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Early Cystic Fibrosis Lung Disease detected by Bronchoalveolar Lavage and Lung Clearance Index

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At a Glance Commentary

Scientific Knowledge on the Subject

Airway infection, inflammation and structural change such as bronchiectasis are present within the first few months of life even in newborn-screened infants with cystic fibrosis (CF). A decline in lung function has also been reported particularly in infants infected with *Pseudomonas aeruginosa*. As these early pathologic events occur mostly in the absence of symptoms, the role of bronchoalveolar lavage (BAL), infant lung function testing (ILFT) and high resolution computed tomography (HRCT) for surveillance of early CF lung disease are under evaluation. The lung clearance index (LCI), a simple measure of ventilation inhomogeneity reflecting small airways disease, has been shown to sensitively identify abnormal lung function in preschool and older children with cystic fibrosis. However its role as a non-invasive marker of early small airway disease in infants and young children with CF and its variability in this younger age group has not been determined.

What this Study Adds to the Field

The LCI is a repeatable measure of small airway function in healthy infants and young children and similarly aged children with CF. The LCI is elevated in stable, well nourished newborn-screened infants and young children with CF compared to their healthy peers. An abnormal LCI is associated with airway inflammation and *Pseudomonas aeruginosa*. Our findings suggest that the LCI may be a useful marker of early CF lung disease. Furthermore our LCI repeatability data highlight the potential role of the LCI as an objective outcome measure for future intervention trials involving young children with CF.

ABSTRACT

Rationale: Unrecognised airway infection and inflammation in young children with cystic fibrosis (CF) may lead to irreversible lung disease therefore early detection and treatment is highly desirable.

Objectives: To determine whether the lung clearance index (LCI) is a sensitive and repeatable non-invasive measure of airway infection and inflammation in newborn-screened children with CF.

Methods: Forty-seven well children with CF (mean age, 1.55 years) and 25 healthy children (mean age, 1.26 years) underwent multiple-breath washout testing. LCI within and between-test variability was assessed. Children with CF also had surveillance bronchoalveolar lavage (BAL) performed.

Measurements and Main Results: Mean (SD) LCI in healthy children was 6.45 (0.49). LCI was higher in children with CF, 7.21 (0.81), $P < 0.001$. The upper limit of normal for LCI was 7.41. Fifteen (32%) children with CF had an elevated LCI. LCI measurements were repeatable and reproducible. Airway infection was present in 17 (36%) children with CF including 7 (15%) with *Pseudomonas aeruginosa.* Polymicrobial growth was associated with worse inflammation. LCI was higher in children with *Pseudomonas,* 7.92 (1.16), than in children without *Pseudomonas,* 7.02 (0.56), $P = 0.038$. LCI correlated with BAL interleukin-8, $R^2 = 0.20$, $P = 0.004$ and neutrophil count $R^2 = 0.21$, $P = 0.001$. A LCI below the upper limit of normality had a high negative predictive value (93%) in excluding *Pseudomonas.*

Conclusions: LCI is elevated early in CF especially in the presence of *Pseudomonas* and airway inflammation. LCI is a feasible, repeatable and sensitive non-invasive marker of lung disease in young children with CF.

Word count: 250

Key words: lung clearance index, bronchoalveolar lavage, cystic fibrosis, *Pseudomonas aeruginosa*

INTRODUCTION

Cystic fibrosis lung disease is characterised by unremitting cycles of airway infection and inflammation which begin early in life and are often clinically inapparent.[1] During infancy airway inflammation may be associated with significant structural change including bronchiectasis.[2] As early childhood is a crucial period of rapid lung development, unrecognised airway disease may have irrevocable consequences for later respiratory health.[3] Therefore investigations which can sensitively identify early disease and which are also non-invasive, repeatable and simple to apply clinically may have the greatest potential to improve outcomes.[4]

Oropharyngeal cultures,[5;6] chest x-rays[7] and serum antibodies[8] are insensitive to detect early CF lung disease. Therefore the indications for bronchoalveolar lavage (BAL), high resolution computed tomography (HRCT) and infant lung function testing (ILFT) are currently being determined. Additionally clinically relevant outcome measures are needed to evaluate new therapies in trials involving younger children with CF.[9-11] Bronchoalveolar lavage is the present standard for diagnosing lower airway infection and inflammation in young children.[12;13] However BAL remains invasive and limited sampling may underestimate infection or inflammation due to regional variability.[14;15] Few centres perform surveillance BAL in young children with CF.[16;17]

Given the inherent invasiveness of BAL, the optimum interval between bronchoscopic sampling in order to monitor early disease is unknown. Similarly HRCT, which has sensitively revealed structural abnormalities in infants as young as 3 months,[2] has cumulative radiation exposure concerns which may hamper its role as a serial outcome measure of early CF lung isease.[18]

In contrast, ILFT has demonstrated both an ability to non-invasively identify early airway disease in cross-sectional studies[19-21] and to define its evolution over time.[16;22] Both the raised volume rapid thoraco-abdominal compression technique[21] and the multiple-breath washout (MBW) method[20] have detected abnormal lung function in infants with CF. However a recent multicentre study using the raised volume rapid thoraco-abdominal compression technique identified "poor feasibility, low repeatability and the need for large sample sizes to detect reasonable treatment effects" as important constraints with this technique in clinical trials involving infants with CF.[23]

The lung clearance index (LCI), a commonly reported MBW outcome measure, may more sensitively detect early functional pulmonary impairment, as it reflects ventilation inhomogeneity due to small airway pathology, the hallmark of early CF lung disease. The LCI has identified early disease more sensitively than spirometry in preschool children with CF.[24] However the ability of the LCI to detect presymptomatic disease in newborn-screened infants and young children with CF, as well as its variability in this age group, remains unknown.

The aims of the current research therefore, were to evaluate the feasibility and repeatability of the LCI in infants and young children with and without CF and to determine the association between the LCI and airway inflammation and infection. Hence our objective was to determine the utility and sensitivity of the LCI as a non-invasive measure of early lung disease in infants and young children with CF. We hypothesised that the LCI would be elevated in presymptomatic/minimally symptomatic newborn-screened infants and young children with CF when compared to their healthy peers reflecting early subclinical lung disease. Some of the results of this study have been previously reported in the form of abstracts.[25;26]

METHODS

Subjects

Children with CF less than 3 years of age, admitted for an annual BAL to The Sydney Children's Hospital, Randwick, between June 2004 and August 2009 as part of an early disease surveillance programme, were recruited for MBW testing. Infants were identified through newborn screening or by meconium ileus presentation and the diagnosis confirmed by sweat chloride concentration > 60 mmol/mL or by CF genetic mutation analysis. Exclusion criteria were (1) respiratory infection within 3 weeks and (2) co-existing cardiac, renal, neuromuscular conditions or lung disease of prematurity. Parents completed a detailed symptom and history questionnaire, (E1 in the online supplement).

Control subjects were infants and young children attending The Sydney Children's Hospital, Randwick, between April 2005 and April 2009, either for a sedated echocardiograph, where normal cardiac structure and function were found, or a dimercaptosuccinic acid scan for previous urinary tract infection. Parental consent was obtained for MBW testing as an add-on procedure. Exclusion criteria were (1) presence of cardiac, respiratory, or neuromuscular disease (2) prematurity (3) respiratory hospitalisation (4) history of asthma, wheezing, breathlessness, chronic cough or use of anti-asthma medication and (5) respiratory infection within 3 weeks.

Multiple-breath washout test

Multiple breath washout testing was performed at the bedside in the paediatric medical procedures unit (Medical Day Unit) using a commercially available mainstream ultrasonic flowmeter (Exhalyzer ® D, Eco Medics AG, Duernten, Switzerland) with sulphur hexafluoride as the tracer gas. The LCI was determined by dedicated data acquisition and

analysis software, (WBeath, version 3.19.8.0, ndd Medical Technologies, Switzerland). Children were examined, weighed and measured and then sedated with oral chloral hydrate as per guidelines.[27;28]

All children, (CF and non-CF) were tested using the same equipment and technique. However the dose of chloral hydrate in the non-CF children scheduled for a dimercaptosuccinic acid scan was lower (30-50 mg/kg compared to 50-80 mg/kg) according to the hospital's sedation protocol for that procedure. The MBW equipment was leak tested and calibrated prior to each patient assessment.

MBW was performed with the child in the supine position during quiet sleep after regular tidal breathing was established (usually 1-2 minutes). The wash-in was initiated if there was no evidence of mask leak or an unstable end-expiratory level. A minimum of two (ideally 3-5) complete wash-in/wash-out curves were obtained for each child without adjusting the mask or body position. This formed the first set of measurements and was usually complete within 10-15 minutes. After a 5-10 minute interval and mask repositioning a second set of curves was obtained to allow assessment of between-test LCI reproducibility.

All wash-in/wash-out curves were saved but only recordings which met acceptability criteria were used to derive the LCI.[29] Hence the LCI was determined from wash-out curves in which there was no evidence of leak, sighs, hiccoughing, swallowing or arousal and in which the functional residual capacity measurements differed less than 10% in relation to the lower value of the other curves within the set.[29] The mean LCI was determined from 3 (minimum 2) acceptable wash-out curves within each set.[29]

A chest x-ray was performed after MBW testing once the child was awake. Children remained overnight (for 24-hour pH monitoring) and underwent BAL on the following day or

were readmitted 48 hours later to the Day Surgery Unit for this. They were subsequently discharged home after a 4-hour period of observation post-bronchoscopy.

