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

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Key Words:	cystic fibrosis, newborn screening for cystic fibrosis, infant pulmonary function testing, lung clearance index, plethysmography, raised volume technique

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

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**Author contributions:**

JS, AB, AW and IBL were responsible for the conception and design of the study; JS and AFH were responsible for supervision of the study and together with JC, for research governance issues including ethics committee approval. Infants with CF were recruited by the paediatric respiratory consultants participating in the LCFC, including AB, IBL, SC, HW, CW and PA. AFH, JC LPT and LB recruited the healthy infants, AFH, JM, LB and LPT undertook lung function measurements and analysis. GD, JK, SL and JS, calculated and interpreted lung function results. PC, SLee and SL managed the research database. GD and AW performed statistical analyses; GD, JS, AW and AB drafted the manuscript; all remaining authors revised and approved the manuscript for intellectual content before submission.

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**120 character summary:** Lung function changes in newborn screened infants with Cystic Fibrosis are mild and transient during the first 2 years of life.

**Word count:** 3716

This article has an online data supplement.

ABSTRACT

With the advent of novel designer molecules for cystic fibrosis (CF) treatment, there is huge need for early life clinical trial outcomes, such as infant lung function (ILF). We investigated the degree and tracking of ILF abnormality during the first two years of life in CF newborn screened infants.

Forced Expiratory Volume (FEV<sub>0.5</sub>), lung clearance index (LCI), and plethysmographic functional residual capacity (FRC<sub>pleth</sub>) were measured at ~3months, 1yr and 2yrs in 62 infants with CF and 34 controls.

By 2yrs there was no significant difference in zFEV<sub>0.5</sub> between CF and controls, whereas mean LCI z-score (zLCI) was 0.81(95% CI: 0.45;1.17) higher in CF. However, there was no significant association between zLCI at 2yrs with either 3month or 1yr results. Despite minimal average group changes in any ILF outcome during the second year of life, marked within-subject changes occurred. No child had abnormal LCI or FEV<sub>0.5</sub> on all test occasions, precluding the ability to identify ‘high-risk’ infants in early life.

In conclusion, changes in lung function are mild and transient during the 1<sup>st</sup> 2yrs of life in newborn screened infants with CF when managed according to a standardised UK treatment protocol. Their potential role in tracking disease to later childhood will be ascertained by ongoing follow up.

Abstract word count: 206

**Key words:** Cystic fibrosis, newborn screening, infant lung function testing, lung clearance index, plethysmography, raised volume technique.

INTRODUCTION

The advent of gene mutation class specific therapies for cystic fibrosis (CF) and demonstration of their efficacy in older children and adults has led to demands to introduce them in infancy, before significant lung damage occurs. However, if such trials are to be undertaken, highly sensitive trial end-points are needed to monitor both for efficacy and safety during this vulnerable period of rapid lung growth. A recent European Cystic Fibrosis Society Clinical Trial Network consensus concluded that infant lung function (ILF) testing using the raised volume rapid thoracic compression (RVRTC) technique should not be used as a primary outcome in clinical trials of infants with CF until further evidence is available[1]. Similar recommendations have been made with respect to infant multiple breath washout (MBW)[2].

The London Cystic Fibrosis Collaboration (LCFC; (<http://www.ucl.ac.uk/london-cystic-fibrosis>)) is following up a newborn screened (NBS) CF cohort and contemporaneous controls. We have previously reported that at 3 months, Forced Expired Volume in 0.5 seconds (FEV<sub>0.5</sub>) was reduced in 25%(17/68) of infants while Lung Clearance Index (LCI) was elevated in 21%(15/70)[3]. ILF at 1yr was predicted by that at 3mth and impressively FEV<sub>0.5</sub> improved over the first year of life with standard UK treatment[4]. We suggested that those infants with abnormal ILF (e.g. an elevated LCI) in early life may be a sub-group in whom specific treatment interventions would make the greatest difference. Yet if innovative therapies with the potential to modify disease are to be deployed, there must be both clinical need and the ability to assess outcome. If our observed improvement in ILF was sustained between 1-2yrs with standard treatment alone in NBS infants, this would have

significant implications for clinical trial design involving novel treatments for CF in early life when the developing lung is potentially at its most vulnerable[5].

The aim of this observational study was to investigate the tracking of ILF in the first 2 years of life in CF NBS infants managed with standard UK therapy, and consider the implications of this in relation to use of such outcomes as endpoints in clinical trials conducted over this time period. Based on our previous findings at 1yr, we hypothesised that group stability of ILF would be maintained to 2yrs in NBS CF infants. Some results presented here have been published as abstracts[6-8].

## MATERIAL AND METHODS

Full details of both the recruitment of the LCFC cohort of infants with CF diagnosed by NBS and contemporaneous healthy controls, and the prospective observational study design have been published[3, 4]. In brief, 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. Healthy controls were recruited contemporaneously from Homerton University Hospital, East London. Infants were ineligible if born <36 weeks gestation or had coexisting congenital abnormalities. 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 at each subsequent clinic visit. CF infants were started on multivitamins and vitamin E, pancreatic enzyme replacement therapy where appropriate and, in accord with UK CF Trust guidelines, oral prophylactic flucloxacillin, according to a standardised treatment protocol[4]. All subjects in the LCFC NBS CF cohort attending

between 1.5- 2.4yrs with a previous visit at 3mths and/or 1yr were included in this 2yr follow-up. Subjects were free from acute respiratory tract infection for at least 3 weeks prior to testing. MBW, plethysmography and RVRTC were performed on each test occasion at the ILF laboratory at UCL Great Ormond St Institute of Child Health as described previously[3, 4]. The main ILF outcomes were LCI, plethysmographic functional residual capacity (FRC<sub>pleth</sub>) and FEV<sub>0.5</sub>. Oral sedation with chloral hydrate (60-100mg/kg; maximum 1000mg) was administered prior to each occasion. MBW results were analysed using custom-made software (P.Gustafsson, version 2012). ILF data were electronically exported to a research database (Re-Base software, Re-Base, UK) where data for each child were linked according to test occasion with demographic and clinical information. ILF outcomes were converted to z-scores using published reference equations derived from healthy infants and young children using identical equipment and protocols[9-11]. Clinical information was collected at routine clinical visits and at each test occasion, including history of intravenous antibiotics and airway microbiology results (from cough swabs or from bronchoalveolar lavage at 1yr).

*Statistical analysis*

Differences between control and CF groups at each test occasion were compared using unpaired t-tests. We planned to study at least 60 NBS CF children and 30 healthy controls at ~2yrs of age to provide 80% power to detect differences of at least 0.725 z-scores in the three primary ILF outcomes (90% power to detect differences of 0.825 z-scores) at the 5% significance level.

Individual line plots over time were used to illustrate change within-subject between-tests. Paired t-tests were used to quantify average change between any two test occasions within

groups and the SD to quantify variability of those changes. Unpaired t-tests were used to compare change over time between groups. To further investigate tracking of ILF in children with CF, abnormalities on each test occasion were defined as  $>1.96z$ -scores for LCI and  $FRC_{pleth}$  or  $\leq -1.96z$ -scores for  $FEV_{0.5}$ .

Regression was used to investigate any relationship between change in ILF and interval between tests. To investigate change over time according to disease status and treatment severity, CF subjects were grouped according to whether they had received intravenous antibiotics or isolated relevant pathogens (*Pseudomonas aeruginosa* (PsA), *Staphylococcus aureus* (SA) or *Haemophilus influenzae* (HI)) in respiratory culture by the time of their 2yr test. Sample estimates (differences between groups, changes over time) are presented with 95% confidence intervals (CI) to facilitate interpretation.

## RESULTS

62 NBS CF infants and 34 healthy controls with prior ILF results had tests repeated at ~2yrs of age. The study population is summarised in Table E1 (online supplement, OLS). The proportion of subjects with technically satisfactory ILF results at each test occasion ranged from 77-98% (Table E2, OLS). Results presented here focus on the relationship between ILF results at 1 and 2yrs, as tracking between 3mths to 1yr has been published[4].

### ***Cross sectional data***

Cross-sectional results for both CF and healthy infants at 2yrs are summarised in Table 1, with results at 3mths and 1yr in Table E3(OLS). At 1yr, there were significant differences between CF and control groups for all three primary LF outcomes (Table E3b). However by

2yrs, there was no significant difference in FEV<sub>0.5</sub> between CF and controls. Over the same time period, differences between CF and controls increased slightly by 0.12 z-scores for LCI, with a mean (95% CI) difference of 0.81(0.45;1.17) z-scores between groups by 2yrs, (Fig 1 and Table 1) whereas difference in zFRC<sub>pleth</sub> remained stable. The impact of using recently updated published RVRTC reference equations is summarised in the OLS.

**Change over time**

There was no significant group change over time for any ILF outcome in controls. By contrast, as reported previously[4], there was a significant improvement in FEV<sub>0.5</sub> in CF infants, particularly in the first year of life (Table E4). A comparison of changes in lung function during the second year of life between CF and controls is summarised in Table 2. While mean changes in ILF were minimal during the second year of life, the relatively wide SDs reflect marked within-subject change between test occasions.

**‘Abnormal’ lung function**

The proportion of CF infants with ‘normal’ or ‘abnormal’ results for each ILF outcome at each test occasion is shown in Table 3. At the time of the 2yr test, abnormal results were only detected in 15%(9/61) of CF infants for LCI, 19%(11/57) for FRC<sub>pleth</sub> and 7%(4/56) for FEV<sub>0.5</sub>. No child had abnormalities in all three outcomes on any test occasion. No child had an abnormal LCI or FEV<sub>0.5</sub> on all test occasions, and only 2/44(5%) CF infants had an abnormal FRC<sub>pleth</sub> on all test occasions.

The association of results between different test occasions within each of the primary ILF outcomes is shown in Fig E1. In contrast to the highly significant relationship in both FRC<sub>pleth</sub> and FEV<sub>0.5</sub> across all test occasions in infants with CF, LCI at 2yr was not predicted by that measured at either 3mth or 1yr. Of the 10 CF infants with an abnormal LCI at 1yr, all but two

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3 had a result within the normal range by ~2yr (Fig E1). Similarly, only 2/9 infants with  
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5 abnormal LCI at 2yr also had an abnormal 1yr result.  
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### 8 ***Clinical status***

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11 Line plots demonstrating change over time of zLCI at the individual level are shown in Fig 2,  
12  
13 with CF infants separated according to PsA status. Similar plots for other ILF outcomes are  
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15 shown in Figure E2(OLS), along with a comparison of results according to whether infants  
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17 had ever received IV antibiotics. Considerable within-subject change in LCI occurred  
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19 between tests even in health and the magnitude of such change was not related to either  
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21 the isolation of PsA or treatment with IV antibiotics by the final ILF test in those with CF (Fig  
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23 2, Fig E2 and Table E6(OLS)). The lack of relationship between the magnitude or direction of  
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25 change in LCI with respect to PsA status or treatment with IV antibiotics is also illustrated in  
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27 Figure E3(OLS). A similar pattern was observed for FRC<sub>pleth</sub> and FEV<sub>0.5</sub>, with the exception of  
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29 the greater improvement in FEV<sub>0.5</sub> during the 1<sup>st</sup> year of life in infants who did not isolate  
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31 PsA by their final ILF test (Table E6(OLS)). Similarly, the magnitude or direction of change  
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33 between tests was not related to whether or not infants had isolated *either* SA or HI, or *any*  
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35 major CF Pathogen (PsA, SA or HI) by 2yr (data not shown). Results of regression analysis  
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37 revealed no significant association between magnitude of within-subject, between-test  
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39 change in ILF and interval between tests.  
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## 51 **DISCUSSION**

### 52 **Summary of main findings**

We report for the first time that in a NBS CF cohort treated with UK standardised therapy, there was no significant difference in FEV<sub>0.5</sub> between healthy controls and CF infants by 2yrs of age. In contrast to reports by the Australian Respiratory Early Surveillance Team for CF (AREST–CF)[12], NBS CF babies in our cohort did not experience deteriorating lung function over the first 2yrs of life. While mean zLCI and zFRC<sub>pleth</sub> were significantly higher in CF infants than controls at 2yrs, neither increased significantly during the second year of life. Those individuals who did exhibit abnormal ILF at any one point often reverted to normality on subsequent testing, suggesting that during infancy such changes are reversible rather than progressive. Within our NBS CF cohort we could not identify any individual with consistently abnormal LCI or FEV<sub>0.5</sub> who could thus be preferentially selected to receive novel therapies. In contrast to the relationship between 3mth and 1yr results previously reported in this cohort [4], there was no significant association between zLCI at 2yrs and that at either 3mth or 1yr.

