Online data supplement

Pulmonary function deficits in newborn screened infants with cystic fibrosis managed with standard UK care are mild and transient

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This supplement includes additional results to compliment the main manuscript

Table E1a. Study population and background demographics

	CF (n=62)	Healthy controls (n=34)	Δ (95% CI) CF–controls
Male	29 (47%)	17 (50%)	
Gestational age (GA), weeks	39.1 (1.5)	40.4 (1.2)	**-1.21 (-1.81 to -0.61)**
Birth weight*, z-score	-0.5 (1.0)	0.2 (0.8)	**-0.69 (-1.09 to -0.28)**
White mother	55 (89%)	30 (88%)	
Cystic fibrosis infants only			
Postnatal age at diagnosis (weeks)[$]	3.7(3.3-4.4)		
p.Phe508del**, n= 60	54 (90%)		
Presented with meconium ileus	8 (12.9%)		
Pancreatic sufficient	6 (9.7%)		

Results presented as n(%) or mean(SD) unless stated otherwise. *z-scores calculated according to Cole *et al* [E1] (infants with GA <37 weeks calculated using UK-WHO-preterm; ≥37 weeks UK-WHO-term). **homozygous or heterozygous. [$]median(interquartile range).

Table E1b: Additional clinical details for CF Infants in relation to test occasion

	By 1yr test (n=60)	By 2yr test (n=62)
Respiratory symptoms		
Physician diagnosed wheeze (ever)	20/60 (34%)	25/62 (40%)
Additional treatment (n(%))		
rhDNase (ever)	6/59 (10%)	9/51 (18%)
IV antibiotics (ever)	16/60 (27%)	27/58(47%)
Gastroesophageal reflux disease treatment	29/56 (52%)	31/61 (51%)
Airway microbiology (ever)		
Pseudomonas aeruginosa	19 (31%), n=1 chronic	32/62 (52%), n=4 chronic
Staph aureus	10 (16%)	18 (29%), n=1 chronic
Haemophilus influenza	15 (24%)	21 (34%)

Of the 27 children who had received intravenous (IV) antibiotics by their two year test, 22 (81%) had isolated *Pseudomonas aeruginosa (*PsA) on at least one occasion. All nine children who had received nebulised rhDNase by the 2yr test had also received at least one course of IV antibiotics. Fourteen children received IV antibiotics between their 1yr and 2yr test.

Table E2. Technically satisfactory infant lung function results on each test occasion

	3 months		1 year		2 years		1yr & 2yr		3mth & 2yr	
	CF (n=61)	**Controls (n=33)**	**CF (n=60)**	**Controls (n=32)**	**CF (n=62)**	**Controls (n=34)**	**CF (n=60)**	**Controls (n=32)**	**CF (n=61)**	**Controls (n=33)**
LCI	57 (93)	30 (91)	59 (98)	32 (100)	61 (98)	33 (97)	58 (97)	31 (97)	56 (92)	29 (88)
FRC_{pleth}	50 (82)	29 (88)	59 (98)	32 (100)	57 (92)	31 (91)	54 (90)	30 (94)	47 (77)	26 (79)
$FEV_{0.5}$	59 (97)	32 (97)	58 (97)	32 (100)	56 (90)	28 (82)	54 (90)	27 (84)	53 (87)	27 (82)

Results are presented as n (%) successful measurements according to outcome.
Abbreviations: LCI= lung clearance index; FRC_{pleth} = plethysmographic functional residual capacity; $FEV_{0.5}$= forced expiratory volume in 0.5 seconds.
Of the 62 CF infants tested at ~2year, all been assessed previously on at least one occasion (61 at 3 months and 60 at 1 year). Similarly, of the 34 healthy infants studied at ~2yr, 33 had been tested at 3mth and 32 at 1yr. Technically satisfactory results in all three infant lung function outcomes (LCI, FRCpleth and $FEV_{0.5}$) were obtained in 47 CF infants at 3mths, 57 at 1yr and 54 at the 2 year test occasion.

Comparison of previously reported outcomes at 3 months and 1 year for the NBS cohort

Recently published equations for the calculation of z-scores from the RVRTC technique have improved the confidence with which we can accurately detect and quantify abnormality in infant lung function (ILF) tests [E2, E3], particularly for infants in the first few months of life. As these new equations have been used for analyses presented within this paper, a comparison with our previously published results obtained using older equations [E4] was undertaken. Cross sectional results for CF infants and controls at 3mth and 1yr were largely consistent with previous reports [E5, E6, E7, E8]. Minor discrepancies using the new equations included mean $FEV_{0.5}$ in CF infants being slightly less 'abnormal' at three months of age.

Had we applied the reference equations described by Lum et al [E4], the mean(SD) $FEV_{0.5}$ at 3mth for the infants included in this 2yr follow –up would have been -1.20(1.05) z-scores, rather than - 0.86 (0.99) as detailed in Table E3. This is virtually identical to the $FEV_{0.5}$ at 3mth originally reported both for the entire NBS LCFC cohort and for those followed up to 1yr of age[E6, E7] where these equations were used.

