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

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Data Supplement

Study population

The London Cystic Fibrosis Collaboration (LCFC) consists of five participating tertiary paediatric centres in London, UK; namely the Royal Brompton Hospital, Great Ormond Street Hospital for Children, The Royal London Hospital, King's College Hospital and University Hospital Lewisham. UK newborn screening (NBS) for cystic fibrosis (CF) involves an immunoreactive trypsin (IRT) assay on dried heel prick blood spot testing on day 5 of life. Those with raised IRT levels undergo CFTR mutation screening and if CF is suspected a sweat test is performed to confirm the diagnosis. The sensitivity of NBS for CF in the UK is 96% and positive predictive value 0.48[1]. At each centre, children with CF identified by newborn screening between 2009 and 2011 underwent diagnostic pilocarpine iontophoresis (sweat chloride >60 mEq/L) and/or genotype confirmation (two known CF-causing mutations) for diagnosis. Children presenting with meconium ileus were also included. Formal recruitment was at a follow up visit approximately two weeks after diagnosis by their consultant at the participating centres. Contemporaneous healthy control subjects were recruited from the Homerton University Hospital, London U.K. Full details of the infant recruitment process, including inclusion and exclusion criteria have been published previously [2, 3]. For the first two years of this study there was synchronisation of treatment approach across study sites, and from then onwards children were treated according to local protocol.

At preschool age (3-6 years) all children with successful measurements from at least one infant test occasion were invited to return for lung function testing. Control subjects were excluded if they had developed any respiratory condition (such as recurrent wheeze or chronic cough) or had developed any other chronic medical condition that may affect pulmonary function.

Protocol

All subjects were tested when well (free from any new respiratory symptoms for 3 weeks preceding the test date) and were examined by a clinician before lung function measurements. Height and weight were measured and expressed as z-scores according to the WHO reference during infancy and the British 1990 reference for preschool children [4]. A cough swab was taken for microbiological culture on each test occasion from those with CF.

Infant measurements
Infants were invited to attend for lung function measurements on three tests occasions at approximately 3 months, 1 year and 2 years of age. All lung function measurements were made supine at Great Ormond Street Hospital under chloral hydrate sedation as described in detail previously [3, 5]. Multiple breath washout (MBW), whole body plethysmography and the raised volume thoraco-abdominal compression (RVRTC) technique were performed in that order. Lung Clearance Index (LCI) was measured by MBW, using mass spectrometry and customised software, an infant mask interface and 4% sulphur hexafluoride (SF_6) as a tracer gas. Plethysmographic functional residual capacity (FRC_{pleth}) and forced expired volumes ($FEV_{0.5}$) from an inflation pressure of 30 cmH_2O using the RVRTC technique were measured using the Jaeger BabyBody device (CareFusion, San Diego, USA; V.4.65). For the longitudinal follow up, primary outcomes were limited to LCI and FEV_t as these can be obtained at any age, whereas FRC_{pleth} cannot be routinely measured in preschool children [6]. Lung function results during infancy were expressed as z-scores from reference equations derived from healthy infants using identical equipment and protocols [7, 8].

CT & BAL at one year
Volume controlled thin section chest CT was performed under general anaesthesia, as described previously [9]. In brief, inspiratory and expiratory scans were obtained using controlled ventilation. Anonymised scans were scored independently by two specialist paediatric thoracic radiologists (Drs Alan Brody and Alistair Calder) with no knowledge of subject clinical characteristics. The Brody-II CF-CT scoring system was used [10, 11]. Air trapping subscore was used as the primary outcome due to the poor intra- and inter-observer agreement for other CT score

parameters previously reported [9]. Total Brody score and bronchial dilatation were also studied as secondary outcomes. The mean of the two operator scores for each of these parameters were used. Abnormal scores were defined as a total CT score greater than 12 (maximum possible 243), any evidence of bronchial dilatation (maximum possible score 72), or air trapping score greater than six (maximum 27) [9].

