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

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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.

Funding

This study is supported by grants from the UK Cystic Fibrosis Trust; Special Trustees: Great Ormond Street Hospital for Children, London, UK; Smiths Medical Ltd, UK; Comprehensive Local Research Network, UK; and supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London Great Ormond Street Institute of Child Health. GD was supported by a NIHR Academic Clinical Lectureship at University College London. AB is an NIHR Senior Investigator and was supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London.

Disclaimer: This article presents independent research funded by the NIHR. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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 ($FEV_{0.5}$), lung clearance index (LCI), and plethysmographic functional residual capacity (FRC_{pleth}) were measured at ~3months, 1yr and 2yrs in 62 infants with CF and 34 controls.

By 2yrs there was no significant difference in $zFEV_{0.5}$ 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 $FEV_{0.5}$ 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 1st 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; (htttp://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 ($FEV_{0.5}$) 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 $FEV_{0.5}$ 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_{pleth}) and $FEV_{0.5}$. 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 <−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 $FEV_{0.5}$ 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 $zFRC_{pleth}$ 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 $FEV_{0.5}$ 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_{pleth} and 7%(4/56) for $FEV_{0.5}$. No child had abnormalities in all three outcomes on any test occasion. No child had an abnormal LCI or $FEV_{0.5}$ on all test occasions, and only 2/44(5%) CF infants had an abnormal FRC_{pleth} 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_{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 the 10 CF infants with an abnormal LCI at 1yr, all but two

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

Clinical status

Line plots demonstrating change over time of zLCI at the individual level are shown in Fig 2, with CF infants separated according to PsA status. Similar plots for other ILF outcomes are shown in Figure E2(OLS), along with a comparison of results according to whether infants had ever received IV antibiotics. Considerable within-subject change in LCI occurred between tests even in health and the magnitude of such change was not related to either the isolation of PsA or treatment with IV antibiotics by the final ILF test in those with CF (Fig 2, Fig E2 and Table E6(OLS)). The lack of relationship between the magnitude or direction of change in LCI with respect to PsA status or treatment with IV antibiotics is also illustrated in Figure E3(OLS). A similar pattern was observed for FRC_{pleth} and $FEV_{0.5}$, with the exception of the greater improvement in $FEV_{0.5}$ during the 1st year of life in infants who did not isolate PsA by their final ILF test (Table E6(OLS)). Similarly, the magnitude or direction of change between tests was not related to whether or not infants had isolated *either* SA or HI, or *any* major CF Pathogen (PsA, SA or HI) by 2yr (data not shown). Results of regression analysis revealed no significant association between magnitude of within-subject, between-test change in ILF and interval between tests.

DISCUSSION

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 $FEV_{0.5}$ 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 $zFRC_{pleth}$ 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 $FEV_{0.5}$ 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.

Technical success rates for ILF tests in our NBS LCFC cohort continue to be better than other longitudinal studies involving infants with CF (as recently summarised by the European CF Society Clinical Trial Network)[1], particularly when compared with those which involved testing across multiple centres[13].

A range of ILFTs were performed to reflect the various aspects of pathophysiology most commonly reported in CF lung disease, with the primary outcomes of LCI, FRC_{pleth} and $FEV_{0.5}$ selected *a priori* to avoid any risk of data dredging or misinterpretation due to use of an excessive number of measurements. We have not reported the tidal breathing ratio, having previously determined that it is not useful in identifying diminished airway function in infants with CF, nor respiratory rate, which is poorly predictive of diminished airway function in this population[14]. While rarely reported, between-test variability within our control group is in keeping with that in older children, variability of up to 1.2z scores in FEV_1 being recorded in 5-11 year olds over the course of a year[15]. Using an observational study design, Davis et al did not recommend inclusion of $FEV_{0.5}$ or FRC_{pleth} measured during infancy as a primary efficacy endpoint in clinical trials due to within-subject variability between tests, technical challenges and the requirement for large sample sizes to detect efficacy [13].

