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ORIGINAL ARTICLE

Evolution of lung function during the first year of life in newborn screened cystic fibrosis infants

The Thanh-Diem Nguyen,^{1,2} Lena P Thia,¹ Ah-Fong Hoo,^{1,3} Andrew Bush,⁴ Paul Aurora,^{1,3} Angie Wade,⁵ Jane Chudleigh,^{1,3} Sooky Lum,¹ Janet Stocks,¹ on behalf of the London Cystic Fibrosis Collaboration (LCFC)

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¹Portex Unit: Respiratory Physiology and Medicine, UCL Institute of Child Health, London, UK

²Department of Respiratory Medicine, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Quebec, Canada

³Respiratory Unit, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

⁴Department of Paediatric Respiratory Medicine, Imperial College & Royal Brompton & Harefield Hospital NHS Foundation Trust, London, UK

⁵Centre for Paediatric Epidemiology and Biostatistics, UCL Institute of Child Health, MRC Centre for Epidemiology of Child Health, London, UK

Correspondence to

Dr The Thanh Diem Nguyen, Portex Respiratory Unit, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK; t.nguyen@ucl.ac.uk

TT-DN and LPT contributed equally.

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ABSTRACT

Rationale Newborn screening (NBS) for cystic fibrosis (CF) allows early intervention. Design of randomised controlled trials (RCT) is currently impeded by uncertainty regarding evolution of lung function, an important trial end point in such infants.

Objective To assess changes in pulmonary function during the first year of life in CF NBS infants.

Methods Observational longitudinal study. CF NBS infants and healthy controls were recruited between 2009 and 2011. Lung Clearance Index (LCI), plethysmographic lung volume (plethysmographic functional residual capacity (FRC_{pleth})) and forced expired volume (FEV_{0.5}) were measured at 3 months and 1 year of age.

Main results Paired measurements were obtained from 72 CF infants and 44 controls. At 3 months, CF infants had significantly worse lung function for all tests. FEV_{0.5} improved significantly (0.59 (95% CI 0.18 to 0.99) z-scores; p<0.01) in CF infants between 3 months and 1 year, and by 1 year, FEV_{0.5} was only 0.52 (0.89 to 0.15) z-scores less than in controls. LCI and FRC_{pleth} remained stable throughout the first year of life, being on average 0.8 z-scores higher in infants with CF.

Pulmonary function at 1 year was predicted by that at 3 months. Among the 45 CF infants with entirely normal LCI and FEV_{0.5} at 3 months, 80% remained so at 1 year, while 74% of those with early abnormalities remained abnormal at 1 year.

Conclusions This is the first study reporting improvements in FEV_{0.5} over time in stable NBS CF infants treated with standard therapy. Milder changes in lung function occurred by 1 year than previously reported. Lung function at 3 months predicts a high-risk group, who should be considered for intensification of treatment and enrolment into RCTs.

INTRODUCTION

The major cause of morbidity and mortality in cystic fibrosis (CF) is pulmonary disease. Until recently, treatment has been targeted at the downstream consequences of *CFTR* dysfunction, such as bronchial infection, inflammation and mucus retention. A recent paradigm shift has, however, led to development of genotype class-specific therapies, such as PTC₁₂₄ to over-ride premature stop codons^{1 2} and VX-770 for the class 3 mutation G551D.^{3 4} It seems likely that these novel therapies will be most effective in early stage disease, before irreversible airway damage has developed. It is therefore essential to

Key messages

What is the key question?

- Newborn screened cystic fibrosis (CF) infants have abnormal lung function by 3 months of age; how does this change during the first year of life?

What is the bottom line?

- Lung function remained stable or improved in newborn screened CF infants during the first year of life; deficits at 1 year were considerably smaller than previously documented in either screened or clinically diagnosed infants.

Why read on?

- This study, the largest of its kind and the only one with contemporaneous healthy controls, describes early lung development in newborn screened infants with CF; these data will inform the design of interventional trials in these children.

understand the evolution of lung function in newborn screened (NBS) CF infants given standard treatment, in order to determine optimal trial end-points and adequately power intervention studies.

CF infants diagnosed clinically have airflow obstruction at diagnosis, even in the absence of respiratory symptoms, signs or history of infection,⁵ with no improvement in pulmonary function over the ensuing years despite specialist treatment.^{6–8} CF NBS has been introduced in the hope that earlier diagnosis will lead to improved outcomes. Previous longitudinal studies investigating lung function in NBS CF infants reported progressive decline in the early years, despite specialist treatment.^{9 10}

Following recent universal introduction of screening throughout the UK, we recruited a cohort of NBS infants with CF and healthy controls between 2009 and 2011. Disappointingly, only 56% of those with CF had normal pulmonary function tests (PFT) when assessed at 3 months.¹¹ The current manuscript describes follow-up PFTs at 1 year for this cohort. Our primary hypothesis was that lung function would deteriorate further between 3 months and 1 year of age. We also aimed to investigate the determinants of lung function at 1 year, and to

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collect data to assess feasibility of recruiting NBS CF infants to invasive studies¹² and inform future power calculations.

METHODS

NBS CF infants born between January 2009 and July 2011 who were referred to the six specialist CF centres in the London CF Collaboration (LCFC) were eligible for recruitment.¹¹ Healthy controls were recruited contemporaneously from Homerton University Hospital, East London. Infants were ineligible if born <36 weeks gestation or had coexisting congenital abnormalities (see online supplementary data). The study was approved by the North Thames Multi-Centre Research Ethics Committee (#09/HO71/314). Informed written parental consent was obtained.

Participating centres prospectively completed Case Record Forms (CRF) at diagnosis and each subsequent clinic visit (see online supplementary data). CF infants were started on multivitamins and vitamin E, pancreatic enzyme replacement therapy where appropriate and, in accord with UK CF Trust guidelines, prophylactic flucloxacillin, according to a standardised treatment protocol (see online supplementary data).

Infant PFTs

All infants were tested at Great Ormond Street Hospital/UCL Institute of Child Health at around 3 months and 1 year post-natal age. Infants were free of respiratory illness for at least 3 weeks before PFTs. Infants were weighed and examined prior to administering chloral hydrate orally or rectally (60–100 mg/kg). Weight and crown-heel length were expressed as z-scores to adjust for age and sex.¹³ Heart rate and SpO₂ were monitored continuously throughout testing. Infant urine or maternal saliva samples were collected for cotinine assay to validate maternal report of smoking. PFTs were undertaken according to international guidelines.^{14–15} Lung Clearance Index (LCI), a measure of ventilation inhomogeneity was measured by multiple breath washout (MBW), using mass spectrometry and customised software.¹⁶ Plethysmographic Functional Residual Capacity (FRC_{pleth}) and forced expired volumes (FEV_{0.5}) and flows (FEF₇₅) from an inflation pressure of 30 cm H₂O using the raised volume technique were measured using the Jaeger BabyBody device (CareFusion, San Diego, USA; V4.65).¹⁵ PFT results were electronically exported to a research database (Re-Base software, Re-Base, UK), which contained all relevant demographic and clinical details. PFT results were expressed as z-scores to adjust for body size, sex and age, using reference equations derived from healthy infants studied with identical equipment and protocols.^{11–17–19} Abnormal PFTs were defined as results outside the 95% limits of normal: that is, >1.96 z-scores (>97.5th centile) for LCI and FRC_{pleth} or <−1.96 z-scores (<2.5th centile) for FEV_{0.5}. Results were reported to the physicians responsible for the clinical care of each child, and subsequently discussed with parents.

Statistical analysis

Data were inspected for distribution and calculation of descriptive statistics (PASW Statistics V18, Chicago, Illinois, USA). Significance was taken as $p < 0.05$. Lung function results at 3 months, at 1 year and changes between 3 months and 1 year were compared between groups using Student t-test. Multivariable linear regressions were used to investigate how lung function variables at 1 year, and change in lung function between 3 months and 1 year, varied according to potential determinants (background characteristics, clinical symptoms, antibiotic treatment and microbiological results, see online supplementary data for details). Model estimates and differences

between groups are presented with 95% CIs. Multiple imputations were used to impute values for any failed PFTs at 3 months (see online supplementary data). Taking into account three primary outcomes (LCI, FRC_{pleth} and FEV_{0.5}), a sample size of 72 infants with CF and 44 controls at 1 year (equivalent to 53/group if equal groups) allows detection of differences between groups equivalent to 0.66 z-scores at the 5% significance level with 84% power.^{20–22}

RESULTS

The screening, recruitment and follow-up of subjects are shown in figure 1. Paired measurements at 3 months and 1 year were obtained from 72 of 101 CF NBS infants, (90% of those tested at ~3 months of age). Inspection of CRFs and regular communication with consultants revealed excellent adherence to treatment protocols. Details of additional treatment are provided in the online supplementary data. Paired measurements were obtained from 44 contemporaneous controls (81% of those tested at 3 months). CF infants were born slightly earlier with lower birth weight than controls, but background characteristics were otherwise similar (table 1). There was no difference between groups regarding change in weight between birth and first PFTs at ~3 months (mean difference CF-controls: −0.14 (95% CI −0.56 to 0.29) weight z-scores).