Flexible bronchoscopy was performed after the CT scan under the same general anaesthetic. A 2.8 mm bronchoscope was inserted via an endotracheal tube. ERS taskforce guidelines for BAL in children were followed [12]; four aliquots of normal saline at a volume of 1ml/kg were instilled and retrieved using low pressure suction, three from the right middle lobe and one from the lingula. The first returned aliquot from each lobe was sent for bacterial and fungal culture, and viral detection by immunofluorescence. Subsequent samples from the right middle lobe were pooled, centrifuged and the supernatants frozen at -80°C. Cytokines were measured from pooled samples using the Meso Scale Discovery® Multi- Array technology (MesoScale Discovery, Gaithersburg, MD, USA). Free neutrophil elastase (NE) activity was measured using an adapted ELISA (enzyme-linked immunosorbent assay) technique as described by the AREST-CF group [13]. Primary outcomes were NE and interleukin-8.

Preschool measurements

Preschool children attended between 3 and 6 years of age to undertake MBW and spirometry. MBW was performed using the same system as used in infants, except that a larger pneumotach and mask, appropriate for the age and size of the child were used, and measurements were made unsedated while the child sat and quietly watched a cartoon to encourage tidal breathing. LCI was adjusted for height, sex and age by expressing results as z-scores based on published reference equations [7].

Spirometry was performed using incentive game software (SentrySuite Software V2.11.1, Carefusion Corporation, San Diego, California, USA). Following ATS/ERS guidelines for an acceptable test [6], $FEV_{0.75}$ and FEV_1 were reported if the duration of expiration exceeded 0.75 and 1 second respectively. Results were expressed as z-scores using the Global Lung Function Initiative (GLI) 2012 reference equations [14] with an abnormal result defined as -1.96 z-scores. $FEV_{0.75}$ was the primary outcome as some healthy preschool children are not able to obtain forced expiration for one second. Equations for white children were used for all subjects as the majority of subjects were of white European descent and values for other ethnicities are not currently available for $FEV_{0.75}$. There were no significant differences in ethnicity between healthy controls and those with CF (Table 1, main manuscript). Plethysmographic airway resistance (sR_{aw}) was also measured in the preschool children, but is not presented in this paper as a) equivalent results are not available from infant testing; and b) previous LCFC studies have demonstrated that LCI is a better marker of CF lung disease in pre-schoolers than sR_{aw} [15]. All measurements were made during clinical stability. CT and BAL were not repeated at preschool testing as previous results had shown only minor abnormality in CT scores and we did not consider that the required anaesthetic (for BAL) and radiation exposure (for CT) were justified [9].

Statistical analysis (additional details)

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 [4, 7, 14]. The extent to which these z-scores differed from zero according to sex, age, and body size was inspected. An abnormal preschool result was defined by being +1.96 z-scores or above for LCI and -1.96 z-scores or below for $FEV_{0.75}$. Comparisons were made between the CF children and the control group using t-tests and chi-square tests as appropriate. Differences are presented with 95% confidence intervals (CI).

To identify potential predictors of preschool lung function, and estimate the extent to which lung function during infancy might predict that at preschool age after adjusting for other relevant factors, linear regression was undertaken using anthropometric and clinical data from all infant test occasions to quantify associations with LCI and $FEV_{0.75}$ z-scores during the preschool years. A series of univariable regression analyses between preschool lung function and relevant anthropometric and clinical variables were performed before constructing multivariable models to assess the joint associations. In accordance to our study aims, 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 and infant CT provided additional prognostic information. Finally, we analysed the impact of late bacterial infection. Multivariable models were initially developed in a forwards stepwise fashion, starting with those univariably associated, but also verifying that there were not

others that became significant post-adjustment. A backwards stepwise regression analysis was used to confirm that the 'final' model had not omitted any important variables. Model estimates are presented with 95% confidence intervals to show the precision of estimation.

Regression analyses were limited to data from subjects with CF since potential clinical predictors such as disease severity and treatment were not applicable to healthy subjects. To assess whether the time of *Pseudomonas aeruginosa* (*Ps aer*) acquisition was associated with lung function decline by the preschool years, we compared those with very early isolation (defined arbitrarily as during the 1st 6 months) and those with isolation later in infancy (6-24 months) with those who had not had *Ps aer* by the time of preschool test or who had acquired it after 2 years. Analysis was performed in SPSS (version 23).

Power of study

The sample size was opportunistic, and initially based on a power calculation for analysis during infancy [2], 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

Children with CF were on average 4 months older than healthy controls at time of preschool testing (Table 1, main manuscript). This reflects both the need to defer testing in some children with CF due to respiratory exacerbations and the fact that priority was given to recruiting the oldest children with CF in the cohort as soon as funding was approved to ensure that they could be tested before exceeding the specified upper age limit of 6yrs.

