Journal of Cystic Fibrosis 15 (2016) 416–423

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Review

Physiologic endpoints for clinical studies for cystic fibrosis

Sanja Stanojevic, Felix Ratjen *

[a] Division of Respiratory Medicine, Hospital for Sick Children, Toronto, Canada
[b] University of Toronto, Toronto, Canada

Received 1 April 2016; revised 27 May 2016; accepted 28 May 2016
Available online 15 June 2016

Abstract

The cystic fibrosis (CF) drug development pipeline promises many exciting new treatments for patients with CF, all which will require clinical studies to prove their benefits on CF lung disease. Historically many pivotal CF studies have used the Forced Expiratory Volume in 1 s (FEV_1) as the primary outcome measure, and after demonstrating significant improvements in the treatment group relative to placebo have led to regulatory approval of therapies for routine clinical care. Widespread implementation of these therapies has subsequently led to significant improvements in outcomes for patients with CF. While preserving lung function has obvious benefits to CF patients, as more patients maintain FEV_1 in the normal range, it has become increasingly difficult to conduct clinical trials using FEV_1 as the primary outcome measure. With multiple concurrent trials competing to enroll from the same pool of patients, there is a need for novel approaches to study end points as well as new physiological outcomes for CF therapeutic trials. In this review we will discuss some of the limitations of FEV_1 in the current era of CF care, describe alternative physiological endpoints and outline areas for further research.

Keywords: Cystic fibrosis; Lung function; Clinical trials; Outcomes

Contents

* Corresponding author at: Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada. Tel.: +1 416 813 7654x228472; fax: +1 416 813 6246.
E-mail address: felix.ratjen@sickkids.ca (F. Ratjen).

http://dx.doi.org/10.1016/j.jcf.2016.05.014

1. Introduction

The cystic fibrosis (CF) drug development pipeline promises many exciting new treatments for patients with CF, which will require clinical studies to prove their benefits on CF lung disease. While endpoints vary between studies, the most commonly reported surrogate outcome for CF clinical trials to date has been the Forced Expiratory Volume in 1 s (FEV_1), measured by spirometry. Many pivotal studies have used FEV_1 as the primary outcome measure, demonstrating significant improvements in the treatment arm relative to the placebo arm, which in conjunction with supporting evidence from other outcome measures has led to regulatory approval. Implementation of these treatments into routine clinical care has led to significant improvements in outcomes for patients with CF. As a consequence, not only life expectancy, but also clinical severity of lung disease has changed over time [1,2]. Improvements in outcomes have shifted the phenotype of patients with CF across the lifespan, such that many patients now maintain normal lung function well into early adulthood. Preserving lung function has obvious benefits to CF patients, but has made it increasingly difficult to conduct clinical trials using FEV_1 as the primary outcome measure. On one hand larger study populations are needed to demonstrate smaller treatment effects in patients that are already heavily treated. On the other hand proportionally fewer patients are in the range of disease severity commonly included into clinical trials to prove efficacy in CF patients. With multiple concurrent trials competing to enroll from the same pool of patients, there is a need for novel approaches to study end points as well as new physiological outcomes for CF therapeutic trials. In this review we will discuss some of the limitations of FEV_1 in the current era of CF care, describe alternative physiological endpoints and outline areas for further research.

2. FEV_1

Spirometry is the hallmark physiological test for respiratory disease diagnosis, management and research studies. FEV_1 is the primary spirometric output used to monitor patients with CF in clinical practice, and the primary outcome measure in many CF clinical trials. Spirometry equipment is readily available in all CF centers and there are standardized testing protocols and certified commercial devices available [3]. FEV_1, in particular, is a very reproducible and repeatable outcome; however the variability is not constant across all ages, or across the spectrum of disease severity [4]. Most CF patients 6 years or older, the age group in whom the test is performed routinely in the clinic, are familiar with the test, and with appropriate training accurate measurements are easy to obtain. FEV_1 is considered an appropriate surrogate outcome for CF studies since low FEV_1 values are strongly associated with increased mortality, and decreased quality of life [5–7].

3. How much improvement in FEV_1 can we expect in the current era of CF care?

Many of the therapies that are now the standard of clinical care in patients with CF were investigated in randomized trials where the FEV_1 was the primary outcome measure (Table 1). While patient characteristics and treatment duration were fairly comparable, treatment effects have varied and except for the remarkable improvements in FEV_1 observed in patients with class III gating mutations treated with Ivacaftor [8], the magnitude of the FEV_1improvement observed, either in absolute or relative terms, have been smaller than the threshold used to assess short term treatment response to interventions such as bronchodilators in patients with asthma or COPD [9] (Table 1). As lung function of the CF population further improves, and more patients with normal lung function are

Table 1
Summary of treatment responses using FEV_1 as an outcome measure for 6 landmark randomized control trials in patients with CF.