**Strengths and limitations**

The strengths of this study include prospective recruitment of the LCFC NBS cohort and contemporaneous controls, allowing unique insights into the impact of CF on early life pulmonary function. Without controls, an understanding of change over time would be challenging. Results were interpreted using z-scores from recently published reference equations, derived using identical equipment and methodology[9-11]. Inclusion of a control group, who were similar in terms of body size, also ensured that any changes in the physiological dead space to tidal volume ratio over the study period would not influence interpretation of LCI results. The equipment and techniques for measuring ILF remained standardised and constant throughout the study period, as did the sedation protocol.

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3 Technical success rates for ILF tests in our NBS LCFC cohort continue to be better than other  
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5 longitudinal studies involving infants with CF (as recently summarised by the European CF  
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7 Society Clinical Trial Network)[1], particularly when compared with those which involved  
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9 testing across multiple centres[13].  
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12 A range of ILFTs were performed to reflect the various aspects of pathophysiology most  
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14 commonly reported in CF lung disease, with the primary outcomes of LCI, FRC<sub>pleth</sub> and FEV<sub>0.5</sub>  
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16 selected *a priori* to avoid any risk of data dredging or misinterpretation due to use of an  
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18 excessive number of measurements. We have not reported the tidal breathing ratio, having  
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20 previously determined that it is not useful in identifying diminished airway function in  
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22 infants with CF, nor respiratory rate, which is poorly predictive of diminished airway  
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24 function in this population[14]. While rarely reported, between-test variability within our  
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26 control group is in keeping with that in older children, variability of up to 1.2z scores in FEV<sub>1</sub>  
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28 being recorded in 5-11 year olds over the course of a year[15]. Using an observational study  
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30 design, Davis et al did not recommend inclusion of FEV<sub>0.5</sub> or FRC<sub>pleth</sub> measured during infancy  
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32 as a primary efficacy endpoint in clinical trials due to within-subject variability between  
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34 tests, technical challenges and the requirement for large sample sizes to detect efficacy [13].  
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42 Judgements regarding the clinical usefulness of any biomarker requires an assessment of its  
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44 reliability, validity and responsiveness, as summarised in a recent review [16]. However,  
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46 objective assessments of surrogate measures such as lung function require a different  
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48 approach, particularly when such measurements are being undertaken in infants. Sedation  
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50 is required for most infant lung function tests, administration of which is not advised either  
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52 in the presence of an exacerbation or at frequent intervals. Furthermore, sedation may  
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54 cause at least temporary disruption of sleep patterns and the duration of these tests (up to  
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3-4 hours including the period to induce sleep) can place a real burden on parents if they are expected to attend for follow up visits more frequently than 6 monthly, a factor which would reduce compliance in any clinical trial. It is therefore extremely difficult to use ILFTs to assess *acute* response to either exacerbations or treatment reliably. However, by undertaking repeated measurements at approximately 9 monthly intervals during periods of clinical stability, information regarding the magnitude of any changes and extent to which ILF tracks during the first 2 years of life in NBS infants with CF managed on standard care can be obtained, knowledge of which is vital if planning to use such tests in future clinical trials.

In addition to natural variability observed in health, improvement of LF by 2yrs following earlier ‘abnormalities’ in CF infants could reflect treatment intensification in the interim, either in response to symptomatic deterioration or following the identification of pathogens such as PsA. The grouping of infants into those that had and had not isolated PsA or been treated with IV antibiotics was simply to reflect relative disease burden, rather than an attempt to relate ILF to specific clinical events or interventions. Although our study was not designed to determine the dynamic effect of exacerbations or treatment response on ILF, neither the isolation of PsA nor treatment with intravenous antibiotics by 2yrs was associated with the magnitude of ILF variability over this period. The influence of early life exacerbations on childhood lung function may however become detectable at subsequent follow-up [17].

Conclusions from a study such as this might differ in regions with differing treatment protocols, prevalence of CF gene mutations or modifier genes, or environmental exposures. The antibiotic protocols used were defined primarily to standardise care between the LCFC centres in line with current UK practice rather than reflecting any evidence that antibiotic

prophylaxis would impact favourably (or otherwise) on ILF outcomes. Ideally study clinicians would have remained blinded to results, but this was not considered to be ethical in an observational study such as this. While this could be viewed as a potential weakness, given the complete overlap in direction and variability of change in ILF between those with and without prior IV antibiotics or CF pathogens such as PsA, this is unlikely to have influenced our findings. In our study, PsA had been isolated on at least one occasion in 32 (52%) by the time of the 2yr test, though only 4 infants had any evidence of chronic infection. The relatively high frequency of 'PsA ever' by 2years of age is in keeping with recent reports and reflects the fact that this cohort were under close surveillance, PsA being isolated not only from the 1yr BAL but from regular cough swabs throughout the study period. . However, we also accept that diagnosis of lower respiratory infection status in infants is difficult, and incorrect classification remains a possibility.

We performed high resolution computed tomography (HRCT) scans at one year of age, but the changes were so mild, and the variability between observers consequently so great[18], that we did not feel ethically justified in repeating the scan at 2yr unless clinically indicated for an individual; the continued relative normality of lung function is supportive of that decision.

### **Clinical significance**

Our study design was a pragmatic means of informing future clinical trials in that it provides evidence both on the magnitude of ILF abnormalities that might be expected on standard therapy, as well as the extent to which such changes track during the first 2 years of life, in exactly the group of infants who might be recruited to an intervention study. Without

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3 observational data it would be difficult to design a study to evaluate effects of any novel,  
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5 medium-term interventions. This evidence is strengthened by our ability to interpret results  
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7 in relation to the normal variability that occurs in health, since we not only had a  
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9 contemporaneous control group but reference equations derived using identical methods  
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11 and equipment. The clinical importance of ILF tests in CF lung disease will be clarified by the  
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13 continuing follow-up of this cohort into the preschool years[19], which will establish  
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15 whether there is evidence of tracking over a longer time period. Although mean LCI was  
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17 static over the second year of life in our cohort, the small increase in z-scores in comparison  
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19 to controls may represent a degree of deterioration at the group level which may become  
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21 more apparent with subsequent follow-up. The importance of physiological outcomes  
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23 during the preschool years is already recognised, as results can reflect subsequent status at  
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25 school age[20], and a recent study has confirmed that LCI is a useful marker to track early  
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27 disease progression in preschool children with CF [21].  
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29 Clinical efficacy of treatment interventions assessed according to functional trial endpoints  
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31 is possible to demonstrate even when baseline results are within the normal range, as  
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33 illustrated by results from the phase III clinical trial of the cystic fibrosis transmembrane  
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35 regulator potentiator ivacaftor in children aged 6-11yrs with a G551D mutation[22].  
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37 However, in contrast to older children or possibly a clinically diagnosed infant cohort,  
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39 interventions aimed at reducing the rate of decline (rather than improvement) in FEV would  
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41 not be appropriate in an infant NBS population such as ours, since FEV<sub>0.5</sub> improved to  
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43 normal levels during the first two years of life with standard UK care alone. While attempts  
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45 could be made to diminish the mild elevations of LCI or FRC<sub>pleth</sub> observed in CF infants by 2  
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47 years of age, the transient nature of within-subject abnormalities at 3mth or 1yr in infants  
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3 treated with UK standard care alone calls into question the clinical relevance of this  
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5 approach.  
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9 As previously reported for our CF NBS cohort, 1yr ILF was predicted by results obtained from  
10  
11 testing at 3mths[4]. However the combination of relatively normal results at 2yrs and  
12  
13 marked bi-directional within-subject change over time (Fig E2 OLS), question the value of  
14  
15 modelling ILF outcomes at 2yrs. We therefore analysed results at 2yrs with respect to those  
16  
17 at 3mths and 1yr separately, rather than in a repeated measures analysis. Any long-term  
18  
19 value of ILF in terms of predicting later CF disease status will become clearer as our current  
20  
21 cohort is followed up through the preschool years and beyond. The transient nature of the  
22  
23 observed changes in lung function in our cohort is encouraging, but does mean that our  
24  
25 previous proposal to try to identify a 'high-risk' subgroup of NBS CF infants based on  
26  
27 'abnormalities' at either 3mths or 1yr of age is not feasible.  
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### 32 **Comparison with other studies**

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35 To our knowledge, the only other prospective longitudinal study reporting ILF outcomes in  
36  
37 NBS infants with CF is that from the AREST-CF group. Although both AREST-CF and the LCFC  
38  
39 detected deficits in ILF by around 3mths in such infants[3, 12, 23], results from these two  
40  
41 studies are widely discrepant at later time points. Instead of improvement to one year and  
42  
43 maintenance of ILF to 2 years as reported here, lung function appeared to deteriorate  
44  
45 significantly over this period in the AREST-CF infants (mean(SD) FEV<sub>0.5</sub> z-score being -  
46  
47 1.4(1.2), -2.4(1.1) and -4.3(1.6) at around 5mth, 1yr and 2yrs of age)[12]. Of note, however,  
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49 was the conversion of RVRTC outcomes to z-scores using historical control data collected  
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51 with different equipment[24], and the absence of a contemporaneous control group within  
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53 the AREST-CF study. Possible explanations for the differences between results from these  
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two NBS cohorts have recently been summarised by Bush and Sly[25]. Interestingly, continued longitudinal follow-up of the AREST-CF cohort has reported much better lung function at school-age than might be expected from their infant results[26]. Although our results contrast with those reported by AREST-CF, Davis et al also reported normal FEV<sub>0.5</sub> yet high within-subject change between ILF tests in CF infants participating in their inhaled hypertonic saline clinical trial[27]. Continued follow up of both the LCFC and AREST-CF cohorts will provide crucial insights to the natural history of CF lung disease, and allow evaluation of proposed predictors of abnormal lung function or structural changes on chest HRCT in later childhood.

**CONCLUSION**

We show, in contrast to previous studies from AREST-CF, that lung function as assessed by measurements of LCI, FRC<sub>pleth</sub> and FEV<sub>0.5</sub> is well preserved to 2 years of age in our cohort of NBS infants with CF managed with UK standard care. The transient nature of any abnormalities observed during this time period suggests that such changes may remain reversible during early life. Whether these results can be translated to other CF populations, such as infants not receiving prophylactic antibiotic therapy or undergoing regular surveillance by infant lung function, is unclear. Nevertheless, the relative normality of infant lung function to 2 years of age in this prospectively followed cohort of NBS CF infants managed with standard UK care is encouraging.

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## TABLES

**Table 1. Comparison of lung function and nutritional outcomes in infants with Cystic Fibrosis and healthy controls at approximately 2 years**

	Cystic Fibrosis	Healthy Controls	Difference (95% CI)	p
<b>N</b>	62	34		
<b>Age at test, weeks</b>	95.0 (7.3)	96.2 (7.7)	-1.24 (-4.39 to 1.92)	0.44
<b>zHeight</b>	0.48 (1.00)	0.71 (1.18)	-0.23 (-0.69 to 0.22)	0.31
<b>zWeight</b>	0.24 (0.92)	0.40 (0.86)	-0.16 (-0.54 to 0.22)	0.41
<b>zBMI</b>	-0.07 (0.92)	-0.03 (0.90)	-0.04 (-0.42 to 0.35)	0.85
<b>zLCI</b>	0.78 (0.93)	-0.03 (0.64)	0.81 (0.45 to 1.17)	<b>0.001</b>
<b>zFRC<sub>pleth</sub></b>	0.85 (1.30)	0.16 (1.28)	0.69 (0.11 to 1.26)	<b>0.02</b>
<b>zFEV<sub>0.5</sub></b>	-0.35 (1.00)	-0.14 (1.23)	-0.21 (-0.71 to 0.29)	0.41
<b>zFVC</b>	-0.19 (0.94)	0.04 (0.92)	-0.23 (-0.66 to 0.21)	0.3
<b>zFEF<sub>25-75</sub></b>	-0.41 (0.95)	-0.24 (1.15)	-0.17 (-0.64 to 0.3)	0.48

Footnote: Values are mean (SD). For number of subjects with each outcome at each test occasion see Table E2 (OLS). Although not primary outcomes, FVC and FEF<sub>25-75</sub> results are included in this table to allow comparison with the published literature.

Table 2: Comparison of changes ( $\Delta$ ) in lung function and nutritional outcomes over time in infants with Cystic Fibrosis and healthy controls during the second year of life.