Hoo et al reported that at 3mths of age, 25% of CF infants (17/68) had an abnormally low $FEV_{0.5}$ (below -1.96 z-scores) [E6]. Had we used the Lum equations [E4] as applied by Hoo et al, 14/59 (24%) of CF infants in this 2yr cohort would have had an abnormal $FEV_{0.5}$ at 3mths. This suggests that those followed up to two years were representative of the original cohort recruited. When using the updated reference equations, only 8/59 (14%) of CF infants had abnormal $FEV_{0.5}$ at 3mths. Nevertheless, despite this shift in absolute z-scores resulting from use of updated equations, the differences in ILF between the CF and control group reported previously and in

this current paper remain unchanged (Hoo et al reported a mean difference of 0.92 (1.29 to 0.56) z-scores between CF and HC at 3 months, which is virtually identical to that shown below in Table E3 when using the updated equations.

Table E3. Comparison of lung function in infants with CF and healthy controls at approximately 3 months (A) and 1 year (B) of age

A

	Cystic Fibrosis	Healthy Control	Difference (95% CI)	p
N	61	33		
Age at test, weeks	11.2 (2.4)	12.3 (2.1)	-1.05 (-2.04 to -0.07)	**0.04**
zHeight	-0.21 (1.07)	0.83 (0.80)	-1.04 (-1.46 to -0.62)	**<0.001**
zWeight	-0.97 (1.09)	-0.04 (0.89)	-0.93 (-1.37 to -0.49)	**<0.001**
zBMI	-1.20 (0.94)	-0.69 (0.99)	-0.51 (-0.92 to -0.10)	**0.02**
zLCI	0.62 (1.24)	0.28 (0.89),	0.34 (-0.17 to 0.85)	0.19
$zFRC_{pleth}$	0.79 (1.14)	-0.18 (1.07)	0.97 (0.45 to 1.49)	**<0.001**
$zFEV_{0.5}$	-0.86 (0.99)	0.08 (0.84)	-0.94 (-1.36 to -0.53)	**<0.001**
zFVC	-0.97 (1.25)	-0.16 (0.94)	-0.81 (-1.31 to -0.31)	**0.002**
$zFEF_{25–75}$	-0.75 (1.16)	0.24 (0.98)	-0.99 (-1.47 to -0.51)	**<0.001**

B.

	Cystic Fibrosis	Healthy Control	Difference (95% CI)	p
N	60	32		
Age at test, weeks	52.8 (5.3)	53.8 (3.4)	-1.05 (-3.09 to 0.99)	0.31
zHeight	0.47 (0.97)	0.74 (1.09)	-0.26 (-0.71 to 0.18)	0.24
zWeight	0.26 (0.87)	0.44 (0.99)	-0.18 (-0.57 to 0.22)	0.38
zBMI	-0.01 (0.74)	0.03 (0.83)	-0.04 (-0.38 to 0.29)	0.80
zLCI	0.82 (1.16)	0.13 (0.87)	0.69 (0.22 to 1.15)	**0.004**
$zFRC_{pleth}$	0.82 (1.2)	0.14 (1.21)	0.69 (0.16 to 1.21)	**0.01**
$zFEV_{0.5}$	-0.51 (1.04)	0.13 (0.88)	-0.64 (-1.07 to -0.21)	**0.004**
zFVC	-0.51 (1.03)	0.24 (0.94)	-0.75 (-1.19 to -0.31)	**0.001**
$zFEF_{25–75}$	-0.29 (1.15)	-0.01 (0.99)	-0.28 (-0.76 to 0.2)	0.25

Footnote: Values are mean (SD). For number of subjects with lung function outcome at each test occasion see Table E2 OLS. Although not a primary outcome, FVC and FEF_{25-75} results are included in this table to allow comparison with the published literature.

Table E4: Change in lung function z-scores between test occasions in A) infants with CF and B) control infants.

A) Cystic Fibrosis	Mean (SD) difference	95% CI of the Difference	p
LCI			
3 months to 1 year	0.33 (1.19)	0.00 to 0.65	**0.050**
3 months to 2 years	0.16 (1.51)	-0.24 to 0.57	0.427
1 year to 2 years	0.00 (1.37)	-0.36 to 0.36	0.993
FRC_{pleth}			
3 months to 1 year	0.04 (1.19)	-0.31 to 0.38	0.836
3 months to 2 years	0.08 (1.38)	-0.33 to 0.48	0.707
1 year to 2 years	0.02 (1.13)	-0.29 to 0.33	0.901
$FEV_{0.5}$			
3 months to 1 year	0.36 (0.97)	0.10 to 0.62	**0.008**
3 months to 2 years	0.46 (1.13)	0.15 to 0.78	**0.004**
1 year to 2 years	0.19 (1.03)	-0.09 to 0.47	0.179

B) Healthy controls	Mean (SD) difference	95% CI of the Difference	p
LCI			
3 months to 1 year	-0.10 (1.14)	-0.54 to 0.35	0.659
3 months to 2 years	-0.16 (0.83)	-0.48 to 0.15	0.296
1 year to 2 years	-0.15 (0.83)	-0.46 to 0.16	0.327
FRC_{pleth}			
3 months to 1 year	0.29 (1.59)	-0.34 to 0.92	0.348
3 months to 2 years	0.42 (1.58)	-0.22 to 1.06	0.186
1 year to 2 years	0.01 (1.10)	-0.40 to 0.42	0.967
$FEV_{0.5}$			
3 months to 1 year	-0.02 (0.89)	-0.35 to 0.31	0.901
3 months to 2 years	-0.23 (1.11)	-0.67 to 0.21	0.290
1 year to 2 years	-0.20 (1.19)	-0.67 to 0.27	0.386

Footnote: As can be seen, in contrast to infants with CF in whom LCI deteriorated between 3mth and 1yr, $FEV_{0.5}$ improved between both 3mth and 1yr and between 1yr and 2yrs. There were no significant group changes in any infant lung function outcome over time among the control infants.

