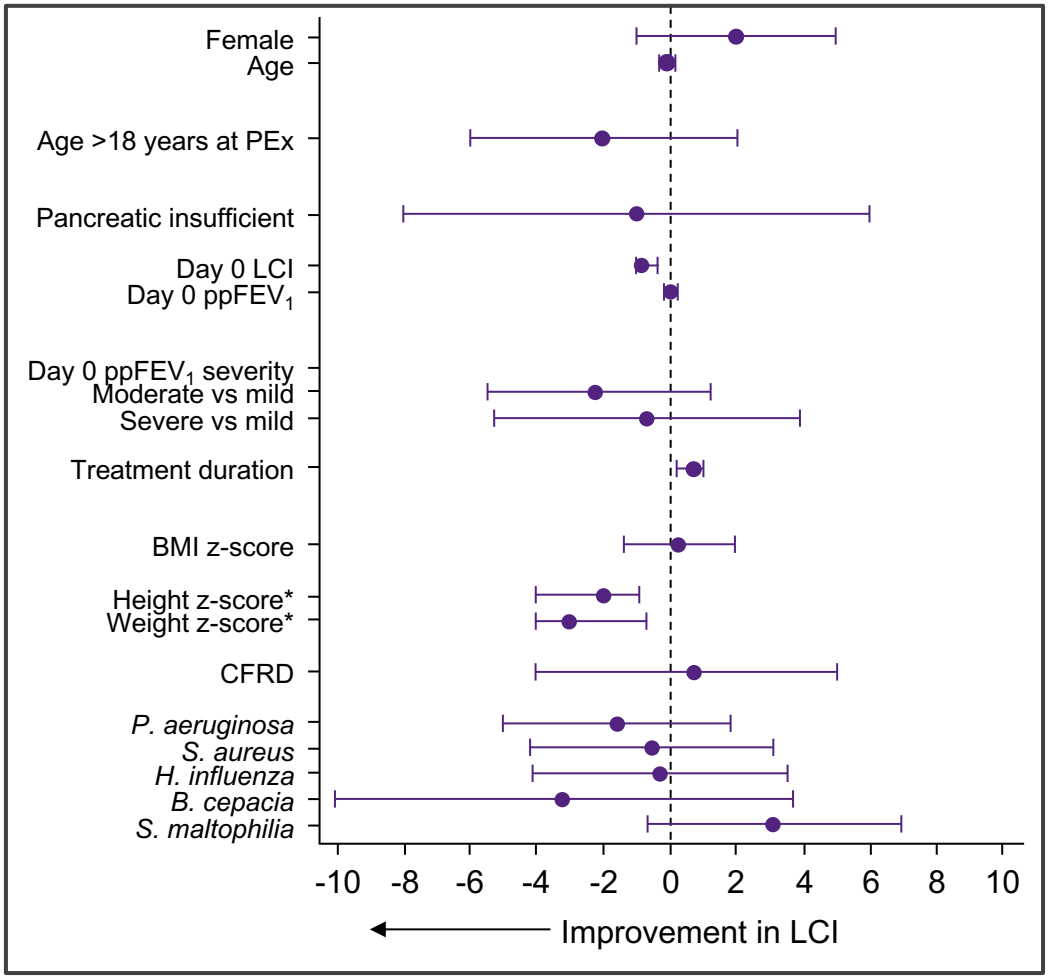
- Twenty-five children with CF, mean (SD) age 13.9 (4.5) years and ppFEV$_1$ 65.4 (22.1)
- Baseline assessments in clinically stable condition were done 3 to 6 months before PEx
- At the time of PEx, mean absolute LCI was significantly increased by 2 units (>10%; *P*<0.001) and returned to baseline at short-term follow-up after antibiotic therapy

*P<0.001 compared with stable CF N_2 group.

Stahl M, et al. *Am J Respir Crit Care Med.* 2017;195(3):349-359.