CF specific chest x-ray scores

Two CF-specific scores, the Brasfield Score (BS)[30] and the Modified Crispin Norman Score (MCNS)[31] were used to assess structural lung disease and were scored by a single paediatric radiologist blinded to the child's clinical status. Severity cut-off values were used to assess "irreversible" lung disease.[32;33] For the BS this was < 21 and for the MCNS this was > 5.

Bronchoalveolar lavage in children with CF

Bronchoalveolar lavage was performed under general anaesthesia within 72 hours of MBW testing. Suctioning through the bronchoscope (Olympus models BF-3C40, BF-3C160, and BF-XP16F, Olympus Corporation of America, New York, USA) was avoided until the tip had passed beyond the carina.

The bronchoscope was sequentially wedged into the right upper lobe (RUL), right middle lobe (RML) and lingula. A single aliquot (1 mL/kg, minimum 10 mL, maximum 20 mL) of warmed non-bacteriostatic sterile saline was instilled into each lobe and BAL fluid immediately aspirated. Three-lobe lavage was performed to optimise detection of airway infection[14] and inflammation.[34] Similarly topical anaesthesia was applied only after BAL samples were collected to prevent bacterial growth inhibition.[35;36]

Pooled BAL fluid samples were processed for cell count and differential, interleukin-8 (IL-8)[37] and the complex of neutrophil elastase with alpha1-protease inhibitor (NE/α1-PI complex). Additionally quantitative bacterial microbiology and viral immunofluorescence and culture were performed.[37]Airway infection was defined as pathogen growth $\geq 10^5$

colony-forming units per milliliter (cfu/mL) of BAL fluid, or a positive viral immunofluorescence or culture.[1]

Statistical analysis

Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). The LCI was the main outcome measure. The mean LCI value from set 1 in each child was used for all analyses. Based on available LCI data in 43 children with CF and 28 healthy controls, we estimated that 25 children per group (healthy and CF) were required to detect a 1 SD difference in LCI with 80% power at the 5% significance level.[38] In addition, an interim analysis of our own data demonstrated that a sample size of 16 per group would be sufficient to detect a 1 SD difference in LCI between infected and non-infected children with CF with 80% power and a 5% significance level.

Lung clearance index within-test repeatability was assessed by the coefficient of variation (CV) of acceptable curves within set 1. The between-test LCI reproducibility was determined by Bland-Altman analysis[39] using the mean LCI values from sets 1 and 2.

A LCI greater than the upper limit of normality (ULN), defined as the mean LCI + 1.96 SD value in healthy children, was classified as abnormal. Categorical data were compared with *chi square* or Fisher's exact tests and continuous data with *t* tests or Mann-Whitney U tests as appropriate. Associations between continuous data such as age, inflammatory markers, chest x-ray scores, pathogen density and LCI were assessed by scatter plot and linear regression. Variables were log transformed if required. Negative BAL cultures were assigned a value of 1 (10^0) cfu/mL to allow log transformation of pathogen density and because the lower level of sensitivity of quantitative culture was 10^1 cfu/mL. Each child contributed only one set of data. Receiver operating characteristic (ROC) curve analysis was used to assess the discriminative ability of the LCI to detect infection and inflammation. A P-value of < 0.05

was considered statistically significant. The study was approved by the South Eastern Sydney Area Health Service Human Research Ethics Committee and registered at the Australian and New Zealand Clinical Trial Register (ACTRN: ACTRN12611000945921).

RESULTS

Sedated MBW testing was attempted in 50 out of 55 eligible children with CF. Parents of 5 children declined lung function. All 50 children with CF were adequately sedated for the duration of MBW testing. However in 2 children, quality criteria for LCI were not met. In one sedated child MBW testing was not performed as this child developed intermittent oxygen desaturation (88-94%) due to upper airway obstruction and required low flow oxygen. Hence 47 children with CF, mean age (range) 1.55 (0.36-3.10) years, had technically acceptable LCI measurements. The mean age (range) of the 8 eligible children who did not provide technically acceptable data was 1.96 (0.13-2.98) years.

Thirty-six healthy non-CF children were given chloral hydrate of which 25, mean age (range) 1.26 (0.32-3.24) years were able to be sedated and had technically acceptable LCI data. Eleven non-CF children, mean age 1.62 (0.50-3.50) years did not sedate in time or woke up prematurely before test completion. Therefore overall 72 of 86 (84%) sedated children provided technically acceptable LCI data.

No major adverse events, defined as termination of procedure, need for resuscitation, intubation for suspected aspiration, need for supplemental oxygen greater than 1 hour, need for positive expiratory pressure, fever $\geq 38.5^0$ or unexpected admission were related to bronchoscopy/BAL.

All children with CF had a normal clinical examination and 45 (96%) were receiving anti-staphylococcal prophylaxis. There was no difference in age, gender, nutritional status, tobacco smoke exposure, family history of asthma or breastfeeding duration between healthy children and children with CF (Table 1).

LCI variability

Within-test repeatability

The mean (SD) CV_{LCI} was almost identical for children with CF and healthy children at 3.9% (2.4), range 0-9.9% and 3.8% (2.5), range 0.5-11.2% respectively. There was no relationship between CV_{LCI} and age in either group, P = 0.16 and P = 0.83 respectively.

Within-test reproducibility

The LCI between-occasion reproducibility (between the first and second set of wash-in/wash-out curves for each child measured 5-10 minutes apart) is shown in the mean-versus-difference (Bland–Altman) plot of Figure 1. The mean (SD) difference in LCI between the two sets in healthy children was -0.07 (0.31) and the 95% limits of agreement were -0.69; 0.54. In children with CF this was 0.11 (0.30) and -0.48; 0.70 respectively demonstrating good and comparable reproducibility with healthy controls.

LCI in children with CF and controls

The mean (SD) LCI in the 25 healthy children was 6.45 (0.49), range 5.42-7.37 with ULN of 7.41. In comparison the LCI was higher in the 47 children with CF, 7.21 (0.81), range 6.19-11.04, P < 0.001. Importantly 15 (32%) children with CF, age range 0.36-2.67 years, had an elevated LCI, (Figure 2).

A difference in LCI between CF and non-CF children was apparent even in infants less than 12 months of age. The mean (SD) LCI in 14 infants with CF was 7.53 (1.10) compared to 6.73 (0.37), in healthy infants, P = 0.022. Six out of 14 (43%) infants with CF had an LCI above the ULN.

In healthy children the LCI was negatively related to age (P = 0.026) but not gender, weight or height. There was no correlation between LCI and age, gender, height or weight in children with CF. Similarly symptoms such as cough or wheeze or risk factors such as parental smoking were not associated with an abnormal LCI, (Table E1, online supplement).

Infection burden in CF

Airway infection ($\geq 10^5$ cfu/mL BAL fluid) was present in 17 (36%) children with CF. In 11 (23.5%) children a respiratory pathogen was present but in low colony densities (10^1-10^5 cfu/mL). In 19 (40.5%) children no pathogens were detected, (Table E2). Seven (15%) children had *P. aeruginosa* infection. A further 3 isolated *P. aeruginosa* in low colony densities.

P. aeruginosa and *H. influenzae* were the most common organisms detected, (Table E2). *Staphylococcus aureus* was uncommon (2%). Fifteen children (32%) isolated one organism (of any colony density). Thirteen children (28%) had polymicrobial growth. In 10 children, 2 organisms were detected and in 3 children, 3 organisms were detected. There was no relationship between increasing age and number of pathogens, P = 0.190. No viruses were identified by immunofluorescence or culture although 2 children were cytomegalovirus polymerase chain reaction positive.

Clinical characteristics including genotype, nutritional status and respiratory symptoms did not distinguish children with or without infection, (Table E3). Similarly previous respiratory

admission or intravenous antibiotic use in the last year were not associated with infection although numbers were small (n = 10 and n = 9 respectively), Table E3.

Airway inflammation in CF

Inflammatory markers were higher in infected BAL samples, (Table E4). There were significant correlations between inflammatory markers and actual pathogen load. Pathogen density explained 56% of the variability in BAL neutrophil percentage, $P < 0.001$ and 44% of the variability in IL-8 levels, $P < 0.001$ (Figures E1 and E2). However there was no association between NE/α1PI complex and infection.

Airway inflammation was also related to the number of pathogens in BAL fluid indicating that polymicrobial growth was associated with worse inflammation in children with CF (Table 2).

Chest x-ray scores

The BS ranged from 19-25 and the MCNS from 0-8 suggesting mild structural lung disease. There was no association between either score and airway infection, $P = 0.138$ and $P = 0.806$, BS and MCNS respectively. Furthermore proposed cut-off scores indicating progression to "irreversible" CF lung disease did not relate to airway infection, BS < 21, $P = 0.237$ and MCNS > 5, $P = 0.417$.

LCI and infection

The mean (SD) LCI was not different between children with and without airway infection, LCI = 7.54 (1.10) versus 7.02 (0.51), $P = 0.083$, (Figure 3). However there was a positive correlation between LCI and pathogen load in children with CF, $R^2 = 0.101$, $P = 0.031$, see Figure 4. Additionally there was a progressive increase in mean LCI when the 3 groups of children were compared, that is, healthy children (6.45 (0.49)) versus uninfected children

with CF (7.02 (0.51)) versus infected children with CF (7.54 (1.10)) see Figure 5. Compared with healthy controls, LCI was significantly elevated in both infected children with CF ($P < 0.001$) and non-infected children with CF ($P < 0.001$).

LCI and inflammation

Figures 6 and 7 demonstrate the significant relationships between LCI, IL-8 and airway neutrophils. Bronchoalveolar lavage IL-8 levels explained 20% of the variability in LCI, $P = 0.004$. The absolute neutrophil count explained 21% of the variability in LCI $P = 0.001$.

