**Early *Pseudomonas aeruginosa* predicts poorer pulmonary function in preschool children with cystic fibrosis**

**Authors:**
Aurora P*[1,2], Duncan JA*[1], Lum S[1], Davies G[2,3], Wade A[3], Stocks J[1], Viviani L[1], Raywood E[1], Pao C[4], Ruiz G[5] and Bush A[6,7], on behalf of the London Cystic Fibrosis Collaboration (LCFC).
*joint first authors

**Affiliations:**
1 Infection, Immunity, Inflammation Research & Teaching Department, UCL Great Ormond Street Institute of Child Health, London, UK.
2 Respiratory Medicine, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK.
3 Clinical Epidemiology, Nutrition and Biostatistics, Population, Policy and Practice Research & Teaching Department, UCL Great Ormond Street Institute of Child Health, London, UK.
4 Department of Paediatric Respiratory Medicine, Royal London Hospital, London, UK.
5 Department of Paediatric Respiratory Medicine, King's College Hospital, London, UK.
6 Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, London, UK.
7 National Heart and Lung Institute, Imperial College London, London, UK.

**Author contributions:**
PA, SL, AW, JS, CP, GR, and AB conceived the study and had input into protocol development. JD and ER collected the data. PA, JD, SL, GD, AW, JS, LV and ER conducted/supervised the analysis. All authors had input into drafting of this manuscript.

**Corresponding author:**
Dr Julie Anne Duncan UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK. julie.duncan@alumni.ucl.ac.uk

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**ABSTRACT**

BACKGROUND
We previously reported relatively normal pulmonary function (2 years of age) and computed tomography (CT, 1 year of age) in cystic fibrosis (CF) newborn screened (NBS) infants. We now report follow up of these children to preschool age.

METHODS
67 NBS children with CF and 41 healthy controls underwent pulmonary function tests in infancy (~3 months, 1 year and 2 years) and at preschool (3-6 years). Broncho-alveolar lavage (BAL) and CT were undertaken in those with CF at 1 year. Primary outcomes at preschool were lung clearance index (LCI) and forced expired volume ($FEV_{0.75}$). Risk factors for lung function impairment were identified by regression modelling, emphasising factors that could be identified or measured in the first 2 years of life.

RESULTS
At preschool age children with CF had poorer lung function than controls, mean(95% CI) difference in LCI z-score: 1.47(0.96;1.97) and $FEV_{0.75}$ z-score -0.54(-0.98; -0.10). Isolation of *Pseudomonas aeruginosa* before 6 months was a highly significant predictor of raised (abnormal) preschool LCI, associated with a mean (95%CI) increase of 1.69(0.43, 2.95) z-scores, compared to those with no *Pseudomonas aeruginosa* during the first 2 years of life. Including 2 year LCI and 1 year CT data in the predictive model increased the $r^2$ from 13% to 61%.

CONCLUSIONS
Lung function deteriorates after 2 years in NBS children with CF. Isolation of *Pseudomonas aeruginosa* before 6 months and minor abnormalities of infant lung function tests and CT in infancy are associated with higher preschool LCI.

**HIGHLIGHTS:**

- Lung clearance index increases in children with CF between 2 years of age and the preschool years
- Growth of *Pseudomonas aeruginosa* in the first six months of life is the strongest clinical predictor of this deterioration in lung function
- We suggest more proactive monitoring and treatment of infants with early signs of CF lung disease

**KEY WORDS:** Cystic fibrosis, paediatric lung function, lung clearance index, pseudomonas aeruginosa

## INTRODUCTION

Life expectancy of individuals born with cystic fibrosis (CF) has dramatically improved recently, largely because of closer monitoring, improved nutrition and more aggressive treatment of lung disease. Recent therapies targeting the primary CF defect are being trialled in infants and young children, potentially enabling a further step change in outcomes [1-4], but this opportunity also brings a challenge. The age of development of significant lung disease is highly variable [5, 6]. Targeting new treatments at infants with higher risk of developing disease could reduce the risk of potential medication side-effects, and save costs, in those for whom there may be little benefit from very early treatment.

The London Cystic Fibrosis Collaboration (LCFC) has followed two cohorts of children with CF before and after the introduction of newborn screening (NBS). The first cohort consisted of children born between 1999 and 2002, who were diagnosed with CF following clinical presentation. These children showed a clear deficit in lung function by preschool age (3-6 years) [7]. In this first cohort, abnormal lung clearance index (LCI) measured at preschool age predicted both poor lung function [8] and abnormalities on computed tomography (CT) in later childhood [9]. The second LCFC cohort consisted of children born between 2009 and 2012, who were diagnosed with CF via newborn screening. In these children we showed that despite a deficit at 3 months of age [10], lung function improved to within the normal range by 1 year [11] and was maintained to 2 years [5] with current management strategies. Furthermore, CT changes at 1 year of age were very minor[12, 13].