LCI Correlates With Pulmonary Exacerbations

Pulmonary Exacerbations and Post-Treatment Resolution (cont)



- Data from 6 published and one unpublished study representing 176 PEx events from 5 pediatric datasets, two with both adult and pediatric data, median age 15 years (range: 5-56)
- Overall LCI decreased by 0.40 units (95% CI, -0.60 to -0.19; *P*=0.004) or 2.5% following treatment with IV antibiotics, but effect size was small
- Relative changes in LCI and ppFEV$_1$ were significantly correlated, but were discordant in 42.5% of subjects
- Minimum clinically important difference in LCI has yet to be defined

Sonneveld N et al. *Eur Respir J.* 2015;46(4):1055-1064. Reprinted with permission.

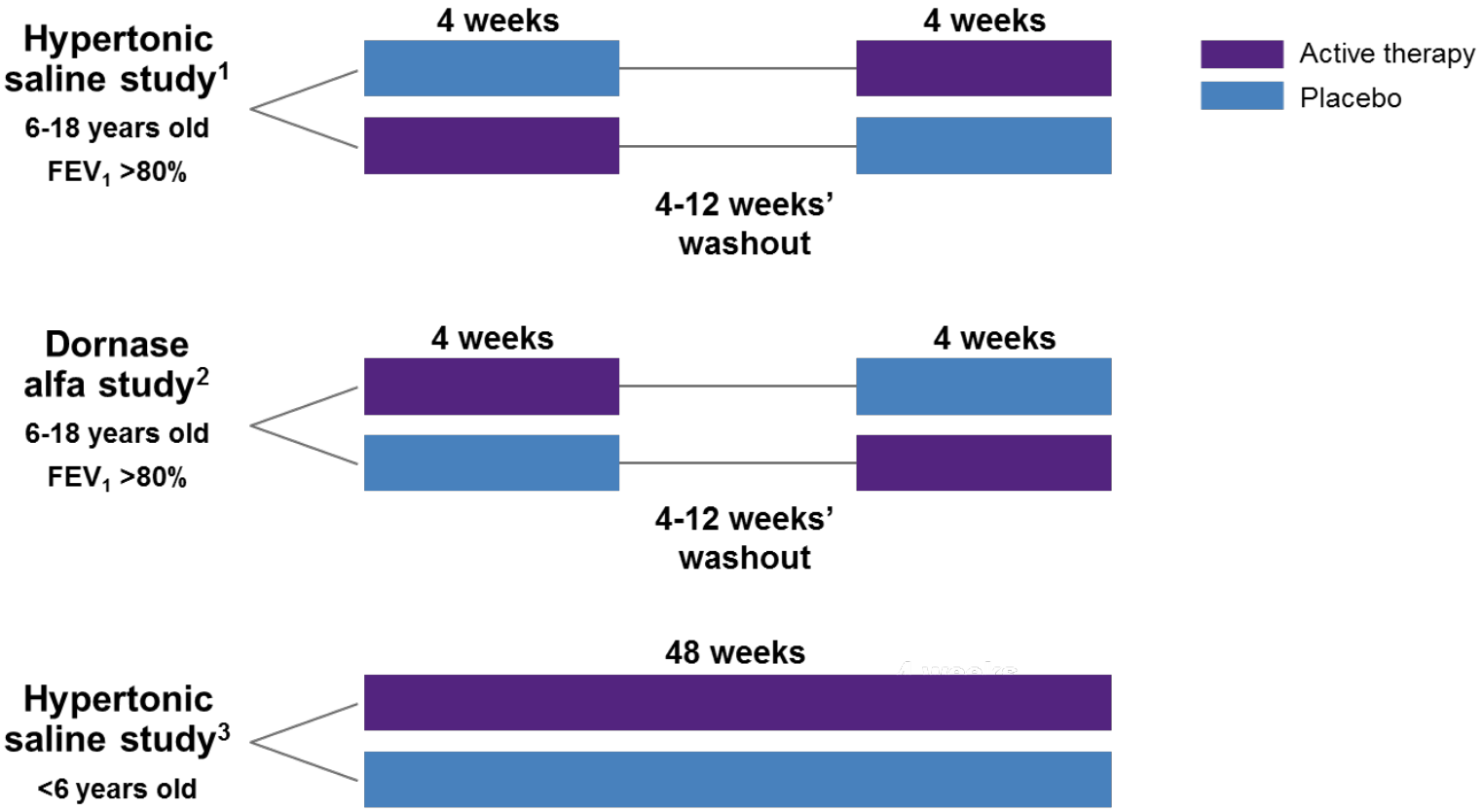
Univariate predictors of LCI change following 2 weeks of IV antibiotic treatment. For continuous variables (age, day 0 LCI, day 0 ppFEV$_1$, treatment duration, and nutritional z-scores), results correspond to the average LCI change following treatment for each 1-unit increase in each continuous variable

