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The Effect of Dornase Alfa on Ventilation Inhomogeneity in Patients with Cystic Fibrosis

Reshma Amin*[1,2] MD MSc, Padmaja Subbarao*[1,2,3] MD MSc, Wendy Lou[2,4] PhD, Alisha Jabar[1] BSc , Susan Balkovec[1] RRT, Renee Jensen[1] RRT, Shawn Kerrigan[1] RRT, Per Gustafsson[5,6] MD PhD, Felix Ratjen[1,2,3] MD PhD

1. Division of Respiratory Medicine, Department of Paediatrics, The Hospital for Sick Children, Toronto, Canada
2. University of Toronto, Toronto, Canada
3. Physiology & Experimental Medicine, Research Institute, The Hospital for Sick Children, Toronto, Canada
4. Division of Biostatistics, Dalla Lana School of Public Health, Toronto, Canada
5. Department of Paediatrics, Central Hospital, S-541 85 Skövde, Sweden
6. The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

*Drs. Amin and Subbarao share first authorship on this manuscript.

Address for Reprints and Correspondence:
Reshma Amin MD
The Hospital for Sick Children
555 University Avenue
Toronto Ontario M5G 1X8
Tel (416) 813 6167
Fax (416) 813 6246
reshma.amin@sickkids.ca

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ABSTRACT

Rationale: Outcome measures to assess therapeutic interventions in cystic fibrosis (CF) patients with mild lung disease are lacking. Our aim was to determine if the Lung Clearance Index (LCI) can detect a treatment response to dornase alfa in paediatric CF patients with normal spirometry.

Methods: CF patients between 6 and 18 years of age with FEV_1% ≥80% predicted were eligible. In a crossover design, 17 patients received 4 weeks of dornase alfa and placebo in a randomized sequence separated by a 4-weekwashout period. The primary endpoint was the change in LCI from dornase alfa versus placebo. A mixed model approach incorporating period-dependent baselines was used. This trial was registered with ClinicalTrials.gov, number NCT00557089.

Results: The mean age ± SD was 10.32 ± 3.35 years. Dornase alfa improved LCI versus placebo (0.90 ± 1.44, p=0.022). A significant improvement in LCI occurred in seven of the seventeen subjects. Small airways flows as measured by forced expiratory flow at 25-75% expired volume (FEF_{25-75}) measured by percent predicted and z-scores also improved in subjects on dornase alfa (6.1% ± 10.34, p=0.03; and 0.28 z-score ± 0.46, p=0.03).

Conclusions: Dornase alfa significantly improved LCI. Therefore the LCI may be a suitable tool to assess early intervention strategies in this patient population.

INTRODUCTION:

Cystic Fibrosis (CF) is a common fatal autosomal recessive disease. Chronic respiratory disease is responsible for the shortened life span in most patients. CF is characterized by an impairment of chloride transport resulting in impaired mucociliary clearance and recurrent pulmonary infections which leads to irreversible structural lung damage. Dornase alfa, a recombinant human DNAse I, is an established therapeutic intervention for CF patients of all lung disease severities (1, 2). Dornase alfa hydrolyzes extracellular deoxyribonucleic acid present in elevated levels in lower airway secretions (3). Dornase alfa has been shown to have a beneficial effect on lung function in patients with mild lung disease but a two year study period and 474 patients were required to detect a treatment benefit (2). Given the constant improvement in the annual decline in lung function in CF patients and the increasing proportion of CF patients with normal pulmonary function due to improved clinical care, more sensitive measures of lung function are needed for clinical studies in CF patients with mild disease.

The Lung Clearance Index (LCI) is a measure of ventilation inhomogeneity determined during Multiple Breath Washout (MBW). Cross-sectional studies in CF patientshave shown that the LCI is more sensitive at detecting lung disease than spirometry (4-9). LCI is determined during tidal breathing and normative values are consistent across the paediatric age range beyond infancy (4-10). A previous trial with hypertonic saline suggested that the LCI could be a potentially useful endpoint for interventional trials in CF patients with mild lung disease but this needs to be confirmed by additional evidence from interventional trials in mild patients (11).