Judgements regarding the clinical usefulness of any biomarker requires an assessment of its reliability, validity and responsiveness, as summarised in a recent review [16]. However, objective assessments of surrogate measures such as lung function require a different approach, particularly when such measurements are being undertaken in infants. Sedation is required for most infant lung function tests, administration of which is not advised either in the presence of an exacerbation or at frequent intervals. Furthermore, sedation may cause at least temporary disruption of sleep patterns and the duration of these tests (up to

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

observational data it would be difficult to design a study to evaluate effects of any novel, medium-term interventions. This evidence is strengthened by our ability to interpret results in relation to the normal variability that occurs in health, since we not only had a contemporaneous control group but reference equations derived using identical methods and equipment. The clinical importance of ILF tests in CF lung disease will be clarified by the continuing follow-up of this cohort into the preschool years[19], which will establish whether there is evidence of tracking over a longer time period. Although mean LCI was static over the second year of life in our cohort, the small increase in z-scores in comparison to controls may represent a degree of deterioration at the group level which may become more apparent with subsequent follow-up. The importance of physiological outcomes during the preschool years is already recognised, as results can reflect subsequent status at school age[20], and a recent study has confirmed that LCI is a useful marker to track early disease progression in preschool children with CF [21].

Clinical efficacy of treatment interventions assessed according to functional trial endpoints is possible to demonstrate even when baseline results are within the normal range, as illustrated by results from the phase III clinical trial of the cystic fibrosis transmembrane regulator potentiator ivacaftor in children aged 6-11yrs with a G551D mutation[22]. However, in contrast to older children or possibly a clinically diagnosed infant cohort, interventions aimed at reducing the rate of decline (rather than improvement) in FEV would not be appropriate in an infant NBS population such as ours, since $FEV_{0.5}$ improved to normal levels during the first two years of life with standard UK care alone. While attempts could be made to diminish the mild elevations of LCI or FRC_{pleth} observed in CF infants by 2 years of age, the transient nature of within-subject abnormalities at 3mth or 1yr in infants

treated with UK standard care alone calls into question the clinical relevance of this approach.

As previously reported for our CF NBS cohort, 1yr ILF was predicted by results obtained from testing at 3mths[4]. However the combination of relatively normal results at 2yrs and marked bi-directional within-subject change over time (Fig E2 OLS), question the value of modelling ILF outcomes at 2yrs. We therefore analysed results at 2yrs with respect to those at 3mths and 1yr separately, rather than in a repeated measures analysis. Any long-term value of ILF in terms of predicting later CF disease status will become clearer as our current cohort is followed up through the preschool years and beyond. The transient nature of the observed changes in lung function in our cohort is encouraging, but does mean that our previous proposal to try to identify a 'high-risk' subgroup of NBS CF infants based on 'abnormalities' at either 3mths or 1yr of age is not feasible.

Comparison with other studies

To our knowledge, the only other prospective longitudinal study reporting ILF outcomes in NBS infants with CF is that from the AREST-CF group. Although both AREST-CF and the LCFC detected deficits in ILF by around 3mths in such infants[3, 12, 23], results from these two studies are widely discrepant at later time points. Instead of improvement to one year and maintenance of ILF to 2 years as reported here, lung function appeared to deteriorate significantly over this period in the AREST-CF infants (mean(SD) $FEV_{0.5}$ z-score being -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, was the conversion of RVRTC outcomes to z-scores using historical control data collected with different equipment[24], and the absence of a contemporaneous control group within the AREST-CF study. Possible explanations for the differences between results from these

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 $FEV_{0.5}$ 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_{pleth} and $FEV_{0.5}$ 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.

ACKNOWLEDGEMENTS:

We thank the infants and parents who participated in this study, and gratefully acknowledge contributions by all members of the London NBS CF Collaboration (Ah-Fong Hoo, Ammani Prasad, Andrew Bush, Angie Wade, Anu Shankar, Catherine Owen, Caroline Pao, Colin Wallis, Deeba Ahmed, Gary Ruiz, Hilary Wyatt, Ian Balfour-Lynn, Jane Chudleigh, Jane Davies, Janet Stocks (Director), John Price, Lena Thia, Lucy Brennan, Mark Rosenthal, Paul Aurora, Ranjan Suri, Richard Chavasse, Siobhan Carr, Sooky Lum, The Thanh Diem Nguyen and Gwyneth Davies) and Per Gustafsson for on-going advice and support with respect to MBW by Mass Spectrometry.