BMI, body mass index; CFRD, cystic fibrosis–related diabetes.
*Calculated for pediatric patients only.
Sonneveld N, et al. *Eur Respir J.* 2015;46(4):1055-1064.

The Use of LCI as an Outcome Measure in CF Interventional Clinical Trials

LCI as an Outcome in CF Therapeutic Trials

Trial Designs

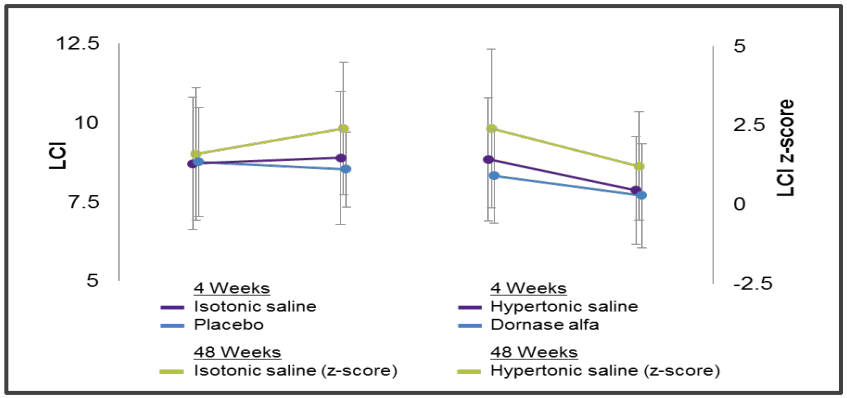


1. Amin R, et al. *Thorax.* 2010;65(5):379-383. 2. Amin, R et al. *Eur Resp J.* 2011;37(4):806-812. 3. Subbarao P, et al. *Am J Respir Crit Care Med.* 2013;188(4):456-460.

LCI as an Outcome in CF Therapeutic Trials

Results

- Data from three trials evaluating impact of 4- or 48-week hypertonic saline (HS) or dornase alpha treatment[1-3]
- Both agents demonstrated statistically significant impacts on reducing LCI[1-3]
- Treatment effects ranged from 0.9 to 2.01[1-3]



Reference	N	Treatment	Age Range	Treatment Effect on LCI	*P* Value
Amin 2010[1]	20	Hypertonic saline	6-18 years (Mean ±SD: 10.5±3.1)	1.16±0.94 (95% CI, 0.27-2.05)	0.016
Amin 2011[2]	17	Dornase alfa	6-18 years (Mean ±SD: 10.32±3.35)	0.90±1.44	0.022
Subbarao 2013[3]	25	Hypertonic saline	<6 years (Median [range]: 2.6 [0.34-4.95])	2.01 (95% CI, 0.26-3.76)	0.025

SAFETY

- Adverse events were more frequent with hypertonic saline than with isotonic saline, including increased sputum production, fever, rhinorrhea, malaise, and cough[1]
- Adverse events reported more frequently with dornase alpha than with placebo were cough (new or increased), pulmonary exacerbation, and dry throat[2]
- No serious adverse events were reported in either study[1,2]

1. Amin R, et al. *Thorax.* 2010;65(5):379-383; 2. Amin, R et al. *Eur Resp J.* 2011;37(4):806-812. 3. Subbarao P, et al. *Am J Respir Crit Care Med.* 2013;188(4):456-460.