Publication	Treatment	Duration (weeks)	Sample Size (N)	Primary Outcome	Secondary Outcomes
Fuchs H.J. et al. NEJM (1994) [10]	Dornase Alfa	24	968	Reduction in pulmonary exacerbations	Relative change in FEV_1 (5.8% ± 0.7SE once daily; 5.6% ± 0.7SE)
Ramsey B.W. et al. NEJM (1999) [11]	Tobramycin	20	520	Relative change in FEV_1% predicted (12%)	
Elkins M.R. et al. NEJM (2006) [12]	Hypertonic Saline	48	164	Linear rate of change in FEV_1 from baseline (0.3 ml/week, 95%CI −1.3; 1.8)	Absolute change in FEV_1 (0.068 L); Relative change in FEV_1 (3.2%)
Saiman L. et al. JAMA (2010) [13]	Azithromycin	24	260	Absolute change in FEV_1 (0.020 L, 95%CI −0.05; 0.08)	Relative change in FEV_1% predicted (2%)
Ramsey B.W. et al. NEJM (2011) [8]	Ivacaftor	24	161	Absolute change in FEV_1% predicted (10.6%)	Relative change in FEV_1 (17.2%); Absolute change in FEV_1 (0.361 L)
Wainwright C.E. et al., NEJM (2015) [14]	Ivacaftor + Lumacaftor	24	1108	Absolute change in FEV_1% predicted (2.8% -3.3%)	Relative change in FEV_1 (4.8% -5.6%)

included in interventional studies, we can expect the magnitude of the treatment effect to be even smaller, and therefore requiring a larger study population to show significant treatment benefit (Fig. 1).

Traditionally, almost all interventional trials have been designed to show a significant improvement in outcomes; however, as patient outcomes continue to improve and more patients maintain lung function in the normal range, there is a growing need to show that treatments prevent deterioration, i.e. preserve lung function. This is also clinically more meaningful as short-term improvements in airway diameter do not necessarily predict long-term benefits in lung function decline. Taken together, smaller expected treatment effects of new interventions, and a healthier patient population are major hurdles to studies using FEV_1 as a primary measure of treatment success in the future.

4. What constitutes a meaningful improvement in lung function?

Estimates of the minimal clinically important difference (MCID) are determined at the individual level, not necessary at the population level; it is therefore difficult to imply these directly to interventional studies [15,16]. The American Thoracic Society/European Respiratory Society suggest that within subject changes of FEV_1 for normal subjects are within 12% (relative change) in short-term trials (weeks) and 15% for long-term trials (1 year) [9]. Thresholds of meaningful changes in FEV_1 ranging from 5 or 10% have been reported in CF trials (Table 1), but these thresholds are well within the inherent variability of the test, which remains relatively poorly defined in CF. Even if the MCID was incorporated at the patient level, the evidence to support a robust estimate of MCID for patients with CF is limited. A meaningful change in FEV_1 may also depend on the disease severity of the patient. For example a relative improvement of 10% in a patient whose lung function is at 30% predicted is not equivalent in absolute terms (Liters) to a 10% improvement for a patients whose lung function is at 80% predicted. On the other hand a small absolute improvement in a patient with severe disease may be equally relevant as a larger absolute change in a patient with milder disease. Since the magnitude of improvement in lung function is sometimes considered in the decision process of public health insurers to regulate accessibility to treatment, the definition of a meaningful change ought to be evidence based. Using pooled data from placebo groups of interventional trials will help to better understand the overall variability of FEV_1, and define its relationship to disease severity.

Given the lack of clearly defined thresholds, interventional studies are considered successful if the differences observed between the treatment and placebo arms are statistically significant. Studies are typically designed to detect a statistical difference based on two key pieces of information, the estimated treatment effect and the variability of the outcome measure, or in the case of studies where the outcome is a change score from baseline, the estimate change from baseline and the variability of the difference between the two measurements. In either case, if we assume that the variability of FEV_1 is constant across studies, the estimated sample size will depend on the magnitude of the treatment effect (Fig. 1). Based on the observed treatment