Therefore we designed a study to test the effect of inhalation of dornase alfa in paediatric CF patients with a FEV_1% predicted ≥80% using the LCI as the primary outcome measure. Our research hypothesis was that amongst paediatric Cystic Fibrosis patients with mild lung disease

(i.e FEV_1% predicted ≥80%), four weeks of dornase alfa inhalation as compared to four weeks of placebo inhalation will improve the Lung Clearance Index.

METHODS:

Participants

Eligible patients had to have a confirmed diagnosis of CF; between 6 and 18 years of age; be able to perform reproducible spirometry; have a baseline FEV_1% predicted greater than or equal to 80% at the screening visit; and an oxyhemoglobin saturation of greater than or equal to 90% on room air. Exclusion criteria included airway cultures yielding *Burkholderia cepacia* complex in the previous 2 years or Non-tuberculous mycobacteria in the past year; current oral corticosteroid use; oxygen supplementation; lung transplantation; intravenous antibiotics or oral quinolones within 14 days of enrolment; or any investigational drugs within 30 days of enrolment.

The study was approved by the institutional review board of the Hospital for Sick Children and by Health Canada. All tests were performed in the pulmonary lung function laboratory at the Hospital for Sick Children, Toronto, Canada, between March 2008 and June 2009.

Study Design

This was a 12-weekcrossover trial consisting of two, 4-week treatment periods separated by a 4-week washout. Study visits occurred at 0, 4, 8 and 12 weeks after randomization.

At a screening visit, demographic characteristics, clinical data, physical examination and spirometry were recorded. Eligible participants were assigned to a treatment intervention (i.e. dornase alfa in period 1 versus placebo in period 1 by means of a concealed, computer generated randomization performed by a research pharmacist not otherwise involved in the study.

Clinicians and research personnel remained unaware of the treatment assignments throughout the study including the primary efficacy analysis.

The solutions were indistinguishable from each other. The solutions were administered using the PARI LC® Star nebulizer (Pari, Midlothian, VA, USA). Patients received either 2.5 ml of dornase alfa or placebo once daily for each 4-week treatment period.

At the first and third visit, spirometry and pulse oximetry were performed at baseline and fifteen minutes after inhalation of the study drug. Participants whose oxyhemoglobin saturation exceeded 90% and whose FEV_1% predicted exceeded 80% of baseline value after inhalation completion were eligible to proceed in the trial. All other treatments were maintained throughout the trial.

Assessment of Outcomes

LCI, spirometry and the Cystic Fibrosis Questionnaire-Revised (CFQ-R) were completed at each study visit (4-6, 9, 12, 13). LCI was performed first followed by the CFQ-R and then spirometry. LCI was the primary outcome. Secondary outcome measures included: Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC), Forced Expiratory Flow at 25% to 75% of the Forced Vital Capacity (FEF_{25-75}) and the Cystic Fibrosis Questionnaire-Revised (CFQ-R).

The MBW setup was identical to the one used in previous publications by Gustafsson et al and Aurora et al with the exception of the pneumotachograph which was replaced by a Hans Rudolph pneumotachograph (Rudolph Linear Pneumotach, Hans Rudolph**,** Shawnee, KS, USA) (4-6, 9). Each MBW test consisted of a wash-in and a washout phase. All MBW tests were performed in the sitting position. Children wore nose clips and were required to breathe through

a small or large mouthpiece (VacuMed mouthpieces # 1000 and 1004, Ventura, CA, USA). We used individual mouthpieces and pneumotachs which were removed and cleaned after individual use. The decision to use the large or small mouthpiece was based on a composite measure of the patients weight (≥ 40kg, large mouthpiece, <40kg, small mouthpiece) as well as mask visit assessed by the LCI technician during the washout. The deadspace of the small mouthpiece was 5ml and the deadspace of the large mouthpiece was 8ml. The post capillary deadspace was 14ml. Therefore, the effective deadspace was less than 2ml/kg for all patients.