Interventional Clinical Trials With LCI as an Outcome Measure Currently Listed on ClinicalTrials.gov

- 21 clinical trials listed in total; 3 have been terminated and 1 has an unknown status
- Of the remaining 17 trials, 10 are interventional clinical trials

ClinicalTrials.gov website search using terms “LCI,” “Cystic Fibrosis,” and “Multiple Breath Washout” (April 2020): https://clinicaltrials.gov/ct2/results?cond=Cystic+Fibrosis&term=LCI%2C+cystic+fibrosis%2C+Multiple+Breath+Washout&cntry=&state=&city=&dist=

VERTEX

Evaluating the Clinical Significance of LCI Outcomes

- A minimum clinically significant difference has yet to be established for LCI[1]
- Consensus statements suggest that treatment effect may be considered clinically significant if it is larger than the difference in LCI measurements observed between repeat measurements without intervention or change in clinical status (ie, “Coefficient of Repeatability” or “Coefficient of Reliability for Replicate Measures”)[1,2]
- The Coefficient of Repeatability for the MBW assay using nitrogen gas and a commercial device is 0.6 in healthy children and 0.96 in children with CF[3]
- Coefficient of variation (CV=SD/mean x 100) expressed as a percentage is also used as a measure of within-test and occasionally between-test repeatability,[2,4,5] and a CV >10% may suggest lack of reliability[2]
- Another variable that is often considered when evaluating the clinical significance of LCI results is how closely they compare with outcomes obtained for drugs known to be efficacious in CF[1]
- Finally, and similar to any outcome measure, an evaluation of clinical significance should take into account trial design, treatment duration, and sustainability of effect[1,6]

Sonneveld N, et al. *ERJ Express.* 2015;46(4):1055-1064. 2. Kent L, et al. *J Cyst Fibros.* 2014;13(2):123-138. 3. Singer F, et al. *Pediatr Pulmonol.* 2013;48(8):739-746. 4. Aurora P, et al. *Am J Respir Crit Care Med.* 2005;171(3):249-256. 5. Horsley AR, et al. *Thorax.* 2008;63(2):135-140. 6. Subbarao P, et al. *Ann Am Thorac Soc.* 2015;12(6):932-939

Summary

- LCI is sensitive to early airway disease before noticeable ppFEV$_1$ decline
- LCI can be performed in infants and young children without the need for sedation and mechanical manipulation
- LCI values have been demonstrated to correlate with
  - Spirometry measures including ppFEV$_1$
  - Lung disease progression
  - Imaging modalities such as HRCT and MRI
  - Inflammatory markers in the BAL such as bacterial colonization, IL-8, CRP, as well as neutrophil and white blood cell counts
- LCI has been evaluated as an outcome measure in several trials in CF, demonstrating significant treatment effects with agents such as dornase alfa and hypertonic saline
- The magnitude of response defined as “clinically significant” must still be established
- LCI is poised to become a valuable tool in the clinic to monitor early-stage CF lung disease, pending validated commercial equipment and standardization of procedures

VERTEX

Backup

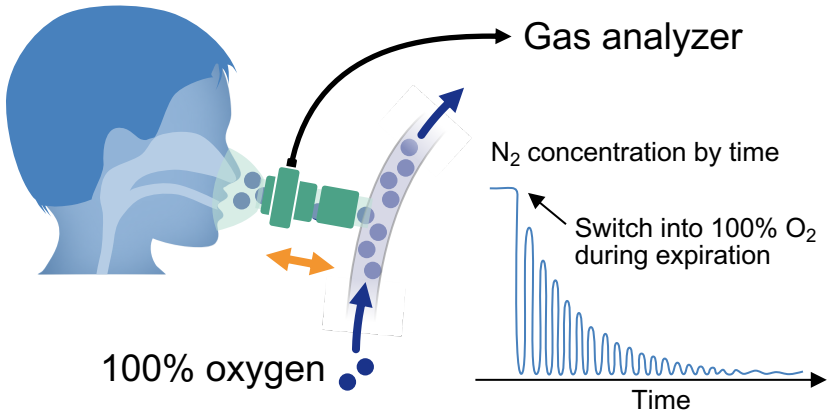
Variables That Impact LCI Results

Variables That Impact LCI Results

- Tracer gas selection
- Equipment used
- Timing of physiotherapy
- Body position of the patient