	Cystic Fibrosis	Healthy Controls	Difference (95% CI): CF-HC
Test interval (weeks)	42.2 (7.8)	42.8 (8.2)	-0.6 (-4.1 to 2.8)
$\Delta z$ Height	-0.04 (0.47)	-0.05 (0.54)	0.01 (-0.2 to 0.22)
$\Delta z$ Weight	-0.08 (0.46)	-0.05 (0.43)	-0.03 (-0.22 to 0.16)
$\Delta z$ BMI	-0.10 (0.61)	-0.06 (0.78)	-0.05 (-0.34 to 0.24)
$\Delta z$ LCI	0.00 (1.37)	-0.15 (0.83)	0.15 (-0.39 to 0.69)
$\Delta z$ FRC <sub>pleth</sub>	0.02 (1.13)	0.01 (1.10)	0.01 (-0.5 to 0.52)
$\Delta z$ FEV <sub>0.5</sub>	0.19 (1.03)	-0.20 (1.19)	0.39 (-0.12 to 0.90)

Footnote: Results are presented as the mean (SD) *change* in z-score for each outcome, between testing at ~1yr and ~2yrs. For number of subjects with each outcome, see Table E2 (OLS). Similar comparisons of changes between 3mth to 1yr, and 3mth to 2yr, are shown in Table E5 (OLS).

**Table 3. Proportion of NBS CF infants with 'normal' or 'abnormal' results on each test occasion, and on more than one test occasion.**

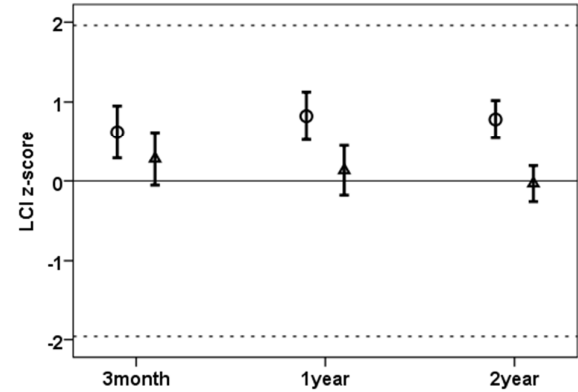
	3mth	1yr	2yr	3mth&1yr	3mth&2yr	1yr&2yr	All test occasions
<b>Total n with LCI</b>	57	59	61	54	56	58	53
<b>Normal LCI</b>	48 (84%)	49 (83%)	52 (85%)	41 (76%)	39 (70%)	41 (71%)	34 (64%)
<b>Abnormal LCI</b>	9 (16%)	10 (17%)	9 (15%)	5 (9%)	1 (2%)	2 (3%)	0 (0%)
<b>Total n with FRC<sub>pleth</sub></b>	50	59	57	47	47	54	44
<b>Normal FRC<sub>pleth</sub></b>	41 (82%)	49 (83%)	46 (81%)	35 (74%)	31 (66%)	38 (70%)	27 (61%)
<b>Abnormal FRC<sub>pleth</sub></b>	9 (18%)	10 (17%)	11 (19%)	4 (9%)	4 (9%)	3 (6%)	2 (5%)
<b>Total n with FEV<sub>0.5</sub></b>	59	58	56	56	53	54	52
<b>Normal FEV<sub>0.5</sub></b>	51 (86%)	54 (93%)	52 (93%)	46 (82%)	43 (81%)	46 (85%)	39 (75%)
<b>Abnormal FEV<sub>0.5</sub></b>	8 (14%)	4 (7%)	4 (7%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)
<b>Total n with results from all*</b>	47	57	54	44	41	51	39
<b>All Normal*</b>	27 (57%)	36 (63%)	35 (65%)	23 (52%)	14 (34%)	20 (39%)	12 (31%)
<b>All Abnormal*</b>	0 (%)	0 (%)	0 (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Footnote: \* All= LCI, FRC<sub>pleth</sub> and FEV<sub>0.5</sub>. 'Abnormal' and 'normal' defined on basis of 1.96 z score threshold for LCI and FRC<sub>pleth</sub>, and -1.96 for FEV<sub>0.5</sub>.

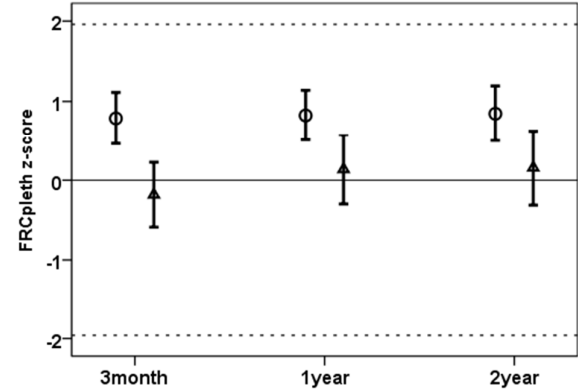
FIGURES

Figure 1. Lung function across the first two years of life in healthy infants and those with CF.

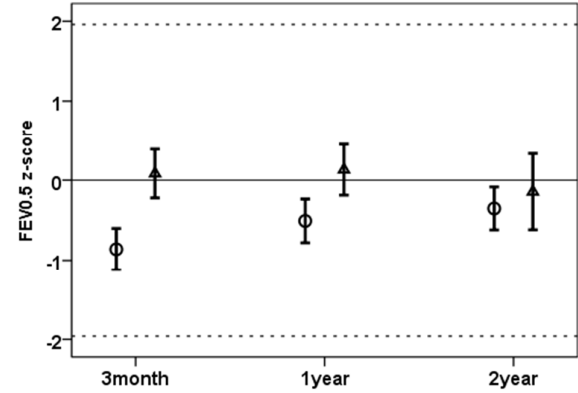
A. LCI



B. FRC<sub>pleth</sub>

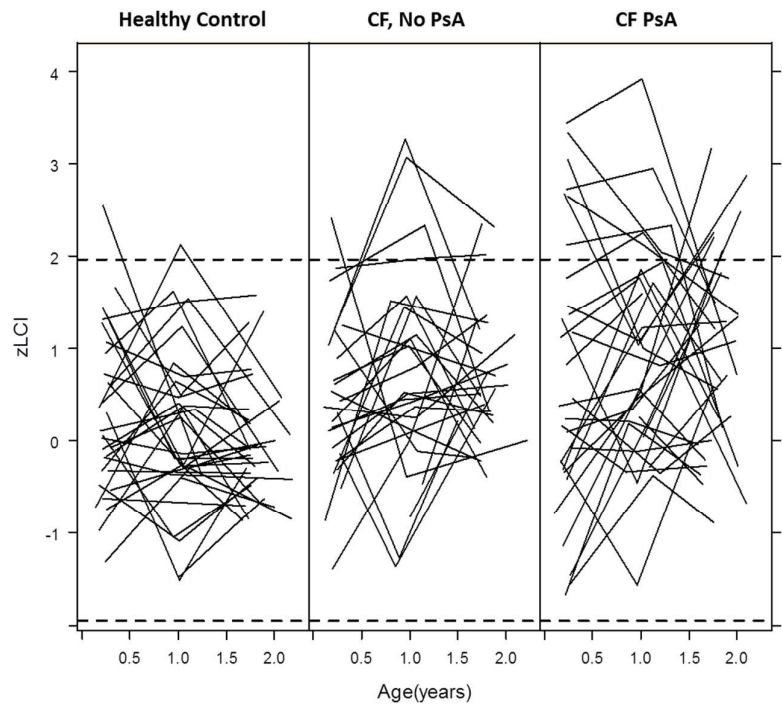


C. FEV<sub>0.5</sub>



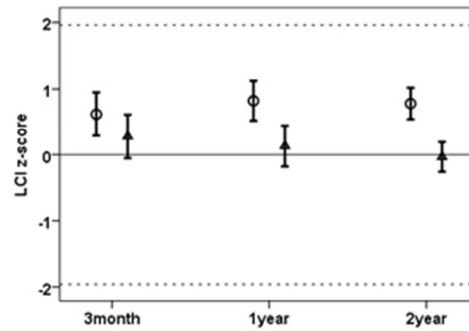
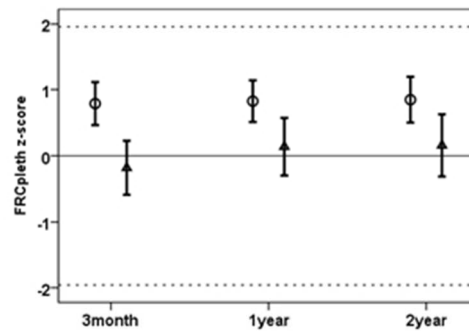
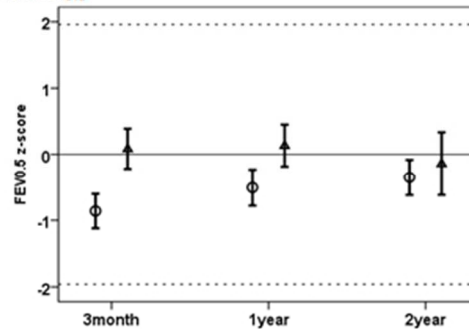
**Figure 1 Legend:** Data plotted represent mean (95% confidence interval) z-score at the 3 month, 1 year and 2 year test occasions for LCI (A),  $FRC_{pleth}$  (B) and  $FEV_{0.5}$  (C). Open circles represent NBS infants with CF, triangles represent healthy controls. Limits of normality are represented by the dashed lines at  $\pm 1.96$  z-scores. More detailed results, together with the comparison between CF and controls at each time point are presented in Table 1 and Table E3 (OLS).

**Figure 2.** Comparison of within-subject change for LCI over the first two years of life in healthy controls, in CF infants without *Pseudomonas aeruginosa* (PsA), and in CF infants in whom PsA had been isolated on at least one occasion prior to their 2yr infant lung function test.



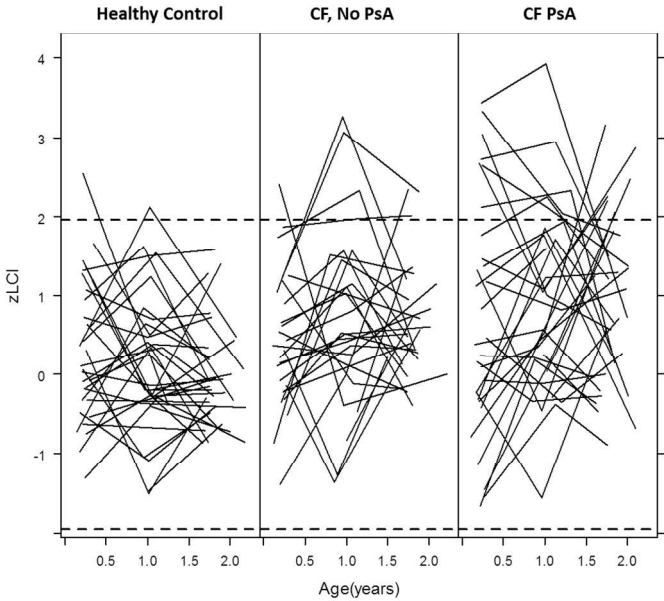
**Figure 2 legend:** Each subject is represented by an individual line. Z-scores for LCI are plotted against actual age at lung function test. Limits of normality are represented by the dashed lines at +/- 1.96 z-scores. Infants with cystic fibrosis are separated according to PsA status at the time of their 2 year infant lung function test ('CF PsA' = isolated PsA in culture on at least one occasion by their two year test). Similar plots for FRC<sub>pleth</sub> and FEV<sub>0.5</sub> and for change over time according to whether the child had received intravenous antibiotics by their 2yr test are presented in Fig E2(OLS).

## A. LCI

B.  $FRC_{pleth}$ C.  $FEV_{0.5}$ 

**Figure 1. Lung function across the first two years of life in healthy infants and those with CF.** Data plotted represent mean (95% confidence interval) z-score at the 3 month, 1 year and 2 year test occasions for LCI (A),  $FRC_{pleth}$  (B) and  $FEV_{0.5}$  (C). Open circles represent NBS infants with CF, triangles represent healthy controls. Limits of normality are represented by the dashed lines at  $\pm 1.96$  z-scores. More detailed results, together with the comparison between CF and controls at each time point are presented in Table 1 and Table E3 (OLS).

93x202mm (96 x 96 DPI)



**Figure 2. Comparison of within-subject change for LCI over the first two years of life in healthy controls, in CF infants without *Pseudomonas aeruginosa* (PsA), and in CF infants in whom PsA had been isolated on at least one occasion prior to their 2yr infant lung function test.**

Each subject is represented by an individual line. Z-scores for LCI are plotted against actual age at lung function test. Limits of normality are represented by the dashed lines at +/- 1.96 z-scores. Infants with cystic fibrosis are separated according to PsA status at the time of their 2 year infant lung function test ('CF PsA' = isolated PsA in culture on at least one occasion by their two year test). Similar plots for FRC<sub>pleth</sub> and FEV<sub>0.5</sub> and for change over time according to whether the child had received intravenous antibiotics by their 2yr test are presented in Fig E2(OLS).

