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Variability of clinically measured lung clearance index in children with cystic fibrosis

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Abstract

Rationale

The lung clearance index (LCI) is increasingly being used in the clinical surveillance of patients with cystic fibrosis (CF). However, there are limited data on long-term variability and clinically relevant changes in LCI during routine clinical surveillance.

Objectives

To evaluate long-term variability of LCI and propose a threshold for a physiologically relevant change.

Methods

Children with CF aged 4-18 years performed LCI measurements every three months as part of routine clinical surveillance during 2011-2020 in two centers. The variability of LCI during periods of clinical stability was assessed using mixed-effects models and was used to identify thresholds for clinically relevant changes.

Results

Repeated LCI measurements of acceptable quality (N= 858) were available in 100 patients with CF, for 74 patients 399 visits at clinical stability were available. Variability of repeated LCI measurements over time expressed as coefficient of variation (CV%) was 7.4%. The upper limit of normal (ULN) for relative changes in LCI between visits was 19%.

Conclusion

We report the variability of LCI in children and adolescents with CF during routine clinical surveillance. According to our data, a change in LCI beyond 19% may be considered physiologically relevant. These findings will help guide clinical decisions according to LCI changes.

Introduction

The multiple breath washout (MBW) is a sensitive method to detect early small airway disease in patients with cystic fibrosis (CF) [1]. The main outcome parameter, the lung clearance index (LCI), quantifies the efficiency of gas distribution within the lungs. Muco-obstructive lung disease in the small airways leads to uneven gas distribution, reflected by an elevated LCI [2-4]. Despite being a sensitive and feasible parameter in the assessment of CF lung disease [2, 5, 6], lacking knowledge on clinically relevant changes in routine measurements limits its clinical application [7, 8].

Short-and mid-term variability of LCI has been examined previously and has reported changes in LCI above 17-27% to be considered clinically relevant [8-10]. However, these studies generally included small numbers, narrow age ranges, or did not include data from clinical routine visits and did not extend beyond two years of observation time, which limits their direct application in clinical settings. As LCI has been shown to increase with age [11], it is important to know how variability of LCI evolves in routine clinical measurements extending beyond two years of observation time. In addition, the recently described substantial sensor-crosstalk error in a commercially available and widely used multiple-breath washout (MBW) device (Exhalyzer D, Eco Medics AG, Duernten, Switzerland) and its associated software

Spiroware 3.2.1 [12] could highly influence short,-mid and long-term variability of LCI and limits the comparability of older variability analyses for current measurements with the updated software version Spiroware 3.3.1.

Since 2011, we have collected quarterly MBW measurements in children with CF as part of the routine clinical surveillance program at the University Children's Hospitals Bern and Zürich. We reanalyzed every measurement in the corrected and updated software version Spiroware 3.3.1. We aimed to evaluate the long-term variability of LCI and define thresholds for physiologically relevant changes in LCI from MBW measurements collected in the clinical setting.

Methods

Study design

This was a longitudinal, observational, multi-center study in children with CF between four to 18 years attending quarterly outpatient clinical surveillance visits at the Bern University Children's Hospital, Switzerland, and the Zurich University Children's Hospital, Switzerland, between 01/01/2011 and 01/01/2020 (STROBE guidelines [13] in Online Supplement). Written informed consent was obtained from patients and caregivers and the study was approved by the local ethics committees (EKNZ 2017-00088).

Clinical data in children with CF

Clinical data and information on respiratory symptoms were collected at outpatient visits conducted by a pediatric pulmonologist in a standardized way. Data analysis was performed retrospectively by structured chart review. Pulmonary exacerbations

were defined if at least two of the modified Fuchs criteria (change in sputum volume or color, increased cough, increased malaise, fatigue or lethargy, anorexia or weight loss, increased dyspnea or decrease in FEV_1 > 10%) provided by the EuroCareCF working group were present (see Online Supplemental). [14]. Pulmonary exacerbations were assessed independently of the consecutive treatment decision. Further, we assessed whether the definition of clinical stability by the modified Fuchs criteria influences variability by calculating variability indexes for visits without respiratory symptoms (cough-negative visits).

Lung function

Nitrogen MBW tests were performed in routine clinical surveillance using the Exhalyzer D MBW device (Eco Medics AG, Duernten, Switzerland) and reanalyzed using the most recent Spiroware software version 3.3.1 according to current consensus [12, 15] (For further details, see Online Supplemental). Spirometry (Jaeger MasterScreen, CareFusion, Hochberg, German) was performed after MBW following ATS/ERS guidelines [16, 17].

1.1.1 Statistical analysis

To determine long-term variability of LCI in the overall cohort, we included only patients contributing at least two consecutive visits (8-16 weeks apart, reflecting a standard routine observation interval).

To determine variability in clinically stable patients, we included only patients with at least one period at clinical stability, defined as at least two consecutive visits (8-16 weeks apart) without pulmonary exacerbations (Figure 1). We used mixed-effects linear regression models to calculate variability for LCI and FEV_1 indices (Table E1),

corrected for correlation of repeated measurements within individuals (see Online Supplemental). To assess variability for absolute changes in LCI, linear mixed models were fitted for absolute differences of subsequent measurements to obtain mean and standard deviation and the coefficient of repeatability (CR). We used the estimated intercept and residual standard deviation to calculate the coefficient of variation (CV%) and intraclass correlation coefficient (ICC). To assess variability for relative changes in LCI, we calculated upper limit of normal (ULN) for relative differences (95% quantile of a normal distribution), based on a log-linear model, indicating a threshold below which 95% of the relative differences are expected to fall. Changes above these thresholds could be considered beyond test variability and thus physiologically relevant.