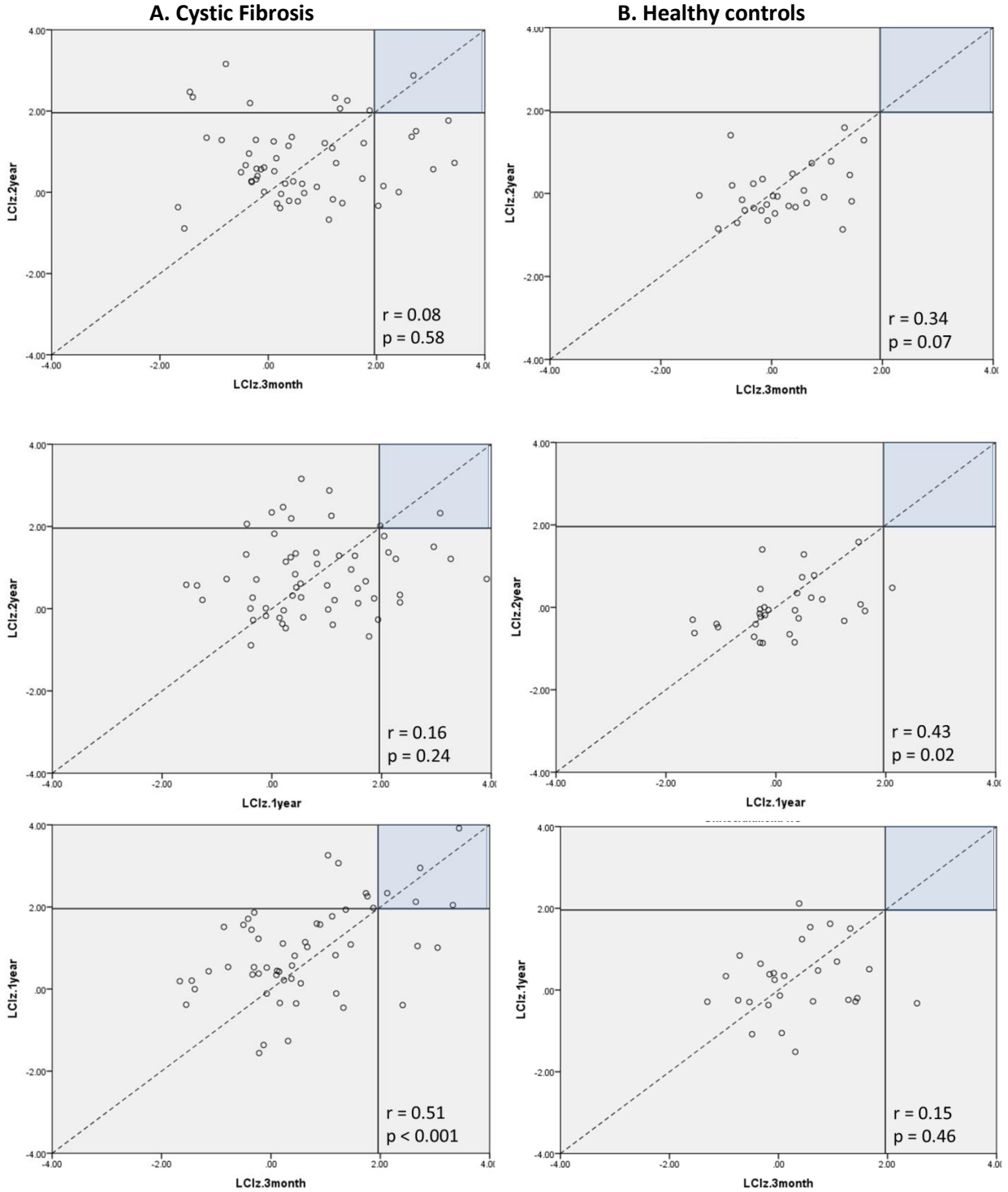
Table E5: Comparison of changes (Δ) in lung function and nutritional outcomes over time in infants with cystic fibrosis (CF) and healthy controls (HC) between approximately 3 months and 1 year, and between 3 months to 2 years of age.

	3 months to 1 year			3 months to 2 years		
	Cystic Fibrosis	**Healthy controls**	**Difference in change (95% CI)**	**Cystic Fibrosis**	**Healthy controls**	**Difference in change (95% CI)**
Interval (weeks)	41.6 (5.0)	41.5 (3.8)	0.1 (-1.9 to 2.1)	83.7 (7.8)	83.7 (7.7)	0.1 (-3.3 to 3.4)
Δ zHeight	0.75 (0.70)	-0.07 (0.71)	**0.82 (0.51 to 1.13) ****	0.69 (0.89)	-0.1 (0.91)	**0.79 (0.41 to 1.18)****
Δ zWeight	1.30 (0.80)	0.46 (0.79)	**0.84 (0.49 to 1.19) ****	1.21 (1.02)	0.46 (0.84)	**0.75 (0.34 to 1.16)****
Δ zBMI	1.23 (0.89)	0.68 (0.92)	**0.55 (0.15 to 0.95) ****	1.12 (1.15)	0.67 (1.22)	0.45 (-0.06 to 0.95)
Δ zLCI	0.33 (1.19)	-0.10 (1.14)	0.42 (-0.12 to 0.97)	0.16 (1.51)	-0.16 (0.83)	0.33 (-0.27 to 0.93)
Δ $zFRC_{pleth}$	0.03 (1.19)	0.29 (1.59)	-0.26 (-0.91 to 0.39)	0.08 (1.38)	0.42 (1.58)	-0.34 (-1.05 to 0.36)
Δ $zFEV_{0.5}$	0.36 (0.97)	-0.02 (0.89)	0.38 (-0.04 to 0.80)	0.46 (1.13)	-0.23 (1.11)	**0.70 (0.17 to 1.23)***

