



ORIGINAL ARTICLE

Lung function is abnormal in 3-month-old infants with cystic fibrosis diagnosed by newborn screening

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ABSTRACT

Background Long-term benefits of newborn screening (NBS) for cystic fibrosis (CF) have been established with respect to nutritional status, but effects on pulmonary health remain unclear.

Hypothesis With early diagnosis and commencement of standardised treatment, lung function at ~3 months of age is normal in NBS infants with CF.

Methods Lung clearance index (LCI) and functional residual capacity (FRC) using multiple breath washout (MBW), plethysmographic (pleth) FRC and forced expirations from raised lung volumes were measured in 71 infants with CF (participants in the London CF Collaboration) and 54 contemporaneous healthy controls age ~3 months.

Results Compared with controls, and after adjustment for body size and age, LCI, FRC_{MBW} and FRC_{pleth} were significantly higher in infants with CF (mean difference (95% CI): 0.5 (0.1 to 0.9), $p=0.02$; 0.4 (0.1 to 0.7), $p=0.02$ and 0.9 (0.4 to 1.3), $p<0.001$, z-scores, respectively), while forced expiratory volume ($FEV_{0.5}$) and flows (FEF_{25-75}) were significantly lower (-0.9 (-1.3 to -0.6), $p<0.001$ and -0.7 (-1.1 to -0.2), $p=0.004$, z-scores, respectively). 21% (15/70) of infants with CF had an elevated LCI (>1.96 z-scores) and 25% (17/68) an abnormally low $FEV_{0.5}$ (below -1.96 z-scores). While only eight infants with CF had abnormalities of LCI and $FEV_{0.5}$, using both techniques identified abnormalities in 35% (24/68). Hyperinflation ($FRC_{pleth} >1.96$ z-scores) was identified in 18% (10/56) of infants with CF and was significantly correlated with diminished FEF_{25-75} ($r=-0.43$, $p<0.001$) but not with LCI or $FEV_{0.5}$.

Conclusion Despite early diagnosis of CF by NBS and protocol-driven treatment in specialist centres, abnormal lung function, with increased ventilation inhomogeneity and hyperinflation and diminished airway function, is evident in many infants with CF diagnosed through NBS by 3 months of age.

INTRODUCTION

The prognosis of cystic fibrosis (CF) has improved dramatically over the years due to implementation of aggressive treatment to optimise nutrition and pulmonary health following diagnosis and increasing global uptake of newborn screening (NBS) for CF.

Key messages

What is the key question?

► Is lung function normal at 3 months of age in infants with cystic fibrosis (CF) diagnosed via newborn screening?

What is the bottom line?

► Despite early diagnosis of CF by newborn screening and protocol-driven treatment in specialist centres, abnormal lung function is evident in many screened infants with CF by 3 months of age.

Why read on?

► This study, the largest of its kind and the only one with contemporaneous healthy controls, describes early lung development in newborn screened infants with CF.

Despite convincing evidence of the long-term benefits of NBS for CF with respect to improved nutritional status,^{1,2} the extent to which pulmonary outcomes have improved remains controversial. Although some studies have failed to demonstrate any benefits with screening,³ others have reported significantly less pulmonary disease on chest radiography⁴ and stable lung function (LF) with less marked decline over time in the screened group.⁵⁻⁸ The Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF) group initially reported normal values of forced expired volume in 0.5 s ($FEV_{0.5}$) in NBS infants with CF in the first 6 months of life,⁹ whereas a more recent report using slightly different methodology found significant reductions in forced expired flows and volumes during the same period.¹⁰

Even in the absence of any clinical respiratory symptoms, infants with CF diagnosed clinically have impaired LF shortly after diagnosis^{11,12} and this impairment persists into school age despite treatment in specialist CF centres.¹³⁻¹⁵ We have previously shown that the lung clearance index (LCI) measured by multiple breath washout (MBW) is a more sensitive measure of early lung disease in preschool and young school-age children with CF than spirometry,¹⁶⁻¹⁸ and that

measurements during the preschool years are highly predictive of LF at 6–10 years of age.¹³ During infancy, complementary information is provided if the forced expiratory manoeuvres¹⁹ and MBW are undertaken.²⁰ Nevertheless, the extent to which infant LF tests should be used to help guide CF management during infancy remains controversial.²¹

Since universal CF NBS was implemented in the UK in October 2007, the median age of CF diagnosis has fallen to ~1 month.²² However, there have yet to be any large randomised controlled trials of treatment in this age group. For these to be initiated, it is vital to have a greater understanding of the natural history of lung disease in such infants. This study aimed to determine baseline LF within the first 3 months of age in NBS infants with CF and compare findings to prospectively recruited healthy infants of similar age. We hypothesised that, with early diagnosis and commencement of standardised specialist treatment, there would be no difference in LF by 3 months of age in NBS infants with CF compared with healthy babies.

METHODS

As part of a longitudinal collaborative research program of infants with CF diagnosed by NBS (see online supplement), this study received ethical approval (#09/HO71/314) from the North Thames Multi-Centre Research Ethics Committee (REC) and the local RECs of the participating specialist centres.

NBS infants with CF born between January 2009 and July 2011 were eligible for recruitment to the London CF Collaboration (LCFC). Healthy full-term infants of similar age were recruited prospectively from a London community (for details, including eligibility criteria, see online supplement, section 2). Lung function tests (LFTs) at 3 months were completed.

Study protocol

Clinical data

With parental consent, participating centres prospectively completed case record forms (CRFs) at diagnosis and each subsequent clinic visit, documenting mode and date of diagnosis, presentation, genotype, history of respiratory symptoms and/or infection, microbiology, treatment, somatic growth and additional investigations undertaken for clinical purposes. These forms enabled auditing and tracking of participating CF centres' adherence to a standardised study treatment protocol (online supplement, section 6) in accordance to the UK CF Trust guidelines.²³

Following diagnosis, all infants were commenced on multivitamins, pancreatic supplements if appropriate, and prophylactic flucloxacillin according to the standardised study treatment protocol (online supplement, section 6). Cough swabs were taken every 2–3 months at clinics and whenever symptomatic, using a standardised protocol for collection, storage and analysis of samples.²⁴

Lung function testing and anthropometry

All infants were tested at Great Ormond Street Hospital/UCL Institute of Child Health at ~3 months postnatal age, when clinically stable and at least 3 weeks after any respiratory tract illness. Infants were weighed and examined, oxygen saturation (SpO₂) levels were measured (Masimo Radical-7 pulse oximeter, Irvine, California, USA) and vital signs assessed prior to administering chloral hydrate orally (60–100 mg/kg). Weight and crown-heel length were expressed as z-scores to adjust for age and sex.²⁵ LFTs were performed during epochs of quiet sleep with the child lying supine. Heart rate and SpO₂ were moni-

tored continuously throughout testing. Infant urine or maternal saliva samples were collected for cotinine assay to validate parental reported smoking habits (online supplement, section 5, table E4). Parents provided informed written consent and were present throughout measurements.

LCI, a measure of ventilation inhomogeneity, and functional residual capacity (FRC_{MBW}) were measured with MBW, using a respiratory mass spectrometer and customised software.²⁰ The Jaeger BabyBody device (V.4.65; Care Fusion, San Diego, California, USA) was used to measure plethysmographic FRC (FRC_{pleth}),^{26,27} total respiratory compliance (C_{rs}) and resistance (R_{rs}),²⁸ forced expiratory volume (FEV_{0.5}), forced vital capacity (FVC) and forced expiratory flows (FEF₇₅, FEF_{25–75}) from an inflation pressure of 30 cmH₂O using the raised volume technique.¹⁹ Measurements were always performed in that order. Details of data collection and analysis as described previously^{20,27–30} are summarised in the online supplement. Results were expressed as z-scores to adjust for body size, sex and age if appropriate using reference equations derived from up to 140 healthy white infants studied in our department over the past decade using identical equipment and protocols^{19,31,32} (online supplement, section 3). Abnormal lung function was defined as results outside the 95% limits of normal (ie, below –1.96 z-scores for FEV_{0.5} or >1.96 z-scores for LCI (<2.5th or >97.5th centile respectively).

Statistical analysis and power of study

Comparisons of group differences were performed using student t tests for continuous variables (age, body size and LFT z-scores) or χ^2 analyses for categorical variables (sex, ethnicity, maternal smoking, parental occupation and asthma) (PASW Statistics V.18). Within the CF group, the relationship between LFT outcomes was quantified using Pearson correlations. Multiple linear regression analyses were used to quantify impact of maternal smoking (data not shown) and the extent to which potential clinical determinants (CF genotype, respiratory symptoms, cough swab culture and antibiotic use) are associated with LFT outcomes within the CF group after adjustment for sex and current body size. Model estimates and differences between groups are presented with 95% CIs. Taking into account two primary outcomes (LCI and FEV_{0.5}) a sample size of 70 infants with CF and 50 controls (equivalent to 58 in each group) will allow detection of differences equivalent to 0.58 or 0.66 z-scores at the 5% significance level with 80% or 90% power, respectively.³³ Statistical significance was taken as $p < 0.05$.

RESULTS

During the study period, 110 infants screened positive for CF, of whom successful LFT measurements were obtained in 79 (81% of those eligible). Inspection of the prospective CRFs, and regular discussion with consultants, suggested that the standardised treatment protocol had been adhered to in all infants at the time of testing. Details are summarised in figure 1, including success rates for each LFT. For clarity, the results presented here are limited to those without meconium ileus (n=71), although including such infants did not affect the results (data not shown). Of the 274 families with potentially eligible healthy infants whom we contacted, 39 (14%) were ineligible. Of the remaining 235, 54 (23% of those eligible) attended for LFTs (figure 1, details in figure E1, online supplement).

Median (IQR) age at diagnosis for the CF infants was 3.6 (3.1–4.4) weeks; the majority were either homozygous (59%) or heterozygous (32%) $\Delta F508$ and 8 (11%) were pancreatic

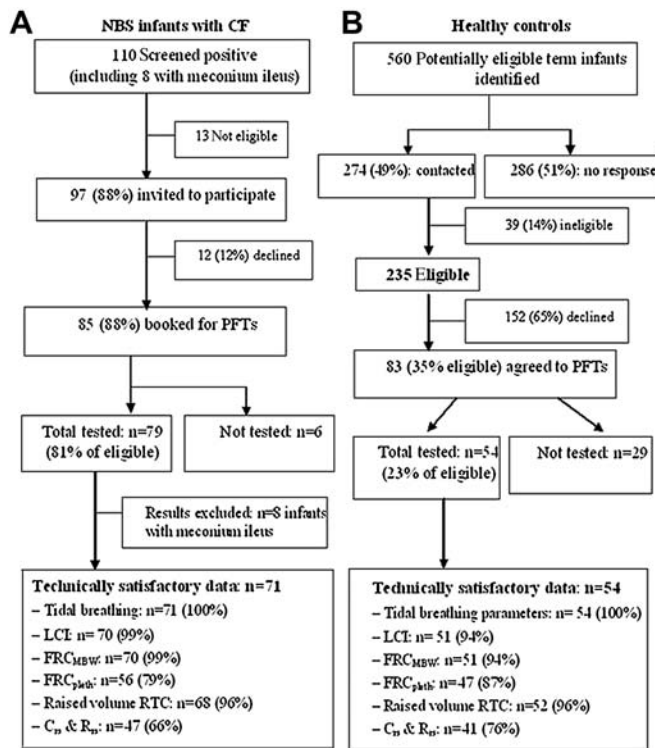


Figure 1 Flow diagram showing success rates in relation to recruitment and achievement of technically acceptable infant lung function outcomes in NBS infants with CF (A) and healthy controls (B). CF, cystic fibrosis; C_{rs}, total respiratory compliance; FRC, functional residual capacity; LCI, lung clearance index; MBW, multiple breath washout; NBS, newborn screening; PFT, pulmonary function test; pleth, plethysmographic; R_{rs}, total respiratory resistance; RTC, Rapid Thoraco-abdominal Compression.

sufficient. Sixty five (92%) NBS infants with CF had had no respiratory or only mild coryzal symptoms prior to LFTs (figure 2). The remaining 6 (8%) had had significant cough and/or wheeze, 16 (23%) had had at least one positive cough swab by ~3 months of age (two with *Pseudomonas aeruginosa*; 10 with *Staphylococcus aureus* and four with *Haemophilus influenzae*). These children were prescribed antibiotics according to a standardised treatment protocol (online supplement, section 6) in addition to the prophylactic flucloxacillin prescribed for all infants on diagnosis. No infant had positive cough swab culture for *Stenotrophomonas maltophilia*; *Burkholderia cepacia complex* or *Aspergillus fumigatus*. Additional antibiotics were also prescribed for 36 infants with negative cough swabs, 32 (89%) of whom had had prior cough and/or wheeze. Thus in total, 52 (73%) NBS infants with CF received *additional* antibiotics prior to their first LFTs at ~3 months (figure 2), but all were asymptomatic at the time of testing. Of the 46 infants with CF who had cough swabs taken within 10 days of LFTs, only three yielded positive growth (two for *S aureus* and one for *H influenzae*).