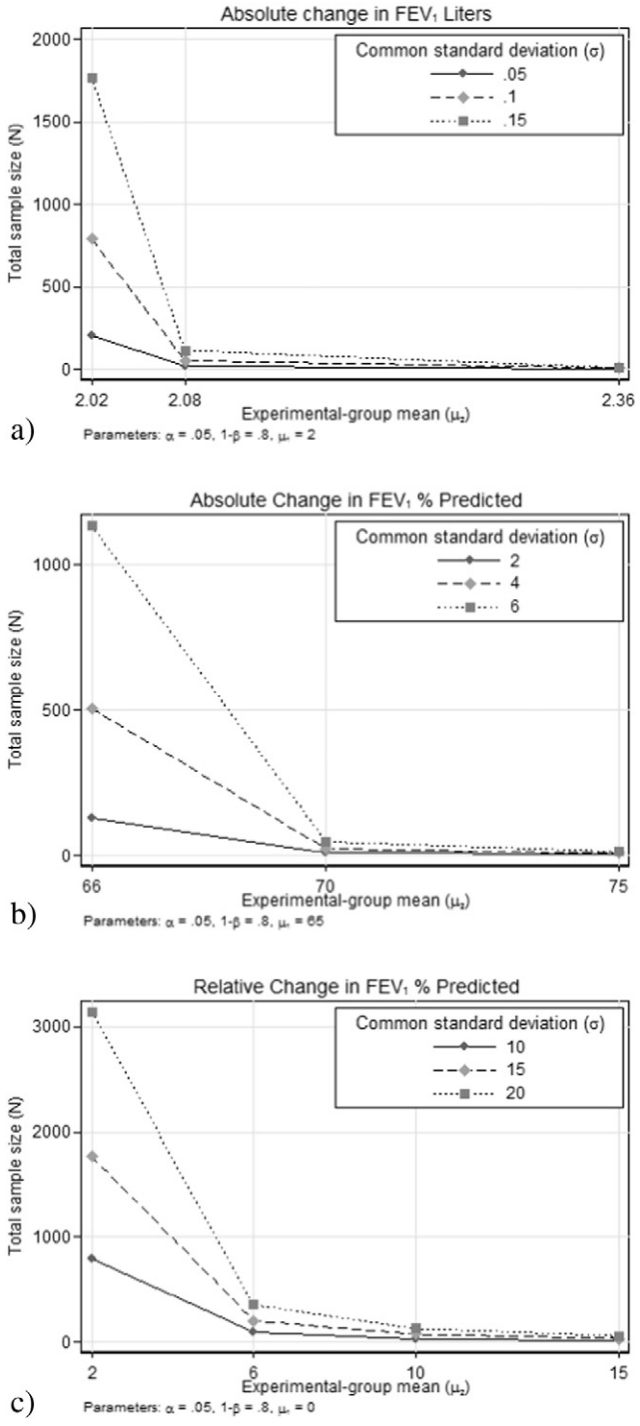

Fig. 1. Sample size estimates (significance level = 0.05, power = 80%) based on the observed treatment effect and common standard deviation reported in the studies presented in Table 1. a) absolute change in FEV_1 Liters where the average FEV_1 in the placebo group was 2 L and sample size was estimated for improvements of 20 ml, 80 ml and 360 ml, b) Absolute change in FEV_1% predicted where the placebo group has an average FEV_1 of 65% predicted and sample size was estimated for a 1%, 5% and 10% absolute improvement and c) Relative change in FEV_1 where the placebo group has an average change of 0% and sample size was estimated for a 2, 6, 10 and 15% improvement in FEV_1.

effects (Table 1), much larger studies are necessary to show modest improvements in FEV_1. With a limited CF population and dozens of studies running concurrently, and even more planned in the near future, there is a need to employ novel study designs and novel outcomes that can demonstrate benefit in smaller populations of patients.

5. Lack of standardized reporting

Direct comparisons between the landmark interventions summarized in Table 1 is almost impossible with some studies reporting improvements from baseline in absolute values of FEV_1 (Liters), others reporting relative differences or a percentage change from baseline. In some instances absolute values of FEV_1 are converted to percent predicted adjusting for age, sex and height; but the reference equations used to convert absolute values to percent predicted are not standardized. Interpretation of absolute changes in FEV_1 are further complicated in pediatric studies, since normal growth and development can led to an underestimation of treatment effects. Another challenging issue is if and how baseline values of lung function are adjusted for in the analysis. The strengths and limitations of end point analysis compared with change score analysis also need to be considered, in particular the correlation between two measurements will likely depend on the time interval between measurements [17]. It is common practice to determine the analytical method a priori within the study design; however secondary analyses are often presented in publications further complicating the interpretation of outcomes between different studies. Moving forward, it would be preferential to provide data for both absolute and relative changes in FEV_1 for all clinical trials to facilitate the comparison of effect sizes among studies.

6. FEV_1 across the age range

Spirometry while easy to perform does require that subjects actively cooperate during testing to ensure both the inspiration to total lung capacity and the forced exhalation reflect their maximal effort. Most pulmonary function laboratories are staffed with trained and certified personnel, but this is not always the case for clinical trials, which may increase the variability of the FEV_1 and reduce the statistical power to detect a treatment effect (Fig. 1). Effort dependency becomes even more of an issue in younger children. The vast majority of published interventional studies in the CF population are limited to children over the age of 6 years and adults. Increasingly it is becoming evident that early intervention is critical to slow the progression of CF lung disease [18]. Until the early 2000s it was largely thought that FEV_1 could only be obtained in children older than 6 years of age. Several studies have shown that it is feasible, with adaptations to protocols and outcomes, to measure spirometry in preschool children (2.5–6 years of age) [19–22]. The physiological properties of the dysynapsis between airway and lung growth in early childhood mean that young children empty their lungs in less than 1 s and outcomes such as $FEV_{0.75}$ in preschool children need to be reported [23]. While $FEV_{0.75}$ is correlated with FEV_1there are no studies that demonstrate the relationship between these outcomes and whether they can be interpreted interchangeably. Despite this advance in technology, not a single clinical trial has been published in preschool children with CF where $FEV_{0.75}$ or FEV_1 was used as the primary outcome measure and very few pediatric pulmonary function laboratories routinely perform spirometry in preschool children.