***P. aeruginosa* and LCI**

Ten children isolated *P. aeruginosa* in any colony density, of which 7 (15%) had growths $\geq 10^5$ cfu/mL. In the 3 children with low *P. aeruginosa* colony counts ($10^1 - 10^5$ cfu/mL), BAL neutrophil percentage counts were 42%, 43%, and 81%, respectively indicating a vigorous neutrophilic response even with low bacterial loads of this organism. There was no age difference between children with *P. aeruginosa*, mean (SD) age 1.75 (0.84) years and children without *P. aeruginosa*, aged 1.50 (0.74) years, $P = 0.369$. The youngest child with *P. aeruginosa* was 4 months old and one child had the mucoid phenotype.

As we would treat the first or early isolation of any growth of *P. aeruginosa* to prevent chronic infection, we examined the effect of *P. aeruginosa* in any colony count on airway inflammation. Children with *P. aeruginosa* had significantly increased inflammatory markers compared with children with other non-pseudomonal pathogens, (Table 3).

Furthermore, in children with CF, the LCI was significantly higher in those with *P. aeruginosa* than in subjects without this pathogen, 7.92 (1.16) versus 7.02 (0.56), $P = 0.038$ (Figure 8). ROC curve analysis compared the discriminative ability of the LCI and BAL

markers of inflammation to detect any growth of *P. aeruginosa* (Figure 9). LCI had similar ability to detect *P. aeruginosa* as increased BAL neutrophils and IL-8 levels.

Based on ROC curve analysis, a BAL IL-8 level of 672 pg/mL had the best combination of sensitivity and specificity to detect the presence of any colony count of *P. aeruginosa* (sensitivity = 100% and specificity = 40%). Similarly the value of 40% neutrophils had the best combination of sensitivity (100%) and specificity (74%) for detecting any growth of *P. aeruginosa*. A LCI ≥7.41 (the upper limit of normality) had a sensitivity of 67%, specificity of 80% and a positive predictive value of 47% to detect *P. aeruginosa* whereas an LCI < 7.41 had a negative predictive value of 93%.

LCI and airway inflammation by pathogen group

To test the hypothesis that the ability of the LCI to detect *P. aeruginosa* was related to this organism's greater potential to induce inflammation, children with CF were assigned to 3 groups based on their culture results (Table 5). Group 1 comprised of children with "nil growth/growth of commensals", Group 2 consisted of children who isolated a pathogen, multiple or otherwise, in any colony count but excluded *P. aeruginosa,* and Group 3 comprised of children who isolated *P. aeruginosa* (Table 5). Neutrophil percentage (72% (22) v 42% (29), P < 0.05) and LCI (8.0 (1.1) v 7.0 (0.6), P <0.01) were significantly higher in infants and children with *P. aeruginosa* compared to infected children without *P. aeruginosa*, suggesting that isolation of *P. aeruginosa* is associated with worse inflammation and greater ventilation inhomogeneity.

DISCUSSION

The results of the present study demonstrate important relationships between airway infection, inflammation and ventilation inhomogeneity assessed by BAL and LCI in young children with CF. The LCI was elevated in presymptomatic/minimally symptomatic newborn-screened infants and young children with CF especially in the presence of airway inflammation and *P. aeruginosa*. LCI measurements were repeatable both in healthy non-CF infants and young children and in similar-aged children with CF, using a portable MBW system in a clinical setting. This study highlights the feasibility, reproducibility and sensitivity of the LCI as a non-invasive measure of small airway function and marker of early lung disease in children with CF.

We established a mean (SD) LCI value of 6.45 (0.49) with an ULN of 7.41 for healthy children aged 0.32 to 3.24 years using a mainstream ultrasonic flowmeter. This is consistent with the literature reporting LCI for healthy preschool children[19] and healthy school-aged children assessed by mass spectrometry;[40] healthy children assessed by a side-stream ultrasonic flowmeter;[41] and healthy children and adults assessed by a photoacoustic analyser,[42] see Table 6. As such it validates the use of our non-CF group as healthy controls and confirms the constancy of LCI values across the age spectrum in healthy individuals whether assessed by mass spectrometry, photoacoustic gas analysis or an ultrasonic flowmeter.

We found a negative relationship between the LCI and age in healthy, non-CF infants and young children. A longitudinal study assessing ventilation inhomogeneity in preterm and term healthy control infants followed from the newborn period to 15-18 months of age also found a significant decrease in LCI between these two time points.[43] During early infancy rapid alveolarisation is associated with dysanaptic growth, that is the growth and development of the alveoli is greater than the growth of the airway, and this physiologic process may contribute to greater ventilation inhomogeneity during this period.[43] The lack of

a similar fall in LCI during infancy in children with CF may reflect persisting ventilation inhomogeneity due to evolving early airway disease. Larger studies of ventilation inhomogeneity in healthy infants and young children assessed longitudinally from birth through to 2-3 years may be needed to confirm our observations and precisely define the normal range for LCI in very young subjects thereby clarifying the relationship between LCI and normal postnatal lung growth.

This study has also shown that the LCI is a highly repeatable measure of lung function in young children. We demonstrated a within-test $CV_{LCI} < 5\%$ in both healthy subjects and in children with CF. This agrees with reports of LCI within-test repeatability in preschool children with CF,[19] older children[40] and adults with CF[42] and studies using different methods of inert gas analysis, see Table E5. For example, the mean (SD) CV_{LCI}, was 7.8% (5.4) in awake children with CF, aged 2-6 years measured by MS,[19] 6.2% (2.9) in CF children aged 6-16 years also measured by MS,[40] and 4.4% (2.8) in adult CF subjects, aged 17-49 years, measured by the photoacoustic gas analyser.[42] We found no relationship between age and CV_{LCI}, a finding also reported by Aurora *et al* in school aged children using MS.[40]

The short-term (over 5-10 minutes) reproducibility between mean LCI measurements in set 1 and set 2, reported in the current research, has not been previously determined in children with CF of this age group. This study found a 5-10 minute between-test reproducibility of approximately ± 0.60 units in both healthy children and children with CF. The latter is an important finding as the minimal clinically important difference for the LCI is not known for young children with mild disease and therefore our data potentially defines the physiologic variation in LCI for this age group. We did not assess the day-to-day LCI reproducibility as we could not justify repeat sedation for multiple testing occasions over short time periods.

No major adverse events were related to either BAL or MBW testing. Safety of sedation for ILF testing has been long established.[44] However one child with unsuspected upper airway obstruction developed intermittent oxygen desaturation requiring low flow oxygen. This child subsequently underwent adenotonsillectomy. Similarly the procedure of bronchoscopy/BAL performed under general anaesthesia is generally safe, well tolerated[45] and acceptable to parents.

Important strengths of this study were that children with CF were identified by newborn screening and had been segregated from birth into cohort groups according to infection status determined by annual BAL. This is the first study to demonstrate an elevated LCI in clinically well infants and young children with CF diagnosed early though newborn screening. Both BAL and LCI were performed as elective procedures when the child was well with no clinical evidence of a respiratory exacerbation. The LCI was elevated in 32% of children despite early diagnosis (mean age 3.8 weeks), normal nutrition, regular clinical assessment and absence of respiratory symptoms, highlighting the subclinical onset of early CF lung disease. In the only other study which has assessed the LCI in infants with CF, subjects were diagnosed after symptomatic presentation and were lighter and shorter than their healthy peers indicating clinically established disease.[20]

In this study, proportionally fewer children with CF had an abnormal LCI and the difference in LCI between children with CF and healthy controls was modest compared to values reported in successively older CF cohorts, (Table 6).[19;38;40;42;46] This suggests that the LCI reflects disease progression as well as sensitively identifying early lung disease although longitudinal studies are required to confirm this.

A major strength of this study was the assessment of the LCI in young children with CF in which concurrent lower respiratory infection and inflammation were quantitatively

determined. In addition our three-lobe BAL method which included the RUL and preceded topical anaesthesia, may have optimised the assessment of both airway infection and inflammation.

The results of the present study demonstrate a significant infection burden in non/minimally symptomatic, well nourished, screened infants and young children with CF assessed electively. Airway infection was present in 36% of children with CF. *P. aeruginosa* infection was present in 15% and polymicrobial growth was present in 28%. *S. aureus* detection was infrequent which, given the high adherence to anti-staphylococcal prophylaxis, supports the preventative role of this strategy for early infection.

Clinical parameters such as symptoms of cough and wheeze in the month prior to bronchoscopy or previous admission for a respiratory exacerbation were not associated with infection or an abnormal LCI. Conversely 24% of children with demonstrated airway infection and 60% of children with an abnormal LCI had no cough or wheeze within 12 months prior to their BAL underscoring the lack of sensitivity of symptoms in the detection of early CF lung disease.

The strong association between airway infection and inflammation has been previously reported[1;37] and is confirmed in this study. However a novel finding was that undiagnosed polymicrobial growth, present in almost one-third of children, was associated with significantly greater airway inflammation.

In this study the LCI was not higher in children with CF and airway infection. However the LCI was positively correlated with pathogen density suggesting that the LCI does reflect the impact of early infection.

Perhaps the most important finding was the strong association between the LCI and the presence of *P. aeruginosa* in the lower airways with a mean difference of 1.03 units between young children with and without this organism. Early *P. aeruginosa* isolation may be asymptomatic yet associated with airway inflammation and structural disease: its initial low colony density and non-mucoid status may present a window of opportunity for eradication. Therefore its isolation in any amount has clinical relevance as an eradication protocol would most likely be initiated.[47] Hence we evaluated the results of all 10 children with *P. aeruginosa* irrespective of pathogen load.