The aims of this study were to a) determine whether children from the second LCFC cohort maintained near normal lung function at preschool age, and b) if not, to identify factors measured *in infancy* (up to 2 years of age) that could predict lung function abnormality at preschool age. Specifically, we wished to examine predictors that could be obtained by routine clinical monitoring early in life, that give insight into development of early CF lung disease. We also aimed to determine whether lung function, chest CT, or inflammation detected by bronchoalveolar lavage (BAL) during infancy provided additional prognostic information. We hypothesised that we would detect only minor abnormalities of lung function at preschool age but, despite this, we would be able to identify early predictors of impairment.

## METHODS

*Study population*

96 infants with CF diagnosed by newborn screening were recruited following referral to any of the five CF centres in London, UK. 62 contemporaneous healthy control (HC) infants were recruited from the Homerton University Hospital in London. Infants underwent pulmonary function testing at approximately 3 months, 1 year and 2 years of age. All children were invited to return at 3-6 years for preschool lung function tests. Full details of the recruitment process and eligibility criteria have been published previously [10]. The study was approved by the London National Research Ethics Committee (12/LO/1668), and parental consent was obtained for all procedures.

*Lung function testing*

All measurements were made at Great Ormond Street Hospital for Children, London, UK. In 'infancy' (3 months, 1 year and 2 year tests), LCI - by multiple breath washout (MBW) using a mass spectrometry system, plethysmographic functional residual capacity ($FRC_{pleth}$) and forced expired volumes ($FEV_{0.5}$) were measured supine while the child was sedated. At preschool age (3-6 years) LCI was measured by

the same mass spectrometry system with a preschool mask interface, and spirometry including forced expired volumes ($FEV_{0.75}$) using MasterScreen (Carefusion Sentry Suite V2.11). All infant and preschool lung function tests conformed to published American Thoracic Society/European Respiratory Society criteria [14-16]. If preschool children were unable to perform technically satisfactory tests on their first visit, they were invited to re-attend within six months for repeat assessment.

*Clinical data and additional assessments*
Clinical information was obtained via a proforma completed by the treating clinicians or specialist nurse from the child's chart. Infants with CF (but not controls) underwent BAL and CT at 1 year[13]. Air trapping was the primary CT outcome, with bronchial dilatation and total CT score as secondary outcomes (see OLS). Previous work found these variables to be the only reproducibly scored CT outcomes in this cohort [12, 13]. Primary BAL outcomes were free neutrophil elastase (NE) and interleukin-8 (IL8). Microbiology data consisted of BAL results; cough swabs[17] taken at time of study visits; and regular cough swabs /sputum samples collected and processed by the child's clinical team (every two months). CT and BAL were not repeated at preschool test as CT abnormalities were mild and poorly reproducible in LCFC infants[12]. BAL required general anaesthesia and children had regular airway surveillance with cough swabs are more applicable to clinical practice.

*Data analysis*
Standard software packages were used for data inspection, distribution, and descriptive statistics (IBM SPSS Statistics, v23.0). Anthropometry and lung function results were expressed as z-scores using published prediction equations [18-21]. Comparisons between children with CF and healthy controls were made using unpaired T-tests with a significance level of $p<0.05$.

Univariable regression analyses between preschool lung function and relevant anthropometric and clinical variables were performed before constructing multivariable models to assess the joint associations and develop a prediction model.

To assess whether the time of *Pseudomonas aeruginosa* (*Ps aer*) or other microbiological pathogen acquisition during the first 2 years of life was associated with lung function decline by preschool years, we compared those with very early isolation with those with isolation later in infancy (6-24 months), using those in whom these organisms were never isolated during these periods as the baseline. There is no agreed definition of early isolation, and we chose 6 months arbitrarily, as we had data available by that time point. These analyses were repeated with the addition of a) lung function; b) CT; and c) BAL inflammation (NE and IL8) data. Multivariable regression models were used to investigate associations. All CT and BAL data were categorical or non-linear. Data were Ln transformed where necessary before regression analyses. Model parameter estimates are presented with 95% confidence intervals to show precision of estimation. As preschool tests were performed over a relatively large age range, age at time of test was included in the modelling. Primary analysis was performed for preschool LCI, and secondary analysis for preschool $FEV_{0.75}$. More detail of the methods, including power calculation, is presented in the on-line supplement (OLS).