For CF NBS infants, the median (IQR) age at diagnosis was 3.6 (3.0–4.4) weeks with 7 (10%) infants presenting with meconium ileus. Since inclusion of these infants did not affect the results (data not shown), they were included in the analysis. At 1 year PFTs, cough-swab cultures had been positive on at least one occasion for *Pseudomonas aeruginosa* in 25 (35%), and for other significant bacteria in 17 (24%).

Anthropometry and lung function results

Comparison between infants with CF and healthy controls

Success in obtaining technically satisfactory PFTs were similar between groups, but varied by age and outcome, being lowest for FRC_{pleth} at 3 months (76%) and highest for LCI ($\geq 93\%$ on both occasions; figure 1 and see online supplementary table E1). At 3 months, CF infants had significantly lower weight, height and body mass index (BMI); higher LCI and FRC_{pleth}; and lower FEV_{0.5}, forced vital capacity (FVC) and FEF₇₅ compared with controls (table 2). Significant increases in z-scores for somatic growth were observed in both groups between 3 and 12 months, but changes were significantly greater in CF infants, such that there were no between-group differences by 1 year. All PFT results remained stable in healthy infants during the first year of life, as did LCI, FRC_{pleth} and FVC in those with CF. However, FEV_{0.5} and FEF₇₅ z-scores improved between test occasions in CF infants (table 2, see online supplementary figure E1): differences in FEF₇₅ no longer being significant by 1 year when compared with controls. Although not one of the selected primary outcomes, there was a significant increase in gas trapping (as reflected by Δ FRC, ie, the within-subject difference in FRC assessed using plethysmography and MBW) in CF infants during the first year of life (table 2).

Determinants of lung function at 1 year

On linear univariable analysis, LCI, FRC_{pleth} and FEV_{0.5} z-scores at 1 year were significantly associated with CF status and 3 months PFT (see online supplementary table E3). Using multivariable linear regression, significant determinants of 1 year LCI z-score were: CF status (regression coefficient (95%

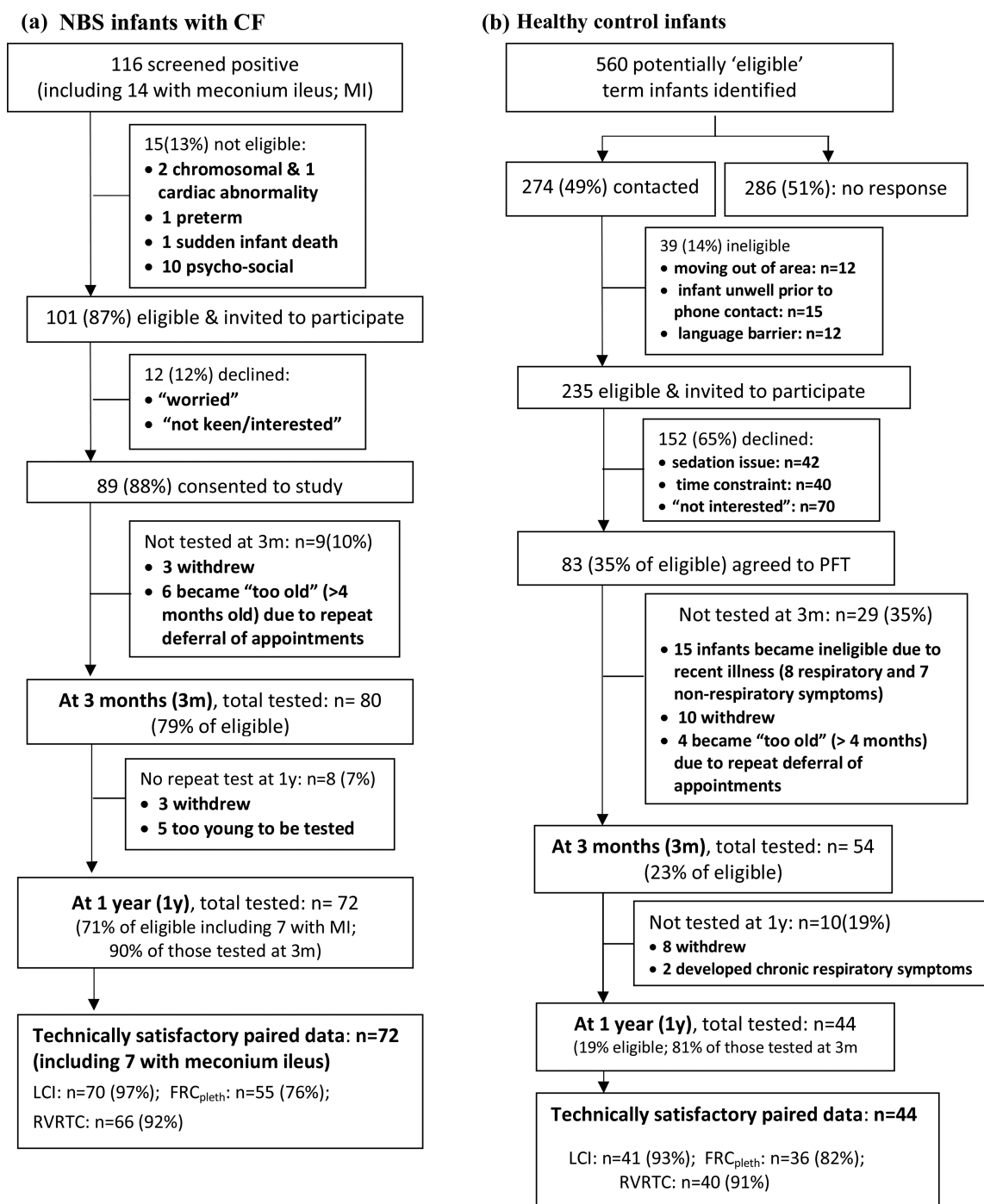


Figure 1 Success rates for recruitment and achievement of technically acceptable infant pulmonary function data. NBS, newborn screened; PFTs, pulmonary function tests; LCI, lung clearance index; FRC, functional residual capacity; MBW, multiple breath inert gas washout; pleth, plethysmographic technique; RVRTC, Raised Volume Rapid Thoraco-abdominal Compression, from which forced expired flows and volumes were derived. See online supplementary table E1 for details of PFT success on each test occasion.

CI) 0.48 (0.04 to 0.93)), 3 month LCI (0.24 (0.07 to 0.41) per unit z-score), history of clinician-diagnosed wheeze (0.59 (0.05 to 1.12)) and change in weight z-score between birth and first PFT (-0.18 (-0.35 to -0.01) per unit z-score). For 1 year FRC_{pleth} z-score, determinants were: 3 months FRC_{pleth} (0.43 (0.27 to 0.59) per unit z-score), history of P_sA infection (0.71 (0.24 to 1.17)) and change in weight z-score between 3 and 12 months (-0.20 (-0.41 to 0.0) per unit z-score change), whereas 1 year FEV_{0.5} z-score was only significantly associated with 3 months FEV_{0.5} on multivariable analysis (-0.18 (-0.35 to -0.01) per unit z-score).

Relationship between PFT results at 3 months and 1 year

At 3 months of age, LCI, FRC_{pleth} and FEV_{0.5} were abnormal in 17% (12/71), 16% (9/57) and 26% (18/68) of CF infants, respectively. By 1 year, the percentage with abnormal FEV_{0.5} had decreased to 9% (6/69) (mean difference (95% CI) -18% (-30% to -5%)) whereas those with abnormal LCI (18% (13/71)) and FRC_{pleth} (16% (11/70)) remained virtually unchanged. Significant correlations were found between PFTs at 3 months and 1 year (figure 2 and see online supplementary table E2). Of the 52 infants in whom all three PFTs were technically successful on both occasions, abnormalities were observed in 33% (17/

Table 1 Characteristics of CF and healthy controls infants with paired lung function at 3 months and 1 year

	CF (n=72)	Controls (n=44)	Δ (95% CI) CF– controls
Male, n (%)	34 (47)	21 (48)	–1% (–19 to 18)
Gestational age, weeks	39.1 (1.4)	40.3 (1.1)	–1.1 (–1.6 to –0.6)
Birth weight, z-score*	–0.64 (0.84)	0.12 (0.81)	–0.76 (–1.07 to –0.45)
Birth weight below 10th percentile*, n (%)	13 (18)	2 (5)	14% (1 to 24)
White mother, n (%)	61 (85)	38 (86)	–2% (–14 to 13)
Maternal smoking during pregnancy, n (%)	8 (11)	3 (7)	4% (–8 to 15)
Current maternal smoking†, n (%)	9 (13)	5 (11)	1% (–13 to 13)
Maternal asthma, n (%)	14 (19)	8 (18)	1% (–14 to 15)
Cystic fibrosis infants only			
Age at diagnosis, postnatal age (weeks)	3.9 (1.7)		
CFTR genotype (classes I–III)‡	59 (82%)		
Presented with meconium ileus	7 (10%)		
Pancreatic sufficient	5 (7%)		
Respiratory symptoms ever prior to 1 year PFTs			
Wheeze, physician diagnosed	24 (33%)		
Crackles, physician diagnosed	6 (8%)		
Cough within 3 weeks of 1-year PFT	15 (21%)		
Bacterial growth on cough swab, ever§ prior to 1 year PFTs			
<i>Pseudomonas aeruginosa</i> , PsA¶	25 (35%)		
Other significant bacterial growth**	17 (24%)		
No growth††	30 (42%)		
Additional treatment‡‡ prior to 1 year PFTs			
rhDNase	6 (8%)		
Intravenous antibiotics, number of courses	0 (0; 3)§§		
GERD treatment	38 (53%)		

Data shown as mean (SD) for continuous and n (%) for categorical variables unless otherwise stated.