There are also comparable techniques to measure spirometry in infants. The raised volume thoracic compression technique (RVRTC) was designed to produce a flow volume loop in infants similar to those obtained by spirometry [24]. The methodology of the RVTLC has been summarized and its merits as an outcome for clinical trials have recently been reviewed [25,26]. Briefly, infants are sedated and the lungs are inflated to near total lung capacity, then a positive pressure is applied to the trunk of the infant at the end of inflated inspiration using an inflatable jacket to force exhalation. As mentioned above emptying of the lung during a forced expiration is age dependent and faster the younger the patient is; thus $FEV_{0.5}$ is used as the equivalent of FEV_1 in infant studies. A longitudinal observational study conducted in the US did provide data for feasibility of RVTLC in a setting of a clinical trial, but also demonstrated that differences between CF and healthy infants were rather small leading to a prohibitive sample size for studies using this parameter as a primary outcome measure [27]. As part of the inhaled hypertonic saline in infants and young children (ISIS) trial to assess the efficacy of hypertonic saline in infants, 15 centers across North America perform the RVRTC measurements [28]. Within these centers 73 infants performed RVRTC, which represents one quarter of the total number of children randomized to hypertonic saline or isotonic saline. A borderline statistically significant treatment effect was observed for the $FEV_{0.5}$ (0.038 L (95%CI 0.001; 0.076)), but only two thirds of the collected data were usable. The need to sedate infants during the test also makes it practically difficult, not only for families, but also from cost of having trained health professionals available for all tests. In addition, using different outcome measures of the forced expiratory maneuver across the age ranges complicates the interpretation of longitudinal spirometry measures when patients move from one age group to the next.

Table 2
Summary of interventional studies using LCI as an outcome measure.

Publication	Treatment	Duration (weeks)	Sample size (N)	Absolute change in LCI
Amin R. et al. Thorax (2010) [49]	Hypertonic saline	4	20 (19 analyzed)	−1.16 ± 0.94 (−2.05; −0.27)
Amin R. et al. ERJ (2011) [50]	Dornase alfa	12	19 (17 analyzed)	−0.90 ± 1.44
Subbarao P. et al. AJRCCM (2013) [51]	Hypertonic Saline	48	27 (25 analyzed)	−1.43 (−2.99; 0.13)
Davies J et al., Lancet Resp Med (2013) [52]	Ivacaftor	4	21 (17 analyzed)	−2.16 (−2.88; −1.44)

7. Alternative spirometry outcomes

FEV_1 is one of many outcomes available from the spirometry test. It has been suggested that mid-expiratory flows (MMEF, $FEF_{25–75}$) may be more sensitive at detecting obstruction of the small airways [29], even though this has been challenged recently and much of the information may be captured in the FEV_1/FVC ratio [30,31]. On a population level $FEF_{25–75}$ often demonstrates a bigger difference between groups, but the measure is more variable and therefore requires larger sample sizes to detect a statistically significant difference [32]. Forced Vital Capacity (FVC) is also largely ignored in CF clinical trials, but may be particularly relevant in patients with severe disease as suggested by studies in patients with COPD [33].

8. The lung clearance index

In the past 5 years there has been a growing interest in the lung clearance index (LCI) as an outcome for clinical trials in CF patients [34]. The LCI is the primary and simplest measure of the Multiple Breath Washout (MBW) test. The MBW test was developed in the 1960s and has been studied by many groups over the years. The availability of commercial MBW equipment in recent years now means that the MBW can be performed in multi-centre clinical trials, and potentially as part of clinical care. The LCI measures ventilation inhomogeneity, is able to distinguish between health and disease, and is more sensitive at detecting early lung changes than the FEV_1 [18,32,35–40]. In addition, greater ventilation inhomogeneity, higher LCI values, are correlated with structural damage measured by high resolution CT in both pediatric and adult patients with CF [18,41–44]. Since elevated LCI and structural damage are often observed in patients whose FEV_1 is in the normal range there is the potential to identify early lung disease and intervene before permanent lung damage occurs. The MBW test is also feasible to perform across all age groups, including infants and preschool children [32,36,45–48].

As the observational evidence in favor of the LCI as standard measure of CF lung disease accumulates, several interventional studies have also demonstrated the ability of the LCI to detect treatment effects of known therapeutic interventions, as well as new therapies (Table 2). Of particular relevance for the design of interventional studies, significant improvements in LCI have been reported with relatively small number of patients. The published treatment effects for LCI range from 0.9 to 2.2 units (Table 2), with the greatest treatment effect observed for Ivacaftor in patients with CFTR gating mutations. However, what defines a clinically relevant change, or warrant changes in clinical practice are still unknown. More recently the US CF Foundation Therapeutics Development Network and the European Clinical Trials Network have supported the training and certification of more than 80 CF centers around the world to be able to use the MBW test for interventional studies. Alongside standardized commercial equipment the MBW has been included as an outcome in more than a dozen ongoing interventional studies.