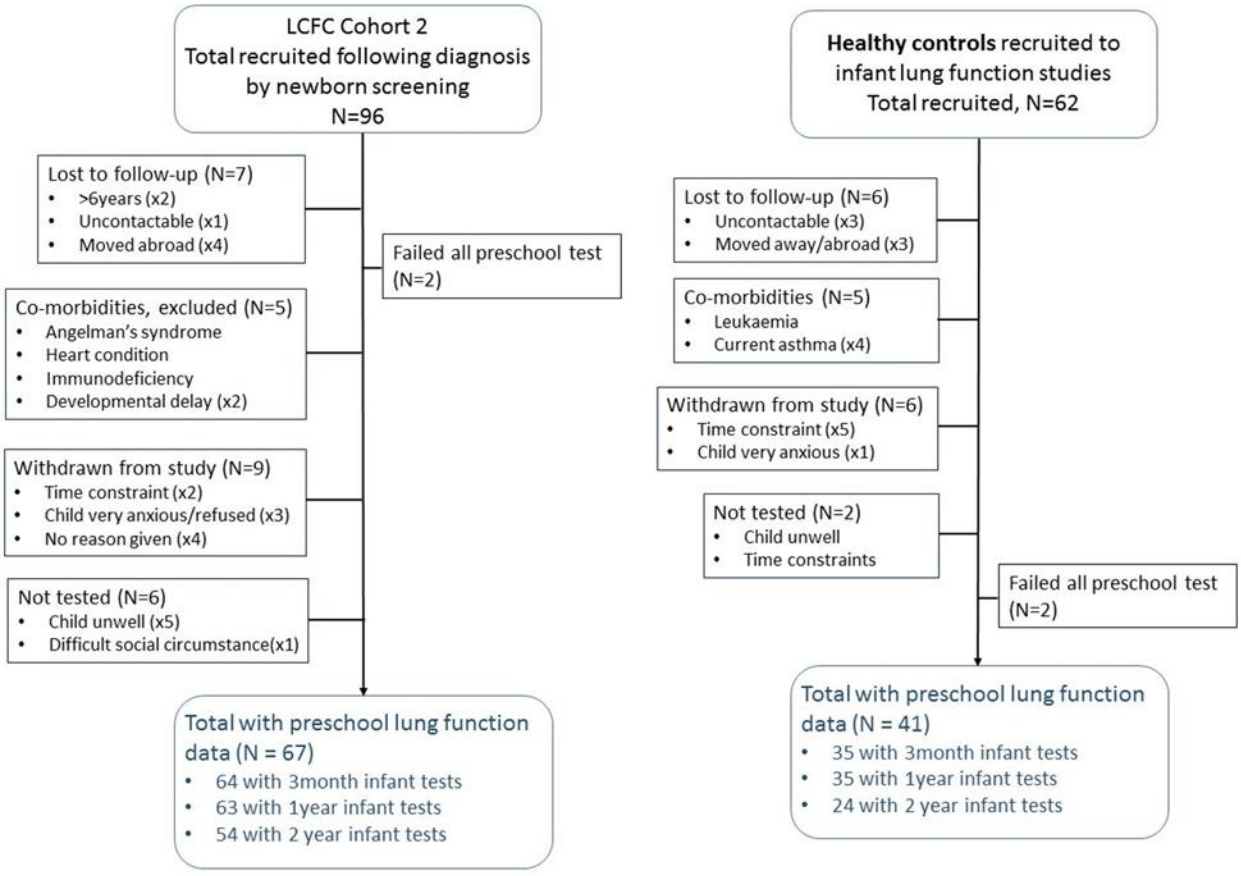
*Power of study*
The sample size was opportunistic, and initially based on a power calculation for analysis during infancy [11], at which time predicted lung function differences were smaller than those predicted at preschool age. Follow up of 60 newborn-screened children with CF and 30 healthy controls at preschool age would

provide 80% power at the 5% significance to detect a difference of at least 0.7 z-scores in the two primary outcomes ($FEV_{0.75}$ and LCI), numbers that were exceeded in this follow up (table E1).

**RESULTS**

*Cross-sectional comparison of preschool children with CF and healthy controls*:
158 children (96 CF and 62 controls) were tested as infants. After exclusions for co-morbidity, loss to follow up or withdrawal from study, and failed preschool testing, 108 children (67 CF, 41 HC) were included in the longitudinal analysis at preschool age (Figure 1).

**Figure 1: Recruitment and accrual for cohort 2 follow up at preschool age**




Mean age at testing was 4.6 years (range 3.1-6.0y), with children with CF being slightly older (4.8y) on average than HC (4.4y). The two groups had similar sex and ethnicity distributions. Preschool children with CF were shorter and lighter than controls, with no difference in body mass index (BMI). Success rates for lung function were similar and improved with age (see OLS; Tables E1- E3). Children with CF had significantly poorer pulmonary function than healthy controls (Table 1). By cross-tabulation, 26/66 (39%) of the children with CF had abnormal LCI (*i.e.* greater than 1.96 z-scores); whilst 7/62 (11%) had abnormal $FEV_{0.75}$. There were no significant differences in background demographics or lung function at ~3m in children with CF who were and were not followed up at preschool age (Table E4).