As one considers visit-to-visit changes within individuals in clinical practice, we explored between-visit changes in LCI for each patient individually. We calculated ULN for relative changes for every patient with at least two or at least three consecutive stable visits. These limits are the variance of the log-transformed outcome (LCI) and the 95% quantile of a normal distribution. They represent the threshold below which 95% of relative differences between measurements in a given patient are expected to fall; thus indicating changes above this threshold being clinically relevant for this patient. Statistical analyses were performed using Stata 16.0 (StataCorp 2019), graphs created using Stata 16.0 or Graph Pad Prism.

Results

1.1.2 Study population

One hundred children with CF (Bern: N=75, Zurich: N=25) between four and 18 years were followed clinically and performed MBW measurements between January 2011 and January 2020. Only nine patients were on highly effective modulator therapy (2 Ivakaftor, 7 Ivakaftor/Tezakaftor). Each patient was followed clinically for an average of 4.8 years (range 0.2 – 8.2 years). The feasibility of MBW measurements was 83% (1188/1428 visits). In total, 858 visits from 100 patients met the inclusion criteria of at least two consecutive measurements 4-16 weeks apart (Figure 1). From these data, 533 (62%) visits were at clinical stability and 325 (38%) were during pulmonary exacerbations. Seventy-four patients contributed at least one period of clinical stability (≥ 2 visits without pulmonary exacerbation), leading to 147 stable periods (duration on average 28 weeks (range 8.8 – 80.9 weeks, consisting of 2-7 visits). Number of visits and patients contributing to a stable period are summarized in Supplemental Figure 1. Demographical characteristics are summarized in Table 1.

Long-term variability of LCI and clinically relevant changes

Variability for repeated LCI measurements for the overall CF cohort and only for patients at clinical stability is summarized in Table 2. The absolute mean difference in LCI between visits was 0.1 LCI units (1.1 SD) for all visits and 0.02 (0.6) for clinically stable periods, which indicates a high reproducibility of quarterly LCI measurements. The ICC (range 0-1) was 0.8 for all visits and 0.9 for clinically stable periods, reflecting high similarity between measurements within one individual. The variability around

the mean LCI across all patients over the entire study period is quantified by the CV. The CV in our dataset was 9.8% in all visits and 7.4% in clinically stable periods.

For absolute changes in LCI, the CR indicates a threshold below 95% of the absolute differences between measurements are expected to fall. In clinically stable CF patients, CR was 1.4 LCI units, suggesting that a change higher than 1.4 LCI units should be considered to be above test variability.

In clinically stable CF patients, we found the upper limit of normal for relative changes in LCI to be 19%, suggesting that relative changes above 19% should be considered to be above test variability and thus physiologically relevant. Including only patients with longer observation periods (≥3 visits) resulted in slightly lower variability with a ULN of 17% for relative changes between visits. Considering only cough-negative visits as clinically stable led to comparable variability indexes with a ULN for relative LCI changes between visits of 22%.

Variability of LCI within centers (Bern and Zurich) was comparable, however slightly lower in Zurich (Supplemental Table E2).

Variability of LCI for individual patients

Between-visit changes in LCI for individual patients expressed as relative change (calculated as upper limit of normal (95% quantile)) showed a wide range between 0.02 and 76% in patients with two measurements (Figure 2a). When including only patients with longer periods of LCI data at clinical stability (three or more consecutive visits) (Figure 2b), the range of individual variability decreased to between 1.4 and 37%.

Interpreting LCI in absolute or relative changes

Figure 3a shows a weak dependency of the within-subject between test SD of absolute LCI changes on the magnitude of LCI (r^2=0.1, p< 0.001). Within-subject between test SD of relative changes in LCI showed no association with the magnitude of LCI (r^2=0.001, p= 0.71)(Figure 3b). Hence, interpretation of LCI as relative changes between visits is favorable being independent of the magnitude of LCI and thus applicable over the whole spectrum of LCI values. Within-subject between-test variability was independent of the within-subject within-test variability (Supplemental Figure 2), as the weak association is mainly driven by outliers.

Long-term variability of spirometry in comparison to LCI

Variability of repeated spirometry measures for the overall CF cohort and only for patients at clinical stability is summarized in Supplemental Table E3. The ICC for FEV_1 z-scores (ICC 0.9) indicates high correlation between individual measurements. Variability around the mean FEV_1 across all patients over the entire study period was 8.3% CV (clinically stable periods), compared to 7.4% for LCI. The CR for absolute FEV_1 changes at clinically stable periods (in z-scores) was comparable to LCI (FEV_1 0.9, LCI 1.4). ULN for relative changes in FEV_1 was 16% (clinically stable periods) compared to 19% for LCI.

Discussion

In this study, we assessed variability in LCI measured in clinical routine with an observation period extending beyond twelve months and covering the whole age range from early childhood into adolescence in a large dataset of children with CF

from two tertiary centers. We provide a comprehensive summary of LCI variability measures and recommendations on how to interpret between visit changes in clinical routine. We suggest that a 19% relative change in LCI between visits is beyond test variability and thus can be considered physiologically relevant.