Tracer Gas Selection

Nitrogen (N_2)

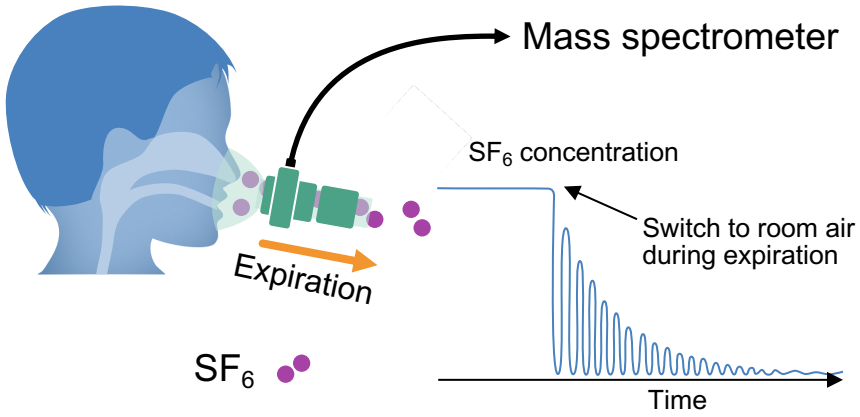


Wash-in
None (N_2 present in the lungs)[1]

Washout
Subjects breathe 100% oxygen (O_2)[1]

Limitation
In infants, O_2 may cause changes in breathing patterns and retinopathy in subjects at risk[2]

Sulfur Hexafluoride (SF_6)



Wash-in
SF_6 until equilibrium is reached at a known gas concentration[1]

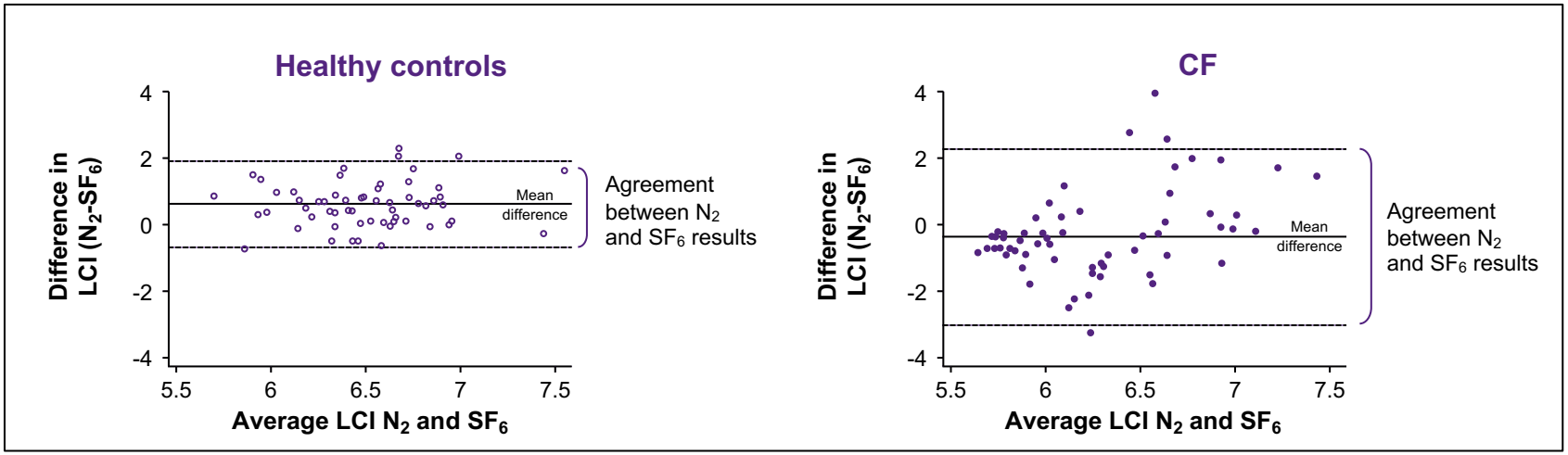
Washout
Subjects breathe room air[1]