*Calculated according to Cole *et al.*¹³

†Objectively validated by the analysis of cotinine levels.²³

‡10% were classes IV–V and 8% unknown (or not classified).

§Swabs collected routinely in clinic at least every 2 months, prior to PFT and also when symptomatic.

¶Definition of colonisation according to Lee *et al.*²⁴ Only 1 infant had chronic PsA.

**Significant bacterial infection with no previous PsA ever included 12 (17%) with methicillin-sensitive *Staphylococcus Aureus*, 14 (19%) with *Haemophilus Influenzae*, 3 (4%) with *Stenotrophomonas maltophilia*, 2 (3%) with *Achromobacter xylosoxidans*, 3 (4%) with methicillin-resistant *Staphylococcus Aureus* and 2 (3%) with *Aspergillus fumigatus*.

††Included those with no growth, upper respiratory tract flora or isolated *E. Coli* only.

‡‡In addition to the prophylactic flucloxacillin prescribed for all CF NBS infants from diagnosis.

§§Median (range).

Δ, mean difference between groups; CF, cystic fibrosis; GERD, Gastro-oesophageal reflux disease, n, number; NBS, newborn screened; PFT, pulmonary function test.

52) at first test, 10 (59%) of whom remained abnormal at follow-up. Of the 35 (67%) with entirely normal results at 3 months 25 (71%) remained so at 1-year.

Identification of a high risk group of NBS CF infants

Based on results from LCI and FEV_{0.5} which were the most feasible outcomes at 3 months (see online supplementary table E1), we attempted to delineate a subgroup of infants who would be at high risk of having abnormal lung function at 1 year, and who thus might be suitable candidates for an intervention study. Among the 64 CF infants in whom acceptable LCI and FEV_{0.5} results were obtained on *both* occasions, abnormalities (elevated LCI or diminished FEV_{0.5}) were identified in 19 (30%) at ~3 months, of whom 14 (74%) remained abnormal at 1 year. Among CF infants with entirely normal PFTs at 3 months from these two tests (n=45), 36 (80%) remained so at 1 year (see online supplementary data for details). There were no significant differences at 1 year in FEV_{0.5} (–0.36 (–0.9 to 0.17) z-scores) or LCI (0.46 (–0.13 to 1.05) z-scores) between CF infants with normal 3 months PFTs and healthy controls. By contrast, when compared with controls at 1 year, LCI was 1.33 (0.6 to 2.1) z-scores higher and FEV_{0.5} –0.8 (–1.5 to –0.1) z-scores lower in those with abnormal PFTs by 3 months (see online supplementary table E3).

DISCUSSION

Contrary to our hypotheses, forced expired flows and volumes improved by 1 year of age, with stability of other PFTs in NBS CF infants. This is the first time such improvement has been reported in an observational longitudinal study of NBS CF infants. The number of CF infants with abnormal LCI and FRC_{pleth} at 1 year was similar to that at 3 months, while there was a significant reduction in those with abnormal FEV_{0.5} during this period. Impaired lung function at 1 year was predicted by lung function at 3 months and associated with clinician-diagnosed wheeze (LCI), poor weight gain (LCI and FRC_{pleth}) and prior *P. aeruginosa* (FRC_{pleth}).

Strengths and limitations

The major strengths of this study are that longitudinal assessments of lung function were undertaken in a large cohort of NBS CF infants within a single location, results being directly compared with healthy controls. Attrition was minimal,¹² with no bias between those who did and did not complete the study. Selection of various PFTs enabled different aspects of pathophysiology to be assessed.¹¹ Appropriate reference equations for infant PFTs,^{18–19} including LCI, which has now been shown to be dependent on body size during early life,¹⁷ facilitated accurate interpretation of results. Limitations are that, in an

Table 2 Comparison of anthropometry and pulmonary function at ~3 months and 1 year in CF NBS infants and healthy controls (HC)

	3 months			1 year			Change over time (1 year–3 months)		
	CF (n=72)	HC (n=44)	CF–HC*	CF (n=72)	HC (n=44)	CF–HC*	CF: Change 1 year–3 months†	HC: Change 1 year–3 months†	Difference in change‡ CF–HC
Age at test, weeks§	11.2 (2.3)	12.1 (2.1)	–1.0 (–1.8 to –0.1)	52.4 (5.3)	53.7 (4.4)	–1.3 (–3.1 to 0.5)	41.2 (37.3 to 43.1)	41.6 (38.6 to 43.6)	–0.36 (–2.30 to 1.58)
Weight z-score¶	–0.89 (1.03)	0.01 (0.97)	–0.90 (–1.27 to –0.52)	0.32 (0.90)	0.55 (1.21)	–0.23 (–0.64 to 0.19)	1.21 (1.02 to 1.40)	0.54 (0.28 to 0.80)	0.67 (0.35 to 0.99)
Length z-score¶	–0.21 (1.01)	0.73 (0.92)	–0.94 (–1.30 to –0.58)	0.47 (1.01)	0.76 (1.20)	–0.28 (–0.71 to 0.15)	0.68 (0.52 to 0.85)	0.03 (–0.19 to 0.25)	0.66 (0.38 to 0.93)
BMI z-score¶	–1.08 (0.99)	–0.55 (0.96)	–0.53 (–0.90 to –0.16)	0.08 (0.83)	0.18 (1.12)	–0.10 (–0.49 to 0.29)	1.15 (0.95 to 1.37)	0.72 (0.43 to 1.01)	0.44 (0.07 to 0.80)
LCI z-score	0.83 (1.32)	0.36 (0.85)	0.47 (0.06 to 0.87)	1.05 (1.23)	0.25 (0.95)	0.80 (0.40 to 1.21)	0.24 (–0.12 to 0.59)	–0.09 (–0.46 to 0.28)	0.33 (–0.18 to 0.84)
FRC _{pleth} z-score	0.75 (1.07)	–0.01 (1.08)	0.77 (0.32 to 1.22)	0.75 (1.14)	–0.05 (0.96)	0.80 (0.40 to 1.20)	–0.04 (–0.32 to 0.23)	–0.04 (–0.45 to 0.36)	0.00 (–0.48 to 0.49)
ΔFRC z-scores (pleth – MBW)	0.59 (0.96)	0.22 (0.94)	0.37 (–0.32 to 0.77)	1.21 (0.86)	0.46 (0.69)	0.75 (0.44 to 1.06)	0.58 (0.26 to 0.89)	0.30 (–0.09 to 0.68)	0.28 (–0.21 to 0.77)
FVC z-score	–0.50 (1.03)	0.23 (0.67)	–0.74 (–1.06 to –0.41)	–0.43 (1.16)	0.23 (0.94)	–0.66 (–1.05 to –0.26)	0.06 (–0.18 to 0.29)	–0.02 (–0.31 to 0.28)	0.08 (–0.29 to 0.45)
FEV _{0.5} z-score	–1.23 (1.07)	–0.16 (0.76)	–1.07 (–1.42 to –0.73)	–0.41 (1.03)	0.12 (0.92)	–0.52 (–0.89 to –0.15)	0.83 (0.56 to 1.09)	0.24 (–0.07 to 0.56)	0.59 (0.18 to 0.99)
FEF ₇₅ z-score	–0.76 (1.25)	–0.07 (0.96)	–0.69 (–1.11 to –0.27)	–0.09 (0.93)	0.09 (0.91)	–0.18 (–0.54 to 0.18)	0.84 (0.48 to 1.19)	0.20 (–0.17 to 0.58)	0.63 (0.12 to 1.14)

Data shown as mean (SD) or mean difference (95% CI) between: *Groups, †Test occasions.