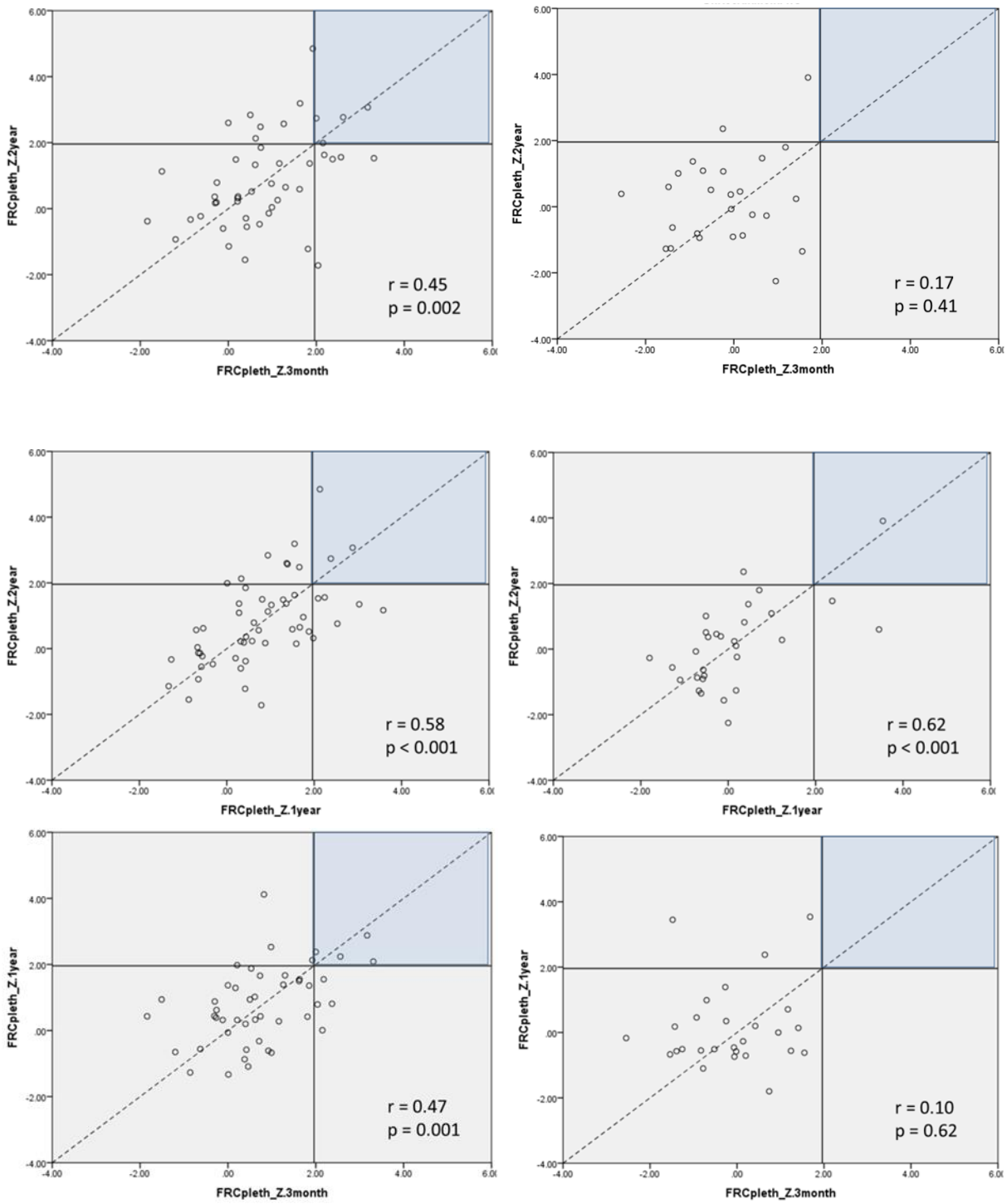
Footnote: Results are presented as the mean (SD) change in z-score for each outcome, between testing at approximately 3 months and either 1 or 2 years of age. *p<0.05, **p<0.01. For number of subjects with each outcome, see table E2. The comparison of changes in lung function and nutritional outcomes over time in infants with Cystic Fibrosis and healthy controls during the second year of life is presented in the main MS (Table 2).