9. What is a clinically meaningful LCI change?

The variability of the LCI in healthy subjects is remarkably small; the between-subject standard deviation is less than 0.5 units. Even within the same subject, either on the same test occasion, or between test occasions the variability remains small [34]. However, all measures of the variability (both within and between tests) are much greater in patients with CF. In particular, these is some evidence that the variability is dependent on the severity of disease (i.e. patients with higher LCI values have more variable values) [53]. Thus, any sample size estimates calculated from a healthy population will be grossly underpowered to detect changes in a CF population (Fig. 2). Moreover, LCI values, and the within-subject variability of these values depends on disease severity, such that pediatric patients have less variable values than adults [54]. Therefore, interventional studies should be stratified either by disease severity or pre-treatment LCI values to avoid unbalanced groups. Important observation data on the natural variability of the LCI across the spectrum of the CF population are necessary to define the treatment effect that can be expected in an interventional trial. Better understanding of the relationship between LCI and other surrogate outcomes, and eventually survival, will help to define the role of LCI in clinical practice as well as an interventional study outcome.

10. MBW is an effort independent test

Compared to spirometry and plethysmography, which require patients to actively co-operate in the test, the MBW test is at least in theory much easier to perform. While this is largely true for the subject undergoing testing, the interpretation of the outcomes relies on the subject breathing in a relaxed tidal volume throughout the test, which in practice may be difficult to do. Deviations from normal breathing at the beginning and end of the test are particularly problematic since calculations of

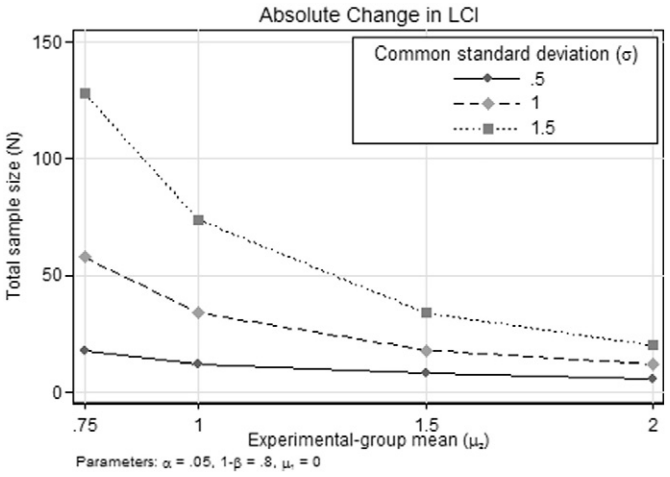

Fig. 2. Sample size estimates (significance level = 0.05, power = 80%) for LCI, where the placebo group has an average LCI change of 0 units, and the sample size is estimated for improvements of 0.75, 1, 1.5 and 2 units based on treatment effect that was observed in Table 2.

the functional residual capacity, a key measure used to calculate the LCI, can be biased and inaccurate. It is also currently not known how much of the observed variability of LCI is due to disease itself, or related to intrinsic properties of the test.

At the moment there are three regional training and certification centers whose role is also to over-read MBW traces for quality for ongoing interventional trials. However, this approach may not be suitable in the long run as more and more centers become proficient in MBW testing and as more interventional studies incorporate the LCI as an outcome measure. Ideally, novel approaches to maintain data quality that can be implemented into the software of devices may facilitate quality control and data analysis in the future.

11. Operational hurdles

An ideal MBW testing environment requires a quiet space where subjects can be coached to breathe in a relaxed manner, but in reality testing often occurs in busy pulmonary function labs where subjects are easily distracted. An appropriate environment ideally separated from the main pulmonary function laboratory, especially for younger children, is the most suitable setting.

There are currently three commercial MBW devices available, but it will not be long before multiple devices are implemented across pulmonary function laboratories. Further work is needed to standardize reporting and interpretation of outcomes, similar to what already exists for spirometry. Nonetheless, LCI differs between different tracer gases [53] and it is important that one standardized approach is used within any given study.

To our knowledge the only clinical trials conducted in children younger than 6 years have been conducted using the custom built mass spectrometer, which uses sulfahexafloride as a tracer gas [51]. There are several ongoing studies that have adapted existing commercial equipment to be able to test preschool children, and infants; however confirmatory work and adaptations to commercial equipment will be necessary before multi-centered studies in infants can be performed.

12. What does the LCI mean to patients and caregivers?

Patients participating in intervention studies are familiar with spirometry testing, and generally, they know that if their FEV_1 decreases that their lung function is declining. The opposite is true for LCI; lower values actually mean that ventilation inhomogeneity is improving. Currently, it is still unknown how much change in LCI is clinically relevant; a one unit change for someone within in the normal range may mean something different compared with a one unit change in someone with severe lung disease. While several studies have measured MBW in healthy subjects, we still do not have a clear understanding of what the normal range for LCI is, nor what magnitude of change is clinically relevant. The fact that we do not have definitive answers to these questions makes it challenging to explain what a given LCI result means to a patient.

Observational and interventional studies are ongoing which are aimed to answer these important questions. At the present time the clinical utility of LCI, in contrast to its role in interventional trials, still remains poorly defined.