Table 1 summarises background characteristics of study participants. With the exception of a slightly lower, but statistically significant, gestational age, both groups were very similar.

Infant details at the time of LFTs are shown in table 2. NBS infants with CF were on average 1 week younger than healthy controls, with a significantly greater proportion having birth weights <10th percentile. After taking age and sex into account, infants with CF were significantly lighter and shorter and had a lower body mass index than the controls. The change in

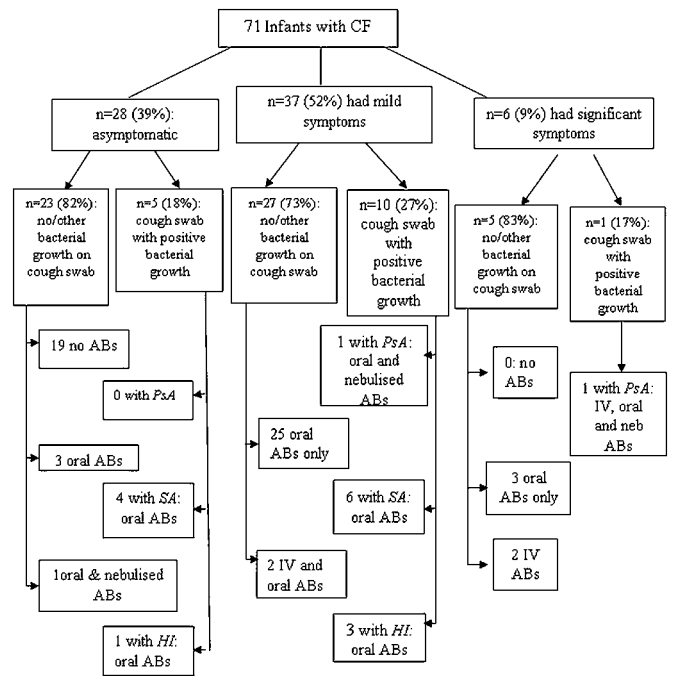


Figure 2 Clinical symptoms and additional antibiotic treatment* of newborn screened infants with cystic fibrosis (CF) prior to lung function tests. Although mothers of infants with CF occasionally reported mild symptoms (slight cough or mild sniffles) in the weeks prior to LFTs, on the day of the test all infants had clear chests on auscultation with no sign of blocked nostrils or cough. Reports of significant symptoms included previous wheeze, crackles, tachypnoea with or without cough and cold. *Antibiotic prescribed in addition to prophylactic flucloxacillin due to respiratory symptoms and/or positive growth on cough swab. See online supplement for standardised treatment protocol. AB, antibiotic; *HI*, *Haemophilus influenzae*; IV, intravenous; *PsA*, *Pseudomonas aeruginosa*; *SA*, *Staphylococcus aureus*.

weight z-score between birth and LFT was significantly lower in infants with CF than in controls (table 2).

Lung function results

Technically satisfactory measurements of LCI and FRC_{MBW} were obtained in 121 infants (97% of those in whom they were attempted), forced expired volumes and flows in 120 (96%), plethysmographic FRC in 103 (82%), C_{rs} and R_{rs} in 88 (70%) (figure 1, table 3). Failures were largely due to infants waking prior to completion of the test protocol or technically unacceptable data. With the exception of tidal volume, which was slightly higher (0.4 z-scores) in those with CF, there were no significant differences for any of the tidal breathing outcomes or passive respiratory mechanics (C_{rs} and R_{rs}). LCI was significantly higher in those with CF, whether expressed as absolute values (online supplement, table E2) or as z-scores (table 3, figure 3). Similarly after adjustment for age, length and sex, FRC_{MBW} was significantly higher (0.38 z-score) in infants with CF. Additional evidence of hyperinflation and gas trapping was seen in those with CF, with FRC_{pleth} and ΔFRC (ie, difference between FRC_{pleth} and FRC_{MBW} z-scores) being significantly elevated (table 3, figure 3). After correction for body size, forced expired flows and volumes were significantly decreased in infants with CF compared with healthy controls, by between 0.5 and 0.9 z-scores, indicating the presence of airways obstruction (table 3, figure 3). The extent to which interpretation of these results would have differed had they simply been

Table 1 Comparison of background characteristics in infants with cystic fibrosis* and healthy controls

	Infants with CF* (n=71)	Healthy controls (n=54)	Δ (95% CI), CF – controls	p Value
Boy, n (%)	33 (46)	27 (50)	–4% (–21% to 13%)	0.696
Gestational age, weeks	39.3 (1.5)	40.2 (1.2)	–0.9 (–1.4 to –0.5)	<0.001
Birth weight, z-score†	–0.34 (0.96)	0.01 (0.84)	–0.35 (–0.67 to –0.03)	0.035
Birth weight below 10th percentile, † n (%)	16 (23)	2 (4)	19% (7% to 30%)	0.003
White mother, n (%)	62 (87)	47 (87)	0% (–12% to 13%)	0.962
Maternal smoking during pregnancy, n (%)	8 (11)	4 (7)	4% (–7% to 14%)	0.456
Current maternal smoking, n (%)	9 (13)	5 (9)	4% (–8% to 15%)	0.540
Mother in non-manual occupation	49 (69)	43 (80)	–11% (–25% to 5%)	0.171
Fathers in non-manual occupation§	41 (59)	40 (75)	–15% (–30% to 2%)	0.055
Maternal asthma, n (%)	13 (18)	10 (19)	0% (–14% to 13%)	0.976

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

*Limited to those without meconium ileus.

†Calculated using UK WHO algorithms.²⁵

§n=69 CF and n=53 control infants.

CF, cystic fibrosis; Δ, difference between groups.

expressed as absolute values or weight-corrected ratios is presented in table E2 of the online supplement.

Additional determinants of lung function

On multivariable analyses, after adjustment for CF, other potential determinants (sex, gestational age, birth weight z-score, pre- or postnatal maternal smoking and maternal asthma) were not significantly associated with any LF z-scores. Among infants with CF, with the exception of a significantly lower FEV_{0.5} (mean (95% CI): –0.70 (–1.29 to –0.10) z-scores) in those who had received any additional antibiotics for symptoms or positive cough swab, there was no significant association between LF outcomes and the infants' genotype, clinical status or treatment prior to LFTs at ~3 months of age (see table E3, OLS).

Relationship between different lung function outcomes in infants with CF

The relationship between selected LF outcomes in infants with CF is shown in figure E2 (online supplement). There was no significant relationship between the two primary outcomes FEV_{0.5} and LCI ($r=-0.10$, $p=0.432$). Twenty-one percent of infants had an LCI above 1.96 z-scores, whereas 25% had an FEV_{0.5} below –1.96 z-scores, while only 12% (8/68) had abnormalities detected by both these tests (figure E2a); if based on either test, 35% (24/68) would be identified with abnormal results. FEF_{25–75} and FEV_{0.5} ($r=0.73$, $p<0.001$) detected a similar proportion of infants outside the normal range (24% and 25%, respectively, figure E2b), whereas FEF₇₅ was less discriminative (only detecting abnormalities in 15% of infants; data not shown). FRC_{pleth} z-score and ΔFRC were highly correlated

($r=0.66$, $p<0.001$, figure E2d), both detecting a similar proportion of infants with abnormally elevated results (18% and 20%, respectively). Forty-four percent (31/71) of NBS infants with CF had at least one abnormal result if based on LCI, FEV_{0.5} or FRC_{pleth}.

DISCUSSION

In this, the largest study of its kind and the only one with contemporaneous healthy controls, we have shown that, by 3 months of age, many NBS infants with CF have reduced forced expired flows and volumes, abnormal gas mixing and hyperinflation, despite early diagnosis and protocol-driven management, which included prophylactic oral flucloxacillin from time of diagnosis.

Strengths and weaknesses

The major strengths of this study include the fact that all lung function measurements were performed to international standards in a single centre by a highly experienced team, thereby minimising methodological or analytical bias. Accurate identification of the extent to which abnormalities in LF were present in individual infants was facilitated by expressing results as z-scores. These were derived from equipment-specific reference equations based on a large group of healthy infants studied in our department with the same equipment and methods over the past decade.^{31 32} The ability to recruit a large group of contemporaneous healthy controls specifically for this study further strengthened the confidence with which we could detect changes due to CF lung disease after adjusting for other relevant determinants such as body size, age and sex.^{34 35}

Table 2 Infant details at time of lung function test

	Infants with CF (n=71)	Healthy controls (n=54)	Δ (95% CI) CF – controls	p Value
Age at test*, weeks	11.4 (2.3)	12.2 (2.0)	–0.82 (–1.59 to –0.06)	0.043
Weight, kg	5.32 (0.84)	6.05 (0.78)	–0.73 (–1.02 to –0.45)	<0.001
Weight, z-score†	–0.75 (1.08)	0.06 (1.93)	–0.81 (–1.17 to –0.45)	<0.001
Length, cm	59.2 (2.7)	61.6 (2.3)	–2.4 (–3.3 to –1.7)	<0.001
Length, z-score†	–0.14 (1.04)	0.72 (0.86)	–0.86 (–1.19 to –0.52)	<0.001
Body mass index	15.1 (1.6)	15.9 (1.4)	–0.8 (–1.3 to –0.3)	0.005
Body mass index, z-score†	–0.94 (1.08)	–0.47 (0.98)	–0.47 (–0.84 to –0.10)	0.013
Change in weight z-score (3 month – birth)	–0.42 (1.06)	0.05 (1.00)	–0.46 (–0.83 to –0.10)	0.014

Unless stated otherwise, data presented as mean (SD).

*Postnatal age at test corrected for gestational age.

†Calculated using UK WHO algorithms.²⁵

CF, cystic fibrosis; Δ, mean difference between groups.