**Table 1: Comparison of background characteristics and lung function in preschool children with CF and healthy controls, together with CT and BAL inflammation outcomes at 1 year of age in those with CF**

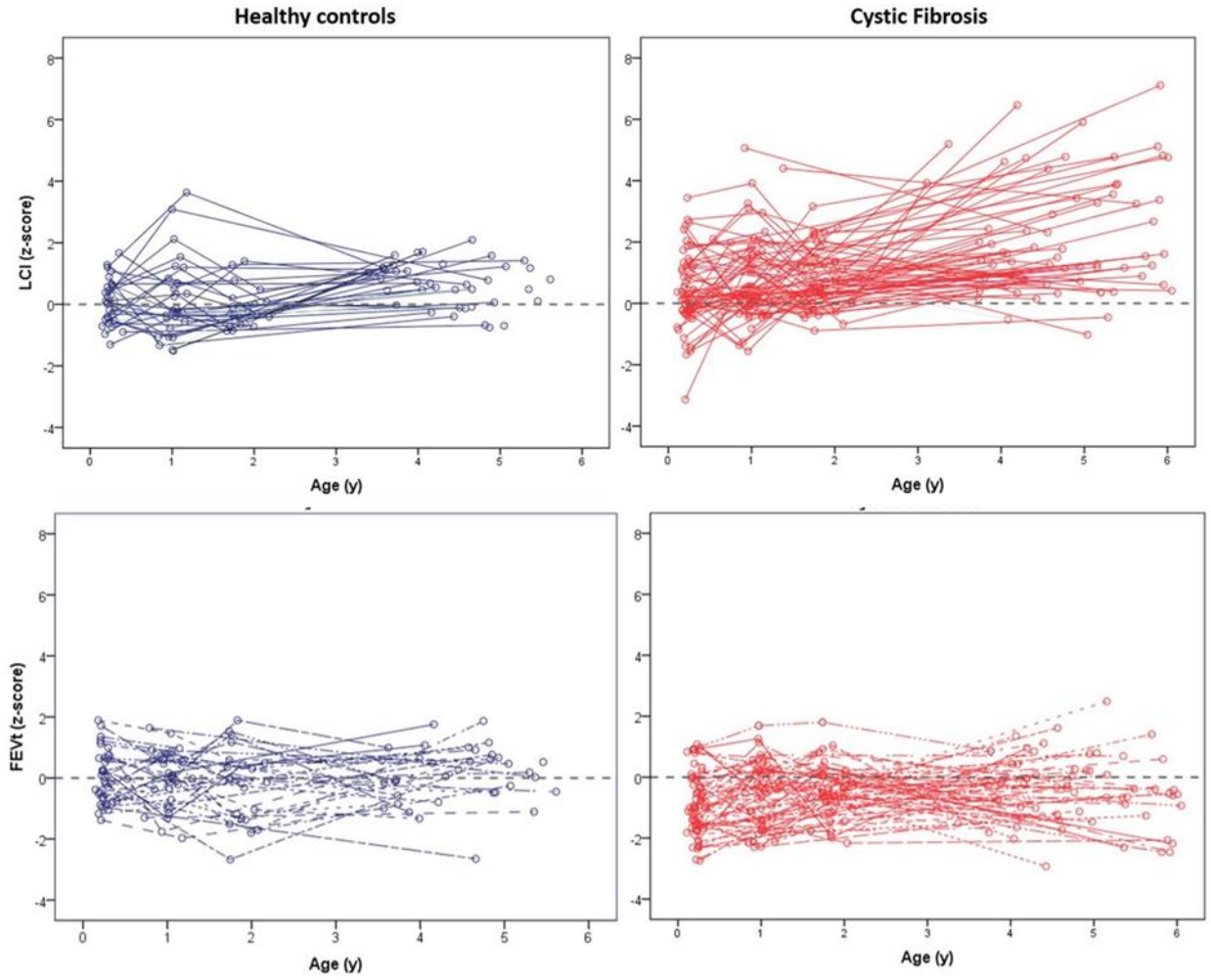
| | Cystic Fibrosis | Healthy Controls | Difference (95%CI) | p |
|---|---|---|---|---|
| **Number of subjects** | 67 | 41 | | |
| **Boys (%)** | 46% | 46% | 0% (19%; 18%) | 0.99 |
| **White (%)** | 87% | 83% | 4% (10%; 19%) | 0.61 |
| **Age at test, year** | **4.76 (0.75)** | **4.41 (0.62)** | **0.35 (0.08; 0.63)** | **0.013** |
| **zBirthweight*** | **-0.38 (0.96)** | **-0.02 (0.74)** | **-0.36 (-0.71; -0.01)** | **0.042** |
| **zWeight*** | **-0.11 (0.84)** | **0.43 (1.15)** | **-0.54 (-0.92; -0.16)** | **0.006** |
| **zHeight*** | **-0.23 (0.87)** | **0.23 (1.21)** | **-0.46 (-0.90; -0.03)** | **0.036** |
| **zBMI*** | 0.09 (0.67) | 0.25 (0.96) | -0.16 (-0.47; 0.15) | 0.313 |
| **LCI** | **8.28 (1.67)[a]** | **7.09 (0.45)[b]** | **1.19 (0.76; 1.63)** | **<0.0001** |
| **zLCI** | **2.14 (1.84)** | **0.67 (0.72)** | **1.47 (0.96; 1.97)** | **<0.0001** |
| **$zFEV_{0.75}$ [$]** | **-0.45 (1.11)[c]** | **0.09 (0.93)[d]** | **-0.54 (-0.98; -0.10)** | **0.016** |
| **$zFEV_1$ [$]** | **-0.31 (1.05)[e]** | **0.15 (0.90)[f]** | **-0.46 (-0.88; -0.03)** | **0.037** |
| **zFVC [$]** | 0.03 (1.03)[g] | 0.25 (0.90)[d] | -0.22 (-0.63; 0.19) | 0.287 |
| **$zFEV_{0.75}$/FVC [$]** | -0.45 (1.11)[c] | 0.09 (0.93)[d] | -0.32 (-0.64; -0.001) | 0.051 |
| **$zFEV_1$/FVC [$]** | **-0.73 (0.77)[e]** | **-0.26 (0.72)[g]** | **-0.47 (-0.79; -0.15)** | **0.004** |
| **Total CT score[#h]** | 2 (0 – 15.5) | | | |
| **CT air trapping subscore[#h]** | 0 (0 – 5.5) | | | |
| **CT bronchial wall dilatation subscore[#h]** | 0 (0 – 3.0) | | | |
| **% with detectable free NE (BAL)[#j]** | 6 (20.7%) | | | |
| **IL8 (BAL)[#j]** | 286 (1 – 5124) | | | |