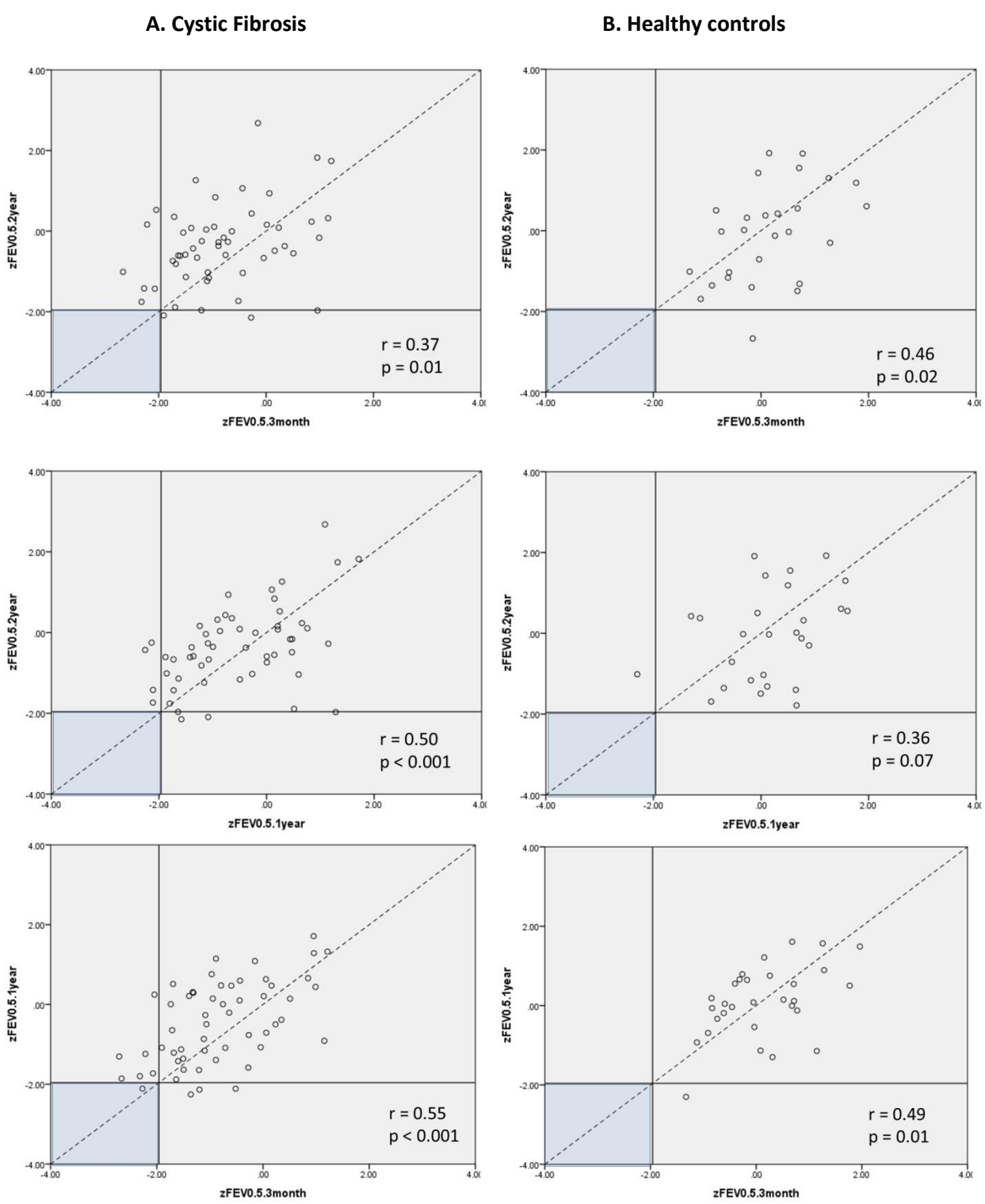
The association of lung function results between different test occasions for each of the primary outcomes is shown in Fig E1. In contrast to the highly significant relationship in both FRC_{pleth} and $FEV_{0.5}$ across all test occasions in infants with CF, LCI at 2yr was not predicted by that measured at either 3mth or 1yr. Of note, the majority of ILF results for CF infants remained within the normal range at both 1 and 2yrs. Of the 10 CF infants with an abnormal LCI at 1yr, all but two had a result within the normal range by ~2yr (Fig 3 and E1). Similarly, only 2/9 infants with abnormal LCI at 2yr also had an abnormal 1yr result. No child had an abnormal LCI on all three occasions (Table 3, main manuscript). Similarly, the majority of CF infants with abnormal FRC_{pleth} at 2yrs had had normal results at 1yr and vice versa (Fig 3 and E1) with only two children having abnormal $zFRC_{pleth}$ on all test occasions. No child with abnormal $zFEV_{0.5}$ at ~2yr had had abnormal results at either 3mth or 1yr (Fig 3 and E1).

Figure E1. Relationship of results between test occasions within each lung function outcome in infants and young children with CF (A) and in healthy controls (B).