96 children with CF and 62 controls were originally recruited during infancy. Longitudinal lung function data were available at preschool age for 67 children with CF who had test results on at least one previous occasion; 64, 63 and 54 of whom had 3 month, 1 year and 2 year infant tests respectively. 41 healthy controls were tested at preschool age; 35 of whom had results at 3 months and 1 year, and 24 at 2 years of age (Figure 1, main manuscript). The proportion of missing LCI and FEV_t data according to test occasion is shown in table E1.

Table E1: Proportion of missing LCI and FEV_t data according to test occasions in CF and Controls

	CF (67 subjects)				Controls (41 subjects)			
Test occasion	**3m**	**1y**	**2y**	**PS**	**3m**	**1y**	**2y**	**PS**
LCI	4/64	0/62	1/54	1/67	4/35	5/35	0/24	2/41
FEV_t	2/64	3/62	6/54	5/67	1/35	2/35	3/24	6/41

Legend: 3m = 3 months, 1y = 1 year, 2y = 2 year, PS = preschool test occasions

Feasibility of study protocol

108 subjects (67 children with CF and 41 healthy controls) attended for lung function measurements at preschool age. Feasibility was calculated for children attempting each lung function test; two children with CF declined to attempt any tests. Of the 108 children attempting lung function measurements, 72 (67%) obtained a valid result for both lung function tests on their first visit; 42 (63%) children with CF and 30 (73%) controls.

Of the 40 children who either declined testing or whose lung function tests were of poor quality on their first visit, 25 children (22 with CF and 3 controls) were able to attend a second test occasion. 21 children (84%), 18 with CF and three controls, then completed the full protocol at second visit. Two children, both with CF, attended a third test occasion and passed both lung function tests on their third visit. The mean age (range) at first test occasion was 4.40 (3.11-6.01) years, at second test 4.90 (3.73-6.05) years and at third test 5.72 (5.49-5.95) years. The median interval (range) between first and second test occasion was 0.8 (0.19-1.68) years and 0.63 (0.27-0.99) between second and

third. If children attended for more than one visit, results for summary data were chosen from the most complete test occasion. Feasibility for each test was similar in children with CF and controls. Success rates were highest for MBW and increased with age between three to six years for both tests (Table E2 and E3).

Table E2: Feasibility for each lung function test and the full study protocol for all children and by diagnosis on first test occasion at preschool testing

	MBW	Spirometry
All children (n=108)	94 (87%)	80 (74%)
CF (n=67)	58 (87%)	48 (72%)
HC (n=41)	36 (88%)	32 (78%)

Legend: Data presented as number of subjects (%).

Table E3: Feasibility of each lung function test and the full study protocol by age at first test (all children)

Age	MBW	Spirometry
3.0-3.9y (n=33)	27 (82%)	19 (58%)
4.0-4.9y (n=55)	47 (85%)	41 (75%)
5.0-6y (n=20)	20 (100%)	20 (100%)

Legend: Data presented as number of subjects, n (%). Abbreviations: y=years

Characteristics of CF subjects

Details of background characteristics, treatment and microbiological status are given in Table 2 of the main manuscript. All but three children with CF were diagnosed before 10 weeks of age. Those with a later diagnosis had equivocal screening results and all were pancreatic sufficient. 7 children (10%) presented with meconium ileus. Children with a later diagnosis or meconium ileus presentation were not outliers in any growth or lung function measurement, and inclusion or exclusion did not change the study results, so they remained in the analysis. 41 children (61%) were homozygous for the DF508 mutation, 20 heterozygote (30%) and 6 (9%) had 2 other mutations. The majority of children (87%) were pancreatic insufficient. In the CF group, five children were of Asian ethnicity, one black African and three mixed ethnic group, and were matched for ethnicity in the control group.

52% of children remained on prophylactic antibiotics at preschool testing, 48% had received mucolytic therapy and only 22% were receiving long term nebulised antibiotic therapy. The total number of intravenous antibiotic courses from birth varied widely (0-9) but most children (78%) had not received a course in the year preceding preschool testing. Five subjects received regular (long term) azithromycin before preschool test. Their preschool lung function was not significantly different to those who did not have long term azithromycin.

By the time of the preschool test most children (73%) had isolated *Ps aer*, and over half *Staphylococcus aureus* (60%) or *Haemophilus influenzae* (57%), with median age at first growth being early in the second year of life. A quarter (24%) had a positive *Stenotrophomonas maltophilia* isolate by test date, with median time for first isolate in the 3rd year, but the age at first isolate varied widely for all organisms.