**Legend:** Data collected at first preschool enrolment study visit and presented as mean (SD) unless otherwise indicated. *according to British 1990 growth reference [19]; [$]according to GLI reference equations [20] using ethnicity as 'white'. Number of measurements as follows: a=66; b=39; c=62; d=35; e=60; f=34; g=64; h=47; j=29. To allow comparison with published literature, spirometry results are converted to z-scores via the GLI-W equations [20]. [#]data presented as median (range). Interleukin 8 (IL8) in pg/ml. Lower limits of detection for IL-8 was 0.04 pg/ml and 200ng/ml for NE. Abbreviations: z=z-score; NE=neutrophil elastase; IL8=interleukin-8

*Comparison of longitudinal changes in lung function to preschool age in healthy children and those with CF:*

Longitudinal changes in LCI and FEV z-scores are presented in Figure 2. $FEV_{0.5}$ was below zero z-scores in most children with CF when first tested at 3m, but $FEV_t$ then improved through infancy, with no difference between groups by 2 years of age and only a small (mean) deficit of ~-0.5 z-scores by the preschool years. In contrast, the difference in LCI between CF and controls increased from the first infant tests to the preschool test.

**Figure 2: LCI and $FEV_t$ z-scores according to age in healthy controls and children with CF**




**Legend:** *$FEV_t$ (z-score) represents $FEV_{0.5}$ z-scores during infant tests and $FEV_{0.75}$ from preschool spirometry. The horizontal dashed line represents 0 z-scores (100% predicted). 95% healthy subjects would be expected to fall between ± 2 z-scores. Six controls with FEV during infancy did not have successful preschool FEV measurements.

*Clinical status of preschool children with CF*

Details of background characteristics, treatment and microbiological status of children with CF are given in Table 2. There were no significant differences in background characteristics of LCFC subjects who participated in this preschool follow up and those who did not (Table E4, OLS).