Comparison with literature

We report the variability of LCI in children with CF over a wide age range in the clinical setting before the era of CFTR modulators started [18, 19]. A small number of studies have assessed the variability of LCI in children with CF and healthy controls in specific age groups or over one year (Table E4) [8-10, 20, 21] but not over a large age range with long-term follow up as in this study. In our clinical dataset, we could replicate the findings of previous research surveillance studies, which allows for a more broad application of these data. In addition, we analyzed our data in the updated Spiroware software 3.3.1 which is corrected for the recently described sensor error [12]. Our findings are comparable to a recently published study reanalyzing clinical trial data in Spiroware 3.3.1 reporting an upper limit for relative changes in LCI of 23.4% [22].

To assess changes in LCI beyond test variability that can be considered clinically relevant, we only included patients at clinical stability, i.e. free of pulmonary exacerbation. While defining pulmonary exacerbations in children is challenging [23], various studies have clearly shown that lung function worsens during pulmonary exacerbations [24-30]. We could show that depending on how rigid an exacerbation was defined and which criteria were used (modified Fuchs criteria, cough negative visits, patients contributing at least three stable visits), variability of LCI changed

slightly, with the highest CV of 9.8% for the overall cohort (including patients with exacerbations) and the lowest CV of 6.9% for those patients with at least 3 consecutive visits and excluding exacerbations based on modified Fuchs criteria. These findings raise the question of whether the modified Fuchs criteria may be too strict for the evaluation of stability in clinical routine.

For absolute changes in LCI between visits, we report 1.4 LCI units to be beyond test variability and thus clinically relevant. However, as we found variability of absolute LCI values to be dependent on its magnitude, we recommend interpreting LCI as relative change between visits (Figure 3) [8, 31]. Thus, in clinical practice, we suggest a 19% change in LCI to be clinically relevant and beyond test variability. Our findings are in line with a large study conducted on CF and healthy subjects [8], which underlines the applicability of our data on a wide spectrum of CF patients.

As clinicians are considering visit-to-visit changes for each patient separately in routine surveillance, we calculated for each individual patient his ULN for relative changes as a potential individual threshold. The high variability we found between individuals appears to be inflated by the inherent sampling variation based on the inclusion of patients contributing only two or three measurements for the calculation [32]. This is further supported by the finding that the ULN for relative changes between visits for the overall population decreases from 19% to 17% when only including patients with ≥3 visits. However, the higher variability in some individuals might also reflect a signal, i.e. individuals with higher variability have a less stable status of their underlying lung disease. To assess whether individual variability is an indicator of disease status not captured by other clinical signs and symptoms or

based on systems dynamics [33], longitudinal studies combining imaging, lung function, and assessment of lower respiratory tract inflammation and infection with longer observation periods are needed to understand the exact cause of this variability. Until further insight into individual variability is provided, we propose, in clinical surveillance, our population-derived limit of 19% change in LCI being considered physiologically relevant but as suggested by Perrem et al., an increase in LCI should always be reviewed in the context of clinical signs and symptoms [34]. An increase in LCI can result from functional (e.g. mucus plugging) or structural impairment within the lungs (e.g. chronic infection leading to bronchiectasis) [2, 4, 35, 36]. If pathological processes are detected, clinicians should consider intensifying and adapting treatment. While population limits might have limited sensitivity in some patients, we still think that our findings are representative of most CF patients as our data emerges from a broad sampling population covering the whole age range from childhood into adolescence with varying disease severity.

Strengths and limitations

The main strengths of the present study are the wide age range of the participants, long periods of follow-up, and the clinical dataset, which allows for a broad application of our findings. We used a commercially available MBW device to perform measurements, applied strict quality control [37-40], and analyzed all data using the corrected commercially available software version Spiroware 3.3.1 to obtain high-quality MBW results. Further, we only included visits during intervals of clinical stability to define clinically relevant changes in LCI. The main limitations of our study are not uncommon in clinical datasets: Patients contributed different

periods of surveillance and visit intervals, physicians were not blinded to LCI results at the clinical visit, and different staff collected MBW data. With our statistical approach of using mixed models the influence of variation in surveillance is minimized. Further, the lack of a healthy control group limits our interpretation of whether the observed variability truly reflects a change in disease state. However, current literature showed that the biological variability of LCI is similar between health and CF and that changes above can be interpreted as clinically meaningful with similar thresholds as in our study [34, 41].

Clinical relevance

Data on variability and clinically relevant changes are essential for the use of LCI in the clinical surveillance of CF lung disease and to guide therapeutic decisions [19]. This study adds important data on the variability of LCI in routine clinical surveillance. Our results emerge from a heterogeneous pediatric CF population across all age groups, allowing the direct comparison and application of our findings with CF patients across most CF clinics. These data can also assist in the design of interventional trials using LCI as an efficacy endpoint. With the continuous improvements in CF care with subsequent preservation of lung function, our results add important information for the implementation of LCI as an additive outcome to traditional lung function parameters [42-44].

Outlook

Our findings need to be validated in a prospective observational study whereby clinicians are blinded to LCI results and pulmonary exacerbations are assessed prospectively using clearly defined criteria. The collation of large datasets from

multiple centres could also help to provide robust limits of variability for the interpretation of LCI [45]. To determine the pathophysiological origins of individual LCI variability, longitudinal comparison with structural and/or functional imaging methods [24, 36], various lung function parameters, and assessment of lower respiratory tract inflammation and infection are needed. Further, it will be crucial to assess how variability of LCI changes in cohorts receiving CFTR modulator therapy.