Table E4 presents a comparison of background characteristics of those children with CF who participated in this preschool follow up and those who did not. There were no significant differences in any parameters.

Table E4: Comparison of background demographics and lung function at ~3m in children with Cystic Fibrosis who were and were not followed up at preschool age

	Tested¥	Not tested#	Difference (95%CI)
Number of subjects	64	21	
Boys (%)	47%	52%	-6% (-28%; 18%)
White (%)	88%	81%	7% (-9%; 28%)
zBirthweight*	-0.36 (0.97)	-0.47 (0.81)	0.11 (-0.36; 0.58)
Genotype (%)			
-DF508: Homozygous	60%	56%	5% (-19%; 29%)
-DF508:Heterozygous	32%	33%	-2% (-27%; 19%)
-Other	8%	11%	-3% (-25%; 9%)
Meconium ileus (%)	11%	10%	1% (-19%; 14%)
Age at test, weeks	12.1 (4.2)	11.5 (1.5)	0.57 (-0.65; 1.80)
zWeight*	-0.78 (1.03)	-0.83 (1.30)	0.05 (-0.50; 0.60)
zHeight*	-0.11 (0.98)	-0.09 (1.19)	-0.02 (-0.53; 0.50)
zBMI*	-1.01 (0.97)	-1.27 (1.40)	0.26 (-0.41; 0.94)
LCI	7.54 (0.76)a	7.89 (0.73)b	-0.34 (-0.73; 0.04)
zLCI	0.40 (1.21)a	0.95 (1.05)b	-0.54 (-1.15; 0.06)
$zFEV_{0.5}$	-0.87 (0.99)c	-0.53 (0.98)d	-0.34 (-0.87; 0.18)
zFVC	-0.91 (1.26)c	-1.06 (1.09)d	0.15 (-0.50; 0.81)

Legend: *Data presented as mean (SD) unless otherwise indicated. *according to British 1990 reference (Cole Stat Med 1998). N measurements as follows: a=60; b=20; c=62; d=18. ¥ Lung function assessments were unsuccessful at ~3 months in 3 of the 67 children who were tested at preschool age. #Data from infants with co-morbidities were excluded (n=5, see Figure 1 for details). Abbreviations: z=z-score; FVC: Forced vital capacity.*

Table E5: Association between preschool lung function and factors during early life in children with CF: univariable regression analysis