Gas concentrations were measured by mass spectrometer (AMIS 2000; Innovision A/S, Odense, Denmark). During the test, the participant was encouraged to watch a video, listen to a portable music device or read a book with the intention of distracting the subject, thus encouraging regular breathing at rest. Patients were asked to tidal breathe a dry gas mixture containing 4% SF_6, 4% He, 21% O_2 and balance N_2 via a flow past system connected to the pneumotachograph until the inspiratory and expiratory SF_6 concentration were equilibrated and stable at 4%. Subsequently, during expiration the flow past system was disconnected and the subject was asked to tidal breathe room air. The washout phase was completed when the end tidal SF_6 concentration was <0.1% for 2 consecutive tidal breaths. Each washout was performed in triplicate at a minimum. The LCI was calculated as the number of lung volume turnovers (cumulative expired volume divided by the FRC) required to reduce end-tidal SF_6 concentration to $1/40^{th}$ of the starting value (4-6). The final LCI value represents the mean of at least three LCI maneuvers calculated from the three technically acceptable washout tests. The following all had to be met for a washout to be considered technically acceptable: appropriate calibration of the mass spectrometer, regular tidal breathing, no evidence of leak, a plateau of SF_6 concentration of 4% at the end of washin, SF_6 concentration of 0% with the first inspiration during the washout

phase, an acceptable capnogram and slope of washout phase on the first exhaled breath, and at least two consecutive breaths with an SF_6 concentration of less than one fortieth (0.1%) the starting concentration at the end of the washout. FRC, tidal volume (Vt) and Vt/FRC were also measured for quality assurance.

Spirometry was performed according to ATS standards using the Vmax systems (VIASYS, Cardinal Health) (12). Reference data was calculated using the Stanojevic reference equations (14). The Quality of life was assessed using the CF specific CFQ-R. One of the three participant formats of the CFQ-R was used depending on the age of the participant: Adolescent and Adults (Patients 14 years old and older), Children Ages 12 and 13 and Children Ages 6-11; a parent questionnaire was completed in addition where appropriate as per the CFQ-R administration guidelines (for Children Ages 6-13 years) (13). The instrument yielded a score of 0 to 100 for each domain with higher numbers indicating better function on various domains (13). The CFQ-R was administered prior to spirometry at each study visit.

Safety and Compliance

Safety was assessed by monitoring adverse events and changes in vital signs, clinical symptoms, physical examination and or spirometry. Patients were withdrawn from the study if they required hospital admission for a pulmonary exacerbation; corticosteroids for a new diagnosis of Allergic Bronchopulmonary Aspergillosis or required oral fluoroquinolones during either of the two study periods. Worsening CF symptoms and/ or the prescription of antibiotics were treated as adverse events. The prescription of antibiotics was at the discretion of the responsible physician in accordance with current CF practice guidelines at our hospital.

Study drug was dispensed at week 0 and week 8 study visits. Used and unused ampoules were subsequently collected to assess treatment compliance at the week 4 and week 12 study visits. Compliance was based on the number of returned ampoules for each study period.

Statistical Analysis

The mixed model approach was used to test the treatment effects of dornase alfa and placebo on the LCI. This method was chosen because all study participants were measured under two different conditions (dornase alfa and placebo treatments). The variables considered in the final fixed subject effects models include treatment type (dornase alfa or placebo), study period (week 0-4 or 8-12), and period-dependent baseline measurements, following Kenward et al. (15) and Jones et al. (16) for obtaining unbiased treatment effects. Similar models were generated for each secondary outcome measure.

Data were analyzed according to the intention-to-treat-principle. Descriptive statistics were used to describe the study population. Normality was determined using the Kolmogorov-Smirnov test.

We calculated the sample size required for testing using dornase alfa as the main exposure variable and the LCI as the primary outcome variable. Our estimate was based on published baseline mean (standard deviation) LCI of 11.54 (2.86) for a population of CF school age children from the UK (5). We estimated a treatment effect of 3.00 +/- 2.86 in the LCI from dornase alfa versus placebo. Assuming a significance level of five percent and a power of eighty percent, 17 study participants would be needed to complete this crossover study (17). Based on an attrition rate of 20% from similar trials from our centre, we aimed to recruit a total of 20

patients or 17 patients that completed the protocol. P-values <0.05 were considered statistically significant.