Although the sensitivity of the LCI to detect *P. aeruginosa* was modest, its high negative predictive value (93%) suggests that the LCI has the potential to rule out *P. aeruginosa* in the majority of well young children with CF. In comparison a recent study using the RVRTC technique was unable to detect lower baseline lung function in the presence of *P. aeruginosa* despite a more rapid decline in $FEV_{0.5}$ at follow-up.[16]

We found that 68% of well young children with CF at elective BAL had airway neutrophil levels which exceeded values reported in healthy young children.[48;49] Airway neutrophils were significantly higher in infected children, in children with two or more pathogens and in children with *P. aeruginosa*. The latter finding suggests that this organism has greater pathogenic potential for inducing airway inflammation.

This study demonstrated significant relationships between the LCI and airway inflammation. Higher LCI values were associated with increased airway neutrophils and IL-8 levels, a finding which has not previously been reported. There are inconsistent reports in the literature regarding the association between markers of airway inflammation and lung function abnormalities in infants with CF. Brennan *et al*[50] demonstrated significant relationships between parenchymal hysteresivity and tissue damping and neutrophilic inflammation whilst

Dakin *et al*[37] reported a significant association between specific respiratory system compliance and both IL-8 and percentage neutrophils. Recently Pillarisetti *et al*[16] reported an association between neutrophil elastase and FVC and $FEV_{0.5}$ but not FEF_{75} and no association between any of these lung function parameters and IL-8, IL-1 or total cell count. Similarly both Nixon *et al*[51] and Linnane *et al*[52] were unable to demonstrate an association between measures of forced expiration and airway inflammation.

Our data suggest that the LCI may be more sensitive than timed expiratory flows to detect early inflammatory disease. It is also possible that our three-lobe lavage technique may have enhanced our ability to detect inflammation. In addition sampling each lobe once and then pooling the resultant lavage fluid is likely to produce a more bronchial sample with higher neutrophil counts.[13] This practice is used by some[37;51;53] but not all centres[16] performing surveillance BAL making comparisons challenging.

We recognise that this study has limitations which include the lack of a robust measure of structural lung disease. The two commonly used CF specific chest x-ray scoring systems did not distinguish between children with or without infection. High resolution CT sensitively detects early structural lung change in infants with CF.[2;17] Recently it has been suggested that the LCI and HRCT have comparable sensitivity to detect lung disease in older (6-10 years), non-screened children with CF.[18] However Hall *et al*[54] reported that the LCI did not relate to the presence of bronchiectasis in infants with CF suggesting that, the LCI may detect early infection and inflammation sensitively but not the onset of structural lung disease.

We acknowledge that our cut-off for airway infection ($\geq 10^5$ cfu/mL BAL fluid) is contentious and that other BAL studies have used lower diagnostic thresholds including $\geq 10^4$ cfu/mL,[17] and $\geq 10^3$ cfu/mL.[53] We chose this threshold as it is commonly used,[1;37;55] consensus

endorsed[12;13] and is based on the marked increase in BAL IL-8 concentrations seen at this pathogen load indicating a significant host response.[5;55]

Furthermore, the concept of lower airway sterility in healthy individuals has recently been questioned with the detection, using sensitive molecular techniques, of a lung microbiome which is indistinguishable from upper respiratory flora except in biomass (lower).[56] Whether this is a transient colonisation or a normal microbiological population of the lower airways in unknown. Additionally it is recognised that the procedure of BAL in itself is not free of contamination risk as the bronchoscope passes through the upper airway, with its myriad of organisms, including common CF respiratory pathogens such as *H. influenzae* and *S. aureus*. Therefore defining a "threshold" colony count which constitutes lower airway infection or clinically relevant disease in CF remains problematic especially in the current era of early disease surveillance. For this reason we considered the effect of any growth of *P. aeruginosa* on airway inflammation and ventilation inhomogeneity.

The role of BAL in young children with CF unable to expectorate has recently been questioned as result of the findings of a randomised controlled trial of Australian newborn screened infants in which BAL-directed therapy did not improve medium term outcomes.[53] In this multicentre study BAL was performed when the child was unwell or had identified *P. aeruginosa* from oropharyngeal cultures. This design was very different from our own study and the results cannot be extrapolated to surveillance BAL performed electively. We acknowledge that, to date, there are no data to demonstrate that our approach of annual surveillance BAL leads to improved outcomes. However, while surveillance BAL, particularly 3-lobe sampling, has not been shown to alter outcomes, we believe that this technique is valuable for identifying clinically inapparent infection and assessing occult lower airway inflammation.

Conclusions

We have provided data demonstrating that the LCI is a feasible, repeatable and sensitive non-invasive marker of early lung disease in well newborn-screened young children with CF. Reproducible measurements of the LCI were achievable in sedated infants and young children using a portable MBW system measured at the bedside in a paediatric medical procedures unit. We acknowledge that our results represent a single time point in the clinical course of each child and that a longitudinal assessment of the LCI with respect to changes in airway infection and inflammation is required to confirm the role of LCI as an early marker of CF lung disease. However our study emphasises that subclinical infection, inflammation and functional lung disease can be evaluated through sensitive techniques such as BAL and MBW in infants and young children with CF and our repeatability data highlight the potential role of the LCI as an outcome measure for future intervention trials involving infants and young children with CF.

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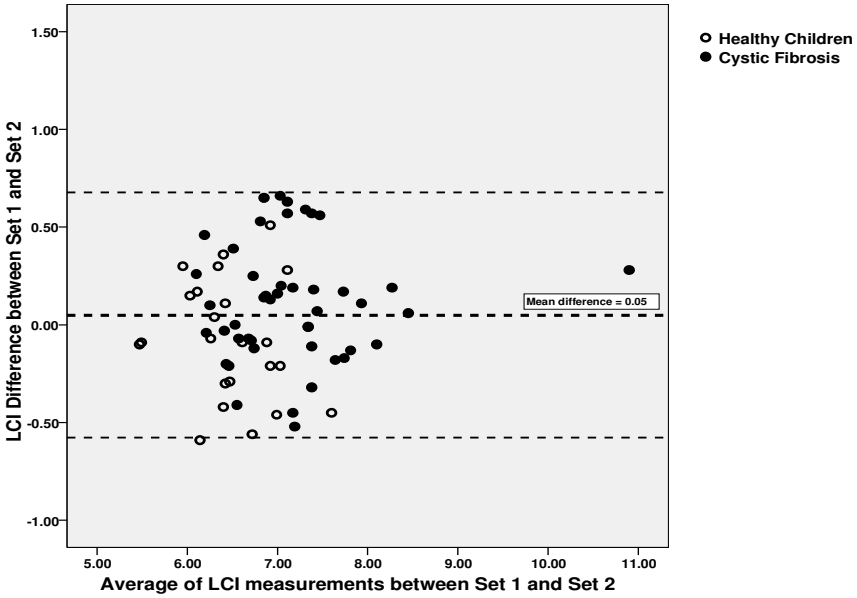
Table 1 Clinical characteristics of the study population

	Healthy Children (n=25)	Cystic Fibrosis (n=47)	P
Male, n (%)	10 (40)	22 (47)	0.580
Age (years) Mean (SD)	1.26 (0.69)	1.55 (0.76)	0.113
Range	0.32 – 3.24	0.36 – 3.10	
Weight (kg) Mean (SD)	10.1 (2.4)	10.8 (2.2)	0.264
Weight < 10th centile, n (%)	3 (12)	6 (13)	1.0
Height (cm) Mean (SD)	77.6 (9.5)	80.9 (9.1)	0.152
Height < 10th centile, n (%)	0	4 (9)	0.2.19
Antenatal smoking, n (%) *	1 (5)	4 (9)	1.0
Household smokers, n (%) *	1 (5)	9 (19)	0.152
Breastfeeding, months Mean (SD)	6.1 (4.8)	4.3 (4.6)	0.158
Range	0 - 14	0 - 15	
Asthma/Atopy 1st degree relatives n % *	13 (59)	31(66)	0.580
Cough** last year, n (%)	0	17 (36)	<0.001
Cough last month, n (%)	0	23 (49)	<0.001
Wheeze last year, n (%)	0	19 (40)	<0.001
Wheeze last month, n (%)	0	10 (21)	<0.001
Respiratory admission, n (%)	0	10 (21)	<0.001
IV Antibiotics last year, n (%)	0	9 (20)	<0.001
Previous *P. aeruginosa* (BAL) n %	0	1 (2)	<0.001

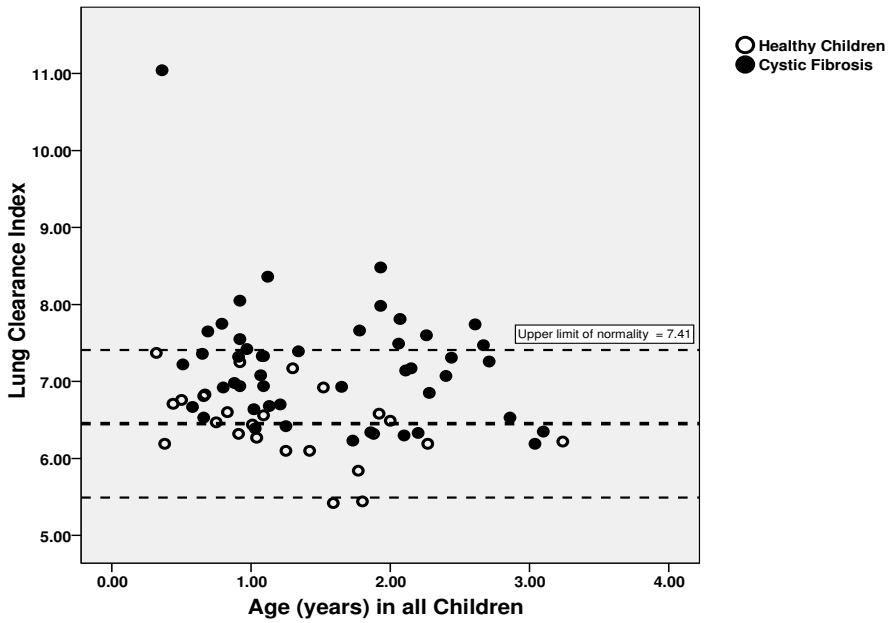
*Legend: * Percentages apply to numbers of responses returned. ** Cough lasting > 3 weeks.*

Figure 1 Bland-Altman analysis of LCI measured 5-10 minutes apart in all children



Legend: The heavy broken line is the mean difference in LCI measured 5-10 minutes apart from all children and the lighter broken lines represent the 95% limits of agreement of this difference (mean ± 1.96SD).