254x190mm (300 x 300 DPI)

**Online data supplement**

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

Gwyneth Davies, Janet Stocks, Lena P Thia, Ah-Fong Hoo, Andrew Bush, Paul Aurora, Lucy Brennan, Simon Lee, Sooky Lum, Philippa Cottam, Joanne Miles, Jane Chudleigh, Jane Kirkby, Ian M Balfour-Lynn, Siobhán B Carr, Colin Wallis, Hilary Wyatt and Angie Wade on behalf of the London Cystic Fibrosis Collaboration (LCFC)

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) <sup>§</sup>	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. <sup>§</sup>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)		
<i>Pseudomonas aeruginosa</i>	19 (31%), n=1 chronic	32/62 (52%), n=4 chronic
<i>Staph aureus</i>	10 (16%)	18 (29%), n=1 chronic
<i>Haemophilus influenza</i>	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)
<b>LCI</b>	57 (93)	30 (91)	59 (98)	32 (100)	61 (98)	33 (97)	58 (97)	31 (97)	56 (92)	29 (88)
<b>FRC<sub>pleth</sub></b>	50 (82)	29 (88)	59 (98)	32 (100)	57 (92)	31 (91)	54 (90)	30 (94)	47 (77)	26 (79)
<b>FEV<sub>0.5</sub></b>	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<sub>pleth</sub> = plethysmographic functional residual capacity; FEV<sub>0.5</sub>= forced expiratory volume in 0.5 seconds.

Of the 62 CF infants tested at ~2yr, 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, FRC<sub>pleth</sub> and FEV<sub>0.5</sub>) 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<sub>0.5</sub> 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<sub>0.5</sub> 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<sub>0.5</sub> 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<sub>0.5</sub> (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<sub>0.5</sub> 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<sub>0.5</sub> 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

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3 this current paper remain unchanged (Hoo et al reported a mean difference of 0.92 (1.29 to  
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6 0.56) z-scores between CF and HC at 3 months, which is virtually identical to that shown below  
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9 in Table E3 when using the updated equations.  
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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 <sub>pleth</sub>	0.79 (1.14)	-0.18 (1.07)	0.97 (0.45 to 1.49)	<0.001
zFEV <sub>0.5</sub>	-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 <sub>25-75</sub>	-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 <sub>pleth</sub>	0.82 (1.2)	0.14 (1.21)	0.69 (0.16 to 1.21)	0.01
zFEV <sub>0.5</sub>	-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 <sub>25-75</sub>	-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<sub>25-75</sub> 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.**

<b>A) Cystic Fibrosis</b>	<b>Mean (SD) difference</b>	<b>95% CI of the Difference</b>	<b>p</b>
<b>LCI</b>			
3 months to 1 year	0.33 (1.19)	0.00 to 0.65	<b>0.050</b>
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
<b>FRC<sub>pleth</sub></b>			
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
<b>FEV<sub>0.5</sub></b>			
3 months to 1 year	0.36 (0.97)	0.10 to 0.62	<b>0.008</b>
3 months to 2 years	0.46 (1.13)	0.15 to 0.78	<b>0.004</b>
1 year to 2 years	0.19 (1.03)	-0.09 to 0.47	0.179

<b>B) Healthy controls</b>	<b>Mean (SD) difference</b>	<b>95% CI of the Difference</b>	<b>p</b>
<b>LCI</b>			
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
<b>FRC<sub>pleth</sub></b>			
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
<b>FEV<sub>0.5</sub></b>			
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<sub>0.5</sub> 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.

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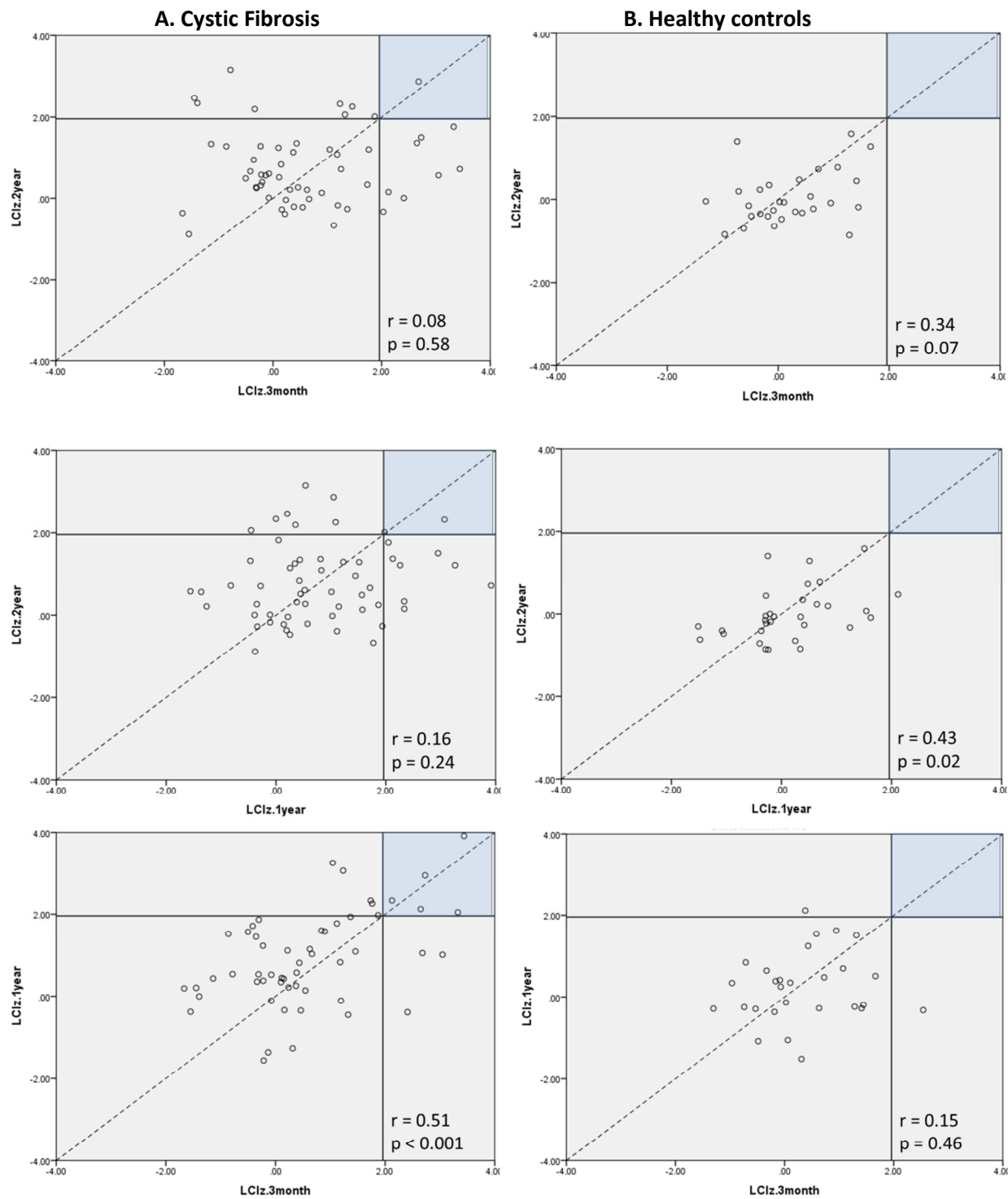
Table E5: Comparison of changes ( $\Delta$ ) in lung function and nutritional outcomes over time in infants with cystic fibrosis (CF) and healthy controls (HC) between approximately 3 months and 1 year, and between 3 months to 2 years of age.

	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)
$\Delta$ zHeight	0.75 (0.70)	-0.07 (0.71)	<b>0.82 (0.51 to 1.13) **</b>	0.69 (0.89)	-0.1 (0.91)	<b>0.79 (0.41 to 1.18)**</b>
$\Delta$ zWeight	1.30 (0.80)	0.46 (0.79)	<b>0.84 (0.49 to 1.19) **</b>	1.21 (1.02)	0.46 (0.84)	<b>0.75 (0.34 to 1.16)**</b>
$\Delta$ zBMI	1.23 (0.89)	0.68 (0.92)	<b>0.55 (0.15 to 0.95) **</b>	1.12 (1.15)	0.67 (1.22)	0.45 (-0.06 to 0.95)
$\Delta$ 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)
$\Delta$ zFRC <sub>pleth</sub>	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)
$\Delta$ zFEV <sub>0.5</sub>	0.36 (0.97)	-0.02 (0.89)	0.38 (-0.04 to 0.80)	0.46 (1.13)	-0.23 (1.11)	<b>0.70 (0.17 to 1.23)*</b>

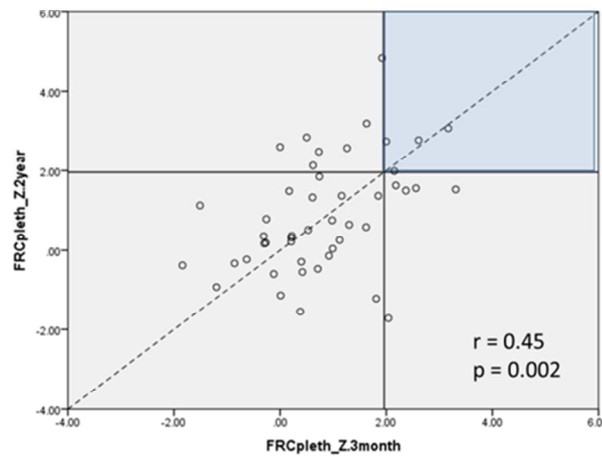
Footnote: Results are presented as the mean (SD) change in z-score for each outcome, between testing at approximately 3 months and either 1 or 2 years of age. \*p<0.05, \*\*p<0.01. For number of subjects with each outcome, see table E2. The comparison of changes in lung function and nutritional outcomes 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).

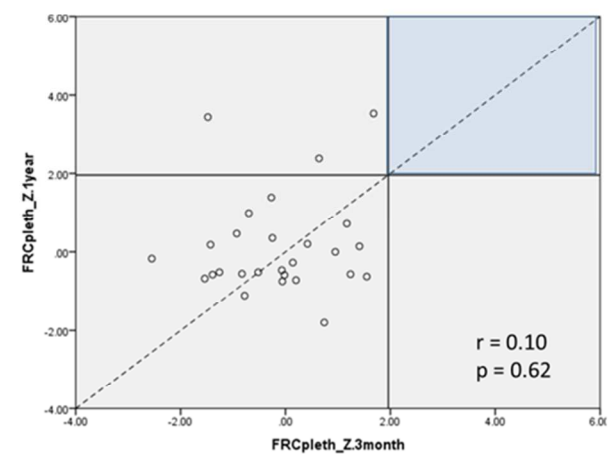
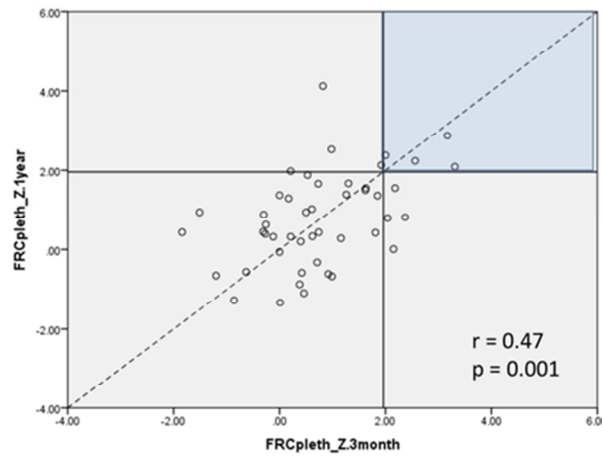
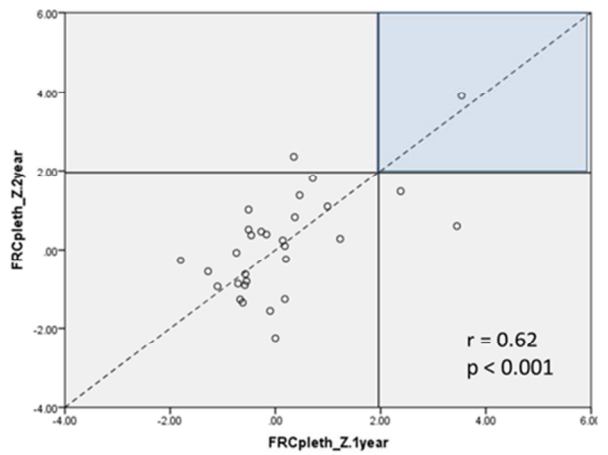
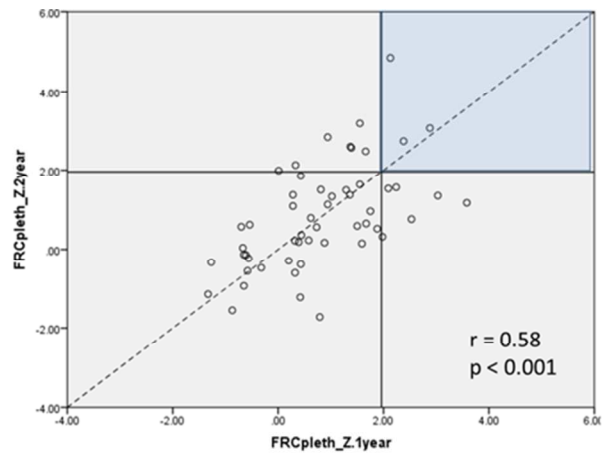
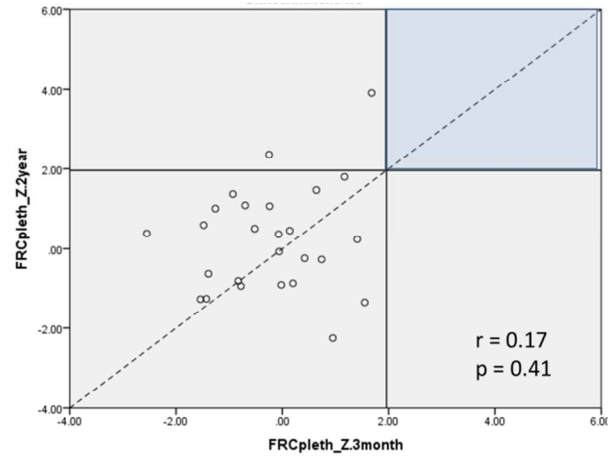
Figure E1. Relationship of results between test occasions within each lung function outcome in infants and young children with CF (A) and in healthy controls (B).