Statistical Analysis Systems software version 9.2 (SAS Institute, Inc., Cary, NC) was used to conduct all analyses.

RESULTS

Nineteen patients entered the study and underwent randomization (Figure 1, Table 1). The LCI results of one patient failed to meet the quality control criteria for one of the four study visits. One other study patient dropped out of the study after two study visits because of a pulmonary exacerbation. Complete crossover data were therefore available for 17 patients.

Table 1: Baseline characteristics of study participants

	Study Participants (n=17)
Age in years (Mean ± SD)	10.3 ± 3.4
Female/Male	11/8
***Pseudomonas aeruginosa* +ve**	3
Pancreatic Insufficient %	95%
ΔF508/ ΔF508 %	37%
ΔF508 compound Heterozygous %	21%
FVC% predicted	97.6 ± 11.4 (82.5, 114.1)
FVC z-score	-0.09 ± 0.9 (-0.5,0.3)
FEV_1% predicted	90.7 ± 9.1 (80.00, 109.78)
FEV_1 z-score	-0.8 ± 0.7 (-1.1, -0.5)
FEV_1/FVC (%)	81.5 ± 7.9 (77.74, 85.22)
FEV_1/FVC z-score	-0.009± 5.5 (-2.6, 2.6)
FEF_{25-75}% predicted	76.9 ± 20.0 (51.7, 119.2)
FEF_{25-75} z-score	-1.1 ± 0.9 (-1.6, -0.6)

FEV_1 denotes forced expiratory volume in one second
FVC forced vital capacity
$FEF_{25–75}$ forced expiratory flow at 25% to 75% of the forced vital capacity

Pseudomonas aeruginosa + defined as two or more positive cultures in the previous year and/or currently on inhaled anti-pseudomonal therapy.

Expressed as Means +/- SD and 95% Confidence Interval

% predicted spirometry values were calculated using the *Stanojevic et al* reference equations (14)

LCI was performed in triplicate during each testing occasion. The intra-visit CV was 6.8% ± 3.9%, 95% CI [6.0, 7.8] (Online Supplement, Table 1). During the course of the study, patients returned for three more visits after the first visit. The two baseline visits for each treatment period were approximately 8 weeks apart (mean [range] 8.3 weeks [6.7-10.1]); the coefficient of variation for these two baseline visits was 10.3% ± 3.9, 95% CI [8.6, 12.1] (Online Supplement Table 1). The variability of the LCI was not related to the magnitude of the LCI for the two baseline visits (r=0.22, p=0.4) (Figure 2).

LCI was measured in 28 healthy Canadian children (18). The mean ± SD for age was 10.1 ± 3.1 years. The mean ± SD for LCI was 6.13 ± 0.41. The upper limit of normal based on 2 standard deviations is 6.95. LCI was significantly higher in our cohort of children with CF compared to aged matched healthy controls (mean difference 2.63 (95% CI 1.96 to 3.31), p<0.001 (18). If an abnormal LCI was defined as a value above +1.96SD of the mean LCI from our healthy control group, all patients had abnormal LCI at the first study visit. In contrast only 3/17 (18%) had abnormal FEF_{25-75} z-score (Online supplement Figure 1).

For LCI, the mean ± SD for each study visit for each subject is shown in Online supplement Table 2. Initial analysis of the data revealed a period effect due to the fact that the baselines were not equal; LCI was significantly worse prior to placebo therapy than dornase alfa, 8.75 ± 1.72 and 8.31 ± 1.48, respectively (p=0.034). Thus a mixed model analysis with fixed subject effects was performed with baselines as a factor which determines the relative improvement in LCI from dornase alfa as compared to placebo. In this model, four weeks of dornase alfa resulted in a significantly improved LCI compared to placebo (0.9 ± 0.34, p=0.02) (Table 2). The spectrum of treatment response to dornase alfa and placebo is shown in Figure 3.

There were no significant differences in FRC (L), Vt (L) or Vt/FRC (%) for either placebo or dornase alfa treatment periods.