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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
N	62	34		
Age at test, weeks	95.0 (7.3)	96.2 (7.7)	-1.24 (-4.39 to 1.92)	0.44
zHeight	0.48 (1.00)	0.71 (1.18)	-0.23 (-0.69 to 0.22)	0.31
zWeight	0.24 (0.92)	0.40 (0.86)	-0.16 (-0.54 to 0.22)	0.41
zBMI	-0.07 (0.92)	-0.03 (0.90)	-0.04 (-0.42 to 0.35)	0.85
zLCI	0.78 (0.93)	-0.03 (0.64)	0.81 (0.45 to 1.17)	**0.001**
$zFRC_{pleth}$	0.85 (1.30)	0.16 (1.28)	0.69 (0.11 to 1.26)	**0.02**
$zFEV_{0.5}$	-0.35 (1.00)	-0.14 (1.23)	-0.21 (-0.71 to 0.29)	0.41
zFVC	-0.19 (0.94)	0.04 (0.92)	-0.23 (-0.66 to 0.21)	0.3
$zFEF_{25–75}$	-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_{25-75} results are included in this table to allow comparison with the published literature.

Table 2: Comparison of changes (Δ) 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)
ΔzHeight	-0.04 (0.47)	-0.05 (0.54)	0.01 (-0.2 to 0.22)
ΔzWeight	-0.08 (0.46)	-0.05 (0.43)	-0.03 (-0.22 to 0.16)
ΔzBMI	-0.10 (0.61)	-0.06 (0.78)	-0.05 (-0.34 to 0.24)
ΔzLCI	0.00 (1.37)	-0.15 (0.83)	0.15 (-0.39 to 0.69)
$\Delta zFRC_{pleth}$	0.02 (1.13)	0.01 (1.10)	0.01 (-0.5 to 0.52)
$\Delta zFEV_{0.5}$	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.

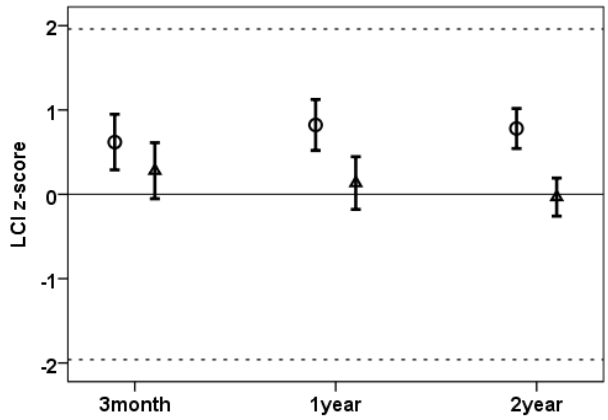
	3mth	1yr	2yr	3mth&1yr	3mth&2yr	1yr&2yr	All test occasions
Total n with LCI	57	59	61	54	56	58	53
Normal LCI	48 (84%)	49 (83%)	52 (85%)	41 (76%)	39 (70%)	41 (71%)	34 (64%)
Abnormal LCI	9 (16%)	10 (17%)	9 (15%)	5 (9%)	1 (2%)	2 (3%)	0 (0%)
Total n with FRC_{pleth}	50	59	57	47	47	54	44
Normal FRC_{pleth}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

Footnote: *All= LCI, FRC_{pleth} and $FEV_{0.5}$. 'Abnormal' and 'normal' defined on basis of 1.96 z score threshold for LCI and FRC_{pleth}, and -1.96 for $FEV_{0.5}$.