Figure 2 LCI and age in all children



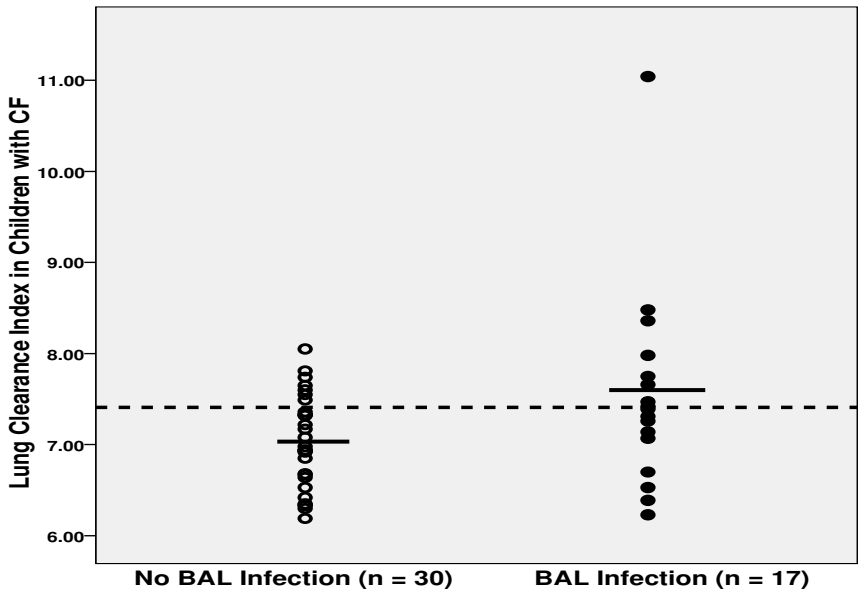
Legend: The broken lines are the mean ± 1.96SD (95% limits of normality) for LCI for healthy children. The ULN is 7.41. There was no relationship between age and the LCI, R^2 = 0.028 P = 0.164 when all children were combined. 15 children with CF had an abnormal LCI (> 7.41)

Table 2 Association between pathogen number and BAL inflammation in children with CF

		≤ 1 Pathogen (n=34)	≥ 2 Pathogens (n=13)	P value
Neutrophils %	Median [IQR]	25 [7-42]	75 [62-91]	**<0.001**
Neutrophil count *	Median [IQR]	32 [10-115]	402 [95-2465]	**0.001**
Total cell count *	Median [IQR]	213 [125-391]	682 [162-2716]	**0.039**
IL-8 pg/mL	Median [IQR]	1144 [387-3048]	4085 [2340-6638]	**0.003**
NE/α1PI complex #	Mean (SD)	126 (114)	146 (121)	0.734

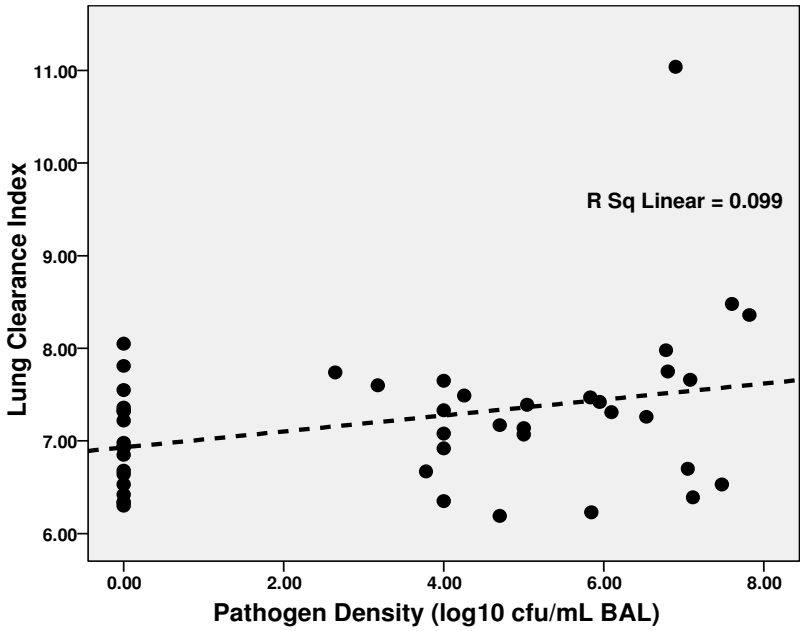
*Legend: * x10^3 /mL. # Neutrophil elastase/alpha 1-protease inhibitor complex,* ng/mL, *n=25.*

Figure 3 LCI and airway infection in children with CF



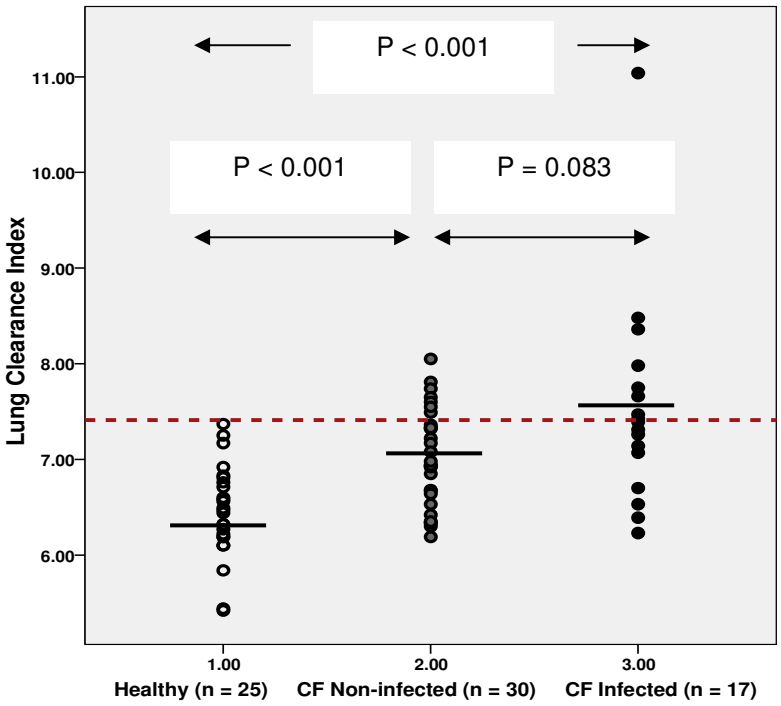
Legend: There was no association between LCI and airway infection in CF children, P = 0.083. The broken line represents the ULN for healthy controls, LCI=7.41. Bars represent the mean (7.02 non-infected versus 7.54 infected).

Figure 4 Association between LCI and BAL pathogen density in children with CF



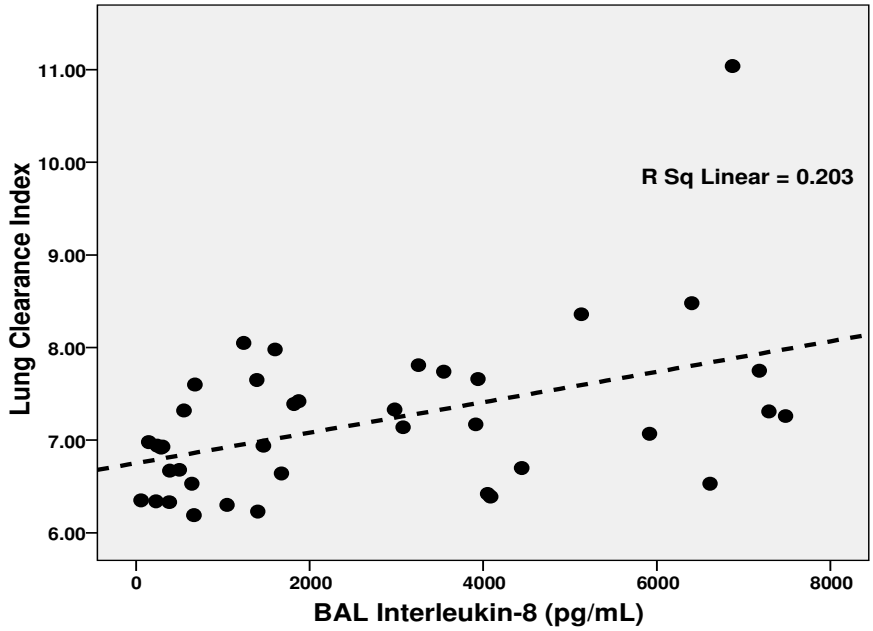
Legend: There was a weak relationship between LCI and pathogen load in young children with CF, R^2 = 0.10, P = 0.031.