Conclusions

We provide comprehensive data on the variability of LCI measured in routine clinical surveillance of children with CF during periods of clinical stability. We recommend that a relative change in LCI beyond 19% should be considered physiologically relevant. Thus, our findings will help guide clinical decisions according to LCI changes.

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References

1. Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, Stroobant J, Carr S, Stocks J, London Cystic Fibrosis C. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. Am J Respir Crit Care Med 2005; 171: 249-56.

2. Ramsey KA, Foong RE, Grdosic J, Harper A, Skoric B, Clem C, Davis M, Turkovic L, Stick SM, Davis SD, et al. Multiple-Breath Washout Outcomes Are Sensitive to Inflammation and Infection in Children with Cystic Fibrosis. Ann Am Thorac Soc 2017; 14: 1436-42.

3. Kieninger E, Yammine S, Korten I, Anagnostopoulou P, Singer F, Frey U, Mornand A, Zanolari M, Rochat I, Trachsel D, et al. Elevated lung clearance index in infants with cystic fibrosis shortly after birth. Eur Respir J 2017; 50.

4. Ramsey KA, Rosenow T, Turkovic L, Skoric B, Banton G, Adams AM, Simpson SJ, Murray C, Ranganathan SC, Stick SM, et al. Lung Clearance Index and Structural Lung Disease on Computed Tomography in Early Cystic Fibrosis. Am J Respir Crit Care Med 2016; 193: 60-7.

5. Gustafsson PM, De Jong PA, Tiddens HA, Lindblad A. Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis. Thorax 2008; 63: 129-34.

6. Yammine S, Summermatter S, Singer F, Lauener R, Latzin P. Feasibility of nitrogen multiple-breath washout in inexperienced children younger than 7 years. Pediatr Pulmonol 2016; 51: 1183-90.

7. O'Neill K, Saunders C. New directions on lung clearance index variability and feasibility. J Cyst Fibros 2018; 17: 137-9.

8. Oude Engberink E, Ratjen F, Davis SD, Retsch-Bogart G, Amin R, Stanojevic S. Inter-test reproducibility of the lung clearance index measured by multiple breath washout. Eur Respir J 2017; 50.

9. Green K, Kongstad T, Skov M, Buchvald F, Rosthoj S, Marott JL, Gustafsson P, Pressler T, Nielsen KG. Variability of monthly nitrogen multiple-breath washout during one year in children with cystic fibrosis. J Cyst Fibros 2018; 17: 242-8.

10. Svedberg M, Gustafsson PM, Robinson PD, Rosberg M, Lindblad A. Variability of lung clearance index in clinically stable cystic fibrosis lung disease in school age children. J Cyst Fibros 2018; 17: 236-41.

11. Frauchiger BS, Binggeli S, Yammine S, Spycher B, Kruger L, Ramsey KA, Latzin P. Longitudinal course of clinical lung clearance index in children with cystic fibrosis. Eur Respir J 2021; 58: 2002686.

12. Wyler F, Oestreich MA, Frauchiger BS, Ramsey KA, Latzin P. Correction of sensor crosstalk error in Exhalyzer D multiple-breath washout device significantly impacts outcomes in children with cystic fibrosis. J Appl Physiol (1985) 2021; 131: 1148-56.

13. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology

(STROBE) statement: guidelines for reporting observational studies. The Lancet 2007; 370: 1453-7.

14. Bilton D, Canny G, Conway S, Dumcius S, Hjelte L, Proesmans M, Tummler B, Vavrova V, De Boeck K. Pulmonary exacerbation: towards a definition for use in clinical trials. Report from the EuroCareCF Working Group on outcome parameters in clinical trials. J Cyst Fibros 2011; 10 Suppl 2: S79-81.

15. 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 single- breath tests. Eur Respir J 2013; 41: 507.

16. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, 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: 1304-45.

17. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012; 40: 1324-43.

18. Short C, Saunders C, Davies JC. Utility of lung clearance index in CF: What we know, what we don't know and musings on how to bridge the gap. J Cyst Fibros 2020; 19: 852-5.

19. Spano J, Milla CE. Defining the Clinical Utility of the Lung Clearance Index. Are We There Yet? Am J Respir Crit Care Med 2021; 203: 937-9.

20. Stanojevic S, Davis SD, Perrem L, Shaw M, Retsch-Bogart G, Davis M, Jensen R, Clem CC, Isaac SM, Guido J, et al. Determinants of lung disease progression measured by lung clearance index in children with cystic fibrosis. Eur Respir J 2021; 58: 2003380.

21. Stanojevic S, Davis SD, Retsch-Bogart G, Webster H, Davis M, Johnson RC, Jensen R, Pizarro ME, Kane M, Clem CC, et al. Progression of Lung Disease in Preschool Patients with Cystic Fibrosis. Am J Respir Crit Care Med 2017; 195: 1216-25.

22. Robinson PD, Jensen R, Seeto RA, Stanojevic S, Saunders C, Short C, Davies JC, Ratjen F. Impact of cross-sensitivity error correction on representative nitrogen-based multiple breath washout data from clinical trials. Journal of Cystic Fibrosis;10.1016/j.jcf.2021.08.033.

