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

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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* (MS*SA*)

(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-1year. 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 1year 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
Pseudomonas aeruginosa	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**

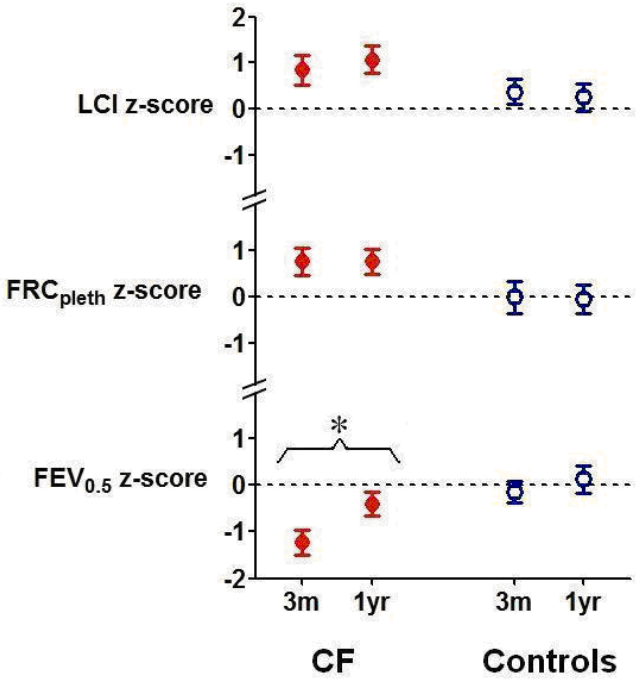
Data shown as mean (95% CI). *extent to which results from each PFT at 3m are associated with those from same test at 1year after adjusting for sex, age and body size, e.g. association between 1yr LCI z-score and 3m LCI z-score Significant associations are shown in bold: Abbreviations: rhDNase= Pulmozyme; 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; ΔWeight (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; ΔWeight (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 ± 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 x2 x100/62) 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 x 2) x (100/62) x (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)**
n	19	45	37		19	45	37	
Age, weeks[§]	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]

EReferences

E1. Chudleigh J, Hoo AF, Ahmed D, et al. Positive parental attitudes to participating in research involving newborn screened infants with CF. *J Cyst Fibros* 2012;12:234-40.

E2. Gibson RL, Emerson J, McNamara S, et al. Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis. *Am J Respir Crit Care Med* 2003;167:841-9.

E3. Lee TW, Brownlee KG, Conway SP, et al. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. *J Cyst Fibros* 2003;2:29-34.

E4. Hoo AF, Thia LP, Nguyen TT, et al. Lung function is abnormal in 3-month-old infants with cystic fibrosis diagnosed by newborn screening. *Thorax* 2012;67:874-81.

E5. Lum S, Hoo AF, Hulskamp G, et al. Potential misinterpretation of infant lung function unless prospective healthy controls are studied. *Pediatr Pulmonol* 2010;45:906-13.

E6. Nguyen TTD, Hoo AF, Lum S, et al. New reference equations to improve interpretation of infant lung function . *Pediatr Pulmonol* 2013;48:370-80.

E7. Lum S, Stocks J, Stanojevic S, et al. Age and height dependence of lung clearance index and functional residual capacity. *Eur Respir J* 2013;41:1371-7.

E8. Stanojevic S, Wade A, Stocks J. Reference values for lung function: past, present and future. *Eur.Respir.J.* 2010;36:12-9.

E9. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95 year age range: the global lung function 2012 equations. *Eur.Respir.J.* 2012;40:1324-43.

E10. Amin R, Subbarao P, Lou W, et al. The effect of dornase alfa on ventilation inhomogeneity in patients with cystic fibrosis. *Eur.Respir.J.* 2011;37:806-12.

E11. Altman, D., Machin D, Bryant TN, et al. 2008. Statistics with Confidence, 2nd ed. BMJ Books, London. 163-164.

E12. Kirkwood BR and Sterne JAC. 2008. Essential Medical Statistics, 2nd ed. Wiley-Blackwell, New Jersey, NJ.

E13. Petrie, A. and C. Sabin. 2005. Medical Statistics at a Glance, 3rd ed. Wiley-Blackwell, New Jersey, NJ. 96-98.

E14. Cole TJ, Wright CM, Williams AF. Designing the new UK-WHO growth charts to enhance assessment of growth around birth. *Arch Dis Child Fetal Neonatal Ed* 2012;97:F219-F222.