Limitation
Potent greenhouse gas – access is limited[1]

1. Subbarao P, et al. *Ann Am Thorac Soc.* 2015;12(6):932-939. 2. Schultzke SM, Frey U. *Eur Respir J.* 2013;41(3):500-502.

Tracer Gas Selection

N_2 produces disproportionately higher results in CF vs healthy controls



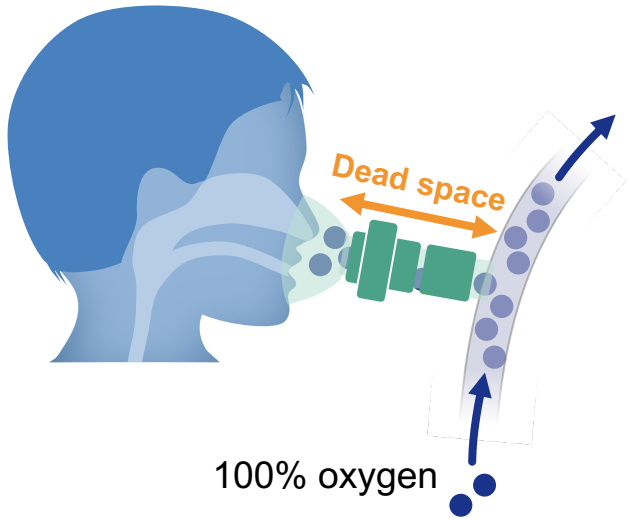
Reprinted from *PLoS One*, 8(2), Jensen R et al, Multiple breath nitrogen washout: a feasible alternative to mass spectrometry, e56868, Copyright 2013.

- On average, N_2 generates higher LCI values than SF_6
- In CF, the difference between the N_2 and the SF_6 systems was double that in health with a clear bias toward higher LCI N_2 compared to LCI SF_6 at higher mean values of LCI

Jensen R, et al. *PLoS One.* 2013;8(2):e56868.