Table 3 Comparison of lung function in 71 infants with cystic fibrosis and 54 healthy controls

Variables according to test order	n	Infants with CF	n	Healthy controls	Δ (95% CI) CF – controls	p Values
Multiple breath washout						
LCI, z-score*	70	0.90 (1.38)	51	0.39 (0.88)	0.51 (0.10 to 0.91)	0.016
FRC _{MBW} , z-score*	70	0.15 (0.95)	51	-0.23 (0.86)	0.38 (0.05 to 0.71)	0.023
Tidal breathing						
Respiratory rate, z-score†	71	-0.01 (1.43)	54	0.02 (1.21)	-0.03 (-0.50 to 0.44)	0.897
Tidal volume, z-score†	71	0.20 (1.19)	54	-0.22 (1.09)	0.42 (0.01 to 0.82)	0.044
t _{PTEF} /t _E , z-score†	71	0.22 (1.05)	54	0.41 (1.0)	-0.19 (-0.56 to 0.18)	0.312
Passive mechanics						
C _{rs} , z-score‡	47	0.22 (1.48)	41	0.14 (0.96)	0.08 (-0.44 to 0.60)	0.763
R _{rs} , z-score‡	47	0.52 (0.94)	41	0.28 (1.04)	0.24 (-0.18 to 0.66)	0.237
Plethysmography						
FRC _{pleth} , z-score‡	56	0.77 (1.15)	47	-0.08 (1.03)	0.85 (0.43 to 1.28)	<0.001
ΔFRC z-scores (pleth – MBW)	55	0.68 (1.10)	45	0.19 (0.92)	0.48 (0.08 to 0.88)	0.018
Raised volume technique						
FEV _{0.5} , z-score‡	68	-1.17 (1.20)	52	-0.25 (0.80)	-0.92 (-1.29 to -0.56)	<0.001
FVC, z-score‡	68	-0.45 (1.07)	52	0.15 (0.71)	-0.60 (-0.92 to -0.28)	<0.001
FEF ₂₅₋₇₅ , z-score‡	68	-1.11 (1.38)	52	-0.46 (1.08)	-0.66 (-1.10 to -0.21)	0.004
FEF ₇₅ , z-score‡	68	-0.74 (1.19)	52	-0.22 (1.11)	-0.53 (-0.95 to -0.11)	0.015

For comparison with previous publications, results expressed as absolute values, or in relation to body weight, are presented in table E2, online supplement.

Data shown as mean (SD).

*According to Lum *et al.*³¹

†According to Nguyen *et al.*³²

‡According to Lum *et al.*³⁴

CF, cystic fibrosis; CI, confidence interval of the difference; Δ, mean difference between groups.

CF, cystic fibrosis; C_{rs}, total respiratory compliance; FEF₇₅, forced expiratory flow at 75% of vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of vital capacity; FEV_{0.5}, forced expiratory volume in 0.5 s; FRC, functional residual capacity; FVC, forced vital capacity; LCI, lung clearance index; MBW, multiple breath washout; pleth, plethysmographic; R_{rs}, total respiratory resistance; t_{PTEF}/t_E, ratio of the time to reach peak tidal expiratory flow in relation to expiratory time.

Inevitably, it was necessary to approach a large number of families of healthy infants to recruit appropriate numbers for comparison, with only 25% of those eligible and whom we could contact actually attending for the tests. This could potentially introduce some bias, especially as due to data protection issues, it was not possible to document background data from those not responding or agreeing to participate. However, it was reassuring to find no significant differences with respect to potential determinants of infant LF such as ethnicity, maternal history of asthma, socioeconomic status (based on parental occupation) or exposure to tobacco smoke between the controls and infants with CF, suggesting that the group were representative of the local population. Furthermore, when expressed as z-scores, both anthropometry and LF were very similar in this current healthy control group as in those previously recruited in London by our team.^{15 20} Travel expenses were reimbursed, but no incentives were provided to encourage attendance for either group, thereby removing a potential source of bias. Infants with CF were, on average, born 1 week earlier than the controls, a pattern that we have noted in previous studies.¹¹ Any potential group differences due to developmental changes were avoided by studying all infants within a narrow age range; mean difference at time of test being less than a week.

Of the 110 infants screening positive for CF over the 2.5-year recruitment period, 12% were excluded due to coexistent morbidity or psychosocial reasons, with tests not possible in a further five infants before 4 months of age due to recent exacerbations and deferment of appointments. If anything, the results from this study therefore underestimate the degree of morbidity by 3 months of age. Nevertheless, with only 13% of parents declining participation (including one family that withdrew after initial consent), we were able to test 79/110 (72%) of the entire cohort and 81% of those eligible.

Since lung function was assessed in the first few months of life, we excluded results from those presenting with meconium

ileus to preclude any adverse effects related to surgery, although subsequent sub-analysis indicated that the results were not affected by including such children. All infants were tested at least 3 weeks after a respiratory illness when free of symptoms, in an attempt to minimise impact of acute exacerbations. This did mean that we had to exclude several healthy controls in whom it was impossible to rearrange appointments within the designated age range for this study of 'early LFT' (figure E1, online supplement). While it could be argued that the observed differences in LF between CF and controls may simply represent post-respiratory tract infection changes in those with CF, this is unlikely given the lack of association between LF and respiratory symptoms in those with CF, except for a significant reduction in z-FEV_{0.5} when infants had received additional antibiotics (table E3, online supplement).

To optimise recruitment to this observational study and to ensure initial LFTs could be undertaken within the first months of life, routine bronchoscopy and bronchoalveolar lavage were not included within this study protocol but, together with chest high-resolution CT scans, will be included when these children are reassessed at 1 year of age.

A further strength of this study was the wide range of tests applied, minimising the chance that early changes in LF would be missed. As in a previous study of infants,²⁰ but in contrast to findings in older children,^{13 16 17 36} we found that assessments of lung disease based on forced expiratory manoeuvres and ventilation inhomogeneity were necessary to identify early lung disease during the first year of life, with additional useful information regarding hyperinflation being obtained from plethysmographic lung volumes. The slightly lower absolute mean LCI observed in infants with CF in this study (table E2, online supplement) than in our previous study²⁰ probably reflects the fact that these infants were diagnosed by NBS rather than clinically, and were therefore assessed earlier in the disease process. Had assessments been limited to a single technique,

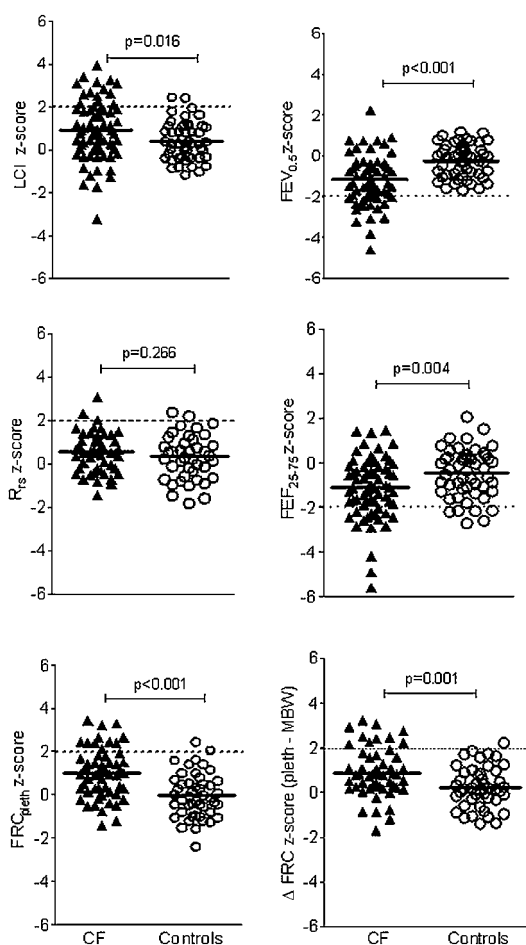


Figure 3 Comparison of lung function outcomes between infants with cystic fibrosis and healthy controls. As expected, approximately 95% of healthy controls had values within ± 1.96 z-scores, with none having an abnormal FEV_{0.5} z-score, 2/51 (4%) having an elevated LCI z-score, and 2/47 (4%) an elevated FRC_{pleth} z-score. FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of vital capacity; FEV_{0.5}, forced expiratory volume in 0.5 s; FRC_{pleth}, plethysmographic functional residual capacity; LCI, lung clearance index; MBW, multiple breath washout; R_{rs}, total respiratory resistance.

abnormalities would only have been detected in ~25% of infants with CF, this proportion rising to 35% when using either of the two primary outcomes (LCI and FEV_{0.5}). The relatively poor correlation between these various outcomes suggests that they are reflecting different aspects of underlying pathophysiology. Despite their relative simplicity, assessments of tidal breathing variables and passive mechanics were far less discriminatory than the other techniques and could be usefully omitted when investigating infants with CF.³⁷ While C_{rs} results were lower and R_{rs} higher in those with CF when expressed as absolute values (table E2, online supplement), this was largely related to body size at the time of testing; C_{rs} increasing and R_{rs} decreasing with somatic growth.^{32–38} In contrast to all other outcome measures investigated, after adjusting for length and/or age, no significant differences were observed with respect to either C_{rs} or R_{rs} (table 3).

Clinical status

Despite early diagnosis and commencement of pancreatic enzyme replacement therapy, vitamin supplement and prophylactic antibiotics, infants with CF experienced significantly

slower growth during the first few months and were significantly lighter and shorter than their healthy peers by the time of the 3-month LFTs. Furthermore, by this age, 61% of the screened infants had had some respiratory symptoms (52% mild, 9% severe), 23% a positive cough swab and 73% had received antibiotics in addition to their routine prophylactic medication. Pulmonary involvement is known to be present early, with some infants with CF having evidence of inflammation in the bronchoalveolar lavage fluid as early as 4 weeks of age.^{39–41} With the exception of a significantly lower FEV_{0.5} in those who had received additional antibiotics for symptoms or positive cough swab, there was no significant association between LF outcomes and the infant's genotype, clinical status, growth trajectory or treatment prior to the LFTs at 3 months of age. Of note, many infants who had been treated aggressively for respiratory exacerbations in the first few months had entirely normal LF by 3 months, whereas others with no prior symptoms or cause for concern had evidence of early lung disease.

Comparison with the literature

The commonest characteristics of LF abnormalities described in CF lung disease during the first years of life have been airway obstruction detected using the raised volume technique,^{10–12 15 20} hyperinflation indicated by elevated resting lung volumes,⁴² increased ventilation inhomogeneity²⁰ and, in infants and slightly older children, gas trapping,^{17 18 43 44} all of which were observed in this study by 3 months of age in infants diagnosed by NBS.

In a US multicentre evaluation of LF in infants with CF aged 4–24 months (21% diagnosed by screening),⁴² elevated lung volumes and diminished forced expiratory flows, but no reduction in FEV_{0.5}, were reported when compared with historical controls. The variability in skill mix and experience of the laboratories participating in that study, together with the lack of contemporaneous healthy controls and different age range studied, may have contributed to differences in findings compared with the current results.³⁵

The Australian AREST-CF study recently published LF results from infants with CF diagnosed by NBS.^{9 10} When using a lung inflation pressure of 20 cmH₂O during forced expiratory manoeuvres, LF was reported to be normal during the first 6 months of life, but thereafter declined at a rapid rate.⁹ However, the number of infants studied during the first months of life was limited. By contrast, a more recent publication from this group,^{9 10} reporting measurements from 28 NBS infants with CF within the first 6 months of life when using an inflation pressure of 30 cmH₂O as recommended by the American Thoracic Society/European Respiratory Society guidelines¹⁹ showed diminished LF at the time of the first test, with continued deterioration over the next 2 years of life when the results were compared with published reference equations.⁴⁵

Clinical implications

The results from this study indicate that despite early diagnosis and rapid implementation of therapy, including prophylactic antibiotics, a substantial number of NBS infants with CF have abnormalities of LF within the first 3 months of life. The apparent wellness of the cohort should not lead to complacency, and prompt and aggressive treatment of any abnormal symptoms or signs is surely vital. Follow-up of this cohort will be essential to ascertain the extent to which early changes in LF persist throughout the first year of life; if there is catch-up growth with conventional treatment, then novel, molecular-

based therapies^{46 47} may be safely deferred; if not, there will be a compelling case for initiating treatment early in these infants, using physiological endpoints to detect benefit.