**Table 2: Background characteristics, treatment and microbiological status for the preschool population with CF**

| | CF (n = 67) |
|---|---|
| Median(range) age at time of diagnosis (weeks) | 3.4 (1.1 – 17.6¥) |
| F508del genotype | |
| • Homozygous | 41 (61.2%) |
| • Heterozygous | 20 (29.9%) |
| • Other | 6 (9.0%) |
| Presented with meconium ileus | 7 (10.4%) |
| Allergic broncho-pulmonary aspergillosis | 1 (1.6%) |
| Pancreatic insufficiency | 58 (86.6%) |
| ***Bacterial growth on cough swab 'ever' prior to preschool test*** | |
| *Pseudomonas aeruginosa*[a] | 49 (74.2%) |
| *Staphylococcus aureus*[b] | 40 (62.5%) |
| *Haemophilus influenzae*[b] | 38 (59.4%) |
| *Stenotrophomonas maltophilia*[b] | 16 (25%) |
| ***Bacterial growth, Median(range) age at 1st isolate (year):*** | |
| *Pseudomonas aeruginosa* | 1.18 (0.12 – 4.34) |
| *Staphylococcus aureus* | 1.38 (0.04 – 5.76) |
| *Haemophilus influenzae* | 1.05 (0.02 – 4.67) |
| *Stenotrophomonas maltophilia* | 2.26 (0.51 – 5.92) |
| ***Additional treatment prior to preschool test**** | |
| DNase | 20 (31.3%) |
| Antacid medication | 26 (40.6%) |
| Hypertonic saline | 11 (17.2%) |
| Ursodeoxycholic acid | 12 (18.8%) |
| ***Prophylactic antibiotics at time of preschool test‡**** | |
| Flucloxacillin | 27 (42.2%) |
| Co-amoxiclav | 8 (12.5%) |
| None | 29 (45.3%) |
| ***Long-term nebulised antibiotics**** | |
| Colistin | 13 (20.3%) |
| Tobramycin | 0% |
| Alternate Colistin/Tobramycin | 2 (3.1%) |
| None | 49 (76.6%) |
| ***Total IV antibiotic courses given since birth (based on n = 65)*** | |
| 9 courses | 1 (1.5%) |
| 5-6 courses | 3 (4.6%) |
| 3-4 courses | 8 (12.3%) |
| 1-2 courses | 30 (46.1) |
| None | 23 (35.4%) |
| ***Total IV antibiotic courses given in the last 12 months prior to preschool test*** | |
| 2 courses | 4 (6.3%) |
| 1 course | 10 (14.9%) |
| None | 50 (78.1%) |

**Legend:** Data presented as n (%). Data on bacterial growth available from: a=66; b=64. ¥ Based on 65 children as confirmation of CF diagnosis was delayed in two children with rare genotypes. *Based on 64 subjects (full data from 3 subjects not available). # Based on 63 subjects (full data from 4 subjects not available). ‡All infants received were prescribed prophylactic flucloxacillin until 2 years of age as part of the then standardised treatment protocol [10, 11]. No subjects isolated methicillin-resistant *Staphylococcus aureus* therefore not included in table

*Predictors of preschool lung function:*
We took a stepwise approach to this analysis. First, we assessed clinical and microbiological variables collected in infancy as predictors of preschool lung function. Second, we assessed whether infant lung function, CT, and BAL inflammation provided additional prognostic information.

*Clinical and microbiological predictors of preschool LCI:*
No significant associations were found on univariable analyses between preschool LCI and height, weight, or clinical variables (genotype, pancreatic insufficiency, and meconium ileus). There was no significant association between LCI and subject age at time of preschool testing. Of microbiological parameters, isolation of *Ps aer* prior to six months of age (n=9) was the most significant univariable predictor of preschool LCI, associated with a mean (95%CI) increase of 1.69 (0.43, 2.95) z-scores compared to those who had not acquired *Ps aer* by that age. In contrast, there was no association between preschool LCI and 'ever' acquisition of *Ps aer*, *Staphylococcus* or *Haemophilus* (*i.e.* a positive culture anytime from birth to time of preschool test) compared to those without. Details of univariable regression analyses (Table E5), and longitudinal plots of LCI according to infection status (Figure E1) are presented in the OLS.

On multivariable analysis, after adjusting for early *Ps aer* acquisition, no significant associations were found for any other clinical or anthropometric predictors or other bacterial acquisition (*Staphylococcus* or *Haemophilus*) during the first 2 years. A multivariable model including *Ps aer* acquisition in the first 2 years of life had $r^2$ of 0.13 (i.e. 13% of preschool LCI can be explained by these predictors), with *Ps aer* acquisition before 6 months as the main predictor (Table 3).

*Infant lung function as a predictor for preschool LCI*
On univariable analysis, each z-score increase in LCI at 2y was associated with a mean (95% CI) increase in LCI of a further 0.89 (0.36; 1.43) z-score at preschool age. There was no association between earlier measurements of LCI (at either 3m or 1y of age) with preschool LCI. There was no association between $FEV_{0.5}$ on any infant test occasion and preschool LCI.

On multivariable analysis, the addition of 2-year LCI to the *Ps aer* model increased $r^2$ to 0.24. In this model early *Ps aer* acquisition remained an independent predictor, and was associated with an average increase of 1.51 LCI z-score compared to those who did not isolate *Ps aer* before 2 years of age. LCI at 2 years was also an independent predictor within this model (Table 3), with each z-score increase in LCI at 2y associated with 0.67 (0.16; 1.17) z-score increase in preschool LCI.

*1-year CT and BAL inflammation as a predictor for preschool LCI*
On univariable analysis, preschool LCI was significantly associated with air trapping and total CT score at 1 year. There was no association between preschool LCI and markers of bronchial dilatation or with BAL IL8 and NE, but only 29 subjects had IL8 and NE results due to processing error.

On multivariable analysis, when air trapping was included in the model, 61% of the variability of preschool LCI was explained (Table 3). LCI at 2 years and air trapping at 1 year were the strongest independent predictors within this model, and the inclusion of *Ps aer* data resulted in minimal change of $r^2$ from 0.6 to 0.61.