Figure 5 LCI in healthy versus non-infected and infected children with CF



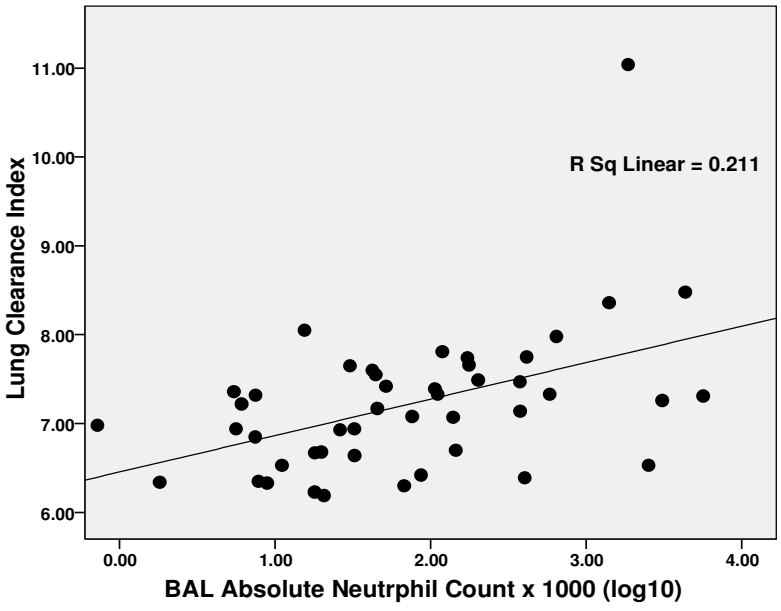
Legend: Healthy vs non-infected CF, LCI = 6.45 (0.49) vs 7.02 (0.51), P < 0.001. CF non-infected vs infected, LCI = 7.02 (0.51) vs 7.54 (1.10), P = 0.083. Healthy vs infected CF, LCI = 6.45 (0.49) vs 7.54 (1.10), P = 0.001. The broken line represents the ULN for LCI=7.41. Horizontal bars represent the mean. Kruskal-Wallis analysis.

Figure 6 Association between BAL Interleukin-8 and LCI in children with CF



Legend: There was a significant relationship between LCI and IL-8 in young children with CF, R^2 = 0.20, P = 0.004.

Figure 7 Association between BAL absolute neutrophil count and LCI in children with CF



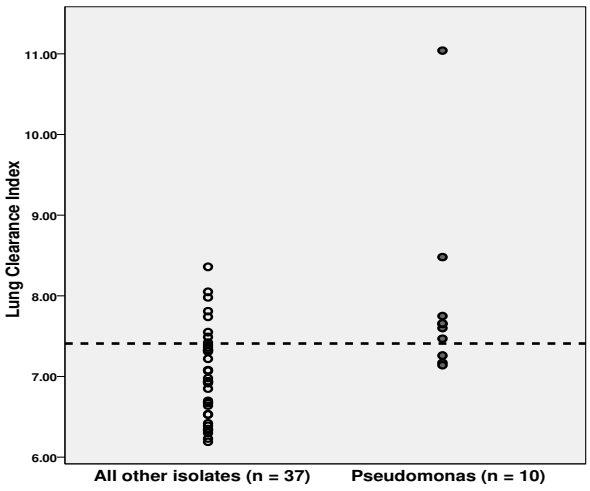
Legend: There was a significant relationship between LCI and absolute neutrophil count in infants and young children with CF, R^2 = 0.21, P = 0.001.

Table 3 Airway inflammation and LCI in CF children with or without *P. aeruginosa* (PsA)

		No PsA Isolated (n=37)	PsA ≥10^1 cfu/mL (n=10)	P
Age (years)	Mean (SD)	1.50 (0.74)	1.75 (0.84)	0.369
Airway Inflammation (BAL)				
Neutrophils %	Median [IQR]	25 [8-47]	77 [45-91]	**<0.001**
Neutrophil count *	Median [IQR]	38 [9-144]	375 [45-2160]	**0.009**
Total cell count *	Median [IQR]	218 [134-490]	470 [92-2366]	0.219
IL-8 pg/mL	Median [IQR]	1435 [387-3671]	3940 [2234-7026]	**0.012**
NE/α1PI complex #	Mean (SD)	125 (100)	174 (158)	0.368
Lung Clearance Index				
Mean (SD) §		7.02 (0.56)	7.92 (1.16)	**0.038**
Median [IQR]		6.94 [6.53-7.38]	7.63 [7.24-7.93]	**0.002**

*Legend: * x10^3 /mL. # Neutrophil elastase/alpha 1-protease inhibitor complex,* ng/mL, *n=25. Values were normally distributed. LCI values were non-normally distributed and hence medians and means are expressed. § t-test equal variances not assumed.*

Figure 8 LCI in CF children with and without *P. aeruginosa* (any growth)



Legend; Children without P. aeruginosa, median LCI = 6.94 [IQR 6.53-7.38] *versus children with P. aeruginosa,* LCI = 7.63 [7.24-7.93], *P = 0.002. This relationship remained significant when the outlier was removed, P = 0.006. The broken line represents the ULN for LCI, 7.41.*

Figure 9 ROC curves for LCI, BAL neutrophils %, and BAL IL-8 for the detection of *P. aeruginosa* (any growth) in children with CF

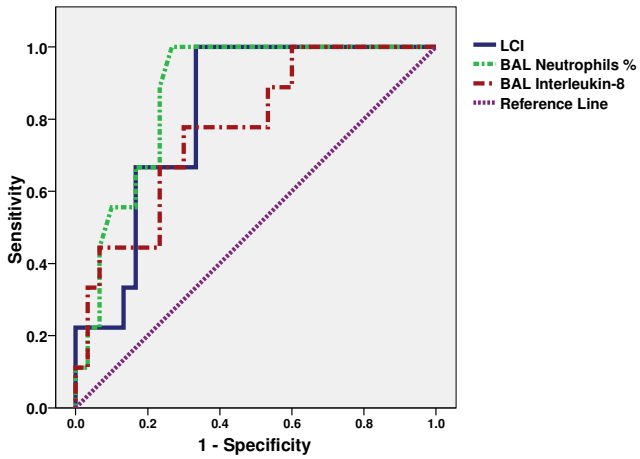


Table 4 Area under the curve (AUC) for LCI, BAL neutrophils % and BAL IL-8 for detection of *P. aeruginosa* in children with CF

	AUC	95% CI	P
BAL Neutrophils %	0.874	0.766-0.982	0.001
LCI	0.819	0.686-0.951	0.004
BAL IL-8	0.774	0.609-0.940	0.014

Legend: The LCI had comparable discriminative ability to detect P. aeruginosa $\geq 10^1$ *cfu/mL as BAL interleukin-8 and BAL percentage neutrophils.*

Table 5 BAL inflammatory markers and LCI by infection group in children with CF

	Group 1 (n=18) BAL negative	Group 2 (n=17) BAL pathogens, PsA negative	Group 3 (n=10) BAL PsA Positive	P
IL-8 pg/mL Median [IQR]	598 [291-1519]	2427 [851-4959]*	3940 [2234-7026]**, ¢	**0.003**
Neutrophils %	14 (12)	42 (29) §	72 (22) §, †	**<0.001**
ANC x 10^3/mL Median [IQR]	13 [6-35]	159 [28-628] §	375 [45-2160] §, ¢	**<0.001**
TCC x 10^3/mL Median [IQR]	0.2 [0.1-0.3]	0.4 [0.2-1.3] #	0.5 [0.1-2.4] #, ¢	0.079
NE/α1PI	74 (78)	148 (103) #	174 (158) #, ¢	0.703
LCI	6.9 (0.6)	7.0 (0.6) #	8.0 (1.1)**, ф	**0.004**

Legend: Data are mean (SD) unless otherwise stated. P values are overall Kruskal Wallis testing and a significant value indicates that at least 2 of the groups were significantly different. ANC = absolute neutrophil count, TCC = total cell count

**P < 0.05 relative to reference group (Group1).*

***P < 0.01 relative to reference group.*

§ P < 0.001 relative to reference group.

 # Not significant relative to reference group.

†P < 0.05 relative to Non-PsA infected group.

ф P < 0.01 relative to Non-PsA infected group.

¢ Not significant relative to the Non-PsA infected group.

¥ P < 0.001 relative to the Non-PsA infected group.