23. VanDevanter DR, Hamblett NM, Simon N, McIntosh J, Konstan MW. Evaluating assumptions of definition-based pulmonary exacerbation endpoints in cystic fibrosis clinical trials. J Cyst Fibros 2021; 20: 39-45.

24. Grasemann H, Ciet P, Amin R, McDonald N, Klingel M, Tiddens H, Ratjen F, Grosse-Wortmann L. Changes in magnetic resonance imaging scores and ventilation inhomogeneity in children with cystic fibrosis pulmonary exacerbations. Eur Respir J 2017; 50: 1700244.

25. Rayment JH, Stanojevic S, Davis SD, Retsch-Bogart G, Ratjen F. Lung clearance index to monitor treatment response in pulmonary exacerbations in preschool children with cystic fibrosis. Thorax 2018; 73: 451-8.

26. Simpson SJ, Ranganathan S, Park J, Turkovic L, Robins-Browne RM, Skoric B, Ramsey KA, Rosenow T, Banton GL, Berry L, et al. Progressive ventilation inhomogeneity in infants with cystic fibrosis after pulmonary infection. Eur Respir J 2015; 46: 1680-90.

27. Sonneveld N, Stanojevic S, Amin R, Aurora P, Davies J, Elborn JS, Horsley A, Latzin P, O'Neill K, Robinson P, et al. Lung clearance index in cystic fibrosis subjects treated for pulmonary exacerbations. Eur Respir J 2015; 46: 1055-64.

28. Stanojevic S, McDonald A, Waters V, MacDonald S, Horton E, Tullis E, Ratjen F. Effect of pulmonary exacerbations treated with oral antibiotics on clinical outcomes in cystic fibrosis. Thorax 2017; 72: 327-32.

29. Vermeulen F, Proesmans M, Boon M, Havermans T, De Boeck K. Lung clearance index predicts pulmonary exacerbations in young patients with cystic fibrosis. Thorax 2014; 69: 39-45.

30. Waters V, Stanojevic S, Atenafu EG, Lu A, Yau Y, Tullis E, Ratjen F. Effect of pulmonary exacerbations on long-term lung function decline in cystic fibrosis. Eur Respir J 2012; 40: 61-6.

31. Robinson PD, Jensen R, Seeto RA, Stanojevic S, Saunders C, Short C, Davies JC, Ratjen F. Impact of cross-sensitivity error correction on representative nitrogen-based multiple breath washout data from clinical trials. J Cyst Fibros 2021;10.1016/j.jcf.2021.08.033.

32. Hoffman L. Multilevel Models for Examining Individual Differences in Within-Person Variation and Covariation Over Time. Multivariate Behavioral Research 2007; 42: 609-29.

33. Thamrin C, Frey U, Kaminsky DA, Reddel HK, Seely AJ, Suki B, Sterk PJ. Systems Biology and Clinical Practice in Respiratory Medicine. The Twain Shall Meet. Am J Respir Crit Care Med 2016; 194: 1053-61.

34. Perrem L, Stanojevic S, Solomon M, Grasemann H, Sweezey N, Waters V, Sanders DB, Davis SD, Ratjen F. Evaluation of clinically relevant changes in the lung clearance index in children with cystic fibrosis and healthy controls. Thorax 2022;10.1136/thoraxjnl-2021-218347.

35. Nyilas S, Bauman G, Pusterla O, Ramsey K, Singer F, Stranzinger E, Yammine S, Casaulta C, Bieri O, Latzin P. Ventilation and perfusion assessed by functional MRI in children with CF: reproducibility in comparison to lung function. J Cyst Fibros 2019; 18: 543-50.

36. Nyilas S, Bauman G, Sommer G, Stranzinger E, Pusterla O, Frey U, Korten I, Singer F, Casaulta C, Bieri O, et al. Novel magnetic resonance technique for functional imaging of cystic fibrosis lung disease. Eur Respir J 2017; 50: 1701464.

37. Jensen R, Stanojevic S, Klingel M, Pizarro ME, Hall GL, Ramsey K, Foong R, Saunders C, Robinson PD, Webster H, et al. A Systematic Approach to Multiple Breath Nitrogen Washout Test Quality. PLoS One 2016; 11: e0157523.

38. Frauchiger BS, Carlens J, Herger A, Moeller A, Latzin P, Ramsey KA. Multiple breath washout quality control in the clinical setting. Pediatr Pulmonol 2021; 56: 105-12.

39. Robinson PD, Latzin P, Ramsey KA, Stanojevic S, Aurora P, Davis SD, Gappa M, Hall GL, Horsley A, Jensen R, et al. Preschool Multiple-Breath Washout

Testing. An Official American Thoracic Society Technical Statement. Am J Respir Crit Care Med 2018; 197: e1-e19.

40. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. Eur Respir J 2013; 41: 507-22.

41. Perrem L, Rayment JH, Ratjen F. The lung clearance index as a monitoring tool in cystic fibrosis: ready for the clinic? Curr Opin Pulm Med 2018; 24: 579-85.

42. Stanojevic S, Ratjen F. Physiologic endpoints for clinical studies for cystic fibrosis. J Cyst Fibros 2016; 15: 416-23.