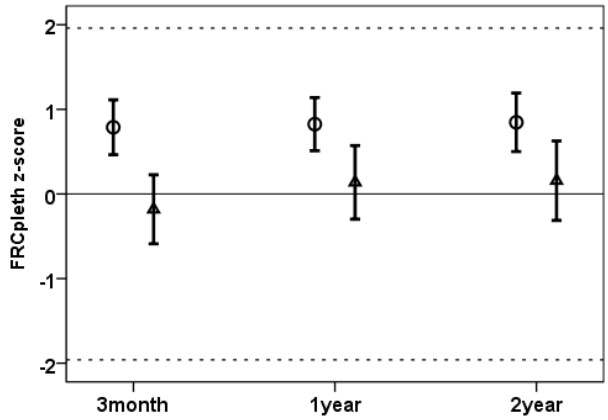
FIGURES

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

A. LCI



B. FRC_{pleth}



C. $FEV_{0.5}$

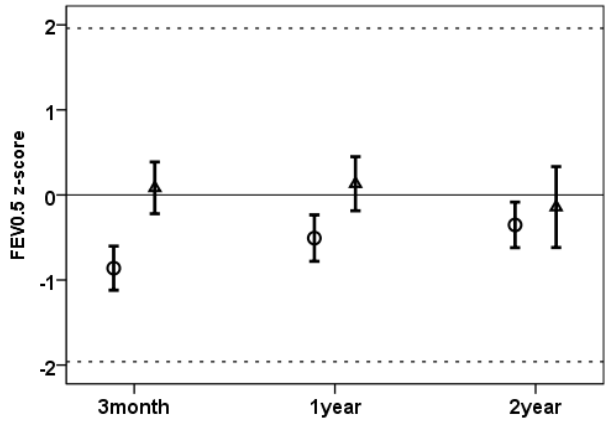


Figure 1 Legend: Data plotted represent mean (95% confidence interval) z-score at the 3 month, 1 year and 2 year test occasions for LCI (A), FRC_{pleth} (B) and $FEV_{0.5}$ (C). Open circles represent NBS infants with CF, triangles represent healthy controls. Limits of normality are represented by the dashed lines at +/- 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.

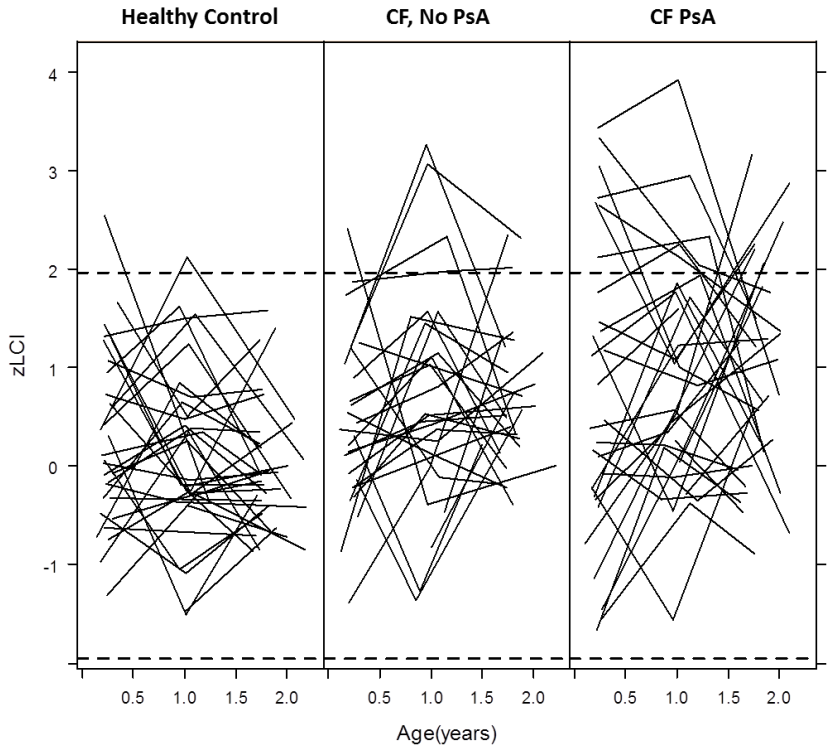


Figure 2 legend: Each subject is represented by an individual line. Z-scores for LCI are plotted against actual age at lung function test. Limits of normality are represented by the dashed lines at +/- 1.96 z-scores. Infants with cystic fibrosis are separated according to PsA status at the time of their 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_{pleth} and $FEV_{0.5}$ and for change over time according to whether the child had received intravenous antibiotics by their 2yr test are presented in Fig E2(OLS).