A. Cystic Fibrosis **B. Healthy controls**



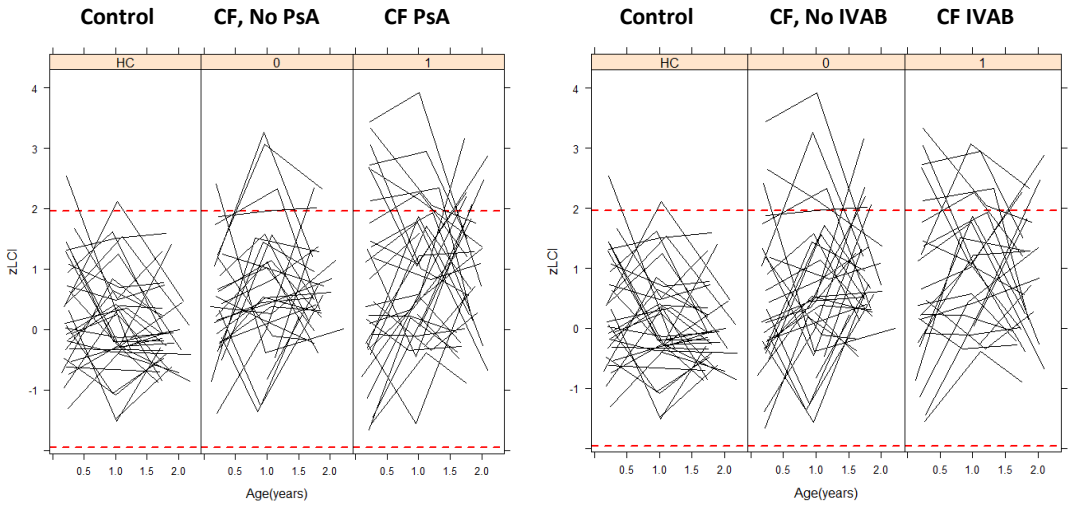


Legend: Relationship of LCI, FRC_{pleth}, and $FEV_{0.5}$ between various test occasions in children with CF and healthy infants. For each outcome, all subjects with results on both test occasions are

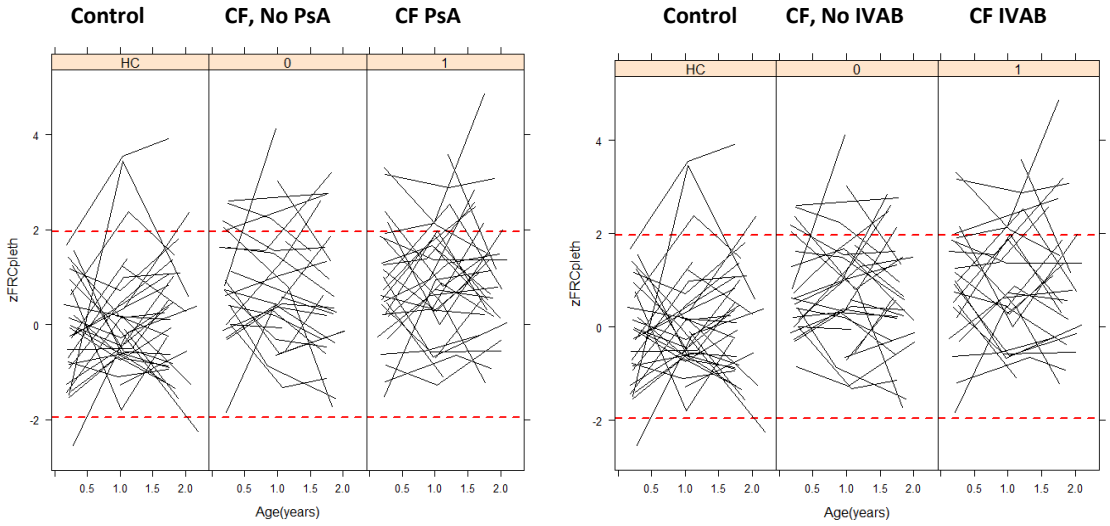
represented by an individual data point. Limits of normality are shown at 1.96 z-scores for LCI and $FRC_{pleth,}$ and -1.96z for $FEV_{0.5}$. The between-test equivalence line is shown on each cross-plot as a dashed line. For LCI and FRC_{pleth}, all values to the right of the vertical line or above the horizontal cut-off were abnormal. Those in the right upper shaded quadrant were abnormal on both occasions (e.g. n=2 subjects with CF for LCI and n=3 with CF for FRC_{pleth} at 2yrs). For $FEV_{0.5}$, values to the left of the vertical line or below the horizontal cut-off were abnormal. The one CF infant with abnormal $FEV_{0.5}$ at both 3 months and 1 year appears in the shaded left lower quadrant.

Fig E2. Within-subject variability for infant lung function outcomes in healthy controls, and for CF infants according to whether they had ever isolated *Pseudomonas aeruginosa*, or received any IV antibiotics by their 2yr infant lung function test.