43. Perrem L, Stanojevic S, Shaw M, Jensen R, McDonald N, Isaac SM, Davis M, Clem C, Guido J, Jara S, et al. Lung Clearance Index to Track Acute Respiratory Events in School-age Children with Cystic Fibrosis. Am J Respir Crit Care Med 2020;10.1164/rccm.202006-2433OC.

44. Voldby C, Green K, Kongstad T, Ring AM, Sandvik RM, Skov M, Buchvald F, Pressler T, Nielsen KG. Lung clearance index-triggered intervention in children with cystic fibrosis - A randomised pilot study. J Cyst Fibros 2020; 19: 934-41.

45. Anagnostopoulou P, Latzin P, Jensen R, Stahl M, Harper A, Yammine S, Usemann J, Foong RE, Spycher B, Hall GL, et al. Normative data for multiple breath washout outcomes in school-aged Caucasian children. Eur Respir J 2020; 55: 1901302.

Figures

Figure 1 Study population and patient selection. For 108 patients 1428 clinical visits along with matching MBW were available. Of these, 240 (17%) needed to be rejected as MBW was not meeting quality control criteria. For 100 patients at least two visits 8-16 weeks apart with good quality MBW were available. To calculate variability measures, at least two visits at clinical stability 8-16 weeks apart were needed for further inclusion, clinical stability was defined according to the modified Fuchs criteria [1].

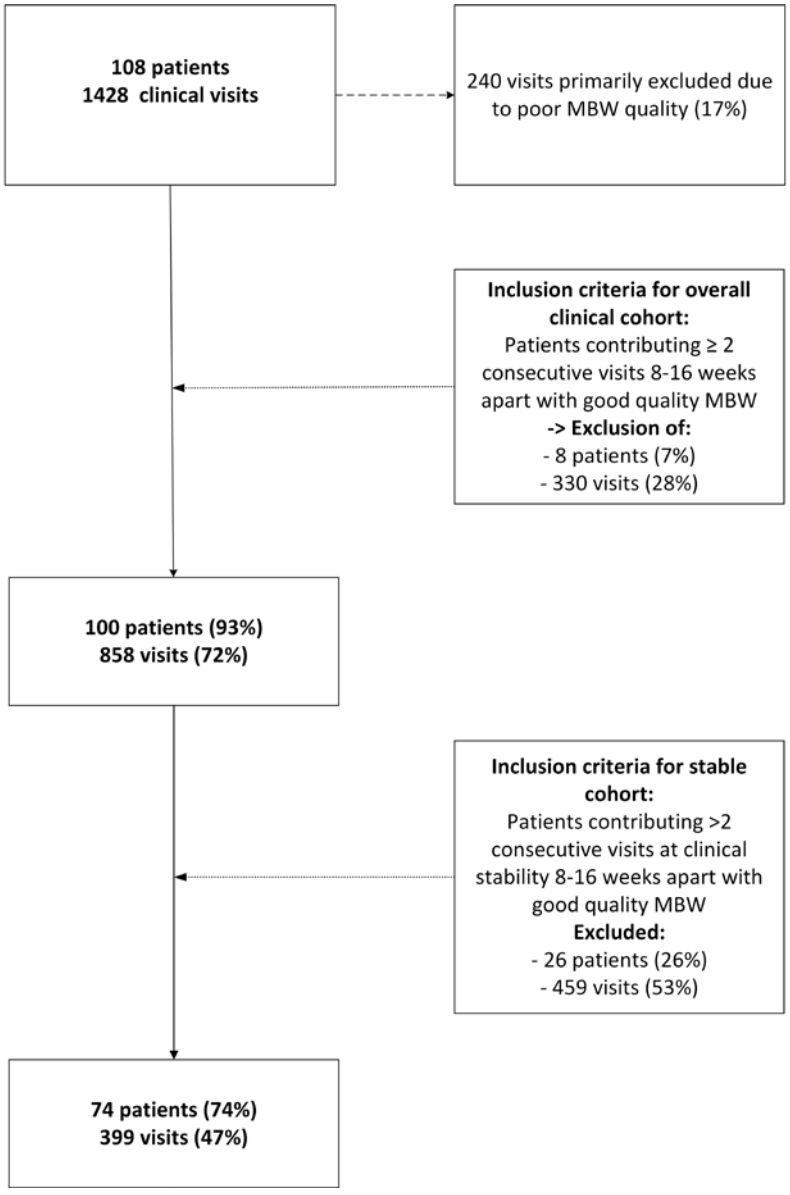
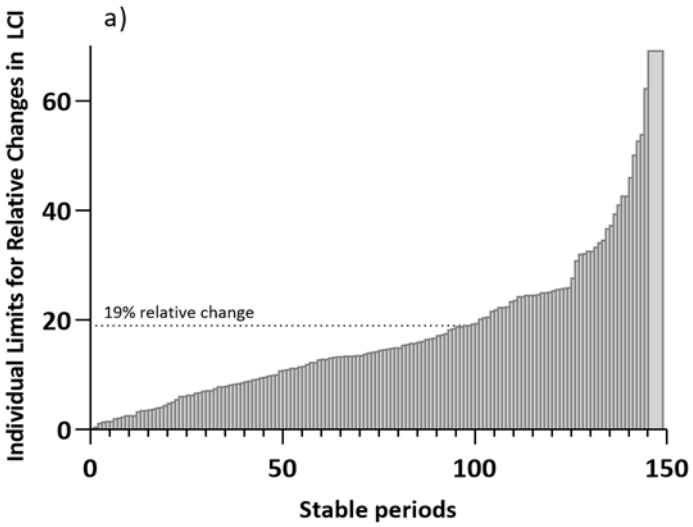