	Preschool LCI z-scores Coefficient [mean (95%CI)]	Preschool $FEV_{0.75}$ z-scores Coefficient [mean (95%CI)]
Growth factors		
zBirthweight	-0.06 (-0.53; 0.42)	-0.09 (-0.39; 0.21)
zWeight at 3m	0.03 (-0.43; 0.50)	-0.01 (-0.28; 0.27)
zWeight at 1y	-0.17 (-0.72; 0.38)	0.12 (-0.21; 0.45)
zWeight at 2y	-0.15 (-0.72; 0.41)	-0.01 (-0.37; 0.34)
zHeight at 3m	0.20 (-0.29; 0.68)	-0.25 (-0.52; 0.03)
zHeight at 1y	0.17 (-0.31; 0.65)	-0.21 (-0.50; 0.07)
zHeight at 2y	0.23 (-0.29; 0.76)	-0.10 (-0.45; 0.26)
zBMI at 3m	-0.12 (-0.61; 0.38)	0.19 (-0.09; 0.48)
zBMI at 1y	-0.51 (-1.1; 0.10)	0.49 (0.14; 0.84)**
zBMI at 2y	-0.48 (-1.05; 0.09)	0.07 (-0.29; 0.43)
Difference in zBMI from birth to 3m	-0.36 (-0.95; 0.24)	0.03 (-0.31; 0.36)
Difference in zBMI from 3m to 1y	-0.36 (-0.95; 0.24)	0.03 (-0.31; 0.36)
Difference in zBMI from 3m to 2y	-0.32 (-0.81; 0.17)	-0.06 (-0.35; 0.23)
Microbiology factors		
Ps aer acquisition "ever" during 1st 2y of life	0.65 (-0.26; 1.55)	0.13 (-0.44; 0.70)
Ps aer acquisition < 6m of life	1.69 (0.43; 2.95)**	0.08 (-0.73; 0.88)
Ps aer acquisition 6 – 24m of life	-0.20 (-1.13; 0.73)	0.15 (-0.43; 0.72)
SA acquisition "ever" in 1st 2y of life	-0.13 (-1.08; 0.83)	-0.21 (-0.81; 0.38)
SA acquisition < 6m of life	-0.12 (-1.18; 0.95)	-0.08 (-0.76; 0.60)
SA acquisition 6 – 24m of life	-0.07 (-1.55; 1.41)	-0.34 (-1.23; 0.55)
HI acquisition "ever" in 1st 2y of life	0.43 (-0.49; 1.35)	-0.18 (-0.76; 0.39)
HI acquisition < 6m of life	0.53 (-0.69; 1.75)	0.20 (-0.65; 1.04)
HI acquisition 6 – 24m of life	0.17 (-0.88; 1.21)	-0.32 (-0.94; 0.30)
SM acquisition "ever" in 1st 2y of life	0.62 (-0.85; 2.10)	-0.89 (-1.76; -0.03)*
SM acquisition < 6m of life	None had acquisition <6m	None had acquisition <6m
SM acquisition 6 – 24m of life (n=7)	0.62 (-0.85; 2.10)	-0.89 (-1.76; -0.03)*
Lung function		
zLCI at 3m	0.15 (-0.30; 0.60)	0.08 (-0.17; 0.34)
zLCI at 1y	0.32 (-0.03; 0.67)	-0.22 (-0.43; -0.02) *
zLCI at 2y	0.89 (0.36; 1.43)**	-0.33 (-0.70; 0.05)
$zFEV_{0.5}$ at 3m	-0.30 (-0.78; 0.17)	0.41 (0.13; 0.69) **
$zFEV_{0.5}$ at 1y	-0.11 (-0.62; 0.39)	0.24 (-0.06; 0.55)
$zFEV_{0.5}$ at 2y	-0.04 (-0.70; 0.63)	-0.00 (-0.44; 0.43)
zFVC at 3m	-0.30 (-0.58; -0.01)*	0.24 (0.02; 0.46) *
zFVC at 1y	-0.29 (-0.63; 0.06)	0.32 (0.06; 0.57) *
zFVC at 2y	-0.07 (-0.54; 0.40)	0.22 (-0.15; 0.60)
$zFEV_{0.5}$/FVC at 3m	-0.07 (-0.37; 0.22)	0.08 (-0.14; 0.31)
$zFEV_{0.5}$/FVC at 1y	0.18 (-0.15; 0.51)	-0.20 (-0.47; 0.07)
$zFEV_{0.5}$/FVC at 2y	0.06 (-0.36; 0.49)	-0.21 (-0.59; 0.17)