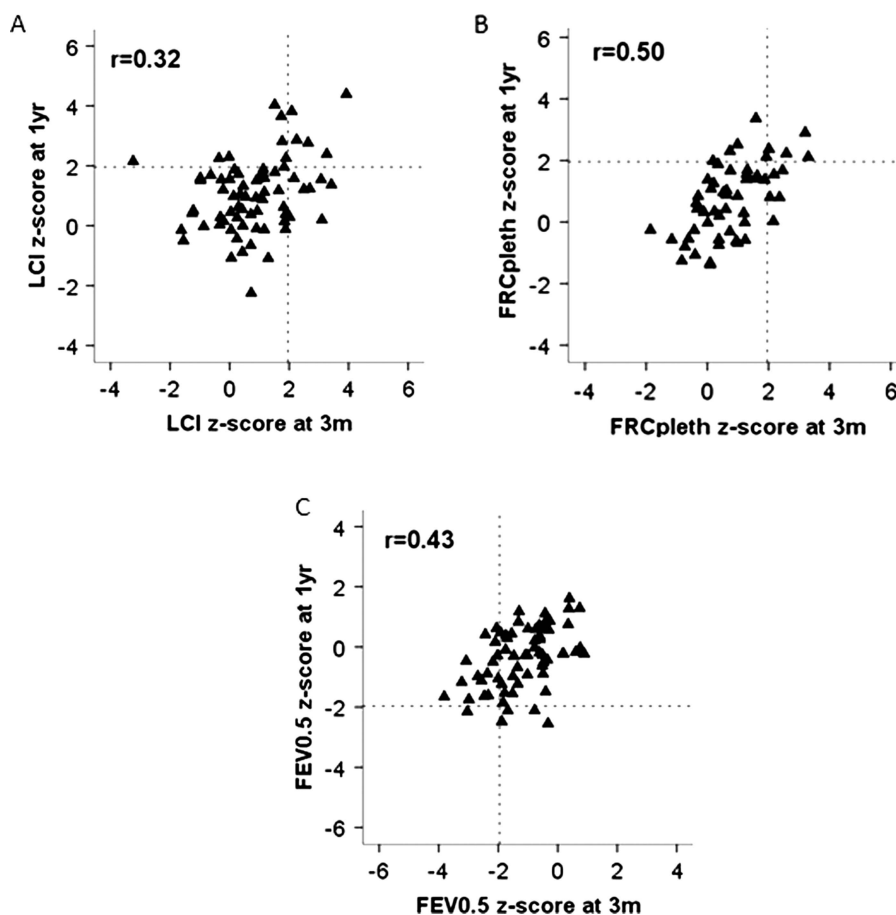
‡Change over time between groups (CF–HC); significant differences (p < at least 0.05) are shown in bold.

§Corrected for gestational age.

¶Calculated according to Cole *et al.*¹³

BMI, Body Mass Index; CF, cystic fibrosis; FRC_{pleth}, plethysmographic functional residual capacity; ΔFRC z-scores (pleth – MBW), difference between FRC_{pleth} and FRC_{MBW} z-scores as a measure of gas trapping; FVC, forced vital capacity; FEV_{0.5}, forced expired volume in 0.5 s; FEF₇₅, forced expired flow when 75% of FVC has been expired; LCI, Lung Clearance Index; MBW, multiple breath inert gas washout; NBS, newborn screened.

Figure 2 Relationship between pulmonary function at 3 months and 1 year in newborn screened CF infants. The 95% limits of 'normal range' (97.5th centile for Lung Clearance Index (LCI) and functional residual capacity (FRC) and 2.5th centile for FEV_{0.5}) are represented by vertical dashed lines at 3 months (3m) and horizontal lines at 1 year (1yr). Those with normal pulmonary function tests on both occasions fall within the lower left quadrant for LCI and FRC, and upper right quadrant for FEV_{0.5}. Infants with abnormal LCI at 3 months but normal LCI at a year, lie within the lower right quadrant (A), while those with abnormal FEV_{0.5} at 3 months which has normalised by 1 year are within the left upper quadrant of (C).



observational study such as this, we can only demonstrate association not causation of potential determinants of 1 year lung function. Computed tomography (CT) and broncho-alveolar lavage were performed at 1 year in CF infants, but not at 3 months, and are therefore not reported in this paper, which focusses on longitudinal changes. Furthermore, structural changes on CT at 1 year were very mild and poorly reproducible.²⁵

Interpretation of PFTs

As reported previously,¹⁶ since the infant PFTs were selected to reflect a wide range of lung pathology, the relatively poor correlations between the different primary outcomes on any one test occasion (see online supplementary table E2) was not surprising. While spirometry is known to be less sensitive than LCI for detection of mild lung disease in preschool children with CF,⁸ during infancy FEV_{0.5} has been shown to be a sensitive outcome in clinically diagnosed CF infants.¹⁶ While this was also observed in this study of NBS infants at 3 months of age,¹¹ by 1 year far fewer NBS infants were identified by the raised volume technique than either plethysmography or LCI. This may reflect the mild nature of lung disease at 1 year in our NBS cohort when compared with those diagnosed clinically and the decreasing sensitivity of forced expiratory manoeuvres to mild lung disease as airway and chest-wall compliance decrease with increasing maturity.²⁶ By contrast with the lack of correlation between FEV_{0.5} and other lung function outcomes on either test occasion, there were significant associations between LCI, FRC_{pleth} and Δ FRC, all of which are thought to be sensitive measures of peripheral airway disease throughout childhood (see online supplementary table E2). Whatever the interpretation of these changes, as discussed

below, they are in sharp contrast with those previously reported in CF infants. Consequently, when selecting outcome measures for intervention trials in NBS CF infants,²⁷ reliance should not be placed solely on the raised volume technique, since measures of LCI appear essential if mild abnormalities are to be detected. While hyperinflation and gas trapping also proved to be sensitive outcomes at 1 year, routine inclusion of these outcomes shortly after birth may be limited by equipment costs and increased failure rate of FRC_{pleth} in young infants. With the exception of a significantly lower FEV_{0.5} (mean (95% CI): -0.70 (-1.29 to -0.10) z-scores) in those who received additional antibiotics for symptoms or positive cough swab, there was no significant association between PFT outcomes and the infants' genotype, clinical status or any acute interventions prior to PFTs at 3 months.¹¹

Comparison with the literature

Results regarding evolution of early lung disease in those diagnosed by NBS have been conflicting (figure 3). The Australian Respiratory Early Surveillance team for CF (AREST-CF) have reported normal and reduced PFTs in such infants within the first 6 months of life,⁹ with further rapid deterioration over the first year of life (mean FEV_{0.5} being -2.4 z-scores by ~ 1 year of age).¹⁰

In the current study, lung function was abnormal by 3 months,¹¹ but stabilised or improved thereafter. As can be seen from figure 3, 1 year-lung function in the LCFC NBS cohort was significantly better than that in previous clinically diagnosed LCFC cohorts^{6 16} or in the AREST-CF NBS cohort at similar age.⁹ The reasons for the discrepancies between our results and those for AREST-CF are unclear. While the standardised protocol adhered to by the LCFC differs in some respects from that used by most centres in the USA, Australia and Europe (eg, use of

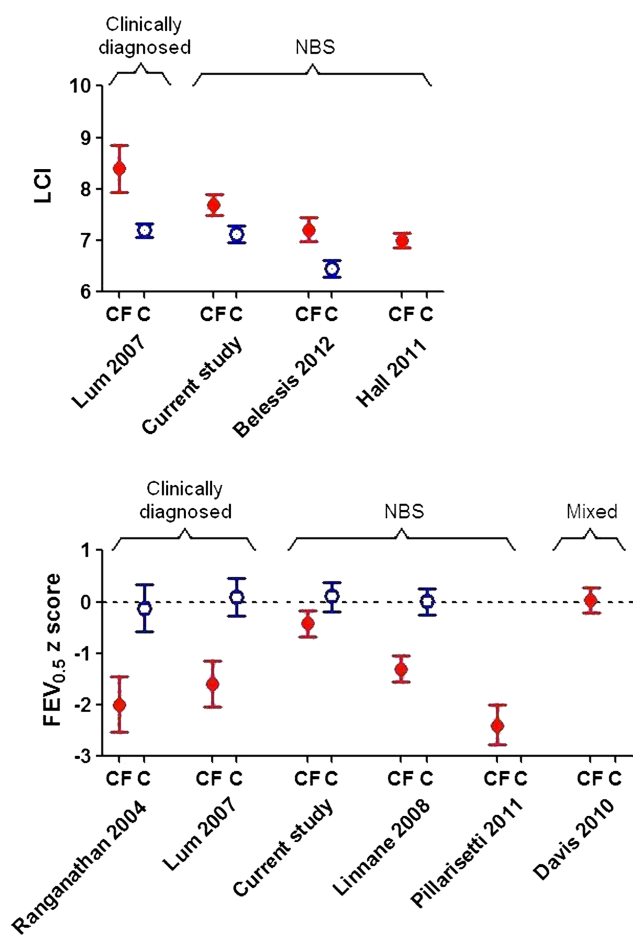


Figure 3 Comparison of current lung function results in infants with cystic fibrosis (CF) and healthy controls (C) at ~1 year of age, with previously published results. Data expressed as mean (95% CI). To allow direct comparison with previously published studies, Lung Clearance Index is presented in absolute units, whereas FEV_{0.5} is expressed as z-scores, based on different reference equations according to each author. The dashed horizontal line at 0 z-scores equates to 100% predicted based on a healthy population. Control data were not available in all studies. NBS, newborn screening.

flucloxacillin prophylaxis), the results should be a benchmark for other centres, and could serve as the basis for quality improvement.²⁸ Median age at first test in this study is younger than that in AREST-CF, which may reflect earlier diagnosis and implementation of treatment within the narrow geographical area of southeast England that we recruited from, thereby halting progression of any early lung disease. It is possible that infants recruited to AREST-CF were sicker, or deteriorated faster due to differences in modifier genes, environment or adherence to treatment, when compared with those in London. Most importantly, by contrast with the current study, AREST-CF data were not compared with contemporaneous controls, historical controls being used initially,⁹ with subsequent results (obtained using higher inflation pressures¹⁰) being interpreted using reference data based on different equipment, which can bias interpretation.^{19 29}

Improvements in lung function following treatment for acute exacerbations in infants with CF have been demonstrated,³⁰ but ours is the first study to document improvements in FEV_{0.5} in infants treated with standard therapy, studied during periods of clinical stability. A recent exploratory study reported greater increases in FEV_{0.5} over a 48-week period in 22 infants and young children treated with hypertonic saline compared with 23

randomised to isotonic saline (mean (95% CI) difference:38 (1 to 76) mL).³¹ However, from the data presented, it is impossible to ascertain whether this reflected stability, improvement or simply less deterioration over time with active treatment, once effects of lung and somatic growth had been accounted for.