*Predictors of preschool $FEV_{0.75}$*

On univariable analysis, $FEV_{0.5}$ at 3m was the strongest predictor of preschool $FEV_{0.75}$. Each z-score decrease in $FEV_{0.5}$ at 3 months was associated with a mean (95%CI) decrease in preschool $FEV_{0.75}$ of 0.4 (0.13; 0.69) z-scores at preschool age ($r^2$=0.134). When *Ps aer* acquisition and $FEV_{0.5}$ z-score at 3m were included in a multivariable model predicting preschool $FEV_{0.75}$, the $r^2$ was 0.155. Addition of CT air trapping to the model increased the $r^2$ to 0.187, with $FEV_{0.5}$ at 3 months remaining the most important predictor (Table 3).

**Table 3: Final multivariable regression models (using factors from early years) for prediction of preschool LCI and $FEV_{0.75}$**

| **Models for zLCI at preschool** | **Clinical (n=65#)** | | **Clinical + LF (n=52)** | | **Clinical + LF + CT (n=38)** | |
|---|---|---|---|---|---|---|
| $r^2$ | 0.134 | | 0.242 | | 0.605 | |
| | **Coefficient** | **95% CI** | **Coefficient** | **95% CI** | **Coefficient** | **95% CI** |
| PsA acquisition age: <6m | **1.85** | **0.64; 3.07**** | **1.51** | **0.10; 2.91*** | 0.72 | -0.51; 1.94 |
| PsA acquisition age: 6 – 24m | 0.23 | -0.62; 1.07 | 0.04 | -0.92; 1.0 | -0.02 | -0.97; 0.93 |
| zLCI at 2y | | | **0.67** | **0.16; 1.17*** | **0.69** | **0.22;1.16**** |
| Air trapping (CT at 1y) | | | | | **0.78** | **0.49; 1.08***** |

| **Models for $zFEV_{0.75}$ at preschool** | **Clinical (n=61)** | | **Clinical + LF (n=56)** | | **Clinical + LF + CT (n=40)** | |
|---|---|---|---|---|---|---|
| $r^2$ | 0.008 | | 0.155 | | 0.187 | |
| | **Coefficient** | **95% CI** | **Coefficient** | **95% CI** | **Coefficient** | **95% CI** |
| PsA acquisition age: <6m | -0.21 | -0.64; 1.06 | 0.09 | -073; 0.92 | 0.07 | -0.88; 1.02 |
| PsA acquisition age: 6 – 24m | -0.18 | -0.42; 0.81 | 0.33 | -0.25; 0.91 | 0.53 | -0.19; 1.25 |
| $zFEV_{0.75}$ at 3m | | | **0.40** | **0.12; 0.68**** | **0.46** | **0.08; 0.84*** |
| Air trapping (CT at 1y) | | | | | -0.06 | -0.30; 0.17 |

**Legend:** Only factors (clinical, early lung function and CT at 1y) which were found to have a strong association with preschool LCI and $FEV_{0.75}$ were included in this model. For these models '*Ps aer* never isolated in the first 2y of life' was used as the baseline. *Ps aer* infection during early life was significantly associated with LCI but not $FEV_{0.75}$ at preschool age. Inclusion of other factors (i.e. somatic growth, microbiology, treatment intensity) did not improve the model fit (not shown).
#Data on *Ps aer* acquisition were unavailable from one CF infant. *p<0.05; **p<0.01; ***p<0.001
Abbreviations: LF: Lung function; PsA: *Pseudomonas aeruginosa*; m: month; y: year.

**DISCUSSION**

We previously demonstrated that this second (NBS) LCFC cohort maintained near normal lung function until 2 years [5], and had near normal CT scores at 1 year[12, 13]. We have now demonstrated a significant deterioration of lung function by preschool age, with mean LCI approximately 1.5 z-scores higher than controls, and $FEV_{0.75}$ approximately 0.5 z-scores lower.

We next analysed predictors of this decline using multivariable modelling. These models provide two sources of complementary information. First, the $r^2$ of the model gives some indication of how valuable these predictors are in combination. Second, the partial coefficients give indication of how much independent contribution different variables provide to the model. We can summarise our analyses as follows:

In our population, acquisition of Ps aer before 6m is an important clinical/microbiological predictor of raised preschool LCI, associated with an increase of ~2 z-scores, albeit with substantial variability between individuals. Addition of infant lung function and CT data improve model fit, to the point where more than 60% of variability of preschool LCI can be explained by measurements during infancy. It should be noted that these latter predictors were measured at 1 and 2 years, so occurred after the subjects did (or did not) acquire Ps aer before 6 months age. Addition of 2 year LCI and 1 year CT scores to these models reduced the independent contribution made by early Ps aer acquisition, implying that the CT and lung function abnormalities were correlated with early infection. We accept that our study was not designed to test these latter relationships/causation, and our data also support the value of one year CT and two year LCI as predictors of subsequent lung function.