13. Pulmonary exacerbations

As lung function has improved in CF, many patients maintain a steady level of lung function (at least as measured by FEV_1) until clinical events happen during which lung function deteriorates and subsequently fails to recover [7,55–57]. These events, pulmonary exacerbations are thus clinically important events for patients with CF, and multiple exacerbations will have negative consequences on the long term prognosis of patients. A complete review of pulmonary exacerbations as an outcome measure in clinical trials is beyond the scope of this article and we will only focus on their relationship and association with functional measures such as FEV_1. There are several definitions of a pulmonary exacerbation, which have also been adapted for use in young children; however there is no standard definition that is used which allows for comparisons between interventional studies [10,58,59]. Often the definition of a pulmonary exacerbation events is linked to patients reporting symptoms, a physician's interpretation of those symptoms and the physician's decision to treat (e.g. IV antibiotics), and therefore can be biased from several perspectives. In some instances the definition includes a quantitative drop in FEV_1 (i.e. 10% drop in FEV_1), in which case all of the limitations of FEV_1 described previously apply. In particular, the relative drop in FEV_1 will depend on numerous factors including the patient's baseline lung function, the time since the previous exacerbation, their age among others [57,60,61]. How baseline lung function is defined may also confound the interpretation of both a drop and improvement in lung function. Furthermore, as more patients live longer with milder lung disease, FEV_1 may not be the adequate measure to define and track these events. When LCI was measured in patients before and after treatment of pulmonary exacerbations and, the overall response was quite variable and not closely correlated to FEV_1 [62]. While this may reflect higher variability of functional measures in patients with more severe disease (the population mostly included in these studies) it could also signify that different functional tests capture different aspects of disease worsening and treatment response; studies matching functional and imaging data may help to clarify this in the future. Given that pulmonary exacerbation are events associated with lung function deterioration that persists in about a third of individuals after treatment [55], and interventional studies assess lung function improvement, it is not surprising that correlation between the two outcome measures is generally poor in clinical trials. Nonetheless both pulmonary exacerbations and functional measures are relevant clinical events and preventing the occurrence of pulmonary exacerbations and/or their frequency is equally important as improving lung function to maintain long term health of patients with CF.

14. Conclusions

The remarkable improvements in survival and lung function in patients with CF mean that it may be necessary to re-evaluate the standard outcomes used for interventional studies. Novel outcomes and creative study designs may help to evaluate new therapies more quickly in the current era of CF disease. The LCI is one of the most promising outcomes, but there is much work to be done before LCI can substitute or compliment FEV_1 in both research and clinical practice.

References

[1] Stephenson AL, Tom M, Berthiaume Y, Singer LG, Aaron SD, Whitmore GA, et al. A contemporary survival analysis of individuals with cystic fibrosis: a cohort study. Eur Respir J 2015;45(3):670–9.

[2] George PM, Banya W, Pareek N, Bilton D, Cullinan P, Hodson ME, et al. Improved survival at low lung function in cystic fibrosis: cohort study from 1990 to 2007. BMJ 2007;342:d1008.

[3] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26(2):319–38.

[4] Taylor-Robinson D, Whitehead M, Diderichsen F, Olesen HV, Pressler T, Smyth RL, et al. Understanding the natural progression in %FEV1 decline in patients with cystic fibrosis: a longitudinal study. Thorax 2012;67(10): 860–6.

[5] Kerem E, Reisman J, Corey M, Canny GJ, Levison H. Prediction of mortality in patients with cystic fibrosis. N Engl J Med 1992;326(18):1187–91.

[6] Dill EJ, Dawson R, Sellers DE, Robinson WM, Sawicki GS. Longitudinal trends in health-related quality of life in adults with cystic fibrosis. Chest 2013;144(3):981–9.

[7] Liou TG, Adler FR, Fitzsimmons SC, Cahill BC, Hibbs JR, Marshall BC. Predictive 5-year survivorship model of cystic fibrosis. Am J Epidemiol 2001;153(4):345–52.

[8] Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 2011;365(18):1663–72.

[9] Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J 2005; 26(5):948–68.

[10] Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme study group. N Engl J Med 1994;331(10):637–42.

[11] Ramsey BW, Pepe MS, Quan JM, Otto KL, Montgomery AB, Williams-Warren J, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic fibrosis inhaled tobramycin study group. N Engl J Med 1999;340(1):23–30.

[12] Elkins MR, Robinson M, Rose BR, Harbour C, Moriarty CP, Marks GB, et al. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. N Engl J Med 2006;354(3):229–40.

[13] Saiman L, Anstead M, Mayer-Hamblett N, Lands LC, Kloster M, Hocevar-Trnka J, et al. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with pseudomonas aeruginosa: a randomized controlled trial. JAMA 2010;303(17):1707–15.

[14] Wainwright CE, Elborn JS, Ramsey BW. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. N Engl J Med 2015;373(18):1783–4.

[15] Cooper PJ, Robertson CF, Hudson IL, Phelan PD. Variability of pulmonary function tests in cystic fibrosis. Pediatr Pulmonol 1990;8(1): 16–22.

[16] Nickerson BG, Lemen RJ, Gerdes CB, Wegmann MJ, Robertson G. Within-subject variability and per cent change for significance of spirometry in normal subjects and in patients with cystic fibrosis. Am Rev Respir Dis 1980;122(6):859–66.

[17] Overall JE, Tonidandel S. Measuring change in controlled longitudinal studies. Br J Math Stat Psychol 2002;55(Pt 1):109–24.