The absolute changes for LCI from placebo and Dornase alfa inhalations are presented in Table 2 Online Supplement. The mean ± SD, 95% CI for absolute change in LCI from placebo: 0.31± 1.36, 95% CI[-0.33, 0.95]. The mean ± SD, 95% CI for absolute change in LCI from dornase alfa: 0. 71± 1.23, 95% CI [0.12, 1.3]. The intervisit coefficient of variation was determined for the placebo period of this study, the placebo period of the hypertonic saline (11) and for the pooled placebo periods of both studies. The mean and 95% CI for intervisit coefficient of variation for the placebo period in this study, the placebo period in the hypertonic saline study and pooled placebo data for both studies are 10.3% [8.6, 12], 7.3% [5.8, 8.7] and 8.8% [7.5, 10] respectively. Therefore, based on the results for the placebo group for the dornase alfa study, 6/17 patients had a significant improvement in the LCI after treatment with dornase alfa inhalation as compared to 7/17 patients based on the pooled placebo data for both studies. Therefore, a significant treatment effect in the LCI of greater than ten percent from baseline could be considered as the minimum clinically important difference (MCID) for future interventional trials powered using the LCI.

Table 2: Summary of Outcome Measures using the mixed model approach with fixed subject effects

	Placebo		Dornase Alfa		Treatment Effect*	P value**
	Pre	Post	Pre	Post		
LCI	8.75 ±1.72	8.52 ±1.19	8.31 ± 1.48	7.69 ± 1.65	-0.90 ± 1.44	0.02
FVC % Predicted	93.08 ± 8.91	95.64 ± 8.60	102.76 ± 12.35	108.08 ± 11.42	-3.61 ± 9.53	0.14

FVC z-score	-0.55 ± 0.69	-0.36 ± 0.69	0.24 ± 0.99	0.66 ± 0.92	-0.28 ± 0.78	0.16
FEV_1% Predicted	89.68 ± 10.68	90.44 ± 7.80	92.09 ± 6.57	97.72 ± 9.96	0.076 ± 8.43	0.97
FEV_1 z-score	-0.84 ± 0.85	-0.79 ± 0.63	- 0.62 ± 0.54	-0.176 ± 0.77	0.015± 0.16	0.93
FEF_{25-75} % Predicted	76.52 ±17.80	84.01 ± 19.94	77.33 ± 23.71	85.54 ± 30.36	6.09 ± 10.34	0.03
FEF_{25-75} z-score	-1.08 ± 0.85	-0.73 ± 0.91	-1.09 ±1.10	-0.76 ± 1.34	0.28 ± 0.46	0.03
CFQ-R Respiratory Domain	79.17 ± 17.68	73.89 ± 16.98	80.56 ± 12.50	70.07 ± 21.24	0.84 ± 17.22	0.87
CFQ-R Parent Respiratory Domain	75.42 ± 12.58	79.53 ± 21.30	80.95 ± 16.27	76.39 ± 22.57	9.78 ± 18.38	0.09

Expressed as means +/- SD and 95% Confidence Interval
* Estimated treatment effects (Placebo versus Dornase Alfa) from the model
** P value for comparison of post Placebo to post Dornase Alfa, while incorporating the period-dependent baseline (pre) measures
% predicted spirometry values were calculated using the *Stanojevic et al* reference equations (14)

For FEF_{25-75}% predicted, the mean ± SD for each study visit for each subject is shown in Online supplement Table 2 and Figure 4. Small airways flows as measured by forced expiratory flow at 25% to 75% expired volume (FEF_{25-75}) as measured in litres/second, percent predicted and z-scores also improved in subjects on dornase alfa compared to those on placebo (0.18L/s ± 0.72, p=0.03; 6.1% ± 10.34, p=0.03; and 0.28 z-score ± 0.46, p=0.03) in the fixed subject effects model (Table 2).