Figure 2 **a)** Individual heterogeneity in limits for relative changes in LCI between visits. Figure a) shows all patients contributing at least 2 visits to a stable period, Figure b) only patients contributing at least 3 visits. Bars represent the individual limits for relative changes at periods of clinical stability (Figure a) N=147, Figure b) N= 64); Individual limits for relative changes at clinical stability range from 0.02% to 76% when including patients with at least 2 stable visits, the range of variability decreases when including only patients contributing at least 3 visits at stability (1.4% to 37%). Individual limits reflect a threshold below which 95% of the relative changes of LCI between visits are expected to fall for the corresponding individual, thus changes above being physiologically relevant for this individual. Dotted line indicates the population-derived physiologically relevant threshold of 19% change in LCI in CF patients at clinical stability. Abbreviations: LCI: lung clearance index. **b)** Individual heterogeneity in limits for relative changes in LCI between visits. Figure a) shows all patients contributing at least 2 visits to a stable period, Figure b) only patients contributing at least 3 visits. Bars represent the individual limits for relative changes at periods of clinical stability (Figure a) N=147, Figure b) N= 64); Individual limits for relative changes at clinical stability range from 0.02% to 76% when including patients with at least 2 stable visits, the range of variability decreases when including only patients contributing at least 3 visits at stability (1.4% to 37%). Individual limits reflect a threshold below which 95% of the relative changes of LCI between visits are expected to fall for the corresponding individual, thus changes above being physiologically relevant for this individual. Dotted line indicates the population-derived physiologically relevant threshold of 19% change in LCI in CF patients at clinical stability. Abbreviations: LCI: lung clearance index



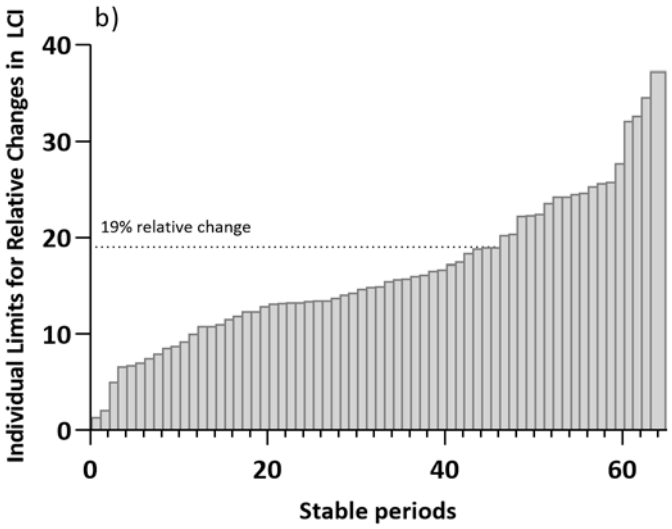
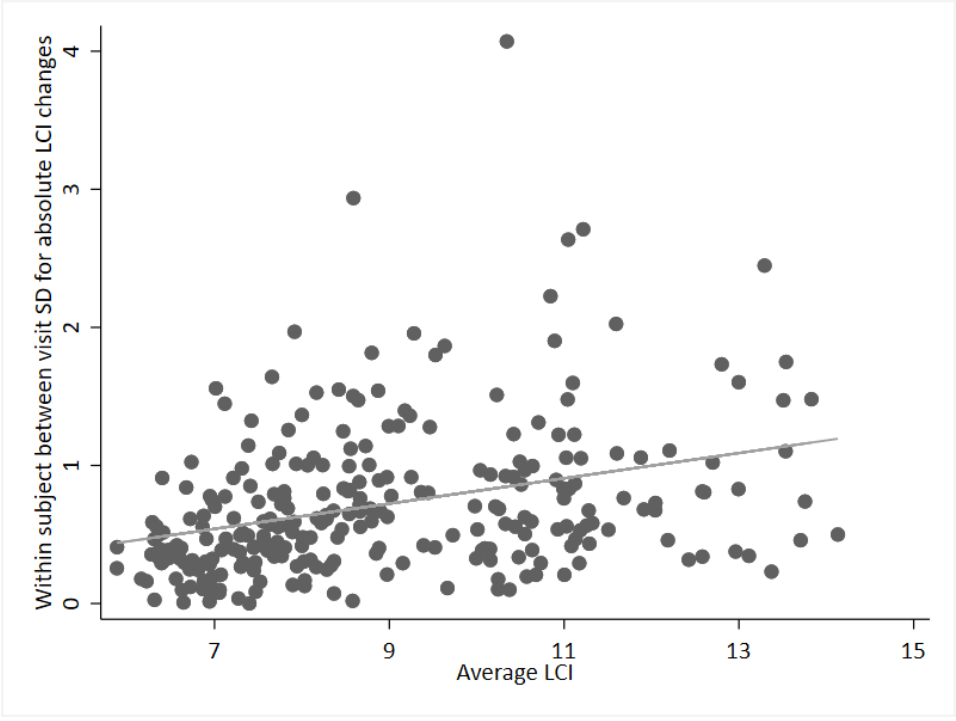