These data add to our growing understanding of the importance of early *Ps aer* infection. The Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF) have also studied NBS CF infants longitudinally from diagnosis. They previously reported an association between lung function decline in infancy and isolation of *Staphylococcus aureus* and *Ps aer* [22]; that *Ps aer* in infancy was associated with increased bronchiectasis score on CT [23]*;* and that pro-inflammatory pathogens in BAL were associated with differences in $FEV_{0.75}$ at early school age [24]. Our study showed poorer preschool lung function in those with *Ps aer* in the first six months, but not in later infancy (after six months to two years), suggesting a particularly detrimental effect of *Ps aer* on the early developing lung.

In our study microbiology data were collected via routine cough swab collection every 2-3 months, plus BAL collected at 1 year. This protocol identified early *Ps aer* acquisition as a predictor of decline, but it could be argued that more aggressive monitoring is justified, for example by more frequent cough swabs or induced sputum [25, 26].

Our previous reports from this cohort demonstrated near normal lung function through infancy, and near normal CT at 1 year. In contrast, the AREST-CF studies reported a sharp decline during infancy, with $FEV_{0.5}$ -4 z-scores by 2 years of age [6]. We have previously discussed the potential causes of the discrepancy between the Australian and London data [27], and how this led to some controversy over the potential value of such early monitoring. Our data agree with a North American study which showed deterioration in LCI over the preschool years (2.5-6 years) using a nitrogen washout technique, but normal spirometry in the same children [28].

The current analysis also shows some discrepancy between London and Australia. In the most recent AREST-CF report of NBS preschoolers with CF [29], 55% (32/58) had abnormal LCI measured using nitrogen washout versus 39% (26/66) in our cohort. This suggests there may still be a difference between the two groups, despite similar treatment protocols. Furthermore, we did not find any association between neutrophil elastase measured in infant BAL and later lung function, unlike the Australian data [6, 22, 23, 30, 31], but inflammatory marker results were only available in 29 subjects in our study. On another important matter our data support their conclusions. The minor lung function and CT deficits that we demonstrated in infancy are strongly associated with subsequent decline, with 61% of the variability of preschool LCI being explained by a combination of 2-year LCI, air trapping on infant CT and early *Ps aer* acquisition.

Our study has important strengths and weaknesses. All lung function measurements were made at the same centre in a specialist laboratory with an experienced team, minimising methodological bias. We did however find that our control group had a higher than expected mean (SD) LCI z-score of 0.67 (0.72). This may be due to a relatively small sample size [32], but we have also recently noted an increase in LCI values for older control subjects [33] and there is a possibility that a laboratory move and refurbishment in 2011 may be associated with a change in computed LCI values. We investigated potential causes in detail, as summarised in the OLS (tables E6-7). We note that this discrepancy was only detectable due to inclusion of healthy control subjects in all our LCFC studies, thus preventing over-estimation of the extent of lung function abnormalities in those with CF, and ensuring that our estimates of both differences at preschool age and change over time are robust.

In our study more than 70% of our cohort had *Ps aer* acquisition by six years of age. We note that this is considerably higher than data reported by the CFFPPR in the United States[34]. It is however consistent with unpublished data from other UK investigators.

As previously reported, the Brody II scores from CT scans in our cohort showed only minor abnormalities at 1 year [12, 13]. It is possible that an alternative scoring system, such as PRAGMA-CF [35] would be more sensitive. It is relevant therefore, that abnormalities of the Brody II air-trapping score at 1 year predicted future lung function decline. Given that we pre-specified Brody II as our scoring system, we considered it inappropriate to change to another score *post-hoc*.

Subject retention in this follow-up study was high for a study involving infants and preschool children, and most dropouts were unavoidable. There was no significant difference in 3-month data between subjects who remained in the study and those who did not (OLS, Table E4). Five children could not be retested because they were unwell at the time of their appointments, and these subjects may have had more severe lung disease. The main weakness of the study is the relatively small numbers who isolated *Ps aer* before 6 months of age, and we did not analyse the effect of treatment of *Ps aer*. Furthermore, data for inflammatory markers were not available for 16 infants due to a processing error of BAL, where

aliquots were frozen before centrifuge which is required before supernatant extraction. This affected our ability to investigate BAL markers as predictors of lung function due to a reduced number of subjects.

In conclusion, these data show that LCI increases between 2 years and preschool age in children with CF, and early growth of *Ps aer* is the strongest clinical predictor of this deterioration. Minor abnormalities of LCI and CT during infancy are also associated with future lung function decline, and may be helpful in identifying children who will benefit from intensified therapy. Our data suggest that this should start before 2 years of age, and that we should not be complacent about the relatively minor changes in the 2-year data, or CT abnormalities at 1 year.

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