[18] Ramsey KA, Rosenow T, Turkovic L, Skoric B, Banton G, Adams AM, et al. Lung clearance index and structural lung disease on computed tomography in early cystic fibrosis. Am J Respir Crit Care Med 2015.

[19] Aurora P, Stanojevic S, Wade A, Oliver C, Kozlowska W, Lum S, et al. Lung clearance index at 4 years predicts subsequent lung function in children with cystic fibrosis. Am J Respir Crit Care Med 2011;183(6): 752–8.

[20] Crenesse D, Berlioz M, Bourrier T, Albertini M. Spirometry in children aged 3 to 5 years: reliability of forced expiratory maneuvers. Pediatr Pulmonol 2001;32(1):56–61.

[21] Marostica PJ, Weist AD, Eigen H, Angelicchio C, Christoph K, Savage J, et al. Spirometry in 3- to 6-year-old children with cystic fibrosis. Am J Respir Crit Care Med 2002;166(1):67–71.

[22] Neve V, Edme JL, Devos P, Deschildre A, Thumerelle C, Santos C, et al. Spirometry in 3-5-year-old children with asthma. Pediatr Pulmonol 2006.

[23] Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, 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(12):1304–45.

[24] Tepper RS, Reister T. Forced expiratory flows and lung volumes in normal infants. Pediatr Pulmonol 1993;15(6):357–61.

[25] Jones M, Castile R, Davis S, Kisling J, Filbrun D, Flucke R, et al. Forced expiratory flows and volumes in infants. Am J Respir Crit Care Med 2000; 161:353–9.

[26] Matecki S, Kent L, de Boeck K, Le Bourgeois M, Zielen S, Braggion C, et al. Is the raised volume rapid thoracic compression technique ready for use in clinical trials in infants with cystic fibrosis? J Cyst Fibros 2016; 15(1):10–20.

[27] Davis SD, Rosenfeld M, Kerby GS, Brumback L, Kloster MH, Acton JD, et al. Multicenter evaluation of infant lung function tests as cystic fibrosis clinical trial endpoints. Am J Respir Crit Care Med 2010;182(11): 1387–97.

[28] Rosenfeld M, Ratjen F, Brumback L, Daniel S, Rowbotham R, McNamara S, et al. Inhaled hypertonic saline in infants and children younger than 6 years with cystic fibrosis: the ISIS randomized controlled trial. JAMA 2012;307(21):2269–77.

[29] Bakker EM, Borsboom GJ, van der Wiel-Kooij EC, Caudri D, Rosenfeld M, Tiddens HA. Small airway involvement in cystic fibrosis lung disease: routine spirometry as an early and sensitive marker. Pediatr Pulmonol 2013;48(11):1081–8.

[30] Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW. Measurement of FEF25-75% and FEF75% does not contribute to clinical decision making. Eur Respir J 2013.

[31] Lukic KZ, Coates AL. Does the FEF25-75 or the FEF75 have any value in assessing lung disease in children with cystic fibrosis or asthma? Pediatr Pulmonol 2015;50(9):863–8.

[32] Lum S, Gustafsson P, Ljungberg H, Hulskamp G, Bush A, Carr SB, et al. Early detection of cystic fibrosis lung disease: multiple-breath washout versus raised volume tests. Thorax 2007;62(4):341–7.

[33] Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, et al. Outcomes for COPD pharmacological trials: from lung function to biomarkers. Eur Respir J 2008;31(2):416–69.

[34] Kent L, Reix P, Innes JA, Zielen S, Le Bourgeois M, Braggion C, et al. Lung clearance index: evidence for use in clinical trials in cystic fibrosis. J Cyst Fibros 2014;13(2):123–38.

[35] Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. Eur Respir J 2003;22(6):972–9.

[36] Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, et al. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. Am J Respir Crit Care Med 2005;171(3):249–56.

[37] Kraemer R, Blum A, Schibler A, Ammann RA, Gallati S. Ventilation inhomogeneities in relation to standard lung function in patients with cystic fibrosis. Am J Respir Crit Care Med 2005;171(4):371–8.