Forced expiratory volume in one second (FEV_1) and Forced Vital Capacity (FVC) in % predicted, litres/second and z-scores as well as CFQ-R respiratory domain and CFQ-R parent respiratory domain scores were not significantly different after dornase alfa inhalation compared to placebo (Table 2, Online Supplement Figure 2). LCI significantly correlated with FEF_{25-75}% predicted, FEF_{25-75} z-score, FEF_{25-75} litres/second, FEV_1% predicted and FEV_1 z-score, The

strongest correlation was found between LCI and FEF_{25-75}% predicted (r= -0.43, p=0.0001) (Online Supplement Table 3).

After administration of the first dose of assigned solution, the FEV_1% predicted fell by a mean of 1 ± 135 ml after placebo inhalation and 39 ± 140 ml after dornase alfa inhalation but there was no significant difference (p=0.47). None of the patients had a drop of FEV_1% predicted $\geq$20%. There were more overall adverse events during the dornase alfa treatment period as compared to placebo period (Online Supplement Table 4). Adverse events included cough, pulmonary exacerbation, increased sputum production, rhinorrhea, dry throat, sore throat, wheeze, and chest pain. Cough was the most common adverse event and was experienced by seven patients during the dornase alfa treatment periods.

Adherence to treatment, as judged by the number of returned ampoules was 88.93 ± 13.42% for the placebo study period and 95.81 ± 4.79 % percent for the dornase alfa study period (p=0.043). Greater than 80% compliance was seen in 79% of patients during the placebo treatment and 100% of patients during treatment with dornase alfa. Treatment allocation sequence was correctly guessed by 9/19 (47%) of the study participants.

DISCUSSION

We have demonstrated that the lung clearance index as well as FEF_{25-75} z-score, % predicted and Litres is able to detect a treatment effect from 28 days of dornase alfa as compared to placebo in seventeen CF patients with mild lung disease. These data add to the evidence that LCI may be a potential outcome measure for interventional trials in patients with preserved lung function.

Although the results regarding the effects on LCI are similar to our recent findings regarding hypertonic saline in a similar patient population, there are some notable differences (11). The magnitude of the treatment effect of the LCI from dornase alfa was smaller than for hypertonic saline. This is surprising given the published literature suggesting the contrary with regards to lung function (19). In this study the FEF_{25-75} parameters also significantly improved with dornase alfa in addition to the LCI thus supporting the LCI as a measure of the small airways. However there was no significant correlation between the changes in these two parameters during treatment suggesting that complementary information can be obtained by performing both tests as previously shown in a cross-sectional study of the LCI and raised lung volume rapid thoraco-abdominal compression in CF infants (10).

Another possible explanation may lie in the different mechanism of action of each of these drugs. Dornase alfa is a mucolytic agent that decreases the viscosity and surface adhesivity of CF sputum and thus improves mucus clearance (3). However hypertonic saline improves mucociliary clearance by restoring the depleted airway surface liquid layer. Therefore the treatment responsiveness of LCI could differ in sensitivity depending on the mechanism of action of the intervention. This raises the question whether LCI may be particularly sensitive to interventions that improve airway surface hydration and mucociliary clearance, an early step of

CF pathophysiology. If confirmed this could potentially have implications for interventional studies of agents targeting the basic defect such as gene replacement therapy, CFTR or non CFTR directed ion channel pharmacotherapy (20).

Finally, the differences between the two interventional studies may be the explained by differing changes in the placebo group. In this study, LCI trended towards improvement during the placebo period (regardless of treatment sequence) while it worsened in the hypertonic saline study (11). Therefore given the cross-over design of both trials, the different magnitudes of the treatment effect may have been driven by the placebo effects going in opposite directions. As placebo effects are difficult to predict in study designs it is reassuring that LCI was able to detect a treatment effect in both studies regardless of the behaviour of the placebo group. In addition when the placebo arm is excluded, the magnitude of the treatment effect from dornase alfa and hypertonic saline (excluding the placebo periods) as measured by the LCI are not different (0.71 ± 0.30 versus 0.88± 0.29, p=0.99). Furthermore, based on the pooled placebo results of this study as well as the authors recent study using hypertonic saline, a ten percent improvement in LCI from baseline should constitute a clinically significant improvement from an intervention based on the limited longitudinal data available at present for the LCI.