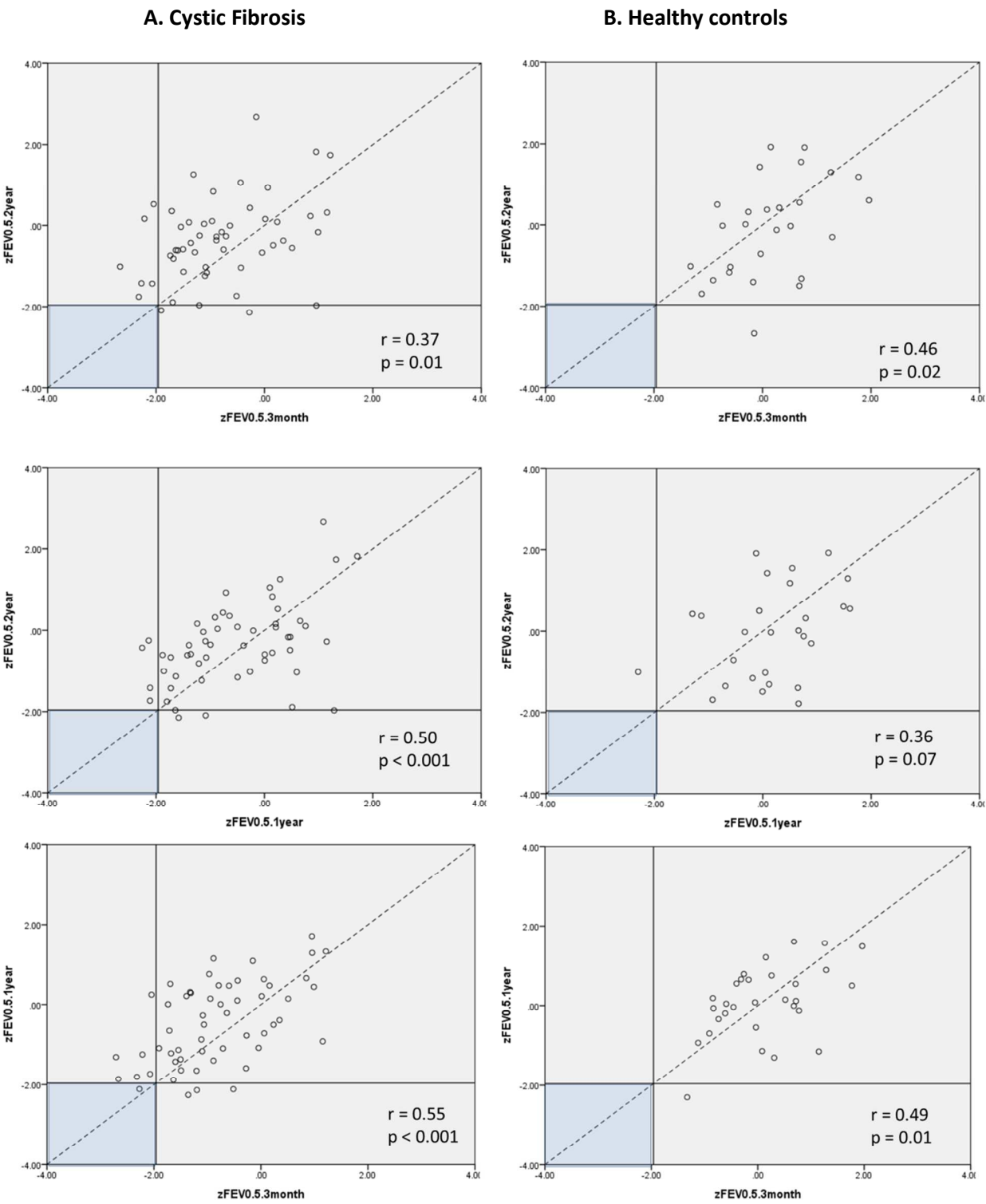


## A. Cystic Fibrosis



## B. Healthy controls



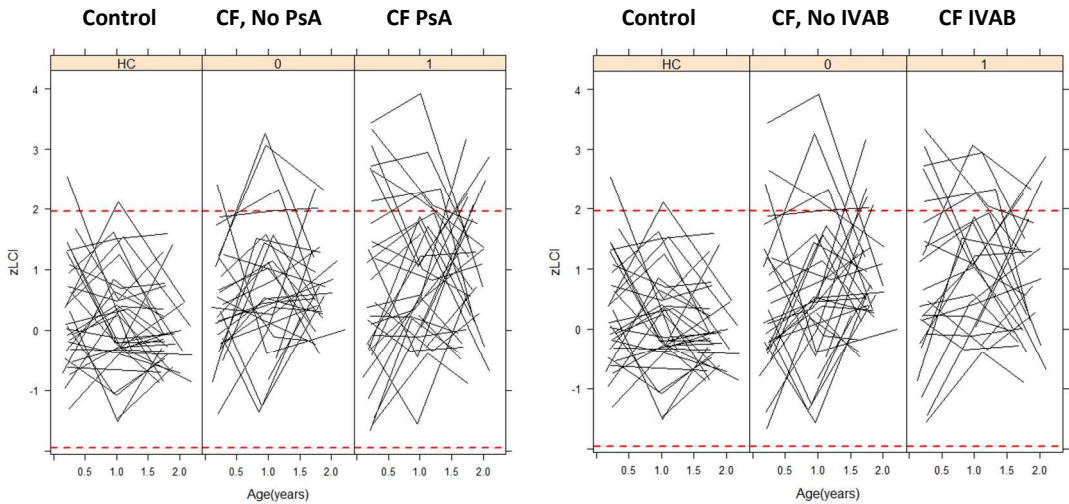


**Legend:** Relationship of LCI, FRC<sub>pleth</sub>, and FEV<sub>0.5</sub> between various test occasions in children with CF and healthy infants. For each outcome, all subjects with results on both test occasions are

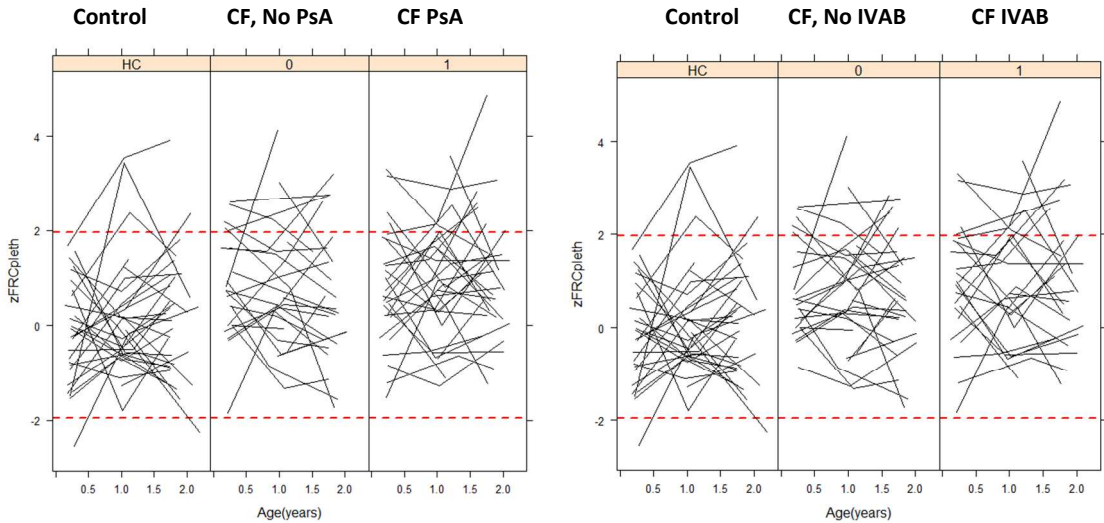
represented by an individual data point. Limits of normality are shown at 1.96 z-scores for LCI and  $FRC_{pleth}$ , and  $-1.96z$  for  $FEV_{0.5}$ . The between-test equivalence line is shown on each cross-plot as a dashed line. For LCI and  $FRC_{pleth}$ , all values to the right of the vertical line 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.

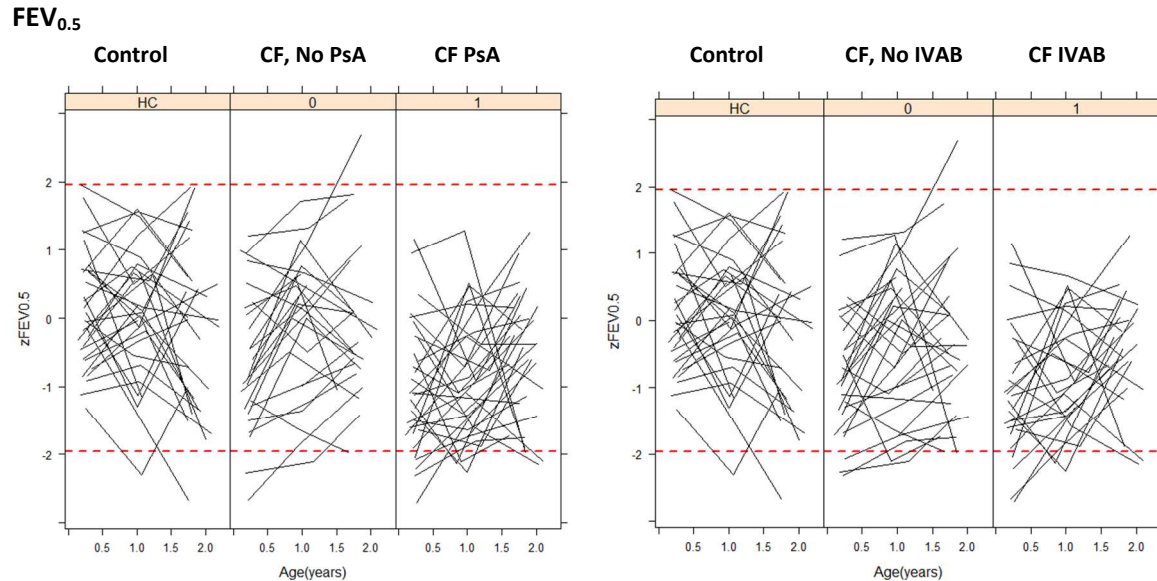
**Fig E2. Within-subject variability for infant lung function outcomes in healthy controls, and for CF infants according to whether they had ever isolated *Pseudomonas aeruginosa*, or received any IV antibiotics by their 2yr infant lung function test.**