Figure 3 **a)** Dependency of within-subject between visit standard deviation of absolute LCI changes on the magnitude of LCI. Within-subject between visit standard deviation of absolute LCI changes is weakly dependent on the magnitude of LCI (r2= 0.1, p< 0.001). Average LCI reflects mean LCI per patient. Abbreviations: SD: Standard deviation, LCI: lung clearance index. **b)** Dependency of within-subject between visit standard deviation of relative LCI changes on the magnitude of LCI. Within-subject between visit standard deviation for relative LCI changes is independent on the magnitude of LCI (r2= 0.006, p= 0.2). Average LCI reflects mean LCI per patient. Abbreviations: SD: Standard deviation, LCI: lung clearance index



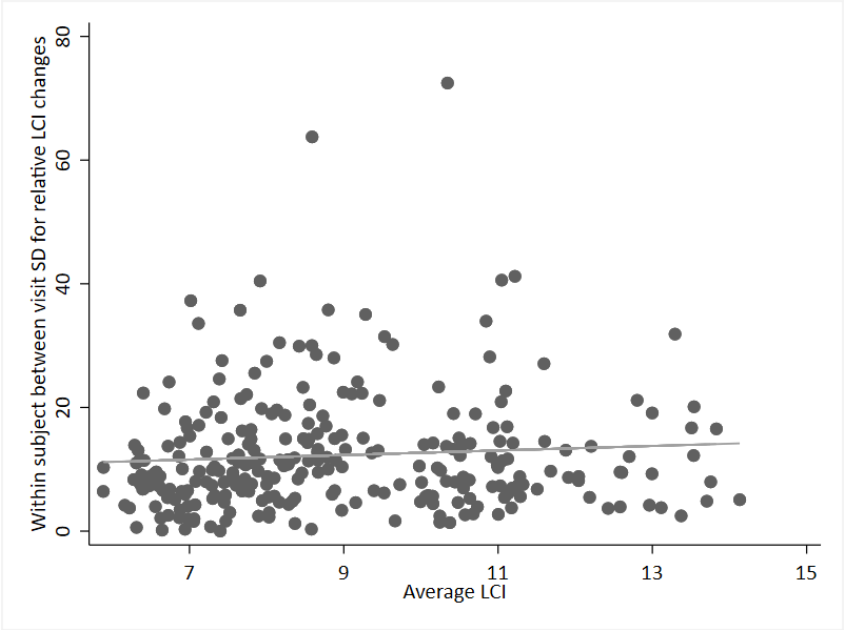


Table 1: Participant demographics at baseline visit

	CF overall cohort	CF stable cohort
Subjects (n)	100	74
Visits (n)	858	399
Female: n (%)	56 (56)	44 (60)
Age (years)	9.1 (4.2; 18.3)	11.7 (4.2; 19)
Weight (z-score)	-0.2 (-4.0;2.5)	-0.2 (-2.4; 2.5)
Height (z-score)	-0.2 (-4.1; 2.4)	-0.4 (-3.2; 2.4)
BMI (z-score)	-0.1 (-2.8; 2.2)	0.0 (-2.6; 2.2)
FEV_1 (z-score)	-0.7 (-5.2; 2.1)	-0.8 (-4.2; 2.1)
LCI 2.5% (units)	8.1 (5.5; 15.0)	7.7 (5.5; 14.1)

FRC (L)	1.2 (0.5; 3.3)	1.3 (0.5; 3.4)
CFTR function		
Minimal	90 (90)	66 (89)
Residual	10 (10)	8 (11)
Pancreatic function		
Insufficient	93 (93)	71 (96)
Residual function	7 (7)	3 (4)

Summary of participant demographics. Data are presented as absolute numbers (%) and as median (range). Children included attended three monthly routine care in the centers Bern and Zurich presented for the overall population and those contributing clinically stable periods. CFTR classifications were considered as minimal and residual function according to cftr2.org [1]. Abbreviations: CF: Cystic Fibrosis; BMI: Body mass index; FEV_1: forced expiratory volume in one second; LCI: Lung clearance index; FRC: Functional residual capacity. CFTR: Cystic Fibrosis Transmembrane Conductance Regulator.

1. The Clinical and Functional TRanslation of CFTR (CFTR2); available at http://cftr2.org.

Table 2: Longterm variability of LCI

Dataset	N (subjects)	N (visits)	Mean absolute Difference (SD)	ICC	%CV	CR	ULN %change (95% quantile)
CF stable cohort	74	399	0.02 (0.6)	0.9	7.4	1.4	19
CF overall cohort	100	858	0.1 (1.1)	0.8	9.8	1.9	24
Cough negative	74	411	0.06 (1.0)	0.9	8.8	1.7	22
Patients contributing ≥3 visits	47	233	0.03 (0.7)	0.9	6.9	1.2	17

Summary of variability measures for repeated clinical LCI measurements. Variability was assessed at three monthly visits either at clinical stability, including all data or during cough negative periods using the residual standard deviation from the random effects of a mixed-effects model to account for repeated measures in each subject. To assess the impact of visits contributed, variability measures were also calculated for patients contributing ≥3 visits. Abbreviations: CF: Cystic Fibrosis; LCI: Lung clearance index; SD: standard deviation; ICC: intraclass correlation coefficient; CV%: coefficient of variation; CR: coefficient of repeatability; ULN: upper limit of normal (95% quantile), % changes refer to relative changes between visits.