There were several limitations of the current study. The mean treatment effect and variability of the LCI from dornase alfa was less than what was anticipated in the sample size calculation *a priori*. The reason for this has been previously explained elsewhere (11). Furtheremore, the baseline LCI measurements before placebo and dornase alfa treatment periods were significantly different. This likely reflects chance variation and the ability of the LCI to measure fluctuations in lung disease in CF patients over time thus supporting the robustness of the LCI as a primary outcome measure. Our study was limited to a patient population that was

able to perform technically adequate spirometry and thus, it has not yet been established as to whether the LCI is a responsive outcome measure for a treatment intervention trial in infants and preschool children. In addition, Type 1 error is always appreciable when making conclusions based on small numbers of patients. Therefore, there is a 5% chance that this is the case. Based on our study results, there was a 1% chance of Type 2 error.

In summary, the LCI is a sensitive and responsive outcome measure that was able to detect a significant treatment effect from dornase alfa in a pediatric cohort of CF patients with mild lung disease. Although there is some overlap, the LCI and the FEF parameters seem to represent different structural and functional abnormalities of the small airways and subsequently identify different treatment responders. Therefore the LCI is a promising outcome measure given the ability to perform clinical testing in patients of all ages and disease severities, to identify treatment responders that would not be identified by other conventional outcomes as well as its potential to facilitate the rapid integration of new therapies into clinical practice.

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Figure 1: Randomization and Enrolment of Study Participants

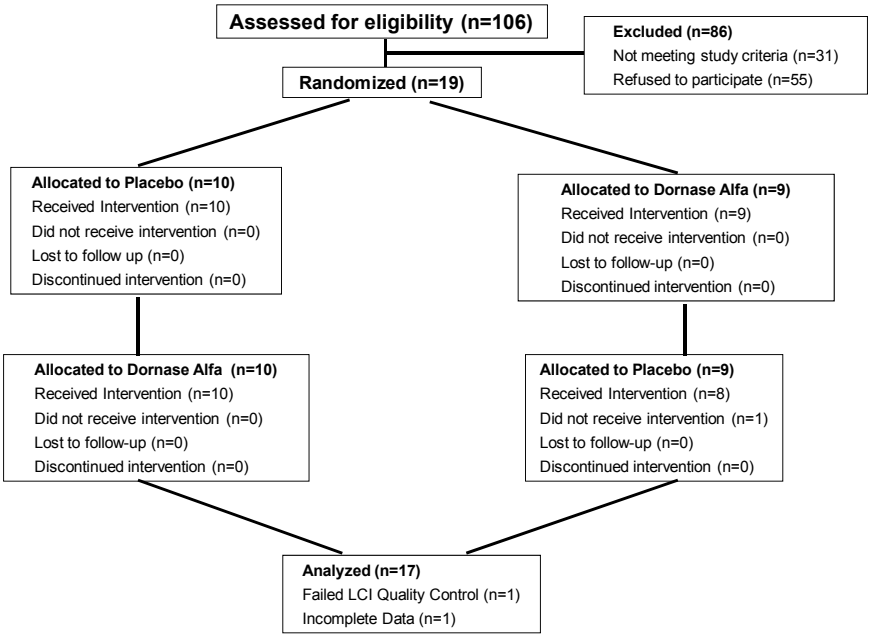

Figure 2: The LCI limits of agreement for the mean of the two baseline LCI measurements versus the difference in LCI of the two baseline measurements. The solid horizontal line represents the mean and the dotted horizontal lines represent the 95% confidence interval.

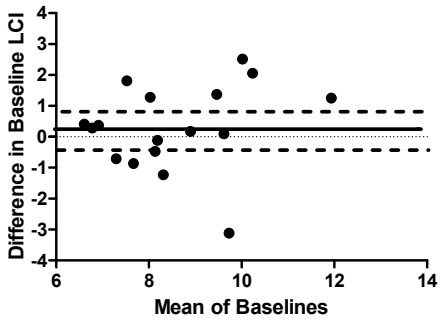

Figure 3: Pre and Post Lung Clearance Index (LCI) for each study participant for the Placebo Treatment period and the Dornase Alfa Treatment Period. Solid circles refer to the Placebo and Hollow circles refer to Dornase Alfa. The p value represents the difference in the treatment effect size of Dornase Alfa versus Placebo calculated using the mixed model approach with fixed subject effects (p=0.022).