**LCI**



**FRC<sub>pleth</sub>**

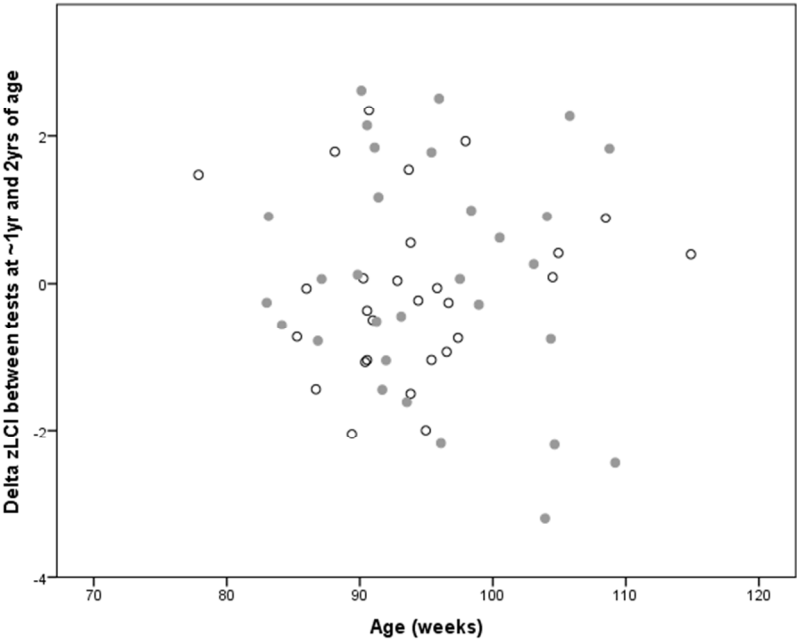




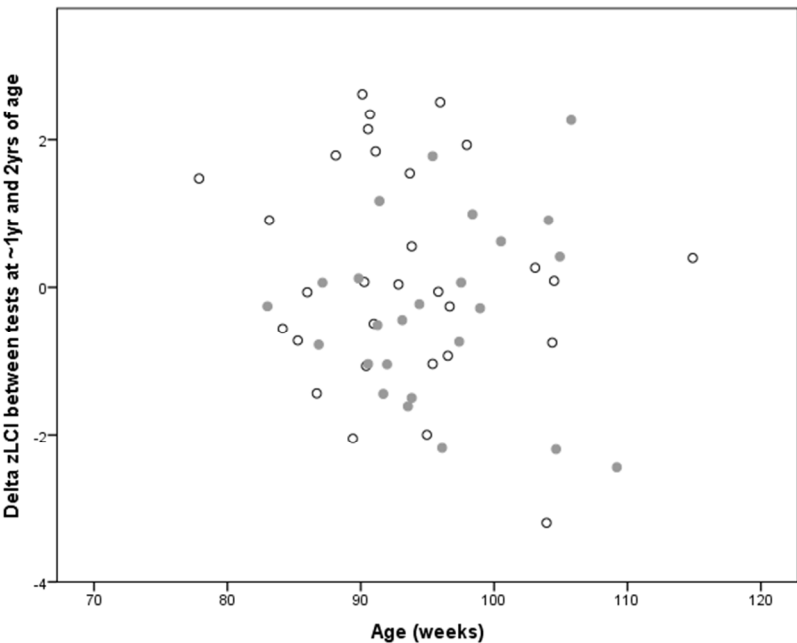
**Legend:** Each subject is represented by an individual line. Z-scores for each infant lung function (ILF) test (LCI, FRC<sub>pleth</sub> and FEV<sub>0.5</sub>) are plotted against actual age at test. Limits of normality are represented by the dashed lines at  $\pm 1.96$  z-scores. For infants with 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<sub>0.5</sub> 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* ( $\Delta$ ) 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)
$\Delta$ zLCI	0.17(1.12), n=29	0.50(1.28), n=25	-0.33(-0.98 to 0.32)
$\Delta$ zFRC <sub>pleth</sub>	0.07(1.16), n=26	0.00(1.24), n=21	0.07 (-0.63 to 0.78)
$\Delta$ zFEV <sub>0.5</sub>	0.11(1.07), n=30	0.64(0.76), n=26	<b>-0.53(-1.03 to -0.02)*</b>
	1 year to 2 years		
	CF PsA	CF No PsA	Difference in change (95% CI)
$\Delta$ zLCI	0.08(1.56), n=30	-0.09(1.16), n=28	0.17(-0.56 to 0.90)
$\Delta$ zFRC <sub>pleth</sub>	0.11(1.25), n=30	-0.09(0.98), n=24	0.20(-0.43 to 0.82)
$\Delta$ zFEV <sub>0.5</sub>	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)
$\Delta$ zLCI	0.05(1.76), n=29	0.28(1.20), n=27	-0.23(-1.04 to 0.59)
$\Delta$ zFRC <sub>pleth</sub>	0.34(1.35), n=25	-0.22(1.37), n=22	0.56 (-0.24 to 1.36)
$\Delta$ zFEV <sub>0.5</sub>	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)
$\Delta$ zLCI	0.48 (1.09), n=23	0.29 (1.30), n=27	0.19 (-0.50 to 0.88)
$\Delta$ zFRC <sub>pleth</sub>	-0.02 (1.20), n=22	0.11 (1.08), n=22	-0.14 (-0.83 to 0.56)
$\Delta$ zFEV <sub>0.5</sub>	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)
$\Delta$ zLCI	-0.33 (1.22), n=25	0.2 (1.48), n=30	-0.53 (-1.27 to 0.22)
$\Delta$ zFRC <sub>pleth</sub>	0.04 (1.28), n=24	-0.03 (1.07), n=27	0.07 (-0.59 to 0.73)
$\Delta$ zFEV <sub>0.5</sub>	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)
$\Delta$ zLCI	-0.10 (1.59), n=24	0.42 (1.51), n=28	-0.52 (-1.39 to 0.34)
$\Delta$ zFRC <sub>pleth</sub>	0.18 (1.27), n=21	0.01 (1.45), n=22	0.18 (-0.67 to 1.02)
$\Delta$ zFEV <sub>0.5</sub>	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 ( $85 \times 2 \times 100 / 62$ ) 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 FEV0.5), 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.

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E7. Nguyen TT, Thia LP, Hoo AF, Bush A, Aurora P, Wade A, et al. Evolution of lung function during the first year of life in newborn screened cystic fibrosis infants. Thorax. 2014;69(10):910-7.

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3 E8. Thia LP, Hoo AF, Brennan L, Nguyen TT, Chudleigh J, Wade A, et al. Stable lung function is  
4 maintained over 2 years in newborn screened (NBS) CF infants (abstract). Eur Respir J. 2013;42 Suppl  
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17 **Pulmonary function deficits in newborn screened infants with cystic fibrosis managed with**  
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23 Gwyneth Davies, Janet Stocks, Lena P Thia, Ah-Fong Hoo, Andrew Bush, Paul Aurora, Lucy  
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25 Brennan, Simon Lee, Sooky Lum, Philippa Cottam, Joanne Miles, Jane Chudleigh, Jane  
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27 Kirkby, Ian M Balfour-Lynn, Siobhán B Carr, Colin Wallis, Hilary Wyatt and Angie Wade on  
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**Table E1a. Study population and background demographics**

	CF (n=62)	Healthy controls (n=34)	$\Delta$ (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%)	
<b>Cystic fibrosis infants only</b>			
Postnatal age at diagnosis (weeks) <sup>§</sup>	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. <sup>§</sup>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)
<b>Respiratory symptoms</b>		
Physician diagnosed wheeze (ever)	20/60 (34%)	25/62 (40%)
<b>Additional treatment (n(%))</b>		
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%)
<b>Airway microbiology (ever)</b>		
<i>Pseudomonas aeruginosa</i>	19 (31%), n=1 chronic	32/62 (52%), n=4 chronic
<i>Staph aureus</i>	10 (16%)	18 (29%), n=1 chronic
<i>Haemophilus influenza</i>	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.

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**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)
<b>LCI</b>	57 (93)	30 (91)	59 (98)	32 (100)	61 (98)	33 (97)	58 (97)	31 (97)	56 (92)	29 (88)
<b>FRC<sub>pleth</sub></b>	50 (82)	29 (88)	59 (98)	32 (100)	57 (92)	31 (91)	54 (90)	30 (94)	47 (77)	26 (79)
<b>FEV<sub>0.5</sub></b>	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<sub>pleth</sub> = plethysmographic functional residual capacity; FEV<sub>0.5</sub>= forced expiratory volume in 0.5 seconds.  
Of the 62 CF infants tested at ~2yr, 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, FRC<sub>pleth</sub> and FEV<sub>0.5</sub>) 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**

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

**B.**

	<b>Cystic Fibrosis</b>	<b>Healthy Control</b>	<b>Difference (95% CI)</b>	<b>p</b>
<b>N</b>	60	32		
<b>Age at test, weeks</b>	52.8 (5.3)	53.8 (3.4)	-1.05 (-3.09 to 0.99)	0.31
<b>zHeight</b>	0.47 (0.97)	0.74 (1.09)	-0.26 (-0.71 to 0.18)	0.24
<b>zWeight</b>	0.26 (0.87)	0.44 (0.99)	-0.18 (-0.57 to 0.22)	0.38
<b>zBMI</b>	-0.01 (0.74)	0.03 (0.83)	-0.04 (-0.38 to 0.29)	0.80
<b>zLCI</b>	0.82 (1.16)	0.13 (0.87)	0.69 (0.22 to 1.15)	<b>0.004</b>
<b>zFRC<sub>pleth</sub></b>	0.82 (1.2)	0.14 (1.21)	0.69 (0.16 to 1.21)	<b>0.01</b>
<b>zFEV<sub>0.5</sub></b>	-0.51 (1.04)	0.13 (0.88)	-0.64 (-1.07 to -0.21)	<b>0.004</b>
<b>zFVC</b>	-0.51 (1.03)	0.24 (0.94)	-0.75 (-1.19 to -0.31)	<b>0.001</b>
<b>zFEF<sub>25-75</sub></b>	-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<sub>25-75</sub> 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	<b>0.050</b>
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 <sub>pleth</sub>			
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 <sub>0.5</sub>			
3 months to 1 year	0.36 (0.97)	0.10 to 0.62	<b>0.008</b>
3 months to 2 years	0.46 (1.13)	0.15 to 0.78	<b>0.004</b>
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 <sub>pleth</sub>			
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 <sub>0.5</sub>			
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<sub>0.5</sub> 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 ( $\Delta$ ) 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.**

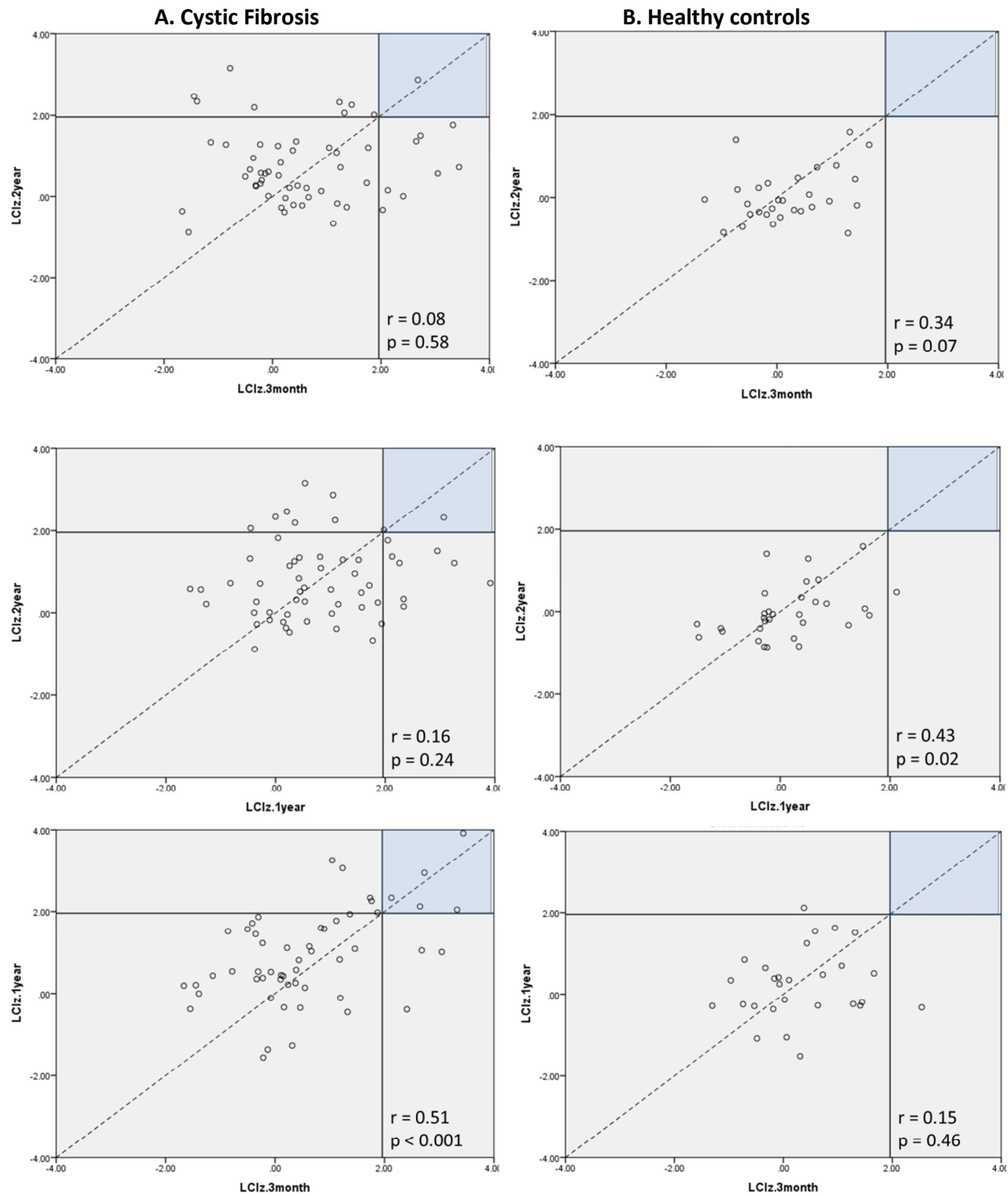
	3 months to 1 year			3 months to 2 years		
	Cystic Fibrosis	Healthy controls	Difference in change (95% CI)	Cystic Fibrosis	Healthy controls	Difference in change (95% CI)
<b>Interval (weeks)</b>	41.6 (5.0)	41.5 (3.8)	0.1 (-1.9 to 2.1)	83.7 (7.8)	83.7 (7.7)	0.1 (-3.3 to 3.4)
<b><math>\Delta</math> zHeight</b>	0.75 (0.70)	-0.07 (0.71)	<b>0.82 (0.51 to 1.13) **</b>	0.69 (0.89)	-0.1 (0.91)	<b>0.79 (0.41 to 1.18)**</b>
<b><math>\Delta</math> zWeight</b>	1.30 (0.80)	0.46 (0.79)	<b>0.84 (0.49 to 1.19) **</b>	1.21 (1.02)	0.46 (0.84)	<b>0.75 (0.34 to 1.16)**</b>
<b><math>\Delta</math> zBMI</b>	1.23 (0.89)	0.68 (0.92)	<b>0.55 (0.15 to 0.95) **</b>	1.12 (1.15)	0.67 (1.22)	0.45 (-0.06 to 0.95)
<b><math>\Delta</math> zLCI</b>	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)
<b><math>\Delta</math> zFRC<sub>pleth</sub></b>	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)
<b><math>\Delta</math> zFEV<sub>0.5</sub></b>	0.36 (0.97)	-0.02 (0.89)	0.38 (-0.04 to 0.80)	0.46 (1.13)	-0.23 (1.11)	<b>0.70 (0.17 to 1.23)*</b>