CONCLUSIONS

Despite early diagnosis of CF by NBS and protocol-driven treatment in specialist centres, abnormal LF, with increased ventilation inhomogeneity and hyperinflation and diminished airway function, is evident in many infants with CF diagnosed through NBS by 3 months of age. CF clinicians should not be lulled into thinking that babies with CF identified by NBS have good pulmonary health in the first few months of life.

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Contributors Conception and design of the study: JS, AB. Supervision of the study: JS. Research governance issues including ethics committee approval: JS, JC. Setting up of recruitment process: AFH, JC. Technical training, supervision and audit of data collection/analyses: AFH. Recruitment of infants with CF: AB, IBL, SBC, HAW, JP, RJC, AS. Recruitment of healthy control infants: KLC, AFH, DA, LPT, TDN, JC. Data collection/analysis and interpretation of results: JS, AFH, LPT, TDN, JC, SL. Statistical analyses: AFH, JS, AW. Drafting the manuscript: AFH, JS, LPT, IBL, AB, CW. Approval for intellectual content: all authors.

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Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was granted by the North Thames Multi-centre Research Ethics Committee (REC) (#09/H071/314) and local REC of the participating specialist centres.

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**LUNG FUNCTION IS ABNORMAL IN 3 MONTH OLD INFANTS WITH CYSTIC
FIBROSIS DIAGNOSED BY NEWBORN SCREENING**

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Online Data Supplement

1. Background

As part of a longitudinal research programme of infants with CF diagnosed by newborn screening (NBS), this study measured lung function at 3 months in NBS infants with CF and contemporaneous healthy controls of similar age (the focus of the current article), with follow up tests at 1 and 2 years of age (to be reported at a later date). Clinical status including use of medications and anthropometry were documented prospectively. This online supplement (OLS) provides additional details regarding recruitment, methods and results, for which there was no space in the main article.

2. Subjects and Methods

Screened infants with CF, referred to 6 tertiary CF centers participating in the London Cystic Fibrosis Collaboration (LCFC) in the Greater London regions, UK, were eligible for recruitment to the study. The LCFC consists of -

- The Respiratory Unit, Great Ormond Street Hospital (GOSH) for Children NHS Foundation Trust, London;
- The Department of Paediatric Respiratory Medicine, Imperial College & Royal Brompton & Harefield Hospitals NHS Trust, London;
- The Department of Paediatric Respiratory Medicine, Barts & The Royal London Hospital, London;
- Queen Mary's Hospital for Children, Epsom & St Helier University Hospitals NHS Trust, Surrey;
- The Department of Paediatric Respiratory Medicine, Kings College Hospital, London;
- The Department of Child Health, Lewisham Healthcare NHS Trust, London.

2.1. Recruitment of screened infants with CF

Infants diagnosed with CF via NBS with an elevated concentration of immunoreactive trypsinogen (IRT) and confirmation of positive CF transmembrane conductance regulator (CFTR) gene mutation profile and/or sweat chloride test

(http://newbornbloodspot.screening.nhs.uk/nat_std_cf_protocol; date accessed: February 2012) referred to the LCFC centers between January 2009 and July 2011, were eligible for recruitment.

Unless there were any special circumstances, parents were invited to participate in the study by their consultants within approximately 4 weeks of diagnosis during a follow-up “Education” visit to the tertiary CF center. The purpose of the study was explained verbally and written information, together with an illustrated leaflet, was given to parents. The family was allowed time to consider the information and ask further questions before giving written consent.

2.1.1. Inclusion criteria for infants with CF

- Infants diagnosed with CF by NBS within the Greater London catchment area.

2.1.2. Exclusion criteria for infants with CF

- Infants with CF born <37 weeks gestation
- Severe congenital disorders, cardio-vascular, skeletal, neuro-muscular or metabolic co-morbidities that could impact on the respiratory system;
- Inability of parents to understand and give informed consent;
- Recruitment contra-indicated on psycho-social grounds;
- History of apnoeic episodes or upper airway pathology;
- Family due to move out of area.

2.2. Recruitment of healthy control infants

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

2.2.1. Inclusion criteria for healthy controls

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

2.2.2. Exclusion criteria for healthy controls

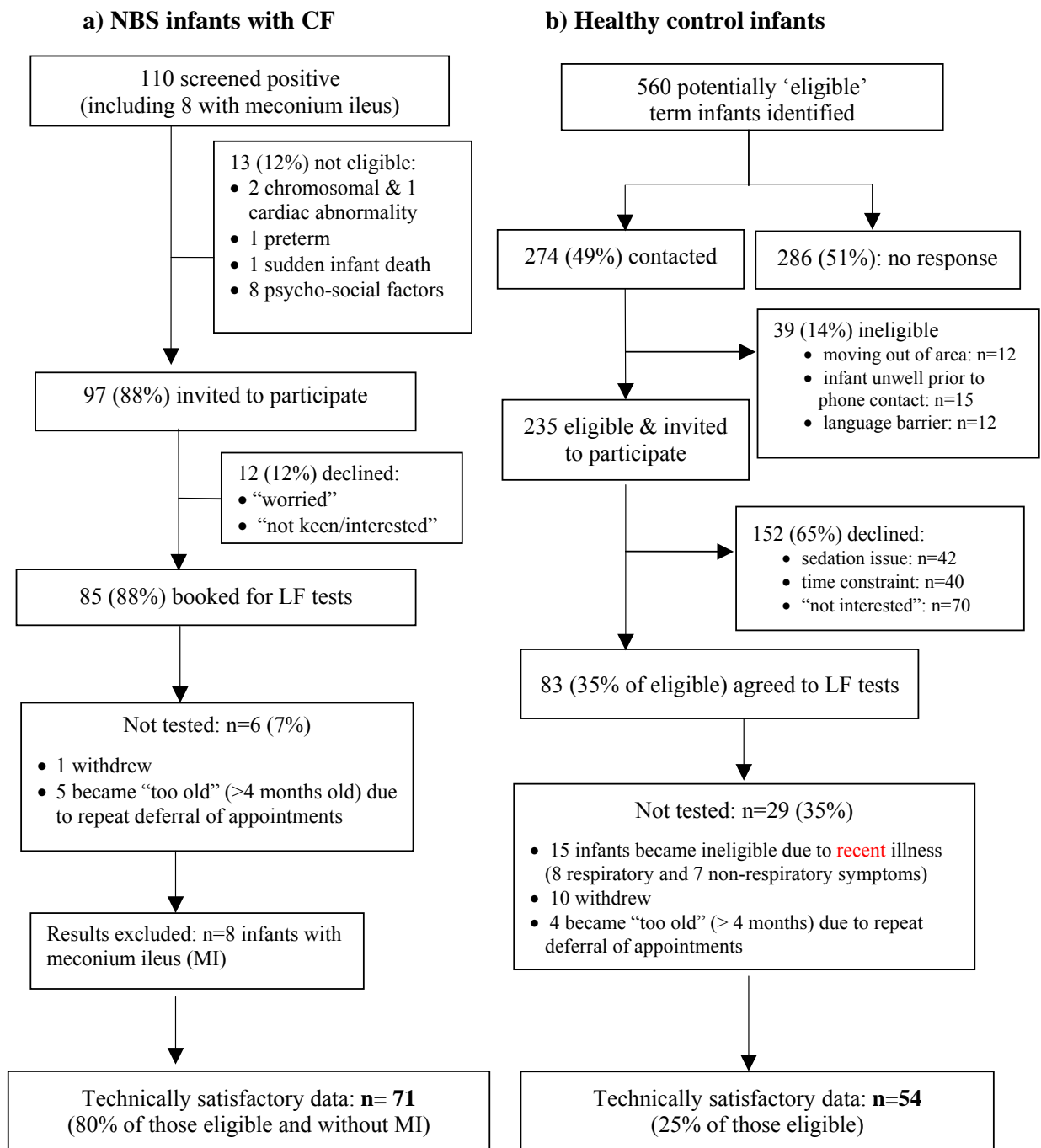
3. Infants born <37 weeks gestation

- Inability of parents to understand and give informed consent;
- Recruitment contra-indicated on medical and/or psycho-social grounds;
- History of apneic episodes or upper airway pathology;

- History of chronic diarrhoea or failure to thrive;
- History of neonatal lung disease, assisted ventilation or co-existent cardio-vascular, skeletal, neuro-muscular, renal or metabolic disorders that could impact on the respiratory system and
- Previous physician diagnosed or hospital admission for lower respiratory tract infections.

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

Figure E1. Flow diagram showing additional details of recruitment process



Footnote: NBS=newborn screen; CF=cystic fibrosis; LF = lung function.

3. Data collection

All lung function tests were performed between January 2009 and October 2011 in a single infant lung function laboratory at the Great Ormond Street Hospital / UCL Institute of Child Health, London. Weight and crown-heel length, measured using an infant stadiometer, were expressed as z (or SD) scores to adjust for age and sex.^{E1}

3.1. Multiple breath inert gas washout (MBW) technique

Lung clearance index (LCI) assesses overall efficiency of ventilation distribution or gas mixing within the lung and provides a measure of early lung disease; functional residual capacity measured using MBW (FRC_{MBW}) represents the resting lung volume that is communicating with the airway opening at time of measurement. The equipment and test procedure for performing MBW in infants were similar to those for preschool children, as described in detail previously (online supplement:

<http://ajrccm.atsjournals.org/cgi/data/171/3/249/DC1/1>)^{E2-4} and summarised below.

Data collection was performed in two stages:

- i) the wash-in phase involved the infant inspiring a bias flow of dry air mixture containing the tracer gas (4% sulfur hexafluoride (SF_6)), 21% oxygen and balance nitrogen, and continued until inspiratory and expiratory SF_6 concentrations were stable and equal at 4% for a minimum of 5-8 breaths, at which time the bias flow was removed;
- ii) the wash-out phase began with the infant inhaling room air and continued until end tidal SF_6 concentration was consistently below 0.1%, i.e., less than $1/40^{\text{th}}$ of the starting concentration.

LCI is defined as the number of lung volume turnovers (or number of FRCs) required to clear the lungs of the inert tracer gas to $1/40^{\text{th}}$ of the starting concentration of the tracer gas. Data were acceptable only if there was no evidence of mask leak or flow through the pneumotachometer (PNT).^{E5} LCI and FRC_{MBW} were calculated as described previously^{E2,E4,E6,E7} and reported as the mean of three technically satisfactory MBW recordings. In exceptional cases, the mean of two technically acceptable recordings was used if results were within 5% of one another. During a recent extensive investigation, the CV (SD) for LCI was found to be 4.1 (2.4)% in healthy infants and children, and 8.9 (1.9)% in those with CF (Robinson, P et al, Pediatric Pulmonology, in press; ‘Abbreviated multi-breath washout for calculation of Lung Clearance Index’). For the current study, only 4% of MBW washouts failed to meet quality control criteria.