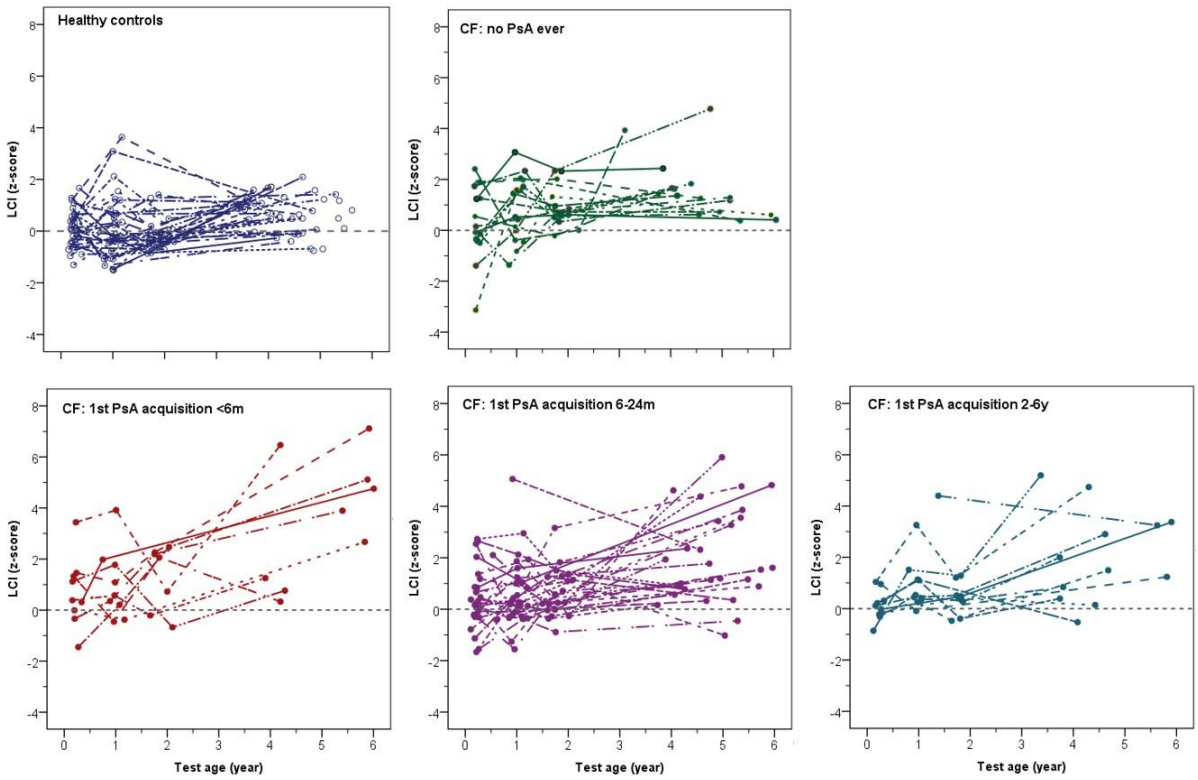
CT / BAL at 1y		
CT air trapping subscores	0.73 (0.40; 1.06)***	-0.13 (-0.37; 0.10)
CT bronchial wall dilatation subscores	0.78 (-0.16; 1.72)	-0.17 (-0.75; 0.41)
Total CT scores	0.34 (0.19; 0.48)***	-0.08 (-0.18; 0.02)
Detectable free NE (BAL)	0.46 (-1.11; 2.04)	0.09 (-0.83; 1.00)
IL8 (BAL)	0.00 (0.00; 0.001)	0.00 (0.00; 0.00)

Legend: *z: z-scores; m: month; y: year; BMI: Body Mass Index; Ps aer: Pseudomonas aeruginosa; SA: Staphylococcal aureus; HI: Haemophilus influenza; SM: Stenotrophomonas Maltophilia; FEV_t: Forced expired volume in t seconds; FVC: Forced vital capacity; NE: Neutrophil elastase; IL8: Interleukin-8; * p<0.05; ** p<0.01; *** p<0.0001*

Time of first *Pseudomonas aeruginosa* acquisition

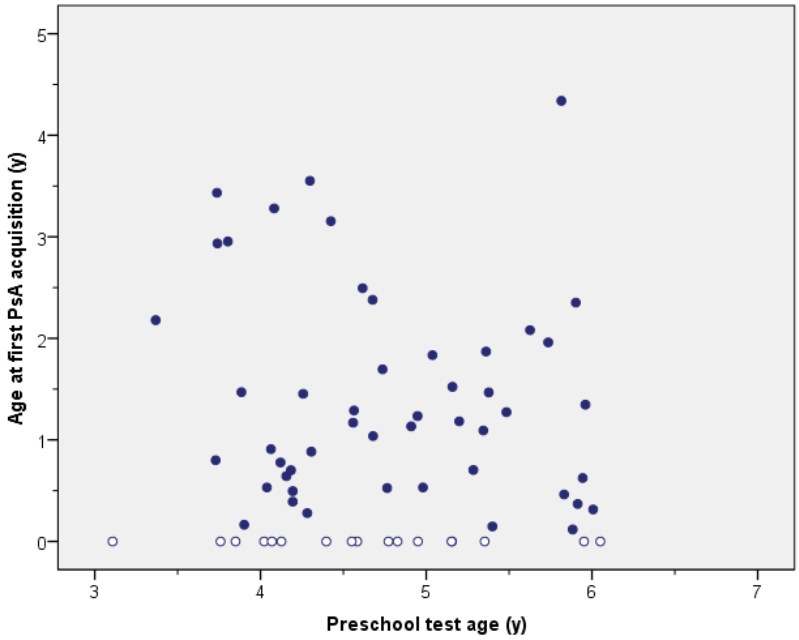
The regression analysis in the main manuscript showed that isolation of *Ps aer* before six months of age was a significant predictor of preschool LCI. Figure E1 demonstrates the pattern identified by the models: namely that early acquisition of *Ps aer* leads to rising LCI in children with CF. Figure E2 shows age of first *Ps aer* acquisition in relation to age at preschool test.