Equipment Used for MBW

Equipment

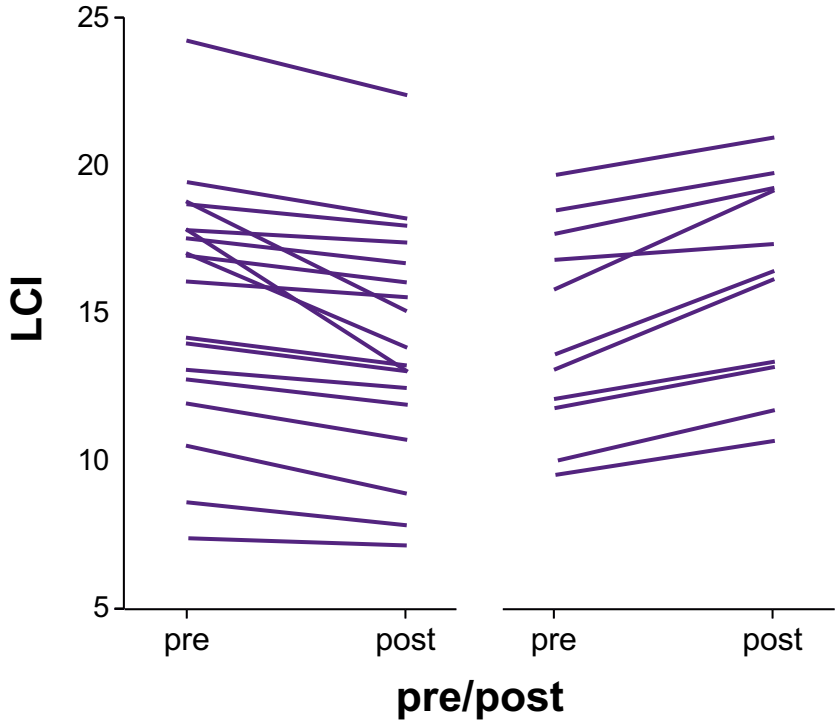


Dead space
Volume of area between subject's mouth and fresh gas stream[1]

- LCI can vary depending on equipment used[1,2]
- N_2 MBW systems have a larger dead-space volume (total 58.2 mL) than SF_6 MBW systems (total 15.4 mL)[1]
- Increased dead space results in higher LCI[1]
- Consensus recommendations pertaining to equipment dead space[2]
  - A facemask is to be used with infants and preschoolers
  - A nose clip and mouthpiece is to be used with older subjects
  - Upper limits for equipment dead space are specified
- It has been suggested that an adjustment be made for equipment dead space when calculating LCI[1]

1. Benseler A, et al. *Respirology.* 2015;20(3):459-466. 2. Robinson PD, et al. *Eur Respir J.* 2013;41(3):507-522.

Effect of the Timing of Physiotherapy on LCI



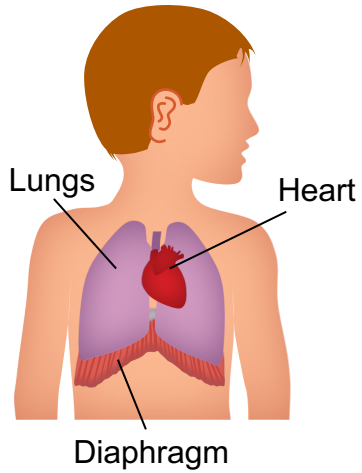
LCI before (pre) and after (post) physiotherapy in 18 patients with a decrease and 11 patients with an increase.

- The short-term effects of physiotherapy on LCI are unpredictable[1]
- One study says that timing of physiotherapy does not change LCI,[2] while another suggests that the short-term impact is variable[1]
- Researchers may reduce variability in LCI measurements by keeping the timing of physiotherapy in relation to MBW procedures consistent[3]

1. Pfleger A, et al. *J Cyst Fibros.* 2015;14(5):627-631. 2. Fuchs SI, et al. *Pediatr Pulmonol.* 2010;45(3):301-306. 3. Welsh L, et al. *J Cyst Fibros.* 2014;13(6):687-691.

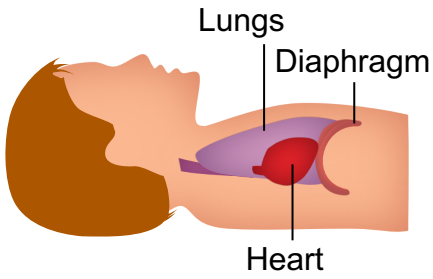
Effect of Body Position of the Patient During MBW on LCI

Body position impacts the pressure within peripheral airways



Upright

- LCI is predominantly generated by intraregional ventilation heterogeneities[1]



Supine

- Functional residual volume is decreased in the supine position[2]
- Ventilation heterogeneity is increased[2] and LCI includes an interregional component[1]

- LCI is affected by body position[1,2]
- Positional changes can impact LCI outcomes in certain situations
  - When comparing LCI in infants vs toddlers
  - When performing longitudinal studies where infants become toddlers

1. Verbanck S, et al. *J Appl Physiol.* 2012;112(5):782-790. 2. Ramsey KA, et al. *J Cyst Fibros*. 2017 Feb 7. pii: S1569-1993(17)30020-6. doi: 10.1016/j.jcf.2017.01.013. [Epub ahead of print]