3.1.1. Prediction equations for lung clearance index and FRC_{MBW}

Until recently, there were no published reference equations for LCI data during infancy, results being reported as absolute values with a fixed upper threshold to identify abnormally elevated results,^{E3,E8} despite the fact that somewhat elevated values have been noted in healthy infants when compared with older children.^{E9} Recent collation of MBW data from 497 subjects (48% boys) on 659 test occasions from birth to 19 years of age collected using identical methods and equipment from 3 centres (London, Sweden and Toronto) has now allowed appropriate reference equations to be developed for both LCI and FRC_{MBW} .^{E10} These reference equations were constructed using the LMS [Lambda (L), Mu (M), Sigma (S)] method as described previously.^{E11, E12} Together the L, M and S coefficients are combined algebraically to convert an individual observation to a z-score.

$$\text{z-score} = [(\text{Measurement}/M)^L - 1] / [L*S]$$

$$\text{Upper Limit of Normal (ULN, i.e. 97.5}^{\text{th}} \text{ percentile)} = M*(1.96*S*L + 1)^{1/L}$$

After adjusting for length or height, age and sex did not contribute to the model when predicting LCI whereas for FRC_{MBW} , length, age and sex all made a significant contribution.

Reference equation for LCI

Table E1: Paediatric reference equations for LCI and FRC_{MBW}

LCI		
L		-0.81
M (Predicted)		$5.99 + (73.86 * \text{height}^{-1})$
S		0.08
ULN		$\text{Predicted} * ((1.96 * 0.08 * -0.81) + 1)^{1 / -0.81}$
FRC		
L		0.19
M (Predicted)		$\exp(-11.10 + (2.12 * \ln \text{Height}) + (0.27 * \text{age}^{0.5}) + (0.04 * \text{sex}))$
S		$\exp(-1.67 + 148.57 * \text{height}^{-2})$
ULN		$\text{Predicted} * ((1.96 * S * 0.19) + 1)^{1 / 0.19}$

*Footnote: Based on these equations, the ULN for LCI can be simplified to Predicted LCI * 1.18; whereas that for FRC_{MBW} becomes $((0.36 * S + 1)$ to the power 5.37) * Predicted FRC.*

Results from this study have been expressed as SD- or z-scores according to these equations, with LCI also being expressed in absolute values where appropriate to facilitate comparison with previous publications (Table E2). A comparison of LCI and FRC_{MBW} z-scores, as well as the difference between plethysmographic and MBW z-scores for FRC (ΔFRC), in infants with CF versus healthy controls is presented in the main article (Table 3, Figure 3).

3.2. Plethysmographic FRC and Tidal Breathing variables assessed using the Jaeger MasterScreen BabyBody system (v4.65; CareFusion)

Measurements of plethysmographic FRC were obtained in accordance with ATS/ERS guidelines.^{E13} After several regular breaths had been recorded, a brief airway occlusion was performed to ensure that there was an airtight seal around the face mask prior to lowering the hood to close the plethysmograph. **Tidal breathing variables** (tidal volume (V_T); respiratory rate (RR); minute ventilation (V_E) and the ratio of time to reach peak expiratory flow/expiratory time (t_{PTEF/t_E}) were recorded for 2-3 minutes while the plethysmograph reached thermal equilibrium. Full details for Jaeger infant box calibration and FRC data collection have been reported previously.^{E14}

The criteria for technical acceptability when collecting FRC_{pleth} data were:

- no evidence of mask leak (no flow through the PNT and no decay of pressure plateau at the airway opening during the occlusion), with the pre-occlusion end expiratory level from the time-based tidal volume trace being re-established within 5–10 breaths post-occlusion;
- ideally, three (minimum two) complete respiratory efforts against the occlusion were recorded. This is essential for adequate correction of the box volume drift, the magnitude and direction of which will change during the airway occlusion, despite establishing a stable trace prior to the occlusion;
- changes in plethysmographic pressure in phase with changes in pressure at the airway opening during airway occlusion, with no evidence of phase lag or “looping” due to glottis closure, leak or poor drift correction.

At least five satisfactory occlusion maneuvers were performed for each infant. The mean of 3–5 measures of FRC_{pleth} (minimum 2 if highly repeatable and of good quality) that were within 10% were reported.

3.3. Total respiratory compliance (C_{rs}) and resistance (R_{rs})

Passive respiratory mechanics (C_{rs} and R_{rs}) was assessed using the single occlusion (SO) technique via the Resistance/Compliance Program of the Jaeger MasterScreen BabyBody system (v 4.65, CareFusion, USA). Details of the protocol for data collection have been described previously.^{E15} After 5–10 regular breaths had been recorded to establish a stable end-established level (EEL), a brief airway occlusion was performed at the end of a tidal inspiration, temporarily maintaining lung volume above the EEL to evoke the Hering Breuer Inflation reflex and hence, respiratory muscle relaxation.^{E15,16} During airway occlusion (i.e., a constant lung volume with no flow), rapid equilibration occurs within the lungs. The pressure plateau recorded at the airway opening (P_{ao}) during such occlusions represents the alveolar pressure, which in turn represents the summed elastic recoil pressure of the lung and chest wall. C_{rs} is calculated as the volume above the relaxed EEL divided by P_{ao} . Provided expiration remains relaxed following release of airway occlusion and the respiratory system can be described by a single time constant, the slope of the relaxed expiratory portion of the flow-volume curve represents the expiratory time constant of the respiratory system (t_{rs}). Since t_{rs} is the product of C_{rs} and R_{rs} , resistance can be calculated as t_{rs}/C_{rs} minus the resistance of the apparatus.^{E16}

Criteria for technically satisfactory data were:

- Smooth expiration, proceeding to within 10% of previous expiration with no evidence of glottic closure, braking or active expiratory efforts;

- Duration of the pressure plateau at the airway opening ≥ 100 ms with variability < 10 Pa;
- Linearity of the flow-volume curve over at least 40% of expiration with $r^2 > 0.99$.

C_{rs} and R_{rs} results were reported using the mean from 3–5 technically acceptable maneuvers.

For the calculation of predicted values and z-scores for FRC_{pleth} , tidal volume (V_T), respiratory rate (RR), the ratio of time taken to reach peak tidal expiratory flow: total expiratory time ($t_{PTEF}: t_E$), passive respiratory mechanics (C_{rs} and R_{rs}), please refer to recently created reference equations derived from healthy infants studied with identical equipment and protocols.^{E17}

3.4. Raised volume (RV) forced expiratory maneuvers

Prior to performing the raised volume forced expiratory maneuvers, tidal forced expiratory maneuvers^{E18} were undertaken to determine the optimal jacket compression pressure (P_j) at which flow limitation was achieved, i.e., the point at which no further increase in expiratory flow was observed despite further increases in applied jacket pressure.^{E18,19} The optimal P_j thus obtained was used for the raised volume maneuvers to obtain “full” or raised volume forced expiratory variables.

Airway function at raised lung volume was assessed as previously described.^{E20,21} Expiration was forced from an augmented lung volume using an inflation pressure of 30 cmH₂O (2.93kPa) and maneuvers repeated until three (minimum of two) technically acceptable and reproducible (sum of $FEV_{0.5}$ and FVC within 10% of each other) flow-volume curves were obtained. $FEV_{0.5}$, FVC and FEF_{75} and FEF_{25-75} were reported from the “best” curve, defined as the technically satisfactory maneuver with the highest sum of $FEV_{0.5}$ and FVC.^{E20} The criteria for technically acceptable forced expired flow-volume curves were: peak expiratory

flow achieved prior to 10% of expired volume, complete expiration towards residual volume (RV) (i.e., no evidence of early inspiration) and no marked flow transients or glottic closure.^{E20} In our laboratory, following quality control, the coefficient of variability for FEV_{0.5} during the first year of life is 3.6% (95% CI: 3.1%; 4.1%). Results were expressed as z-scores to account for sex, age and/or body length, adjusted for the equipment used (Jaeger Masterscreen BabyBody) as described previously.^{E22} Reduced airway function was defined as a z-score below -1.96 (<2.5th centile).

4. Lung Function Results

Table E2 summarises lung function results in absolute values and after adjustment for body weight at the time of tests for both the CF and control groups to allow for comparisons with the previous literature. For comparison, please refer to Table 4 in the main article, where results are presented more appropriately as z-scores, to adjust for sex, age and body size where necessary.

With the exception of a significantly lower FEV_{0.5} in those who had received any antibiotics for symptoms or positive cough swab (i.e., in addition to the routine prophylactic Flucloxacillin that was prescribed for all infants), there was no significant association between lung function outcomes and the infants' genotype, clinical status or treatment prior to the lung function tests at 3 months of age.

Table E3 compares mean z-scores between subgroups with and without homozygous Δ F508, respiratory symptoms, positive culture on cough swab and use of additional antibiotic.

Differences are presented with 95% confidence intervals together with p-values from two sample *t*-tests. FEV_{0.5} z-score was significantly lower in those who had received additional antibiotics (difference: -0.7 (-1.29, -0.1), p=0.049). All other differences were non-

significant. However, note that some confidence intervals were quite wide and we could not exclude some differences of clinical importance. This sub-group analysis is exploratory.

Table E2: Comparison of lung function (absolute values and in relation to weight at test) in infants with CF and healthy controls

		Infants with CF n=71		Healthy controls n=54	Δ (95% CI) CF- controls	p values
Weight at test		5.32 (0.84)		6.05 (0.78)	-0.73 (-1.02; -0.45)	<0.001
Weight z-score		-0.59 (1.1)		0.25 (1.04)	-0.84 (-1.22; -0.46)	<0.001
Lung Function results						
Multiple breath washout	n		n			
LCI	70	7.89 (0.92)	51	7.46 (0.55)	0.46 (0.19; 0.72)	0.002
FRC _{MBW} , mL	70	105.7 (22.0)	51	106.7 (19.8)	-1.2 (-8.8; 6.5)	0.800
FRC _{MBW} , mL/kg	70	20.0 (3.4)	51	18.0 (3.5)	2.0 (0.8; 3.3)	0.002
Tidal breathing						
Respiratory rate, per min	71	37.8 (7.6)	54	36.5 (6.5)	1.3 (-1.2; 3.8)	0.297
Tidal volume, mL	71	51.4 (8.1)	54	54.6 (7.0)	-3.2 (-5.9; -0.6)	0.018
Tidal volume, mL/kg	71	9.7 (1.3)	54	9.1 (1.2)	0.6 (0.2; 1.1)	0.007
t _{PTEF} /t _E , %	71	28.7 (10.1)	54	30.3 (9.4)	-1.6 (-5.1; 1.8)	0.356
Minute ventilation, mL/min	71	1913 (342)	54	1968 (285)	-55 (-166; 57)	0.332
Minute ventilation, mL/min/kg	71	363 (61.1)	54	329 (47.9)	35 (15; 54)	0.001
Passive mechanics						
C _{rs} , mL/kPa	47	67.3 (13.5)	41	73.9 (10.7)	-6.7 (-11.8; -1.5)	0.011
C _{rs} , mL/kPa/kg	47	12.7 (1.9)	41	12.3 (1.8)	0.38 (-0.40; 1.16)	0.331
R _{rs} , kPa/L/s	47	5.50 (1.41)	41	4.83 (1.28)	0.63 (0.06; 1.20)	0.031
Plethysmography						
FRC _{pleth} , mL	56	111.3 (24.7)	47	104.4 (21.2)	7.0 (-2.0; 15.9)	0.127
FRC _{pleth} , mL/kg	56	20.9 (4.0)	47	17.3 (3.5)	3.6 (2.1; 5.0)	<0.001
Δ FRC (pleth-MBW), mL/kg	55	1.1 (4.1)	45	-0.4 (3.2)	1.5 (0.05; 2.9)	0.043
Raised volume technique						
FEV _{0.5} , mL	68	146.2 (27.1)	52	177.3 (25.2)	-31.0 (-40.5; -21.5)	<0.001
FVC, mL	68	172.8 (34.3)	52	208.5 (31.0)	-35.7 (-47.6; -23.9)	<0.001
FEF ₂₅₋₇₅ , mL/s	68	316.0 (87.0)	52	380.2 (85.5)	-64.2 (-95.7; -32.8)	<0.001
FEF ₇₅ , mL/s	68	169.6 (55.5)	52	207.1 (57.2)	-37.5 (-58.2; -16.8)	<0.001

Data expressed as mean (SD)

Δ = mean difference between groups; CI = confidence interval of the difference.