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).

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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).

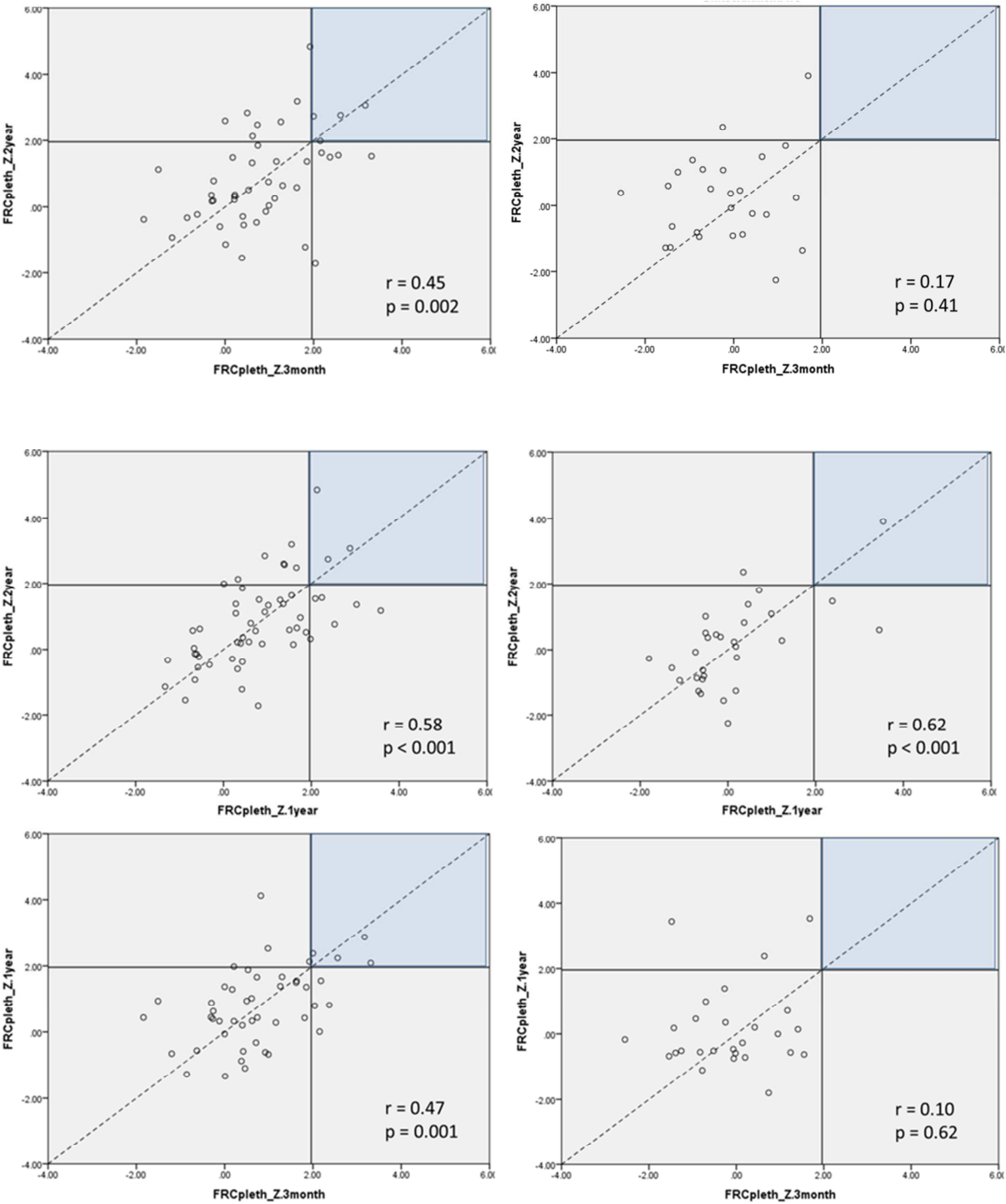
Figure E1. Relationship of results between test occasions within each lung function outcome in infants and young children with CF (A) and in healthy controls (B).



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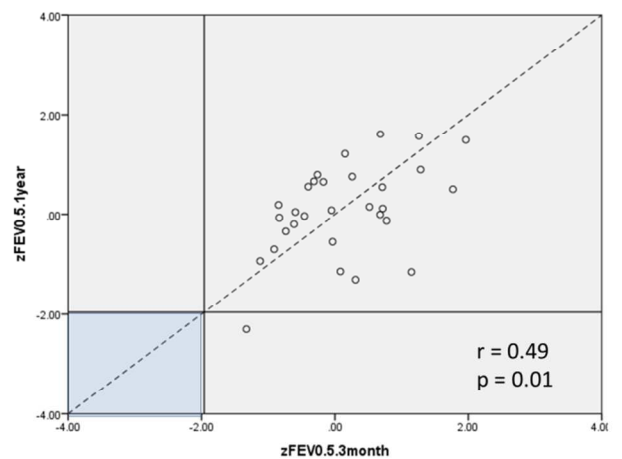
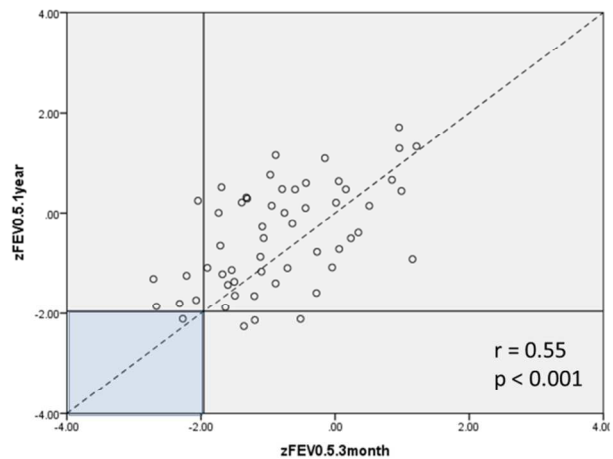
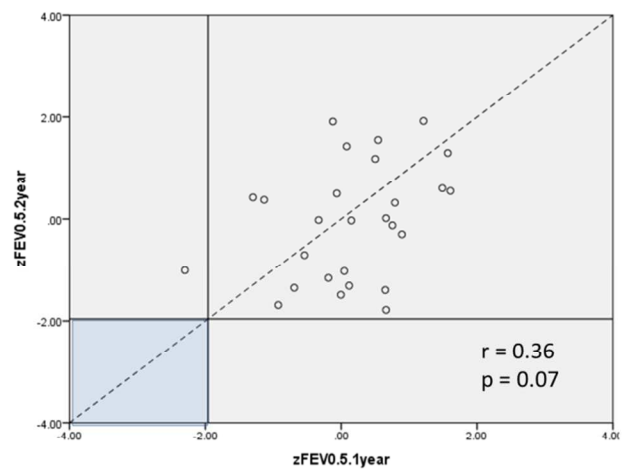
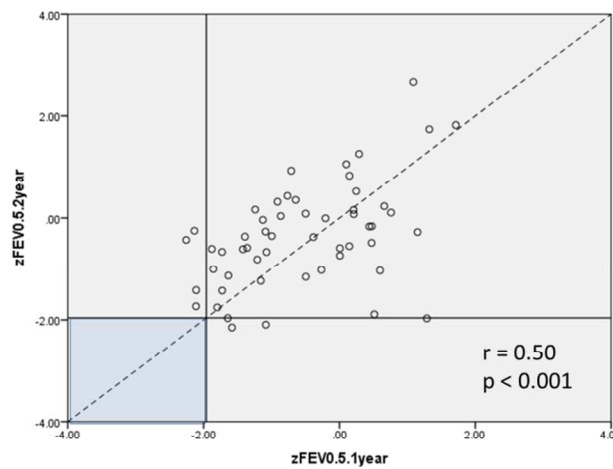
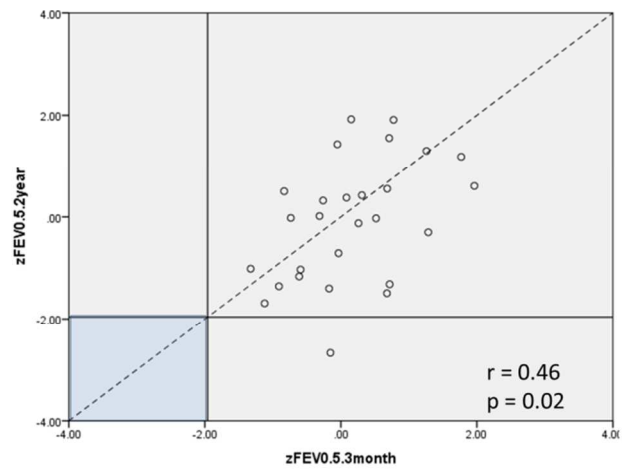
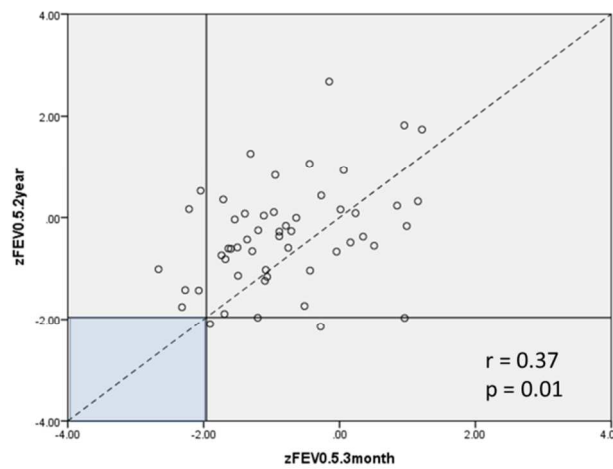
A. Cystic Fibrosis