Table 6 Comparison of data for LCI in CF from infancy to adulthood

Study	Subjects	Age	MBW Equipment	LCI mean (SD)	LCI Mean difference (95% CI)	% CF children with a high LCI
Current study	25 healthy 47 CF (screened)	0.32-3.24 yrs 0.36-3.10 yrs	USFM* mainstream	6.45 (0.49) 7.21 (0.81)	0.76 (0.40 - 1.11)	32%
Lum *et al* 2007[20]	21 healthy 39 CF (non-screened)	15.3-77.9 wks 7.6-94.1 wks	MS#	7.20 (0.30) 8.40 (1.50)	1.20 (0.70 - 1.70)	56.4%
Aurora *et al* 2005[19]	30 healthy 30 CF (non-screened)	4.31 (0.84) yrs 4.43 (0.77) yrs	MS	6.89 (0.44) 9.61 (2.19)	2.72 (1.90 - 3.54)	73%
Gustafsson *et al* 2003[38]	28 healthy 43 CF (non-screened)	4.5-18.7 yrs 3.0-18.2 yrs	MS	6.33 (0.43) 8.33 (2.48)	2.00 (1.06 - 2.95)	63%
Fuchs *et al* 2008[46]	22 healthy 26 CF (non-screened)	6.8-18.9 yrs 4.7-17.6 yrs	USFM sidestream	6.70 (0.50) 10.20 (2.80)	3.5 (2.36 - 4.68)	77%
Aurora *et al* 2004[40]	33 healthy 22 CF (non-screened)	11.3 (3.1) yrs 11.5 (3.2) yrs	MS	6.45 (0.49) 11.53 (2.86)	5.08 (4.07 - 6.10)	95%
Horsley *et al* 2008[42]	12 healthy children 48 healthy adults 33 adult CF	6-16 yrs 19-58 yrs 17-49 yrs	P∞	6.30 (0.50) 6.70 (0.40) 13.10 (3.80)	Not stated	Not stated

*Legend: * USFM = ultrasonic flowmeter, # MS = mass spectrometry, P∞ = photoacoustic. Ages as ranges or mean (SD), non-screened = not diagnosed by newborn screening*

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ONLINE DATA SUPPLEMENT

Early Cystic Fibrosis Lung Disease detected by Bronchoalveolar Lavage and Lung Clearance Index

Yvonne Belessis[1,2], Barbara Dixon[1], Glenn Hawkins[3], John Periera[4], Jenny Peat[5], Rebecca MacDonald[1], Penny Field[1], Andrew Numa[1,2], John Morton[1,2], Kei Lui[2,6] and Adam Jaffe[1,2]

E1. Cystic Fibrosis Data Collection Questionnaire

LUNG FUNCTION TEST DATA COLLECTION SHEET (Page 1)

DEMOGRAPHIC DATA

Date of Study ……Study Number ……

MRN ……

Child's Family Name ……

Child's Given Name ……

Date of Birth ……

Gender Male …. Female……..

CF Genotype DF508/DF508 Yes.

DF508/Other Yes

Other/Other Yes

Sweat Chloride ……(mmol/L)

Ethnicity Caucasian

Asian/Subcontinent

Indigenous Australian

African……

Mother's Full Name ……

Father's Full Name ……

Home Address ……

Contact numbers Home…… Mobile…….

CURRENT SYMPTOMS

In the last year has your child had a cough lasting more than 3 weeks? Yes…No….

If yes, has this cough occurred

Mostly at night?	=1
Night and day?	=2
Mostly with physio?	=3
Mostly with food/meals?	=4
Mostly with exercise?	=5
Mostly with signs of a cold?	=6

LUNG FUNCTION TEST DATA COLLECTION SHEET (Page 2)

In the last 12 months how many times has your child had a cough that lasted more than 3 weeks?

0 1 2 3 more than 3 times

Has your child had a cough in the last month? Yes…. No….

If yes has this been

Mostly at night? =1

Night and day? =2

Mostly with physio? =3

Mostly with food/meals? =4

Mostly with exercise? =5

Mostly with signs of a cold? =6

In the last month is your child's cough

Mostly dry? =1

Mostly wet? =2

Productive of sputum (mucus)? =3

Did your child have any wheezing episodes in their first year? Yes… No…

If yes, how many?……

In the last year has your child ever wheezed? Yes… No…

Less than three episodes in the last year =1

More than three episodes in the last year =2

Has this wheeze occurred

Mostly at night? =1

Night and day? =2

Mostly with physio? =3

Mostly with food/meals? =4

Mostly with exercise? =5

Mostly with signs of a cold? =6

Has your child been wheezing in the last month? Yes…. No….

Mostly at night? =1

Night and day? =2

LUNG FUNCTION TEST DATA COLLECTION SHEET (Page 3)

Mostly with physio? =3

Mostly with food/meals? =4

Mostly with exercise? =5

Mostly with signs of a cold? =6

Has a doctor diagnosed asthma in your child? Yes… No…

In the last year has your child vomited after meals? Yes… No…

Mostly with a gastro-like illness/infection? =1

Mostly when unwell for other reasons? =2

Mostly just related to feeds? =3

Mostly when they have eaten too much? =4

For other reasons? =5

Please state…….

In the last year has your child seemed in pain or uncomfortable after meals? Yes… No…

Mostly with a gastro-like illness/infection? =1

Mostly when unwell for other reasons? =2

Mostly just related to feeds? =3

Mostly when enzymes are forgotten? =4

For other reasons? =5

Please state……

In the last year has your child refused food even when hungry? Yes… No…

Mostly with a gastro-like illness/infection? =1

Mostly when unwell for other reasons? =2

Mostly because they disliked the meal? =3

For other reasons =4

Please state……

Does your child gag or choke with food? Yes… No…

Very rarely? =1

Frequently? =2

Does your child have trouble gaining enough weight? Yes… No…

LUNG FUNCTION TEST DATA COLLECTION SHEET (Page 4)

Has a doctor diagnosed reflux in your child? Yes… No…

Has your child been treated for reflux in the last 12 months? Yes… No…

Does your child have a hoarse or croaky voice? Yes… No…

Does your child snore most nights of the week? Yes… No…

Is the snoring associated with long pauses between breaths? Yes… No…

Does your child work very hard to breathe when asleep? Yes… No…

Does your child have any allergies? Yes… No…

Please describe…….

Has your child had hay fever in the last 12 months? Yes… No…

Has your child had eczema in the last 12 months? Yes… No…

PAST HISTORY

Gestational age Weeks……Days……

Birth weight (g) ……

Birth length (cm) ……

Did your child require any assistance with their breathing at birth? Yes… No…

Resuscitation of baby (tick one or more)

None	=1
Suction	=2
Oxygen given	=3
Bag and mask	=4
Intubation and ventilation	=5
Cardiac Massage	=6

Name of hospital where child was ventilated…….

Did the mother smoke at all during the pregnancy? Yes… No…

If yes, how many cigarettes each day on average during the pregnancy?

None	=1
≤ 10 per day	=2
> 10 per day	=3
Not sure	=4

Duration of breast feeding (months) ……..…

LUNG FUNCTION TEST DATA COLLECTION SHEET (Page 5)

Date of CF diagnosis ……

Mode of Presentation

Newborn screen =1

Meconium ileus =2

Failure to thrive/malabsorption =3

Recurrent chest infections =4

Recurrent wheezy episodes =5

Prolonged jaundice =6

Antenatal bowel pathology =7

Rectal prolapse =8

Family history =9

Infection Episodes ever

Lower respiratory tract infection	☐	No. of episodes?…….	No. admitted?.....
Bronchiolitis	☐	No. of episodes?…….	No. admitted?……
Pneumonia	☐	No. of episodes?…….	No. admitted? ……

Infection Episodes in the last year

Upper respiratory infection	☐	No of episodes?…….	No. admitted?.....
Lower respiratory infection	☐	No of episodes?…….	No. admitted?……
Bronchiolitis	☐	No of episodes?…….	No. admitted? ……
Pneumonia	☐	No of episodes?…….	No. admitted?……

Hospitalisation History:

1.Date of admission
Reason for admission
Days of IV Antibiotics if given

2. Date of admission
Reason for admission
Days of IV Antibiotics if given

3. Date of admission
Reason for admission
Days of IV Antibiotics if given

Does your child have any other medical condition/congenital disease? Yes ☐ No ☐

If yes, please describe ……..……..……..

LUNG FUNCTION TEST DATA COLLECTION SHEET (Page 6)

CURRENT MEDICATIONS/MANAGEMENT

Pulmonary

Type	Name	Strength	Dose
Oral antibiotics i.e. Flopen, Amoxyl, Bactrim			
Inhaled antibiotics i.e. Tobramycin			
Steroids i.e. Predmix/Readipred/ Flixotide/Seretide			
Bronchodilators i.e. Ventolin			

Nutritional

Type	Name	Strength	Dose
Pancreatic enzymes i.e. Creon			
Vitamin supplements i.e. Vitabdeck			
Antacids i.e. Zantac, Losec			

Extra courses of oral antibiotics for CF related conditions

Last year? ……..
Ever? ……..

Extra courses of steroids for CF related conditions

Last year? ……..
Ever? ……..

Number of courses of intravenous antibiotic treatment ever…….

Is your child on daily inhaled antibiotic therapy? Yes… No…

Name and dose of antibiotic……

How often is physiotherapy given?

Never □ Daily □ Weekly □

……..times/day

……..tomes/week

Does your child receive nutritional supplements? Yes □ No □
Type and amount per day ……

Is your child gastrostomy fed? Yes □ No □
Type and amount per day ……

LUNG FUNCTION TEST DATA COLLECTION SHEET (Page 7)

FAMILY HISTORY

Is there anyone else in your extended family that had or has CF? Yes ☐ No ☐

Please describe……..…….

Is there a history of wheeze in any family members? Yes ☐ No ☐

Please describe……..…….

Is there a history of chest trouble of any kind in the family? Yes ☐ No ☐

Please describe……..……

Is there a history of eczema in any family members? Yes ☐ No ☐

Please describe……..……

Is there a history of hay fever in any family members? Yes ☐ No ☐

Please describe……..……..

Does the mother smoke currently? Yes ☐ No ☐

Number of cigarettes per day……

Does the father smoke currently? Yes ☐ No ☐

Number of cigarettes per day……

Number of smokers living at home…….…….…….

Has your child been exposed to cigarette smoke in the last 24 hrs? Yes ☐ No ☐

INVESTIGATIONS

Date(s) of previous chest x-rays ……

Result ……

Date(s) of previous BAL ……

Result ……

Date(s) of pH probe study ……

Result …….