Clinical implications

These results have implications for clinical practice and research. Although PFTs represent only one of the potential outcomes that can be used during early life,²⁷ with additional information gleaned from inflammatory markers and computerised tomography,³² they represent the mainstay of clinical management and a major outcome in randomised controlled trials (RCTs) in children and adults. Since lung function tracks from late infancy into later life, accurate identification of early abnormalities is imperative. Furthermore, given the increasing number of centres undertaking 'clinical' infant PFTs,³³ the current study may facilitate more meaningful interpretation of results by providing vital evidence regarding the natural changes that can occur over time in healthy infants and those with lung disease, in the absence of any specific interventions.

We have shown that lung function and somatic growth during the first year of life are significantly better in infants diagnosed by NBS in the UK than in their counterparts who were clinically diagnosed a decade earlier^{6 16} (figure 3). It is, however, of concern that despite early diagnosis and prompt treatment, LCI remains abnormal at 1 year (figure 3), albeit to a mild degree.¹⁶ Further follow-up is required to establish the extent to which these changes predict later outcome. Nevertheless, in this study, normal lung function was sustained in at least 50% NBS CF infants to 1 year of age. The significant improvement in FEV_{0.5} and stability of sensitive measures of distal airway function during early life when on 'standard therapy', and the relatively small deficits in lung function in CF NBS infants at 1 year also have important implications for design of future randomised intervention trials, which are essential to better define better standards of care in this age group. Despite considerable within-subject variability, the main predictor of lung function at 1 year was that at 3 months, allowing us to identify a 'high-risk' group who could potentially be targeted for future intervention trials.

Using data from this study, results from ~85 infants/arm would be required to detect relatively small differences in lung function (ie, equivalent to 0.5 z-scores) that might occur in response to an intervention if unselected NBS CF were recruited to such a trial. By contrast, were recruitment to such a RCT limited to a 'high-risk group' (ie, abnormal PFTs by 3 months, see online supplementary tables E3 and E4), a larger treatment effect would be expected, with only 22 infants/arm being required to detect a difference of 1 z-score (equivalent to ~9% for LCI), with 90% power. Such an approach could optimise recruitment since parents of infants with early PFT abnormalities would be more likely to consent, and also this approach would minimise exposure of children with potentially little to gain from therapy from unnecessary side effects.

In summary, we have shown that some measures of pulmonary function improve in the year following CF NBS diagnosis, and none deteriorate. Performing randomised intervention studies in an unselected cohort of infants using PFTs as an end point will, therefore, require large sample sizes due to the generally mild changes in lung function observed. Nonetheless, it is possible to identify CF infants with abnormal lung function by 3 months, who represent a high-risk group for persistent

abnormalities at 1 year, and who may benefit from additional treatment during the vital first few years of life.

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Contributors JS and AB were responsible for the conception and design of the study; JS is responsible for supervision of the study and together with JC, for research governance issues including ethics committee approval; A-FH provided technical training, supervision and audit of data collection and analyses; A-FH and JC set up the recruitment process. Infants with CF were recruited by the paediatric respiratory consultants participating in the LCFC, including AB and PA. TT-DN, LPT, A-FH, JC and SL recruited the healthy infants, undertook all lung function measurements and, together with JS, calculated and interpreted lung function results; TT-DN, LPT and AW performed statistical analyses; TT-DN, LPT, AB, PA and JS drafted the manuscript; all remaining authors revised and approved the manuscript for intellectual content before submission.

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Competing interests None.

Patient consent Obtained.

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Evolution of Lung Function during the First Year of Life in Newborn Screened Cystic Fibrosis Infants

Authors: The Thanh-Diem Nguyen, MD*; Lena P. Thia, MBChB*; Ah-Fong Hoo, PhD; Andrew Bush, MD; Paul Aurora, PhD; Angie Wade, PhD, Jane Chudleigh, PhD; Sooky Lum, PhD and Janet Stocks, PhD: on behalf of the London Cystic Fibrosis Collaboration (LCFC)

*Both authors contributed equally

Data Supplement

1. Background

As part of a longitudinal research program of infants with cystic fibrosis (CF) diagnosed by newborn screening (NBS), this study measured lung function at 3 months and 1 year in NBS infants with CF and contemporaneous healthy controls. Clinical status, including use of medications and anthropometry, were documented prospectively. This online supplement (OLS) provides additional details regarding recruitment, methods and results, for which there was no space in the main article. Further details regarding parental attitudes to participating in this research study have been reported recently.[E1]

2. Recruitment of healthy controls

Healthy infants of similar age who met the inclusion criteria (see below) were identified monthly using the birth register from the Homerton University Hospital in East London, UK. Since the majority of mothers with babies were discharged from hospital within 24-48 hours following delivery, their family doctors were contacted by post to check that there were no medical and/or social contra-indications for contacting the families in the community. Once confirmation was received from the family doctors, a postal invitation letter, together with a parental information sheet and leaflet describing the lung function tests, were sent to the appropriate families. A phone call was made 7-10 days afterwards to explain and discuss the study further and answer any questions the parents may have with respect to participation.

2.1 Inclusion criteria for healthy controls

- Healthy infants with no congenital abnormalities, born ≥ 37 weeks gestation at the Homerton University Hospital, East London;
- Families living within reasonable travelling distance of the Infant Lung Function Laboratory at Great Ormond Street Hospital / UCL Institute of Child Health, and

- Parental consent to lung function measurements under chloral sedation.

2.2 Exclusion criteria for healthy controls

- Infants born <37 weeks gestation
- Inability of parents to understand and give informed consent;
- Recruitment contra-indicated on medical and/or psycho-social grounds;
- History of apnoeic episodes or upper airway pathology;
- History of chronic diarrhoea or failure to thrive;
- History of neonatal lung disease, assisted ventilation or co-existent cardio-vascular, skeletal, neuro-muscular, renal or metabolic disorders that could impact on the respiratory system and
- Previous physician diagnosed or hospital admission for lower respiratory tract infections.

Any healthy infant who was recruited to the study but was subsequently admitted to hospital due to respiratory infection, upper airway pathology or who developed chronic diarrhoea or failure to thrive was excluded from the study.

3. Characteristic of CF infants

3.1 CFTR mutations

CFTR classes I-III were identified in 82% of the NBS CF and classes IV-V in 10%. The remaining 8% of patients had unknown mutations (most likely I-III). Since there are over 1800 mutations, some rare mutations are extremely difficult to classify. With so few class IV-V subjects, there was insufficient power to include mutation class in the regression.

3.2 Standardised treatment for newborn infants with CF

Prior to commencing this study, a standardised treatment protocol, as described below, was developed and agreed upon by all participating consultants. This was used throughout the duration of the study.

Following diagnosis, all infants commenced on multivitamins, pancreatic supplements (if pancreatic insufficient as determined from faecal elastase levels) and prophylactic flucloxacillin (25mg/kg twice daily). The extent of adherence to protocol was checked both by regular review of prospectively completed Case Record Forms (CRFs) and by discussions at collaborative meetings of the LCFC. Cough swabs were taken routinely at clinic visits (minimum every 2–3 monthly) and additionally when the infant was symptomatic.

All centres in the UK encourage daily chest physiotherapy to infants and children with CF. Within the London CF Collaboration (LCFC), parents/carers of CF infants and children are taught an appropriate airway clearance technique. Physiotherapy is carried out as appropriate to the child's age and condition and reviewed frequently in conjunction with medical treatment.

3.2.1 Infection with *Pseudomonas aeruginosa* (PsA)

(a) First growth- Monthly cough swabs were collected while on treatment.

- **Well infant (based on clinical judgement)**
 - Home therapy with 3 weeks of oral Ciprofloxacin (15mg/kg twice daily) and
 - 3 months of nebulised Colistin (Colomycin: 1 million units twice daily).
- **Unwell infant (based on clinical judgement)**
 - Hospital admission for 2 weeks of intravenous antibiotics;

- Intravenous Ceftazidime (50mg/kg three times daily) and intravenous Tobramycin (10mg/kg once daily), though this choice could be modified by results from culture and sensitivity;
- Also started on 3 months of nebulised Colistin, initiated whilst in hospital.