Table E3. Associations between potential clinical determinants and lung function outcomes at 3 months of age in infants with CF

Predictor	Outcome			
	Homozygous Δ F508 (n=42/71)	Respiratory symptoms (cough and/or wheeze), ever [†] (n=34/71)	Positive growth on cough swab, ever [†] (n= 17/71)	Received additional antibiotics, included nebulised & intravenous [†] (n=44/71)
LCI z-score	0.04 (-0.71; 0.78) p = 0.921	0.02 (-1.65; 0.68) p = 0.964	-0.40 (-1.22; 0.42) p = 0.335	0.37 (-0.33; 1.06) p = 0.092
FEV _{0.5} z-score	-0.14 (-0.80; 0.53) p = 0.683	-0.52 (-1.09; 0.54) p = 0.075	-0.06 (-0.76; 0.65) p = 0.878	-0.70 (-1.29; -0.10) p = 0.049
FEF ₂₅₋₇₅ z-score	-0.03 (-0.79; 0.74) p = 0.947	-0.25 (-0.92; 0.42) p = 0.458	-0.32 (-1.15; 0.51) p = 0.444	-0.61 (-1.30; 0.09) p = 0.306
FRC _{pleth} z-score	0.27 (-0.43; 0.97) p = 0.443	-0.14 (-0.76; 0.47) p = 0.640	0.08 (-0.68; 0.85) p = 0.829	0.06 (-0.60; 0.71) p = 0.860

Footnote: See Figures 1 and 2 in main article for further details.

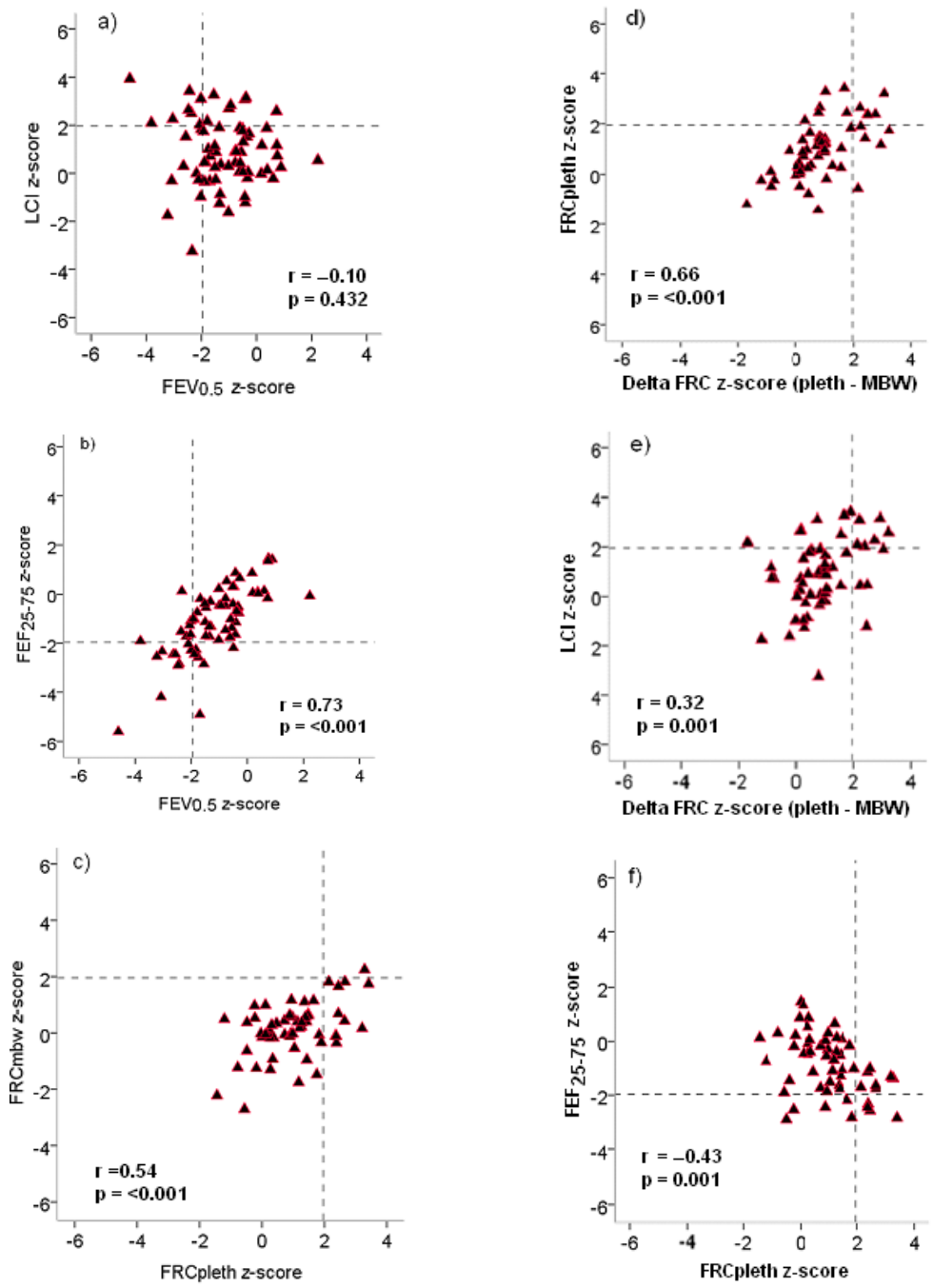
LCI: lung clearance index; FEV_{0.5}: forced expired volume in 0.5s; FEF₂₅₋₇₅: forced expired flow between 25–75% of forced vital capacity; FRC_{pleth}: plethysmographic functional residual capacity.

[†] duration between diagnosis of CF to time of lung function tests

4.1. Associations between different lung function outcomes

For clarity, the following paragraph is reproduced here from the main article in order to explain the graphs in Figure E2, which shows the relationship between selected pulmonary function outcomes in infants with CF. As can be seen, Figure E2a illustrates that there was no significant relationship between the two primary outcome measures, FEV_{0.5} and LCI ($r = -0.16$, $p=0.09$), suggesting that they reflect different aspects of underlying pathophysiology. The RV forced expiratory technique is likely to be most sensitive in early life when the chest wall is highly compliant and airway closure more likely to occur even in the tidal range. Airflow limitation is thus more likely to occur in the presence of relatively mild lung disease than in later life. This situation changes by the preschool years, when spirometry becomes less discriminative in CF lung disease than during infancy despite overall disease progression,^{E23} such that by 4 years of age the LCI is by far the most discriminative test.^{E24} During early infancy, mild airway obstruction in CF may be associated with less uneven distribution of ventilation than in later life, and hence less impact on LCI. In addition, the increased LCI found among younger subjects due to developmental differences^{E9} may make LCI less discriminatory during early life. 21% infants had an LCI above 1.96 z-scores, whereas 25% had an FEV_{0.5} below -1.96 z-scores. If using either test, 34% (24/68) would be identified with abnormal results, whereas only 12% (8/68) had abnormalities detected by both these tests. Both FEV_{0.5} and FEF₂₅₋₇₅ ($r = 0.73$, $p<0.0001$) detected a similar proportion of infants outside the normal range (25% and 24% respectively), whereas FEF₇₅ was less discriminative (only detecting abnormalities in 15% infants) (Figure E2b). FRC_{pleth} z-score and delta FRC were highly correlated ($r = 0.66$, $P<0.001$) (Figure E2d) and both detected a similar proportion of infants with abnormally elevated results (20% and 18%, respectively). 52% (37 /71) NBS CF infants had at least one abnormal result if based on MBW, plethysmography or the raised volume technique.

Figure E2. Association between selected pulmonary function outcomes in infants with CF



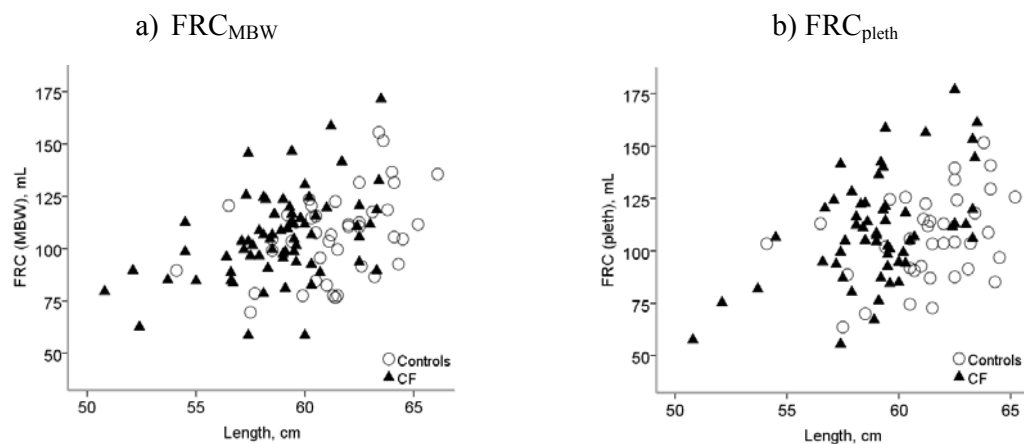
Legend: The horizontal dashed line denotes *either* the upper limit of normality for LCI and FRC (1.96 z-score) *or* the lower limit of normality (-1.96 z-score) for FEF₂₅₋₇₅; the vertical dashed line represents *either* the upper limit of normality for FRC (1.96 z-score) *or* the lower limit of normality (-1.96 z-score) for FEV_{0.5}.

4.2. Impact of CF on lung function

4.2.1. Lung volumes

The relationship between FRC_{MBW} and FRC_{pleth} and body length in infants with CF and healthy controls is shown in Figure E3. It can be seen that despite being of similar age, CF infants tended to be shorter at time of test, and that despite much overlap, FRC_{MBW} was slightly higher at any given length in those with CF (Figure E3a, Table E2) as reflected by the small, but significant increase in FRC_{MBW} , once differences in length are adjusted for by expressing results as z-scores (Table 3, main article). This difference would, however, have been over-estimated had results simply been expressed as a ratio of body weight (Table E2), due to the relative growth restriction in those with CF by 3 months.

Figure E3. FRC measured using the MBW and plethysmograph respectively as a function of crown-heel length at time of test



When FRC_{pleth} was plotted versus body length, despite considerable overlap between the groups, relatively higher values were observed in infants with CF when compared with controls of similar body size. Thus, although there was no significant difference between the groups when results were expressed in absolute terms (Table E2), after adjusting for length by

expressing results as z-scores, FRC_{pleth} was significantly higher in those with CF (Table 3 and Figure 3c in main article). Again, had results simply been expressed as a ratio of body weight (Table E2), this difference would have been over-estimated.