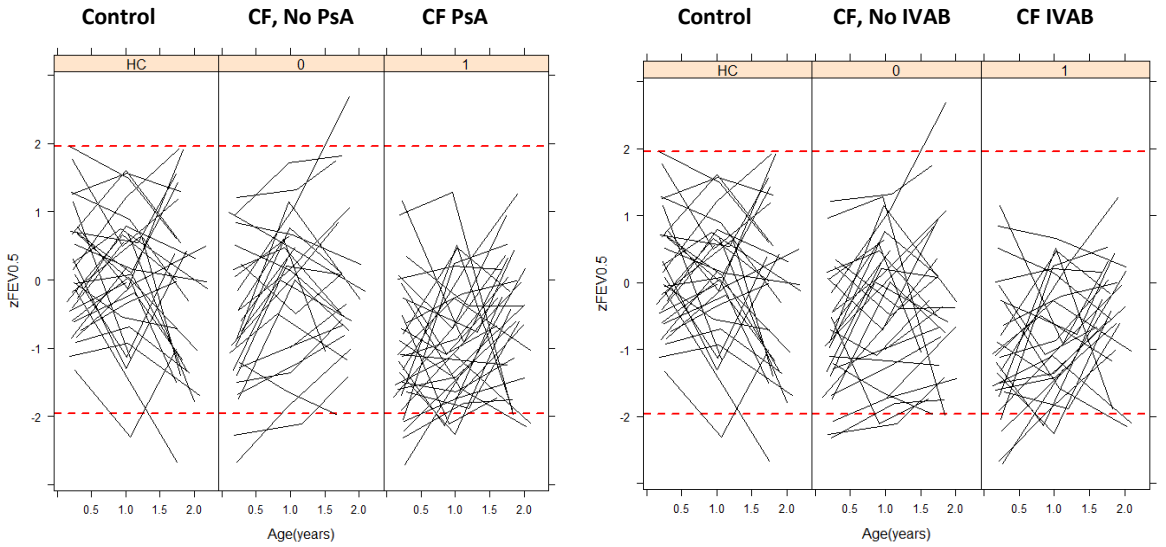
LCI



FRC_{pleth}



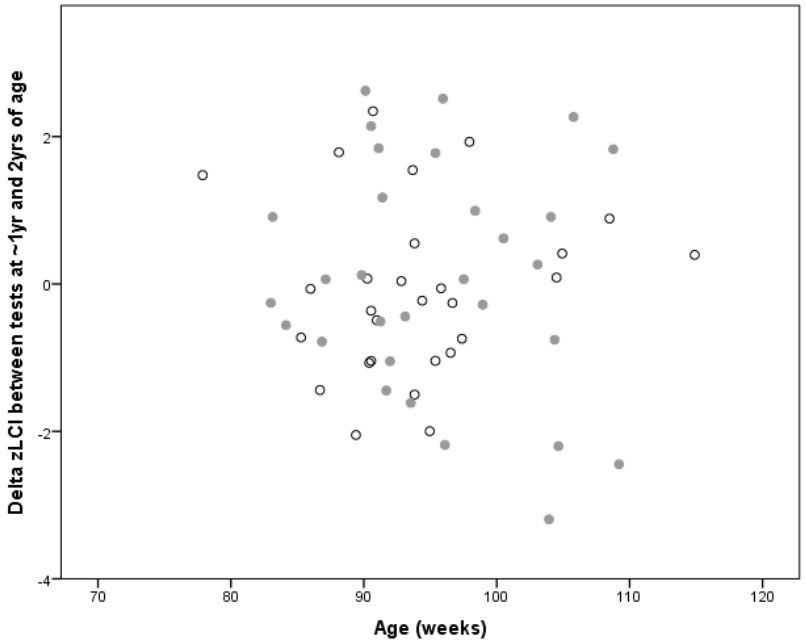
$FEV_{0.5}$



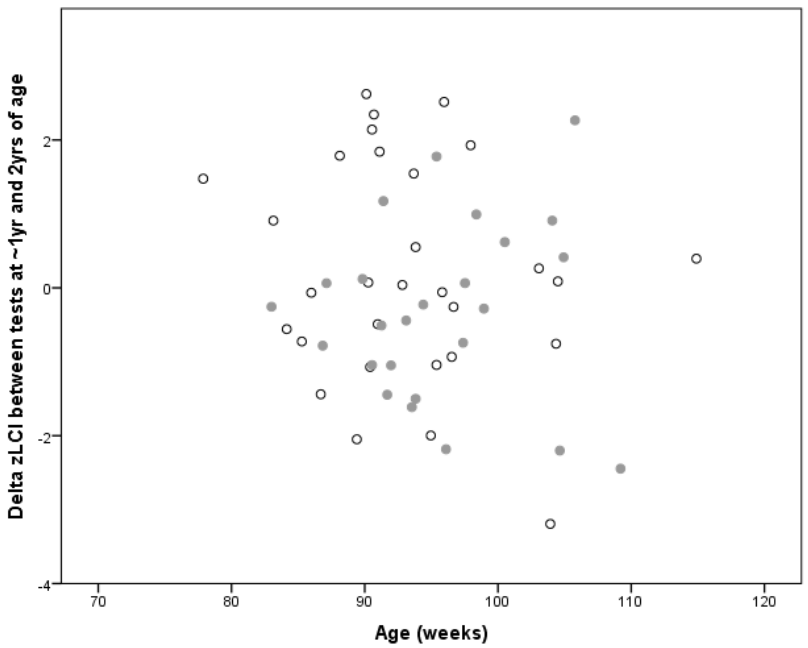
Legend: Each subject is represented by an individual line. Z-scores for each infant lung function (ILF) test (LCI, FRC_{pleth} and $FEV_{0.5}$) are plotted against actual age at test. Limits of normality are represented by the dashed lines at +/- 1.96 z-scores. For infants with cystic fibrosis, plots are separated according to *Pseudomonas aeruginosa* (PsA) status (left hand panel) or whether they had ever received intravenous antibiotics (IVAB) (right hand panel) by their 2yr lung function test at ~2 years. Infants with CF were considered 'CF PsA' if they had ever isolated PsA in culture by their 2yr ILF. Control infants are represented on the left of both panels for ease of comparison. As can be seen, while there was a tendency for those with an abnormally high LCI during the first year of life to improve by 2 years, whereas most infants with abnormal LCI by 2 years had results within the normal range previously, no clear pattern was evident.

Fig E3. Change in LCI between ~1 and ~2yrs of life plotted against age at final (~2yr) test, classified according to Pseudomonas aeruginosa status (A), or treatment with at least one course of intravenous antibiotics by final lung function test (B).

A. *Pseudomonas aeruginosa* status



B. Intravenous antibiotics



Legend: CF infants with LCI measurements at 1 & 2yrs of age are represented with one data point per subject. NOTE: In contrast to spirometric outcomes, an increase in zLCI between tests is suggestive of deterioration whereas a decrease is suggestive of improvement. Infants with CF are classified according to *Pseudomonas aeruginosa* (PsA) status (A), or treatment with at least one course of intravenous (IV) antibiotics by final lung function test (B). In plot A, Infants with CF but no isolations of PsA by their ~2year lung function tests are represented by open circles. Infants isolating PsA on at least one occasion by their final test are represented by grey filled circles. In plot B, infants with CF with no history of ever receiving IV antibiotics by their ~2year lung function tests are represented by open circles. Infants with at least one course of IV antibiotics by their final test are represented by grey filled circles.