(b) Re-growth during the initial 3 month treatment period (whilst still on Colistin)

- **Well infant**
 - Further 3 weeks of oral Ciprofloxacin.
- **Unwell infant**
 - Hospital admission for intravenous antibiotics and a further 3 months of nebulised Colistin. (If second course of intravenous antibiotics was inappropriate, 3 weeks of oral Ciprofloxacin was given instead.)

(c) Regrowth at the end of 3 weeks ciprofloxacin or 3 months nebulised Colistin

- **intravenous antibiotics**, and either
 - a further 3 months of nebulised Colistin, or
 - 28 days of TOBI (300mg twice daily) followed by 3 months of Colistin.

(d) Regrowth after IVs and at least 6 months of nebulised Colistin

- Try 28 days nebulised TOBI™ [E2] and then continuous nebulised Colistin 1 mu bd for a further six months. In practice this is unlikely to arise during the study.

(e) Regrowth > 6 months from first growth

- Treat as for first growth.

(f) Chronic Pseudomonas Infection

Defined for analysis purposes by the Leeds criteria:[E3]

Never	never cultured
Free	cultured previously but not in last year
Intermittent	cultured in < 50% of samples in past year
Chronic	cultured in > 50% of samples in past year

3.2.2 Infection with Methicillin Sensitive *Staphylococcus Aureus* (MSSA)

(a) First growth

- **Well infant**
 - Oral Augmentin duo (400/57) 0.3mL/kg twice daily for 2–4 weeks, *or* an equivalent dose of Co-amoxiclav syrup (0.25mL/kg of 250/62 strength) three times daily for 2–4 weeks based on clinical judgment.
- **Unwell infant**
 - Hospital admission for 2 weeks of intravenous antibiotics;
 - Intravenous Tobramycin once daily and intravenous Teicoplanin 10mg/kg for 2 doses twelve hours apart then 10mg/kg once daily.

(b) Regrowth less than 6 months from first growth

- Oral Flucloxacillin increased from 25mg/kg (prophylactic dose) to 50mg/kg for 28 days.

(c) Further regrowth within 6 months

- Two oral anti-staphylococcal antibiotics for 4 weeks.

(d) Re-growth after more than 6 months from first growth

- Treat as for first growth.

3.2.3 Infection with *Haemophilus influenzae* (HI)

(a) First growth

- **Well infant**

- Oral Augmentin Duo or Co-amoxiclav syrup for 2–4 weeks (based on clinical judgement)

- **Unwell infant**

- Hospital admission for 2 weeks of intravenous Ceftazidime (50mg/kg three times daily) and intravenous Tobramycin (10mg/kg once daily)

(b) Regrowth less than 6 months from first growth or more than 6 months from first growth

- Oral Augmentin duo or Co-amoxiclav syrup for 2–4 weeks (based on clinical judgement)

(c) Further regrowth within 6 months

- Clarithromycin (7.5mg/kg twice daily) for 2–4 weeks

3.2.4 Viral Upper Tract Infections (otherwise well child)

- Oral Augmentin duo (400/57) 0.3 mls/kg bd for 2 (minimum) to 4 weeks (clinical judgment) or equivalent dose of Co-amoxiclav syrup tds

<1 year 0.25ml/kg TDS Augmentin 250/62; >1 - 2 years 5ml TDS Augmentin 250/62
for 2 (minimum) to 4 weeks (clinical judgment)

3.2.5 Respiratory exacerbation with unknown organism, unwell child (clinical judgment)

Depending on severity of exacerbation:

- Oral Augmentin duo (400/57) for 2 to 4 weeks or equivalent dose of Co-amoxiclav syrup tds (as above) OR
- Intravenous Tobramycin 10 mg/kg once daily and Intravenous Ceftazidime 50 mg/kg three times a day for 2 weeks

NOTE: choice of antibiotic may vary from the protocol depending on culture sensitivities

Additional details regarding treatment of CF NBS infants during the first year

Inspection of CRFs and regular communication with consultants revealed excellent adherence to treatment protocols. 17 NBS infants had used an inhaled bronchodilator at some point by 1 year of age, all but one of whom commenced this by 3 months. Of these 17 infants, only 1 still used it regularly by 1 year of age, with very intermittent use by the remaining 16. One infant was on regular inhaled steroid by 1 year of age, while another had had a single course of oral prednisolone for wheeze between 3 months-1 year. Hypertonic saline had been used in three infants by age 1 year, one of whom started this at 3 months. Six patients received treatment with rhDNase between 3 months – 1 year. Within the limited power of study for such sub-group analysis, there was no significant differences for any anthropometric or lung

function measurements at 1 year, nor for the change in any of these measures between 3 months to 1 year between those who did and did not receive rhDNase. However, there was a non-significant tendency for FRC_{pleth} to be higher (mean [95%CI] difference in z-scores: 0.62 (-0.35; 1.59)) and $FEV_{0.5}$ to be lower (-.43[-1.31; 0.44] at 1 year in the 6 infants who had been prescribed rhDNase, suggesting that this was prescribed for children with more severe symptoms.

Although all lung function reports were sent to clinicians within a few weeks of testing so that they could be discussed with parents, this did not impact on treatment unless independently indicated by clinical status. In contrast to studies in older children, there is a paucity of evidence relating to the short- or long-term significance of changes in lung function during infancy and consequently clinical management of the children recruited to the LCFC study continues to be guided by signs and symptoms during the first year of life.

4. Age range at time of lung function measurements

We aimed to test all infants between 2-4 months of age on the first occasion and between 9-15 months on the second occasion, to allow for any cancellations and subsequent rebooking due to upper respiratory infections or respiratory exacerbations. The age ranges at time of tests were as follows:

1st lung function: 5.4 to 17.0 weeks in CF infants and 7.7 to 18.3 weeks in controls

2nd lung function: 42.1 to 68.9 weeks in CF infants and 43.3 to 64.3 weeks in controls

5. Feasibility of lung function measurements

The relative success rate in obtaining technically satisfactory measurements on each occasion is summarised in Table E1. While FRC_{pleth} detected abnormalities of lung function in NBS CF infants as readily as LCI or $FEV_{0.5}$, it was not tolerated as well by the younger infants,

making it less feasible as an outcome variable at 3-months of age, or in longitudinal studies commencing at this age. As reported previously, measures of respiratory compliance and resistance obtained using the single occlusion technique and tidal breathing parameters discriminated poorly between infants with CF and healthy controls.[E4]

Table E1. Technically satisfactory infant PFTs obtained of paired assessments undertaken in 72 NBS CF and 44 healthy control infants

	Results at 3 months		Results at 1 year		Results on both occasions	
	CF	Controls	CF	Controls	CF	Controls
LCI	71 (99%)	41(93%)	71 (99%)	44 (100%)	70 (97%)	41 (93%)
FRC _{pleth}	57 (79%)	38 (86%)	70 (97%)	42 (95%)	55 (76%)	36 (82%)
FEV _{0.5}	68 (94%)	42 (95%)	69 (96%)	42 (95%)	66 (92%)	40 (91%)

Results are presented as n (%) successful measurements according to outcome.

Abbreviations: LCI= lung clearance index; FRC= functional residual capacity; pleth= plethysmographic technique; FEV_{0.5}= forced expiratory volume in 0.5 sec.

Table E2 Association between selected lung function z-scores in infants with CF

	3m LCI	3m FRC_{pleth}	3m ΔFRC (pleth–MBW)	3m FEV_{0.5}	1yr LCI	1yr FRC_{pleth}	1yr ΔFRC (pleth–MBW)	1yr FEV_{0.5}
3m LCI	–	r=0.18 p=0.17	r=0.39 p=0.003	r=-0.09 p=0.47	r=0.30 p=0.012	r=0.25 p=0.035	r=0.29 p=0.017	r=0.04 p=0.75
3m FRC_{pleth}	r=0.18 p=0.17	–	r=0.66 p<0.0005	r=-0.012 p=0.93	r=0.03 p=0.80	r=0.58 p<0.0005	r=0.12 p=0.37	r=0.13 p=0.36
3m ΔFRC (pleth–MBW)	r=0.39 p=0.003	r=0.66 p<0.0005	–	r=-0.03 p=0.80	r=0.18 p=0.19	r=0.20 p=0.15	r=0.11 p=0.45	r=0.06 p=0.65
3m FEV_{0.5}	r=-0.09 p=0.47	r=-0.012 p=0.93	r=-0.03 p=0.80	–	r=-0.34 p=0.004	r=0.01 p=0.94	r=-0.18 p=0.14	r=0.48 p<0.0005
1yr LCI	r=0.30 p=0.012	r=0.03 p=0.80	r=0.18 p=0.19	r=-0.34 p=0.004	–	r=0.07 p=0.55	r=0.55 p<0.0005	r=-0.18 p=0.14
1yr FRC_{pleth}	r=0.25 p=0.035	r=0.58 p<0.0005	r=0.20 p=0.15	r=0.01 p=0.94	r=0.07 p=0.55	–	r=0.47 p<0.0005	r=0.06 p=0.60
1yr ΔFRC (pleth–MBW)	r=0.29 p=0.017	r=0.12 p=0.37	r=0.11 p=0.45	r=-0.18 p=0.14	r=0.55 p<0.0005	r=0.47 p<0.0005	–	r=-0.09 p=0.45
1yr FEV_{0.5}	r=0.04 p=0.75	r=0.13 p=0.36	r=0.06 p=0.65	r=0.48 p<0.0005	r=-0.18 p=0.14	r=0.06 p=0.60	r=-0.09 p=0.45	–

Footnote: Data shown as spearman correlation and p value; Significant differences (p< at least 0.05) are shown in bold.