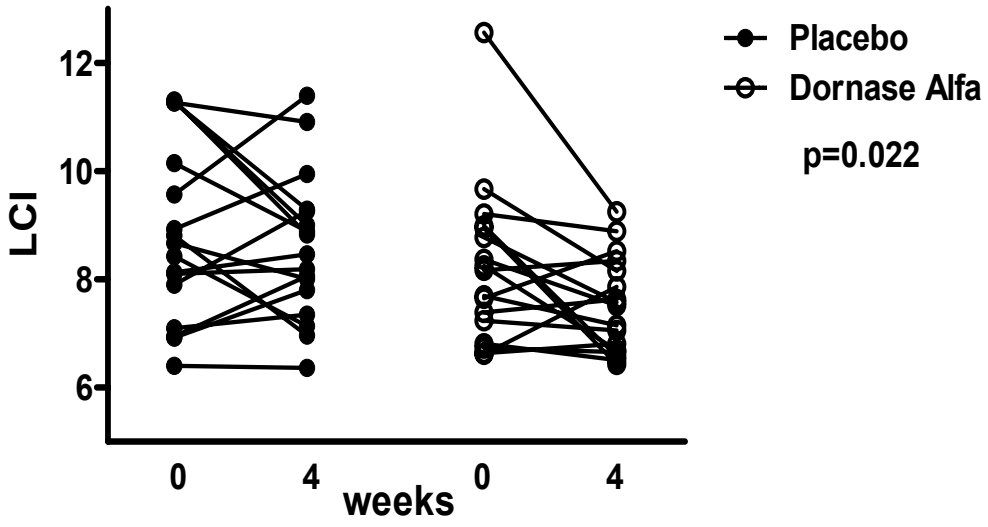

Figure 4: Pre and Post FEF_{25-75} % Predicted for each study participant for the Placebo Treatment period and the Dornase Alfa Treatment Period. Solid circles refer to the Placebo and Hollow circles refer to Dornase Alfa. The p value represents the difference in the treatment effect size of Dornase Alfa versus Placebo calculated using the mixed model approach with fixed subject effects (p=0.029).

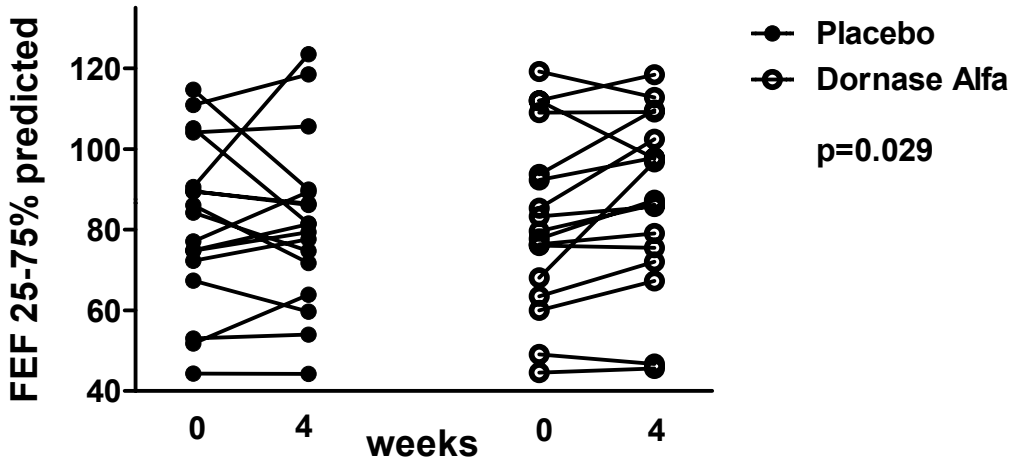

Figure 5: Correlation between the change in LCI and the change in FEF_{25-75} (L/s) from four weeks of Dornase Alfa inhalation