B. Healthy controls



## A. Cystic Fibrosis

## B. Healthy controls



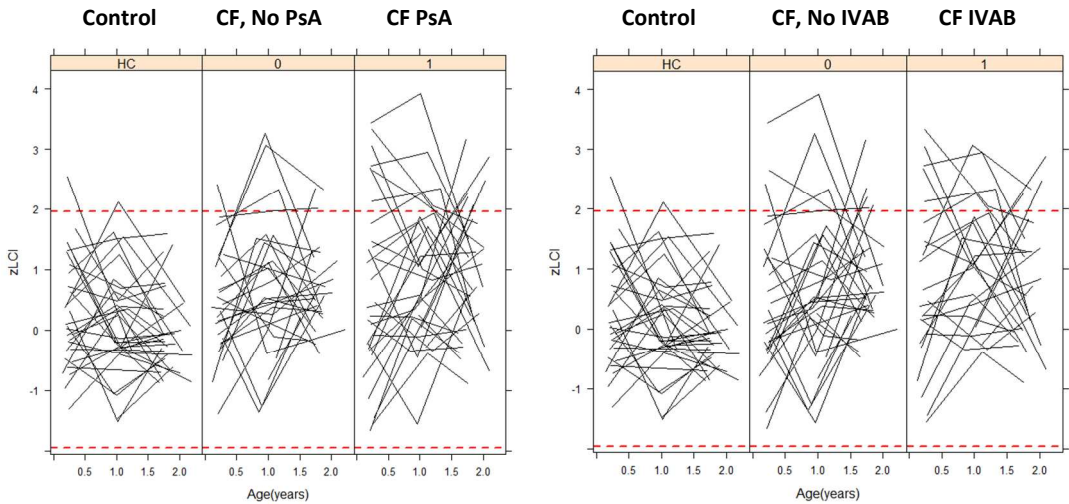
**Legend:** Relationship of LCI,  $FRC_{pleth}$ , and  $FEV_{0.5}$  between various test occasions in children with CF and healthy infants. For each outcome, all subjects with results on both test occasions are

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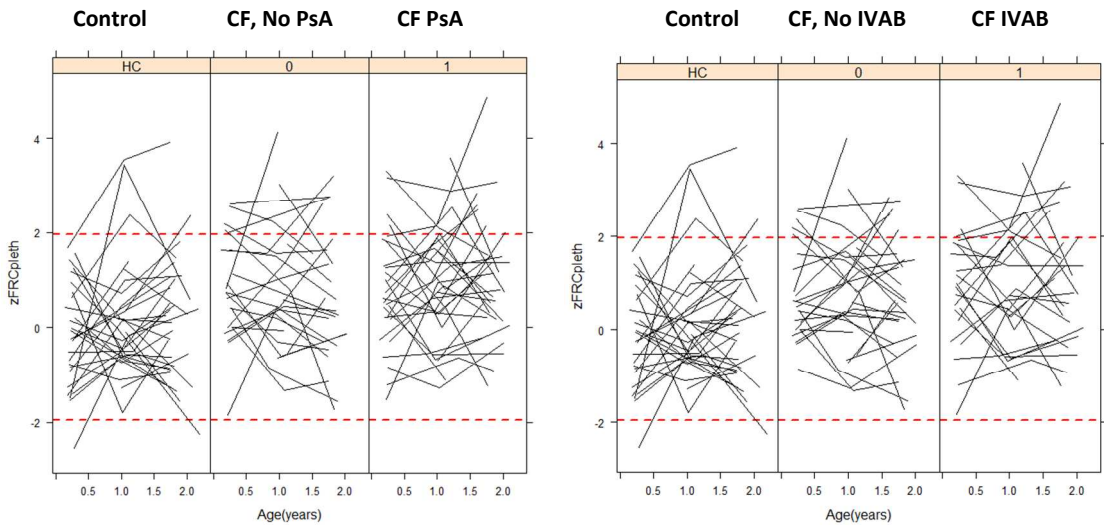
represented by an individual data point. Limits of normality are shown at 1.96 z-scores for LCI and FRC<sub>pleth</sub>, and -1.96z for FEV<sub>0.5</sub>. The between-test equivalence line is shown on each cross-plot as a dashed line. For LCI and FRC<sub>pleth</sub>, all values to the right of the vertical 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<sub>pleth</sub> at 2yrs). For FEV<sub>0.5</sub>, values to the left of the vertical line or below the horizontal cut-off were abnormal. The one CF infant with abnormal FEV<sub>0.5</sub> at both 3 months and 1 year appears in the shaded left lower quadrant.

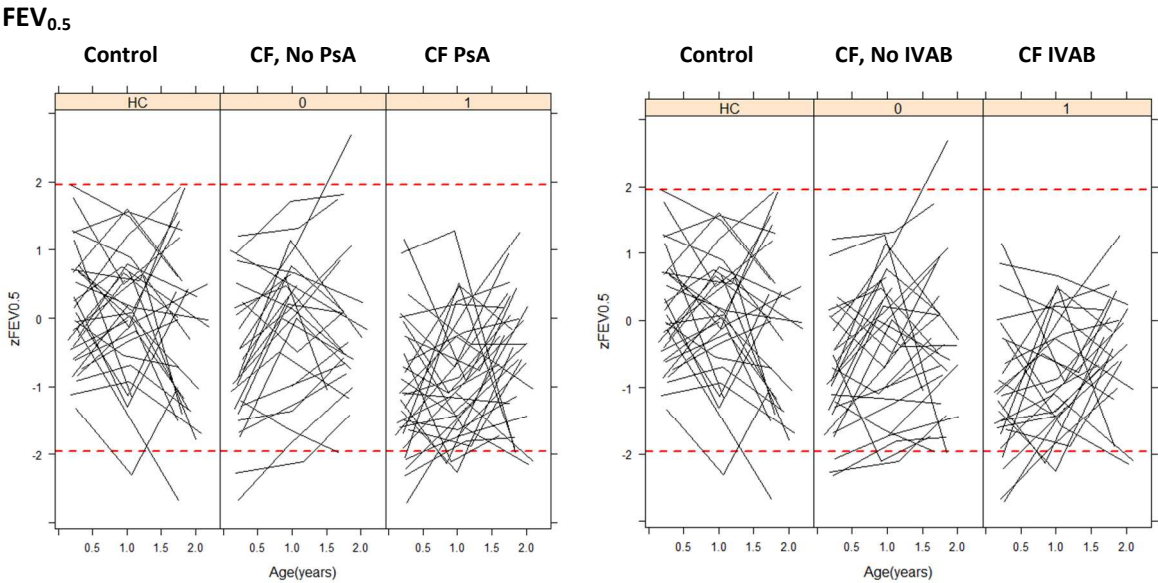
**Fig E2. Within-subject variability for infant lung function outcomes in healthy controls, and for CF infants according to whether they had ever isolated *Pseudomonas aeruginosa*, or received any IV antibiotics by their 2yr infant lung function test.**

**LCI**



**FRC<sub>pleth</sub>**

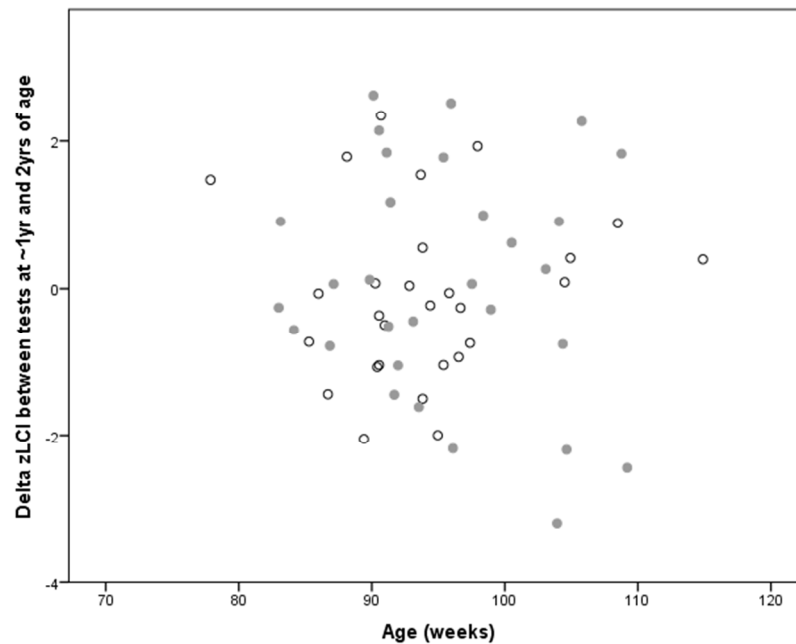




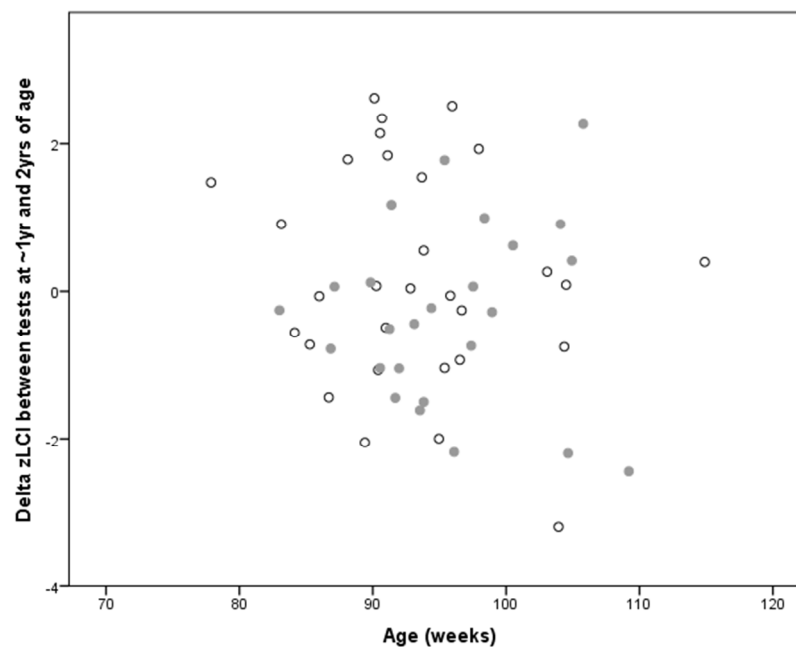
**Legend:** Each subject is represented by an individual line. Z-scores for each infant lung function (ILF) test (LCI, FRC<sub>pleth</sub> and FEV<sub>0.5</sub>) are plotted against actual age at test. Limits of normality are represented by the dashed lines at  $\pm 1.96$  z-scores. For infants with 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



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**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<sub>0.5</sub> 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* ( $\Delta$ ) 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)
$\Delta$ zLCI	0.17(1.12), n=29	0.50(1.28), n=25	-0.33(-0.98 to 0.32)
$\Delta$ zFRC <sub>pleth</sub>	0.07(1.16), n=26	0.00(1.24), n=21	0.07 (-0.63 to 0.78)
$\Delta$ zFEV <sub>0.5</sub>	0.11(1.07), n=30	0.64(0.76), n=26	<b>-0.53(-1.03 to -0.02)*</b>
	1 year to 2 years		
	CF PsA	CF No PsA	Difference in change (95% CI)
$\Delta$ zLCI	0.08(1.56), n=30	-0.09(1.16), n=28	0.17(-0.56 to 0.90)
$\Delta$ zFRC <sub>pleth</sub>	0.11(1.25), n=30	-0.09(0.98), n=24	0.20(-0.43 to 0.82)
$\Delta$ zFEV <sub>0.5</sub>	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)
$\Delta$ zLCI	0.05(1.76), n=29	0.28(1.20), n=27	-0.23(-1.04 to 0.59)
$\Delta$ zFRC <sub>pleth</sub>	0.34(1.35), n=25	-0.22(1.37), n=22	0.56 (-0.24 to 1.36)
$\Delta$ zFEV <sub>0.5</sub>	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)
$\Delta$ zLCI	0.48 (1.09), n=23	0.29 (1.30), n=27	0.19 (-0.50 to 0.88)
$\Delta$ zFRC <sub>pleth</sub>	-0.02 (1.20), n=22	0.11 (1.08), n=22	-0.14 (-0.83 to 0.56)
$\Delta$ zFEV <sub>0.5</sub>	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)
$\Delta$ zLCI	-0.33 (1.22), n=25	0.2 (1.48), n=30	-0.53 (-1.27 to 0.22)
$\Delta$ zFRC <sub>pleth</sub>	0.04 (1.28), n=24	-0.03 (1.07), n=27	0.07 (-0.59 to 0.73)
$\Delta$ zFEV <sub>0.5</sub>	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)
$\Delta$ zLCI	-0.10 (1.59), n=24	0.42 (1.51), n=28	-0.52 (-1.39 to 0.34)
$\Delta$ zFRC <sub>pleth</sub>	0.18 (1.27), n=21	0.01 (1.45), n=22	0.18 (-0.67 to 1.02)
$\Delta$ zFEV <sub>0.5</sub>	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  $(22 \times 2) \times (100/62) \times (100/30)$  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.

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