6. Multiple imputations

Paired data at 3 months and 1 year (Table E1) were achieved for:

70 (97%) CF infants and 41 (93%) healthy controls for LCI,

55 (76%) CF infants and 36 (82%) controls for FRC_{pleth} and

66 (92%) CF infants and 40 (91%) controls for $FEV_{0.5}$.

The imputation procedures used all the known covariates thought to be associated with lung function at 1 year to help to predict the value of any missing data. The incomplete variables were 3-month LCI z-score, 3-month FRC_{pleth} z-scores and 3-month $FEV_{0.5}$ z-score. The observed covariates considered were sex, gestational age, birth weight z-scores, maternal smoking, maternal and paternal occupations, somatic growth (between birth to 1 year and between 3 months to 1 year), microbiology results (*Pseudomonas aeruginosa* (*PsA*), significant bacterial growth ever and no growth/ non-significant bacterial growth ever), respiratory signs (wheeze, crackles and cough) and treatment with rhDNase, intravenous antibiotics for respiratory symptoms or gastro-oesophageal reflux disease. One hundred imputations were performed using PASW Statistics v.18 (Chicago, IL, US). The results using multiple imputations were similar to those obtained using list-wise deletion.

7. Determinants of pulmonary function at one year

Table E3. Univariable linear regression with multiple imputations: determinants of pulmonary function at 1 year

	1yr LCI z-score	1yr FRC _{pleth} z-score	1yr FEV _{0.5} z-score
CF	0.82 (0.39;1.24) p<.0001	0.79 (0.38; 1.20) p<.0001	-0.49 (-0.88;0.10) p=.02
3m LCI z-score	0.32 (0.15;0.50) p<.0001	-0.36 (-0.74; -0.06) p=0.022	0.08(-0.26;0.44) p=0.62
3m FRC _{pleth} z-score	-0.25 (-0.52; 0.07) p=0.13	0.48(0.31; 0.65) p<.0001	0.02 (-0.26; 0.29) p=0.90
3m FEV _{0.5} z-score	-0.12 (-0.56; 0.25) p=0.44	0.14 (-0.24; 0.60) p=0.40	0.42 (0.26;0.58) p<.0001
Male	0.35 (-0.09; 0.78) p=.12	0.39 (-0.03; 0.81) p=.07	-0.01 (-0.39; 0.38) p=.98
Gestational age	-0.02 (-0.17; 0.14) p=.83	-0.11 (-0.26; 0.04) p=0.14	0.15 (0.02; 0.29) p=.02
Birth weight z-score	-0.19 (-0.43; 0.06) p=.14	-0.11 (-0.35; 0.13) p=.37	0.27 (0.06; 0.49) p=.01
Maternal smoking during pregnancy	-0.48 (-1.22; 0.27) p=.21	-0.08 (-0.81; 0.65) p=.83	0.52 (-0.13; 1.18) p=.12
Current maternal smoking	-0.38 (-1.04; 0.29) p=.27	0.24 (-0.42; 0.89) p=.48	0.47 (-0.12; 1.05) p=.12
Mother in non-manual occupation	-0.43 (-0.95; 0.09) p=.10	-0.30 (-0.79; 0.18) p=.22	0.12 (-0.34; 0.57) p=.62
Father in non-manual occupation	-0.22 (-0.70; 0.25) p=.35	0.07 (-0.38; 0.52) p=.76	0.17 (-0.24; 0.58) p=.41
ΔWeight (3m-birth), z-score	-0.19(-0.39;-0.003) p=0.04	-0.01(-0.20; 0.18) p=.93	-0.01 (-0.19; 0.16) p=.90
ΔWeight (1yr-3m), z-score	0.20 (-0.04; 0.45) p=.11	-0.11 (-0.35; 0.13) p=.36	-0.21(-0.42;0.001) p=.05
<i>Pseudomonas aeruginosa</i>	0.53 (-0.03; 1.08) p=.06	0.94 (0.46; 1.42) p<.0001	-0.37 (-0.84; 0.11) p=.13
Significant bacterial growth	0.35 (-0.17;0.87) p=.18	0.22 (-0.26; 0.70) p=.36	-0.18 (-0.64; 0.28) p=.45
Wheeze, ever	0.99(0.48;1.50) p<.0001	0.71 (0.21; 1.21) p=.006	-0.52(-0.98;-0.07) p=.02
Crackles, ever	0.70 (-0.28; 1.68) p=.16	0.85 (-0.08; 1.78) p=.07	-0.60 (-1.44; 0.24) p=.16
Cough, within 3 weeks of 1yr lung function	0.44 (-0.21; 1.09) p=.19	0.61 (-0.01; 1.22) p=.05	-0.71(-1.25;-0.16) p=.01
rhDNase treatment, ever	0.31 (-0.68; 1.30) p=.54	0.92 (-0.01; 1.85) p=0.55	-0.63 (-1.46; 0.21) p=.14
IV antibiotics, number of courses	0.27 (-0.10; 0.64) p=0.16	0.54 (0.21; 0.87) p=.001	-0.28 (-0.59; 0.02) p=.07
GERD treatment, ever	0.59 (0.13; 1.05) P=.01	0.52 (0.08; 0.95) p=.02	-0.44 (-0.84;-0.04) p=.03

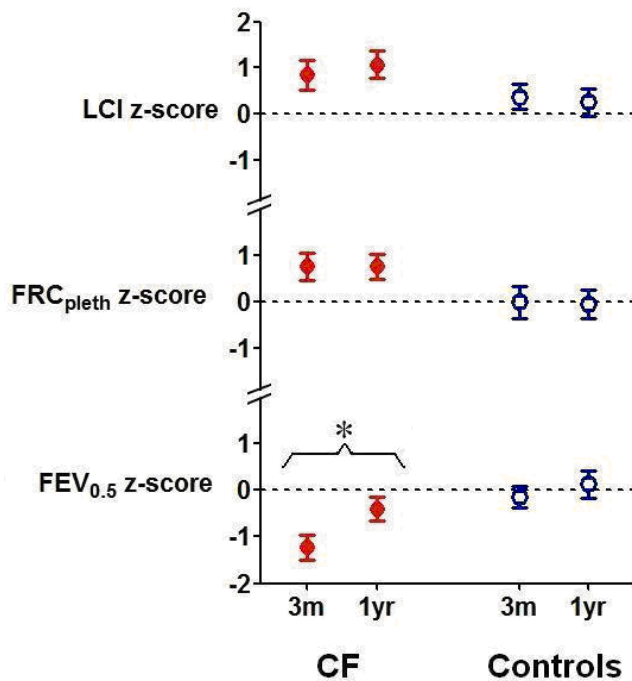
Data shown as mean (95% CI). * extent to which results from each PFT at 3m are associated with those from same test at 1 year after adjusting for sex, age and body size, e.g. association between 1yr LCI z-score and 3m LCI z-score. Significant associations are shown in bold: Abbreviations: rhDNase= Pulmozyme; IV= intravenous; GERD= gastro-oesophageal reflux disease

Table E3 shows linear univariable analysis of LCI, FRC_{pleth} and $FEV_{0.5}$ z-scores at 1 year using multiple imputations. Results were similar with or without multiple imputations. Stepwise, forward selection and backward elimination procedures were used for selecting the best regression model. On multivariable linear regression, lung function at 3 months was shown to be predictive of that at 1 year for all PFT outcomes. Significant determinants of 1-year LCI z-score were: CF status [coefficient (95% CI): 0.48 (0.04;0.93) z-score, $p=.032$]; 3-month LCI [0.24 (0.07;0.41) z-score, $p=.005$]; history of clinician diagnosed wheeze [0.59 (0.05;1.12) z-score, $p=.031$] and change in weight z-score between birth and 3-months; Δ Weight (3m-birth) [-.18 (-0.35;-0.01) z-score, $p=.042$]. FRC_{pleth} at 1 year was significantly associated with FRC_{pleth} at 3 months [0.43 (0.27;0.59) z-score, $p<.0005$], history of *PsA* infection [0.71 (0.24;1.17) z-score, $p=.003$] and change in weight z-score between 3-months and 1 year; Δ Weight (1yr-3m) [.20 (-0.41;0.003) z-score, $p=.054$]. After adjustment for these factors, other variables including CF status were no longer significantly associated with 1 year FRC_{pleth} . Finally, on multivariable analysis, 1-year $FEV_{0.5}$ z-score was only significantly associated with $FEV_{0.5}$ z-score at 3 months [-0.18 (-0.35; -0.01) per unit z-score].