Date(s) of previous CT scan (chest) ……

Result ……

Thank you

Table E1 Clinical variables and an abnormal LCI in children with CF

	CF Normal LCI (≤ 7.41) (n=32)	CF Abnormal LCI (> 7.41) (n=15)	P
Male n (%)	16 (50)	6 (40)	0.522
Age (years) (SD)	1.6 (0.8)	1.5 (0.8)	0.937
Weight (kg) Mean (SD)	10.7 (1.9)	10.8 (2.7)	0.857
Height (cm) Mean (SD)	81.0 (8.5)	80.8 (10.5)	0.932
Antenatal smoking, n (%)	3 (9)	1 (7)	1.0
Household smokers, n (%)	7(22)	2 (13)	0.697
Breastfeeding, months Mean (SD)	4.3 (4.7)	4.2 (4.8)	0.905
Asthma / Atopy 1st degree relatives, n (%)	20 (63)	11 (73)	0.465
Cough* last year, n (%) Cough last month, n (%)	11 (34) 16 (50)	6 (40) 7 (47)	0.708 0.831
Wheeze last year, n (%) Wheeze last month, n (%)	13 (41) 9 (28)	6 (40) 1 (7)	0.968 0.135
Anti-staphylococcus prophylaxis, n (%)	30 (94)	15 (100)	1.0

*Legend: No clinical variables were associated with an elevated LCI in children with CF. * Cough lasting > 3 weeks.*

Table E2 Type and density of the predominant pathogen in BAL in children with CF

	Number (%)	$<10^5$ cfu/mL (n=30)	$\geq 10^5$ cfu/mL (n=17)	Highest Colony Count x 10^5 cfu/mL
*P .aeruginosa**	**10 (22)**	3*	7	400
H. influenzae	**10 (22)**	2	8	660
B. catarrhalis	**3 (6)**	1	2	60
E. coli	**2 (4)**	2	0	0.06
S. pneumoniae	**1 (2)**	1	0	0.16
S. aureus	**1 (2)**	1	0	0.18
S. maltophilia	**1 (2)**	1	0	0.0044
Commensals	**17 (36)**	16	1	12
Nil growth	**2 (4)**	2	N/A	N/A
TOTAL	**47 (100%)**	27	18**	

*Legend: *Included are two Pseudomonas species. Percentages are rounded up / down to total 100%. ** This includes BAL growth of commensals = 12 x 10^5 cfu/mL in one child.*

Table E3 Clinical characteristics of children with CF by infection status

	No Infection (n=30)	Infection (n=17)	P value
Male, n %	14 (47)	8 (47)	0.979
Age (years) Mean (SD) Range	1.45 (0.76) 0.51 – 3.10	1.73 (0.75) 0.36 – 2.86	0.233
Weight (kg) Mean (SD) Weight <10th centile, n, %	10.7 (2.2) 4 (13)	10.9 (2.2) 2 (12)	0.773 1.0
Height (cm) Mean (SD) Height <10th centile, n, %	80.3 (8.7) 2 (7)	82.1 (9.8) 2 (12)	0.505 0.613
Homozygous F508del n, %	19 (63)	10 (59)	0.760
Maternal antenatal smoking, n, %	3(10)	1(6)	1.0
Household smokers, n, %	8 (27)	1 (6)	0.127
Breastfeeding, months Mean (SD) Range	3.9 (4.4) 0 - 15	5.0 (5.1) 0 - 15	0.454
Asthma/Atopy 1st degree relatives n, %	20 (67)	11 (65)	0.892
Cough * last year n, % Cough last month n, %	8 (27) 13 (43)	9 (53) 10 (59)	0.072 0.307
Wheeze last year n, % Wheeze last month n, %	13 (43) 7 (23)	6 (35) 3 (18)	0.589 0.727
Anti-staphylococcal prophylaxis, n, %	29 (97)	16 (94)	1.0
PPIs / H_2 receptor antagonists, n, %	9 (30)	6 (35)	0.708
Respiratory admission ever n, %	9 (30)	1 (6)	0.07
IV Antibiotics last year n, %	7 (23)	2 (12)	0.455

*Legend: Results are presented as mean (SD) or numbers and percentages. * Cough lasting > 3 weeks.*

Table E4 Airway inflammation in CF children with and without infection

		No Infection (n=30)	Infection (n=17)	P value
Neutrophils %	Median [IQR]	19 [5-40]	73 [52-91]	**<0.001**
Neutrophil count *	Median [IQR]	26 [8-72]	402 [142-2185]	**<0.001**
Total cell count *	Median [IQR]	181 [78-283]	682 [338-2391]	**<0.001**
IL-8 pg/mL	Median [IQR]	667 [306-1675]	4788 [2174-6808]	**<0.001**
NE/α1PI complex #	Mean (SD)	125 (116)	151 (117)	0.589

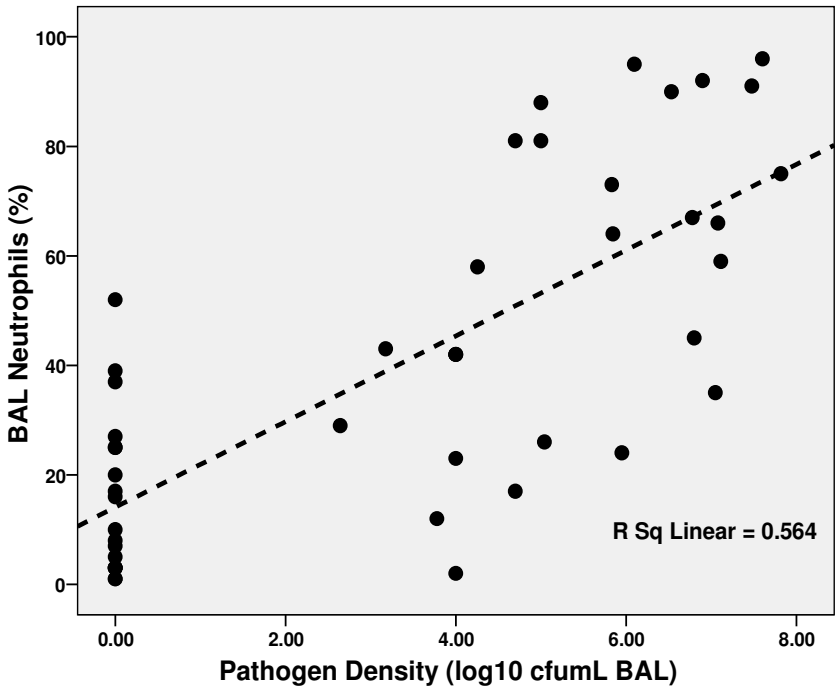
*Legend: * x10^3/mL. # Neutrophil elastase / alpha 1-protease inhibitor complex,* ng/mL, *n=25*

Table E5 Comparison of repeatability and reproducibility data for LCI

Study	Subjects	MBW Equipment	Short-term Repeatability CV*	Reproducibility Mean test difference** (95% LOA$^{\Phi}$)
Aurora *et al* 2004[1]	33 healthy school children 22 CF children	MS	5.2 (2.8)% 6.2 (2.9)%	Not assessed
Aurora *et al* 2005[2]	30 healthy preschool children 30 CF children	MS	5.2 (2.3)% 7.8 (5.4)%	Not assessed
Horsley *et al* 2008[3]	48 healthy adults 12 healthy children 33 CF adults	PA§	3.6 (2.1)% 5.4 (3.8)% 4.4 (2.8)%	-0.20 (-0.78; 0.46) in 16 healthy volunteers
Fuchs *et al* 2009[4]	44 children / adolescents range, 5.3-20.3y	USFM sidestream	5.3%	0.17 (-0.016; 0.348)
Sinhal *et al* 2010[5]	29 unsedated preterm infants (healthy n = 10 RDS$^{\#}$ n = 14 CNLD§§ n = 5)	USFM mainstream	9%	0.19 (-0.33; 0.71)

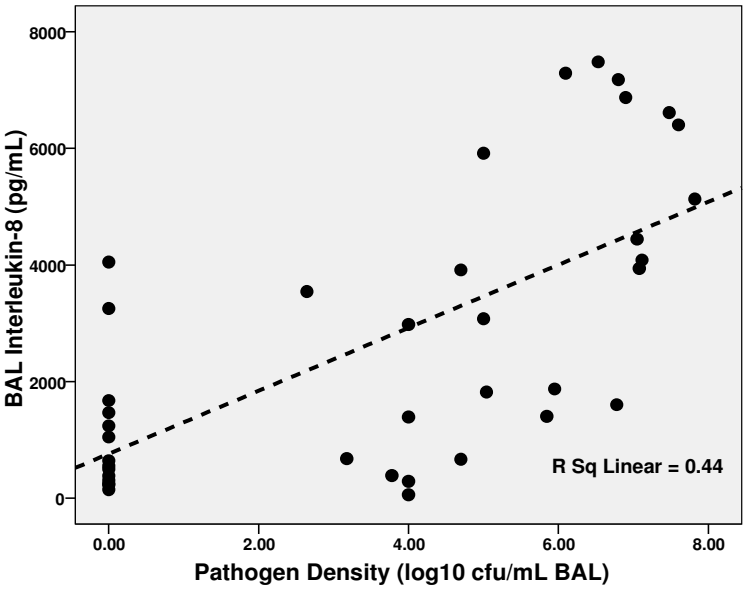
*Legend: * Coefficient of variation, **Bland Altman Analysis. $^{\Phi}$ LOA = limits of agreement § PA = photoacoustic. Results for CV are mean (SD). # Respiratory distress syndrome (resolved). §§Chronic neonatal lung disease.*

Figure E1 Association between BAL neutrophils % and pathogen density in children with CF



Legend: There was a strong relationship between BAL neutrophils % and pathogen load in infants and young children with CF, $R^2 = 0.56$, $P < 0.001$.

Figure E2 Association between BAL IL-8 and pathogen density in children with CF



Legend: There was a strong relationship between BAL IL-8 and pathogen load in infants and young children with CF, $R^2 = 0.44$, $P < 0.001$.

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