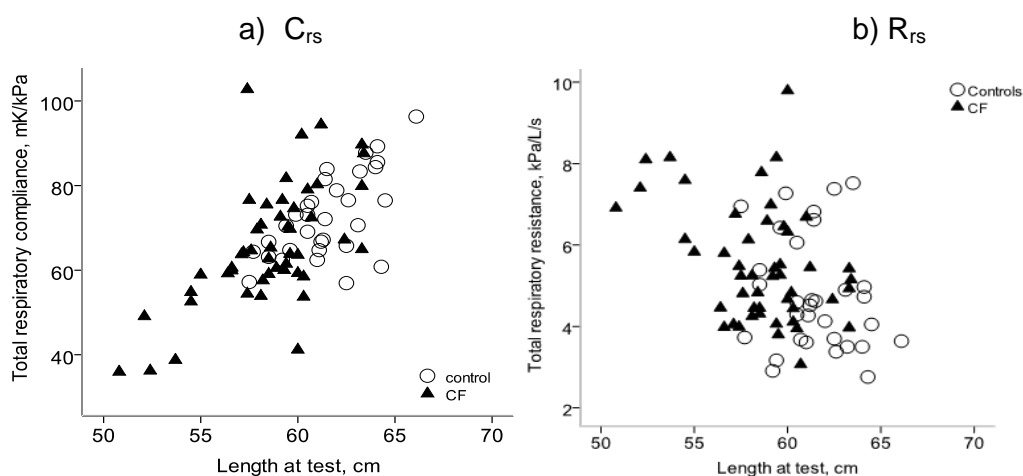
4.2.2. Effect of CF on tidal breathing variables

There was no difference in respiratory rate or $t_{\text{PTEF}/t_{\text{E}}}$ between infants with CF and healthy controls, whether results were expressed in absolute terms or as z-scores (Table E2, and Table 3 in main article). While tidal volume appeared lower in those with CF when expressed in absolute terms (Table E2), once expressed as z-scores to correct for body size and age (section 3.4.1), tidal volume was significantly higher in those with CF (Table 3, main article). Tidal volume was also increased in CF if results were expressed in mL/kg body weight, although this approach is not recommended as it will tend to over-estimate differences in growth restricted infants.^{E17}

4.2.3. Effect of CF on Respiratory mechanics

The relationship between respiratory mechanics and length according to diagnostic group is illustrated in Figure E4. It can be seen that while values of C_{rs} were lower and R_{rs} higher in those with CF when expressed as absolute values (Table E2), this was largely related to body size at time of test; C_{rs} increasing and R_{rs} decreasing with somatic growth. After adjusting for length and/or age by expressing results as z-scores,^{E17} no significant difference was observed between the groups with respect to either C_{rs} or R_{rs} (Table 3 and Figure 3b in main article).

Figure E4. Comparison of: a) C_{rs} and b) R_{rs} in NBS CF infants and healthy controls at 3 months of age



4.2.4. Effect of CF on forced expired flows and volume

As can be seen from Table E2, there were highly significant reductions in FVC, forced expired volume and flows among infants with CF at 3 months when results were expressed in absolute terms ($p < 0.001$ for all). However, given the shorter length for age amongst those with CF, a more accurate estimation of this reduction is obtained once results are expressed as z-scores to adjust for sex and body size, as presented in Table 3 of the main article.

5. Validation of parental report of smoking exposure

In this study, the reported incidence of maternal smoking during pregnancy and postnatally were relatively low ($\leq 13\%$; Table 1, main article), when compared with values from infants in London reported a decade ago.^{E25} Table E4 summarises the level of cotinine concentrations (a metabolite of nicotine) for infant urine and maternal saliva, collected from those whose mothers reported no smoking during pregnancy and postnatally. The results were well below the reported optimum cut-off values to distinguish non-smokers from smokers: i.e., 50 ng/mL

for urine and 12 ng/mL for salivary samples,^{E26} inferring that parents in this cohort were honest when reporting their smoking habit, thus suggesting that passive smoke exposure is not likely to bias interpretation of infant lung function in this study.

Table E4. Urine and salivary cotinine results

	n	Infants with CF	n	Healthy controls	Δ (95% CI) CF- controls
non-smoking mothers	63		50		
Infant urine cotinine, ng/mL	45	1.1 (0.4) (range: 0.1 –3.0)	26	2.8 (5.4) (range: 0.9–24.5)	-1.8 (-4.0; 0.4) p =0.148
Maternal saliva cotinine, ng/mL	8	0.1 (0.01) (range: 0.1 – 0.1)	11	0.9 (2.2) (range: 0.1 – 7.6)	-0.8 (-2.3; 0.7) p =0.276

Data were expressed as mean (SD) unless otherwise stated.

CF= cystic fibrosis; CI=confidence interval.

Δ = mean difference between groups.

6. Standardised treatment for newborn infants with CF

Prior to commencement of this study, a standardised protocol was developed and agreed upon by all participating consultants. This was adhered to throughout the duration of the study.

Following diagnosis, all infants commenced on multivitamins, pancreatic supplements (if pancreatic insufficient) and prophylactic flucloxacillin (25mg/kg twice daily).^{E27} The extent to which protocol was adhered to was checked both by regular review of prospectively completed Case Record Forms (CRF) and by discussions at collaborative meetings of the LCFC. There was no evidence of deviations from this protocol prior to or at the time of the 3 month lung function tests. Cough swabs were taken routinely at clinic visits (minimum every 2–3 monthly) and additionally when the infant is symptomatic. A standardised protocol for collection, storage and analysis of samples was used.^{E28}

Within the UK, all centres encourage daily chest physiotherapy to infants and children with CF. Within the London CF Collaboration, parents/carers of CF infants and children are educated about the importance of physical activity and its benefits on the respiratory system, and they are taught an appropriate airway clearance technique. Physiotherapy is carried out as appropriate to the child's condition and reviewed frequently in conjunction with medical treatment.

6.1. Infection with *Pseudomonas aeruginosa* (PsA)

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

- **Well infant (based on clinical judgement)**
 - Home therapy with 3 weeks of oral ciprofloxacin (15mg/kg twice daily) and
 - 3 months of nebulised Colistin (Colomycin: 1 million units twice daily).^{E29}
- **Unwell infant (based on clinical judgement)**
 - Hospital admission for 2 weeks of intravenous (IV) antibiotics. The choice of antibiotics was guided by results from culture and sensitivity;

- Intravenous Ceftazidime (50mg/kg three times daily) and intravenous Tobramycin (10mg/kg once daily);
- Also started on 3 months of nebulised Colistin, initiated whilst in hospital.

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

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

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

- **intravenous antibiotics**, and either
 - a further 3 months of nebulised Colistin, or
 - monthly alternating nebulised Colistin and Tobramycin (300mg twice daily).

6.2. Infection with *Staphylococcal aureus* (SA)

(a) First growth

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

(b) Regrowth less than 6 months from first growth

- Oral flucloxacillin 50mg/kg for 28 days.

(c) Further regrowth within 6 months

- Two oral anti-staphylococcal antibiotics for 4 weeks.

6.3. Infection with *Haemophilus influenzae* (HI)

(a) First growth

- **Well infant**

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

- **Unwell infant**

- Hospital admission for 2 weeks of intravenous antibiotics

(b) Regrowth less than 6 months from first growth

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

(c) Further regrowth within 6 months

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

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**LUNG FUNCTION IS ABNORMAL IN 3 MONTH OLD INFANTS WITH CYSTIC
FIBROSIS DIAGNOSED BY NEWBORN SCREENING**

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Online Data Supplement

1. Background

As part of a longitudinal research programme of infants with CF diagnosed by newborn screening (NBS), this study measured lung function at 3 months in NBS infants with CF and contemporaneous healthy controls of similar age (the focus of the current article), with follow up tests at 1 and 2 years of age (to be reported at a later date). Clinical status including use of medications and anthropometry were documented prospectively. This online supplement (OLS) provides additional details regarding recruitment, methods and results, for which there was no space in the main article.

2. Subjects and Methods

Screened infants with CF, referred to 6 tertiary CF centers participating in the London Cystic Fibrosis Collaboration (LCFC) in the Greater London regions, UK, were eligible for recruitment to the study. The LCFC consists of -

- The Respiratory Unit, Great Ormond Street Hospital (GOSH) for Children NHS Foundation Trust, London;
- The Department of Paediatric Respiratory Medicine, Imperial College & Royal Brompton & Harefield Hospitals NHS Trust, London;
- The Department of Paediatric Respiratory Medicine, Barts & The Royal London Hospital, London;
- Queen Mary's Hospital for Children, Epsom & St Helier University Hospitals NHS Trust, Surrey;
- The Department of Paediatric Respiratory Medicine, Kings College Hospital, London;
- The Department of Child Health, Lewisham Healthcare NHS Trust, London.

2.1. Recruitment of screened infants with CF

Infants diagnosed with CF via NBS with an elevated concentration of immunoreactive trypsinogen (IRT) and confirmation of positive CF transmembrane conductance regulator (CFTR) gene mutation profile and/or sweat chloride test

(http://newbornbloodspot.screening.nhs.uk/nat_std_cf_protocol; date accessed: February 2012) referred to the LCFC centers between January 2009 and July 2011, were eligible for recruitment.

Unless there were any special circumstances, parents were invited to participate in the study by their consultants within approximately 4 weeks of diagnosis during a follow-up “Education” visit to the tertiary CF center. The purpose of the study was explained verbally and written information, together with an illustrated leaflet, was given to parents. The family was allowed time to consider the information and ask further questions before giving written consent.

2.1.1. Inclusion criteria for infants with CF

- Infants diagnosed with CF by NBS within the Greater London catchment area.

2.1.2. Exclusion criteria for infants with CF

- Infants with CF born <37 weeks gestation
- Severe congenital disorders, cardio-vascular, skeletal, neuro-muscular or metabolic co-morbidities that could impact on the respiratory system;
- Inability of parents to understand and give informed consent;
- Recruitment contra-indicated on psycho-social grounds;
- History of apnoeic episodes or upper airway pathology;
- Family due to move out of area.