There was no relationship between the magnitude or direction of change in LCI between ~1 and ~2 years of life and age at which the final (~2yr) test was performed, nor with either *Pseudomonas aeruginosa* status or history of IV antibiotics by the time of the final ILF visit (Fig E3 and Table E6). While the improvement in $FEV_{0.5}$ during the first year of life was significantly greater in CF infants who did not isolate PsA during this period, than in those that did, catch up in the latter group was faster during the second year of life (Table E6).

Table E6. Comparison of *changes* (Δ) in lung function over time in infants with cystic fibrosis (CF) between test dates according to history of *Pseudomonas aeruginosa* (PsA), (A) or intravenous (IV) antibiotics (B) by final (~2year) infant lung function test.

***A) Pseudomonas aeruginosa* (ever by 2yr test)**

	3months to 1year		
	CF PsA	**CF No PsA**	**Difference in change (95% CI)**
Δ zLCI	0.17(1.12), n=29	0.50(1.28), n=25	-0.33(-0.98 to 0.32)
Δ $zFRC_{pleth}$	0.07(1.16), n=26	0.00(1.24), n=21	0.07 (-0.63 to 0.78)
Δ $zFEV_{0.5}$	0.11(1.07), n=30	0.64(0.76), n=26	**-0.53(-1.03 to -0.02)***
	1 year to 2 years		
	CF PsA	**CF No PsA**	**Difference in change (95% CI)**
Δ zLCI	0.08(1.56), n=30	-0.09(1.16), n=28	0.17(-0.56 to 0.90)
Δ $zFRC_{pleth}$	0.11(1.25), n=30	-0.09(0.98), n=24	0.20(-0.43 to 0.82)
Δ $zFEV_{0.5}$	0.38(1.16), n=30	-0.05(0.82), n=24	0.43(-0.13 to 0.99)
	3months to 2 years		
	CF PsA	**CF No PsA**	**Difference in change (95% CI)**
Δ zLCI	0.05(1.76), n=29	0.28(1.20), n=27	-0.23(-1.04 to 0.59)
Δ $zFRC_{pleth}$	0.34(1.35), n=25	-0.22(1.37), n=22	0.56 (-0.24 to 1.36)
Δ $zFEV_{0.5}$	0.43(1.25), n=29	0.51(1.00), n=24	-0.08 (-0.71 to 0.55)

B. IV antibiotics (ever by 2 yr test)

	3months to 1year		
	CF IVABs	**CF No IVABs**	**Difference in change (95% CI)**
Δ zLCI	0.48 (1.09), n=23	0.29 (1.30), n=27	0.19 (-0.50 to 0.88)
Δ $zFRC_{pleth}$	-0.02 (1.20), n=22	0.11 (1.08), n=22	-0.14 (-0.83 to 0.56)
Δ $zFEV_{0.5}$	0.22 (1.07), n=25	0.51 (0.90), n=27	-0.29 (-0.84 to 0.27)
	1 year to 2 years		
	CF IVABs	**CF No IVABs**	**Difference in change (95% CI)**
Δ zLCI	-0.33 (1.22), n=25	0.2 (1.48), n=30	-0.53 (-1.27 to 0.22)
Δ $zFRC_{pleth}$	0.04 (1.28), n=24	-0.03 (1.07), n=27	0.07 (-0.59 to 0.73)
Δ $zFEV_{0.5}$	0.41 (1.02), n=24	0.05 (1.07), n=26	0.36 (-0.24 to 0.96)
	3months to 2 years		
	CF IVABs	**CF No IVABs**	**Difference in change (95% CI)**
Δ zLCI	-0.10 (1.59), n=24	0.42 (1.51), n=28	-0.52 (-1.39 to 0.34)
Δ $zFRC_{pleth}$	0.18 (1.27), n=21	0.01 (1.45), n=22	0.18 (-0.67 to 1.02)
Δ $zFEV_{0.5}$	0.58(1.13), n=24	0.43(1.18), n=25	0.16 (-0.51 to 0.82)

Footnote: Results are presented as the mean (SD) change in z-score for each outcome, between testing at approximately 3 months and 1 year, 1 year to 2 years, and 3months to 2 years of age. Numbers (n) for each unpaired t-test comparison are shown. *$p<0.05$.

Sample calculations for randomised control trials (RCTs).

The results from the current study indicate that, contrary to our previous suggestions [E6, E7], when studying a NBS cohort of infants with CF managed according to standard UK protocols, it is not possible to identify infants who are at high-risk ' for poor lung function by 2 years of age for selective recruitment into an RCT. The impact that this would have on power calculations for studies intending to use infant LFT as an outcome variable in the first 2 years of life is explained below in two excerpts from Nguyen et al Thorax 2014, which presented results from this cohort at 1yr of age.

Excerpt from discussion in main MS: Nguyen et al 2014 [E7]

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.

From Nguyen et al 2014; OLS Section e [E7]

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 FRCpleth and 15% or 46 mL for FEV0.5. Decisions regarding what constitutes a minimal clinically important difference in intervention trials are complex, but values equating to at least 0.5 SD (or z16 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 FEV0.5), 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.

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