Figure E1: LCI z-scores plotted against test age according to health and time of 1st *Ps aer* acquisition



Legend: *data presented were based on individual subject data at each test and lines denote data from individual subjects. Total n for each CF subgroup are as follows: no Ps aer=17; Ps aer acquisition <6m = 9; Ps aer acquisition 6-24m = 28; Ps aer acquisition 2-6y = 12. Abbreviations: PsA = Pseudomonas aeruginosa; m=month; y=year.*

Figure E2: Age of 1st *Ps aer* acquisition in relation to age at preschool test



Legend: *Filled circles denote data from children with CF who had acquired Ps aer while the open circles denote children with CF who had not 'ever' acquired Ps aer since birth. PsA=Pseudomonas aeruginosa*

There were no differences in the 9 subjects with *Ps aer* in the first six months of life from the rest of the cohort in variables of known clinical significance, such as age of diagnosis, genotype, mode of presentation (1 meconium ileus, the rest NBS), other organisms isolated or treatment (all were on flucloxacillin prophylaxis as was the rest of the cohort, none were on azithromycin, only 1 received a mucolytic). Of these nine subjects, seven had successful eradication of *Ps aer* and two had persistent positive cultures (failed eradication). Preschool LCI was not significantly higher in these two subjects than the others with *Ps aer* in the first six months.

Investigation of raised LCI in control population

LCI was higher than expected in our control population, with mean (SD) LCI z-score of 0.67 (0.72). It is possible that this was a chance finding related to the relatively small sample size [16], but we also investigated potential methodological errors. Data collection and test procedures were the same for this study as in previous preschool studies reported from our centre [15] and laboratory protocols have not changed over time. We identified three potential sources of error, these being:

1. Inter-observer repeatability
2. MBW analysis software changes
3. Relocation of the laboratory

To investigate differences between observers, MBW runs were reanalysed by two different investigators (JD and ER) and the results compared. The MBW runs were analysed in the same software version, with the same settings. Table E6 shows the results of this analysis. Data were available in 36 subjects, initially in 25 controls and then with 11 CF subjects added to include results with higher LCI results (as this reflects a longer MBW trace with more variability). There was no significant difference in LCI analysis between observers. There were no changes in signal linearity, alignment or apparatus dead space after relocation.

Table E6: Inter-observer differences in MBW analysis

	Observer 1	**Observer 2**	**Difference 1-2 (SD of difference)**
Mean LCI (SD)	7.47 (1.15)	7.45 (1.17)	0.03 (0.13)

Legend: *Data between observers was compared with the Wilcoxon paired samples test. Abbreviations: LCI=lung clearance index, SD=standard deviation. There was no significant difference between observers.*

The software used to analyse MBW measurements had undergone minor adjustments to improve the quality and accuracy of analysis between the infant and preschool studies. It is possible that such adjustments could affect computed values of LCI. To investigate any differences, ten MBW datasets collected at preschool were re-analysed in both software versions - software *a)*, current at time of infant MBW analysis, and software *b*), current at time of preschool data analysis - as shown in table E5. There was a non-significant trend to lower LCI values from software version *b.* The difference was however in the opposite direction to that noted in the control population, where preschool control subjects - whose MBW data were analysed in software version *b* - as a group had *higher* LCI results than infant controls.

Table E7: MBW software comparison in both cohorts

	Version *a*	**Version *b***	**Difference a-b (SD of difference)**
LCI (SD)	7.94 (1.47)	7.85 (1.27)	0.09 (0.20)

Legend: *Results for analysis in each software version were compared with the Wilcoxon paired samples test. Abbreviations: LCI=lung clearance index, SD=standard deviation. There was no significant difference between software versions.*

Investigation of raised LCI in control population: Discussion

Despite investigation (which included re-analysis, agreement between observers, and test set-up), the only identified factor potentially associated with this change was relocation of the lung function laboratory, including the mass

spectrometer, within the hospital prior to the preschool visits. We observed a similar magnitude of increase in LCI z-score in contemporaneous healthy controls included in the adolescent follow up for our first LCFC cohort, and in healthy infants who were tested around the same time (unpublished data). Fortunately, inclusion of the contemporaneous healthy control group on all test occasions, allowed unbiased interpretation of result.

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