(d) Relationship between PFT results at 3 months and 1 year:

Figure E1 compares changes in lung function during the first year of life in CF infants and healthy controls. After adjustment for age, sex and body size as appropriate [E5-7] there were no significant changes in any lung function outcome in healthy infants during this period, and LCI and FRC_{pleth} remained stable, albeit somewhat elevated in those with CF. By contrast there was a significant improvement in $FEV_{0.5}$ during the first year of life in NBS CF infants. For further details see Table 2 main manuscript

Figure E1. Lung function at 3 months and 1 year in NBS infants with CF and healthy controls



Footnote: Data are expressed as mean \pm 95% Confidence interval *p<0.05

The horizontal line represents 0 z-scores which equates to 100% predicted or the 50th centile for results derived from a healthy population.[E8]

(e) Sample calculations for randomised control trials (RCTs).

Sample size calculations depend on numerous factors including the magnitude of change/difference to be detected, the number of outcomes under investigation, the between-subject variability for any given outcome, and the confidence (power) that is desired with which to detect such differences. Taking into account the between-subject variability of infant PFTs observed in this and previously published studies[E5-7] a difference of 1 z-score (SD) at 1 year equates to ~ 9% or 0.64 units for LCI, 14.5% or 27 mL for FRC_{pleth} and 15% or 46 mL for FEV_{0.5}. Decisions regarding what constitutes a minimal clinically important difference in intervention trials are complex, but values equating to at least 0.5 SD (or z-

scores) are probably appropriate, to avoid risk of sampling error.[E9] In contrast to studies in older children with CF, in whom larger differences in PFTs may be observed,[E10] the mean difference between the NBS CF infants and healthy controls at one year for the 3 primary outcomes in this study was only 0.5 to 0.8 z-scores (with 95% confidence intervals ranging between 0.2 – 1.2 z-scores, Table 2, main manuscript).

If planning a randomised controlled intervention study with, for example, LCI as a primary endpoint, a sample size of 85 subjects per arm would allow detection of differences in lung function at one year of age equivalent to 0.5 z-scores at the 5% significance level with 90% power, whereas 63 patients per group would provide 80% power to detect the same difference.[E11-13] Given that, despite excellent success rates in PFTs and minimal attrition, paired lung function tests at 1 year were ‘only’ attained in 62% NBS CF infants presenting during the recruitment period (Figure 1), a pool of at least 275 CF infants ($85 \times 2 \times 100/62$) would be required to undertake such a study, increasing further if based on more than one outcome. However, if recruitment were limited to those with evidence of abnormal lung function at 3 months, then the magnitude for potential improvement would be considerably larger. Under these circumstances, an effective intervention in this ‘high risk group’ could improve lung function by at least 1 z-score (Table E4 and E5). Thus a RCT designed to detect a 1 z-score improvement in lung function in response to an intervention would only require 22 infants in each arm for 90% power at the 5% significance level. Nevertheless, since abnormalities at 3 months were only observed in 30% of our infants when based on the 2 most feasible PFTs (LCI and $FEV_{0.5}$), after allowing for attrition and exclusions as discussed above it would still be necessary to access a population of $(22 \times 2) \times (100/62) \times (100/30)$ i.e. ~237 NBS CF infants to obtain 90% power in a RCT. This is more than double the number

identified in the South-East of England over a 2.5 year period during the present study and would hence inevitably require a multi-centre study if to be completed in a timely manner.

Table E4. Summary of anthropometry and pulmonary function at ~3 months and 1 year in CF NBS infants with normal (N) and abnormal (A) lung function on the first test occasion (3 months) versus healthy controls (C).

	At 3 months				At 1 year			
	Abnormal* (A)	Normal† (N)	Controls (C)	p value (ANOVA)	Abnormal* (A)	Normal† (N)	Controls (C)	p value (ANOVA)
<i>n</i>	19	45	37		19	45	37	
<i>Age, weeks</i> [§]	11.1 (2.3)	11.1 (2.2)	11.9 (2.0)	0.22	55.0 (5.1)	51.7 (5.3)	53.5 (4.5)	0.05
Weight, z-score [¶]	-0.67 (0.89)	-1.00 (1.07)	-0.03 (0.97)	<0.001	0.36 (0.80)	0.25 (0.95)	0.51 (1.29)	0.56
Length, z-score [¶]	0.12 (0.92)	-0.40 (0.96)	0.67 (0.93)	<0.001	0.76 (0.98)	0.28 (0.96)	0.73 (1.25)	0.11
BMI, z-score [¶]	-1.02 (0.90)	-1.09 (1.07)	-0.55 (0.95)	0.05	-0.10 (0.72)	0.14 (0.91)	0.14 (1.16)	0.64
LCI, z-score	1.22 (1.85)	0.51 (0.91)	0.37 (0.89)	0.03	1.64 (0.98)	0.78 (1.23)	0.31 (0.97)	<0.001
FEV _{0.5} , z-score	-2.29 (0.79)	-0.71 (0.80)	-0.13 (0.77)	<0.001	-0.67 (0.95)	-0.24 (1.04)	0.13 (0.94)	0.02

Footnote: Comparisons between groups were undertaken using ANOVA. Dataset used for this analysis were limited to those infants with technically successful LCI and FEV_{0.5} results on both test occasions. CI=confidence interval of the difference; BMI= Body Mass Index. * based on those with abnormal LCI and/or FEV_{0.5} at 3 months (i.e. outside the 95% limits of normality found in healthy infants); † based on those with normal LCI and FEV_{0.5} at 3 months. ‡ Based on post-hoc Bonferroni adjustment for multiple t tests between and within groups. The significant difference in anthropometry at 3 months identified by ANOVA was limited to comparisons between healthy controls and NBS CF infants with normal PFTs at 3m. There were no significant anthropometric differences between the two subgroups of CF infants, nor between those with abnormal PFTs and controls at 3 months. § corrected for gestational age; ¶ calculated according to Cole et al[E14]

Table E5. Comparison of anthropometry and pulmonary function at ~3 months and 1 year between CF NBS infants with normal (N) and abnormal (A) lung function at 3 months and healthy controls (C)

	At 3 months			At 1 year		
	Diff (95%CI):A-N* p value	Diff (95%CI):N-C* p value	Diff (95%CI):A-C* p value	Diff (95%CI):A-N* p value	Diff (95%CI):N-C* p value	Diff (95%CI):A-C* p value
Age, weeks [†]	-0.02 (-1.30; 1.25) 0.97	-0.77 (-1.70; 0.15) 0.10	-0.80 (-2.07; 0.46) 0.20	3.3 (0.38; 6.16) 0.028	-1.79 (-3.94; 0.37) 0.10	1.48 (-1.35; 4.31) 0.29
Weight, z-score [‡]	0.34 (-0.19; 0.86) 0.620	-0.97 (-1.42; -0.52) <0.001	-0.64 (-1.16; -0.11) 0.018	0.10 (-0.37; 0.57) 0.66	-0.25 (-0.76; 0.25) 0.32	-0.15 (-0.71; 0.41) 0.59
Length, z-score [‡]	0.52 (0.004; 1.04) 0.048	-1.07 (-1.49; -0.65) <0.001	-0.55 (-1.08; -0.02) 0.041	0.48 (-0.06; 1.02) 0.082	-0.45 (-0.94; 0.04) 0.076	0.03 (-0.59; 0.64) 0.93
BMI, z-score [‡]	0.07 (-0.46; 0.59) 0.80	-0.54 (-0.98; -0.09) 0.018	-0.47 (-0.99; 0.05) 0.078	-0.23 (-0.66; 0.20) 0.28	-0.01 (-0.47; 0.46) 0.97	-0.24 (-0.75; 0.27) 0.35
LCI, z-score	0.71 (-0.21; 1.63) 0.12	0.14 (-0.26; 0.54) 0.49	0.85 (0.12; 1.58) 0.023	0.87 (0.28; 1.45) 0.005	0.46 (-0.02; 0.95) 0.059	1.33 (0.787; 1.89) <0.001
FEV _{0.5} , z-score	-1.57 (-2.01; -1.13) <0.001	-0.59 (-0.93; -0.24) 0.001	-2.16 (-2.61; -1.71) <0.001	-0.44 (-0.98; 0.11) 0.11	-0.36 (-0.80; 0.07) 0.10	-0.80 (-1.34; -0.26) 0.005

Footnote: Dataset used for this analysis were limited to those infants with technically successful LCI and FEV_{0.5} results on both test occasions. CI=confidence interval of the difference; BMI= Body Mass Index; A: CF infants with abnormal LCI and/or FEV_{0.5} at 3 months; N: CF infants with normal LCI and FEV_{0.5} at 3 months; C: controls

*Based on student's t-test; [†]corrected for gestational age; [‡]calculated according to Cole et al[E14]

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