[38] Horsley A, Macleod K, Saunders C, Gray R, Dewar M, Voase N. UK CF Gene therapy consortium tracking study: lung clearance index improves with treatment of an infective exacerbation. Pediatr Pulmonol 2007; 42(S30):335–6.
[39] Gustafsson PM. Peripheral airway involvement in CF and asthma compared by inert gas washout. Pediatr Pulmonol 2007;42(2):168–76.
[40] Horsley AR, Davies JC, Gray RD, Macleod KA, Donovan J, Aziz ZA, et al. Changes in physiological, functional and structural markers of cystic fibrosis lung disease with treatment of a pulmonary exacerbation. Thorax 2013;68(6):532–9.
[41] Amin R, Stanojevic S, Kane M, Webster H, Ratjen F. A randomized controlled trial to evaluate the lung clearance index as an outcome measure for early phase studies in patients with cystic fibrosis. Respir Med 2016;112:59–64.
[42] Fuchs SI, Gappa M, Eder J, Unsinn KM, Steinkamp G, Ellemunter H. Tracking lung clearance index and chest CT in mild cystic fibrosis lung disease over a period of three years. Respir Med 2014;108(6):865–74.
[43] Horsley AR, Gustafsson PM, Macleod KA, Saunders C, Greening AP, Porteous DJ, et al. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. Thorax 2008;63(2):135–40.
[44] Owens CM, Aurora P, Stanojevic S, Bush A, Wade A, Oliver C, et al. Lung clearance index and HRCT are complementary markers of lung abnormalities in young children with CF. Thorax 2011;66(6):481–8.
[45] Kieninger E, Singer F, Fuchs O, Abbas C, Frey U, Regamey N, et al. Long-term course of lung clearance index between infancy and school-age in cystic fibrosis subjects. J Cyst Fibros 2011;10(6):487–90.
[46] Nguyen TT, Thia LP, Hoo AF, Bush A, Aurora P, Wade A, et al. Evolution of lung function during the first year of life in newborn screened cystic fibrosis infants. Thorax 2014;69(10):910–7.
[47] Singer F, Kieninger E, Abbas C, Yammine S, Fuchs O, Proietti E, et al. Practicability of nitrogen multiple-breath washout measurements in a pediatric cystic fibrosis outpatient setting. Pediatr Pulmonol 2013;48(8): 739–46.
[48] Singer F, Yammine S, Schmidt A, Proietti E, Kieninger E, Barben J, et al. Ventilatory response to nitrogen multiple-breath washout in infants. Pediatr Pulmonol 2014;49(4):342–7.
[49] Amin R, Subbarao P, Jabar A, Balkovec S, Jensen R, Kerrigan S, et al. Hypertonic saline improves the LCI in paediatric patients with CF with normal lung function. Thorax 2010;65(5):379–83.
[50] Amin R, Subbarao P, Lou W, Jabar A, Balkovec S, Jensen R, et al. The effect of dornase alfa on ventilation inhomogeneity in patients with cystic fibrosis. Eur Respir J 2011;37(4):806–12.
[51] Subbarao P, Stanojevic S, Brown M, Jensen R, Rosenfeld M, Davis S, et al. Lung clearance index as an outcome measure for clinical trials in young children with cystic fibrosis. A pilot study using inhaled hypertonic saline. Am J Respir Crit Care Med 2013;188(4):456–60.
[52] Davies J, Sheridan H, Bell N, Cunningham S, Davis SD, Elborn JS, et al. Assessment of clinical response to ivacaftor with lung clearance index in cystic fibrosis patients with a G551D-CFTR mutation and preserved spirometry: a randomised controlled trial. Lancet Respir Med 2013;1(8): 630–8.
[53] Jensen R, Stanojevic S, Gibney K, Salazar JG, Gustafsson P, Subbarao P, et al. Multiple breath nitrogen washout: a feasible alternative to mass spectrometry. PLoS One 2013;8(2):e56868.
[54] Lum S, Stocks J, Stanojevic S, Wade A, Robinson P, Gustafsson P, et al. Age and height dependence of lung clearance index and functional residual capacity. Eur Respir J 2013;41(6):1371–7.
[55] Sanders DB, Bittner RC, Rosenfeld M, Hoffman LR, Redding GJ, Goss CH. Failure to recover to baseline pulmonary function after cystic fibrosis pulmonary exacerbation. Am J Respir Crit Care Med 2010;182(5): 627–32.
[56] Sanders DB, Bittner RC, Rosenfeld M, Redding GJ, Goss CH. Pulmonary exacerbations are associated with subsequent FEV1 decline in both adults and children with cystic fibrosis. Pediatr Pulmonol 2011;46(4):393–400.
[57] Waters V, Stanojevic S, Atenafu EG, Lu A, Yau Y, Tullis E, et al. Effect of pulmonary exacerbations on long-term lung function decline in cystic fibrosis. Eur Respir J 2012;40(1):61–6.
[58] Treggiari MM, Rosenfeld M, Mayer-Hamblett N, Retsch-Bogart G, Gibson RL, Williams J, et al. Early anti-pseudomonal acquisition in young patients with cystic fibrosis: rationale and design of the EPIC clinical trial and observational study. Contemp Clin Trials 2009;30(3):256–68.
[59] Bilton D, Canny G, Conway S, Dumcius S, Hjelte L, Proesmans M, et al. 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.
[60] VanDevanter DR, Elkin EP, Pasta DJ, Morgan WJ, Konstan MW. Investigators, et al. changing thresholds and incidence of antibiotic treatment of cystic fibrosis pulmonary exacerbations, 1995-2005. J Cyst Fibros 2013;12(4):332–7.
[61] Wagener JS, Rasouliyan L, VanDevanter DR, Pasta DJ, Regelmann WE, Morgan WJ, et al. Oral, inhaled, and intravenous antibiotic choice for treating pulmonary exacerbations in cystic fibrosis. Pediatr Pulmonol 2013;48(7):666–73.
[62] Sonneveld N, Stanojevic S, Amin R, Aurora P, Davies J, Elborn JS, et al. Lung clearance index in cystic fibrosis subjects treated for pulmonary exacerbations. Eur Respir J 2015.