2.2. Recruitment of healthy control infants

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

2.2.1. Inclusion criteria for healthy controls

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

2.2.2. Exclusion criteria for healthy controls

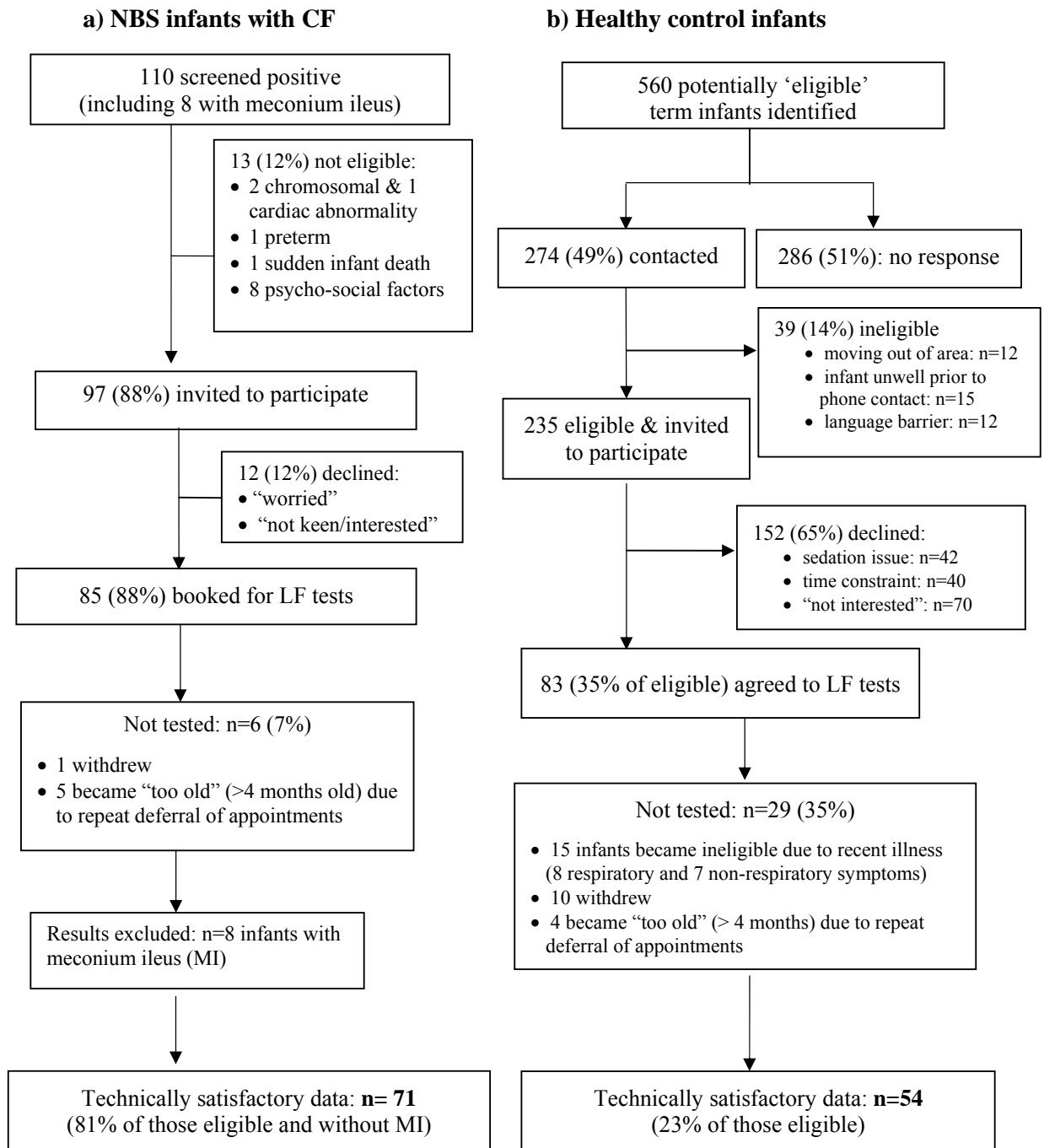
3. Infants born <37 weeks gestation

- Inability of parents to understand and give informed consent;
- Recruitment contra-indicated on medical and/or psycho-social grounds;
- History of apneic episodes or upper airway pathology;

- History of chronic diarrhoea or failure to thrive;
- History of neonatal lung disease, assisted ventilation or co-existent cardio-vascular, skeletal, neuro-muscular, renal or metabolic disorders that could impact on the respiratory system and
- Previous physician diagnosed or hospital admission for lower respiratory tract infections.

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

Figure E1. Flow diagram showing additional details of recruitment process



Footnote: NBS=newborn screen; CF=cystic fibrosis; LF = lung function.

3. Data collection

All lung function tests were performed between January 2009 and October 2011 in a single infant lung function laboratory at the Great Ormond Street Hospital / UCL Institute of Child Health, London. Weight and crown-heel length, measured using an infant stadiometer, were expressed as z (or SD) scores to adjust for age and sex.^{E1}

3.1. Multiple breath inert gas washout (MBW) technique

Lung clearance index (LCI) assesses overall efficiency of ventilation distribution or gas mixing within the lung and provides a measure of early lung disease; functional residual capacity measured using MBW (FRC_{MBW}) represents the resting lung volume that is communicating with the airway opening at time of measurement. The equipment and test procedure for performing MBW in infants were similar to those for preschool children, as described in detail previously (online supplement:

<http://ajrccm.atsjournals.org/cgi/data/171/3/249/DC1/1>)^{E2-4} and summarised below.

Data collection was performed in two stages:

- i) the wash-in phase involved the infant inspiring a bias flow of dry air mixture containing the tracer gas (4% sulfur hexafluoride (SF_6)), 21% oxygen and balance nitrogen, and continued until inspiratory and expiratory SF_6 concentrations were stable and equal at 4% for a minimum of 5-8 breaths, at which time the bias flow was removed;
- ii) the wash-out phase began with the infant inhaling room air and continued until end tidal SF_6 concentration was consistently below 0.1%, i.e., less than $1/40^{\text{th}}$ of the starting concentration.

LCI is defined as the number of lung volume turnovers (or number of FRCs) required to clear the lungs of the inert tracer gas to $1/40^{\text{th}}$ of the starting concentration of the tracer gas. Data were acceptable only if there was no evidence of mask leak or flow through the pneumotachometer (PNT).^{E5} LCI and FRC_{MBW} were calculated as described previously^{E2,E4,E6,E7} and reported as the mean of three technically satisfactory MBW recordings. In exceptional cases, the mean of two technically acceptable recordings was used if results were within 5% of one another. During a recent extensive investigation, the CV (SD) for LCI was found to be 4.1 (2.4)% in healthy infants and children, and 8.9 (1.9)% in those with CF (Robinson, P et al, Pediatric Pulmonology, in press; ‘Abbreviated multi-breath washout for calculation of Lung Clearance Index’). For the current study, only 4% of MBW washouts failed to meet quality control criteria.

3.1.1. Prediction equations for lung clearance index and FRC_{MBW}

Until recently, there were no published reference equations for LCI data during infancy, results being reported as absolute values with a fixed upper threshold to identify abnormally elevated results,^{E3,E8} despite the fact that somewhat elevated values have been noted in healthy infants when compared with older children.^{E9} Recent collation of MBW data from 497 subjects (48% boys) on 659 test occasions from birth to 19 years of age collected using identical methods and equipment from 3 centres (London, Sweden and Toronto) has now allowed appropriate reference equations to be developed for both LCI and FRC_{MBW} .^{E10} These reference equations were constructed using the LMS [Lambda (L), Mu (M), Sigma (S)] method as described previously.^{E11, E12} Together the L, M and S coefficients are combined algebraically to convert an individual observation to a z-score.

$$\text{z-score} = [(\text{Measurement}/M)^L - 1] / [L*S]$$

$$\text{Upper Limit of Normal (ULN, i.e. 97.5}^{\text{th}} \text{ percentile)} = M*(1.96*S*L + 1)^{1/L}$$

After adjusting for length or height, age and sex did not contribute to the model when predicting LCI whereas for FRC_{MBW} , length, age and sex all made a significant contribution.

Reference equation for LCI

Table E1: Paediatric reference equations for LCI and FRC_{MBW}

LCI		
L		-0.81
M (Predicted)		$5.99 + (73.86 * \text{height}^{-1})$
S		0.08
ULN		Predicted * $((1.96 * 0.08 * -0.81) + 1)^{1/-0.81}$
FRC		
L		0.19
M (Predicted)		$\exp(-11.10 + (2.12 * \ln \text{Height}) + (0.27 * \text{age}^{0.5}) + (0.04 * \text{sex}))$
S		$\exp(-1.67 + 148.57 * \text{height}^{-2})$
ULN		Predicted * $((1.96 * S * 0.19) + 1)^{1/0.19}$

*Footnote: Based on these equations, the ULN for LCI can be simplified to Predicted LCI * 1.18; whereas that for FRC_{MBW} becomes $((0.36 * S + 1)$ to the power 5.37) * Predicted FRC.*

Results from this study have been expressed as SD- or z-scores according to these equations, with LCI also being expressed in absolute values where appropriate to facilitate comparison with previous publications (Table E2). A comparison of LCI and FRC_{MBW} z-scores, as well as the difference between plethysmographic and MBW z-scores for FRC (ΔFRC), in infants with CF versus healthy controls is presented in the main article (Table 3, Figure 3).

3.2. Plethysmographic FRC and Tidal Breathing variables assessed using the Jaeger MasterScreen BabyBody system (v4.65; CareFusion)

Measurements of plethysmographic FRC were obtained in accordance with ATS/ERS guidelines.^{E13} After several regular breaths had been recorded, a brief airway occlusion was performed to ensure that there was an airtight seal around the face mask prior to lowering the hood to close the plethysmograph. **Tidal breathing variables** (tidal volume (V_T); respiratory rate (RR); minute ventilation (V_E) and the ratio of time to reach peak expiratory flow/expiratory time (t_{PTEF/t_E}) were recorded for 2-3 minutes while the plethysmograph reached thermal equilibrium. Full details for Jaeger infant box calibration and FRC data collection have been reported previously.^{E14}

The criteria for technical acceptability when collecting FRC_{pleth} data were:

- no evidence of mask leak (no flow through the PNT and no decay of pressure plateau at the airway opening during the occlusion), with the pre-occlusion end expiratory level from the time-based tidal volume trace being re-established within 5–10 breaths post-occlusion;
- ideally, three (minimum two) complete respiratory efforts against the occlusion were recorded. This is essential for adequate correction of the box volume drift, the magnitude and direction of which will change during the airway occlusion, despite establishing a stable trace prior to the occlusion;
- changes in plethysmographic pressure in phase with changes in pressure at the airway opening during airway occlusion, with no evidence of phase lag or “looping” due to glottis closure, leak or poor drift correction.

At least five satisfactory occlusion maneuvers were performed for each infant. The mean of 3–5 measures of FRC_{pleth} (minimum 2 if highly repeatable and of good quality) that were within 10% were reported.

3.3. Total respiratory compliance (C_{rs}) and resistance (R_{rs})

Passive respiratory mechanics (C_{rs} and R_{rs}) was assessed using the single occlusion (SO) technique via the Resistance/Compliance Program of the Jaeger MasterScreen BabyBody system (v 4.65, CareFusion, USA). Details of the protocol for data collection have been described previously.^{E15} After 5–10 regular breaths had been recorded to establish a stable end-established level (EEL), a brief airway occlusion was performed at the end of a tidal inspiration, temporarily maintaining lung volume above the EEL to evoke the Hering Breuer Inflation reflex and hence, respiratory muscle relaxation.^{E15,16} During airway occlusion (i.e., a constant lung volume with no flow), rapid equilibration occurs within the lungs. The pressure plateau recorded at the airway opening (P_{ao}) during such occlusions represents the alveolar pressure, which in turn represents the summed elastic recoil pressure of the lung and chest wall. C_{rs} is calculated as the volume above the relaxed EEL divided by P_{ao} . Provided expiration remains relaxed following release of airway occlusion and the respiratory system can be described by a single time constant, the slope of the relaxed expiratory portion of the flow-volume curve represents the expiratory time constant of the respiratory system (t_{rs}). Since t_{rs} is the product of C_{rs} and R_{rs} , resistance can be calculated as t_{rs}/C_{rs} minus the resistance of the apparatus.^{E16}

Criteria for technically satisfactory data were:

- Smooth expiration, proceeding to within 10% of previous expiration with no evidence of glottic closure, braking or active expiratory efforts;

- Duration of the pressure plateau at the airway opening ≥ 100 ms with variability < 10 Pa;
- Linearity of the flow-volume curve over at least 40% of expiration with $r^2 > 0.99$.

C_{rs} and R_{rs} results were reported using the mean from 3–5 technically acceptable maneuvers. For the calculation of predicted values and z-scores for FRC_{pleth} , tidal volume (V_T), respiratory rate (RR), the ratio of time taken to reach peak tidal expiratory flow: total expiratory time ($t_{PTEF}: t_E$), passive respiratory mechanics (C_{rs} and R_{rs}), please refer to recently created reference equations derived from healthy infants studied with identical equipment and protocols.^{E17}

3.4. Raised volume (RV) forced expiratory maneuvers

Prior to performing the raised volume forced expiratory maneuvers, tidal forced expiratory maneuvers^{E18} were undertaken to determine the optimal jacket compression pressure (P_j) at which flow limitation was achieved, i.e., the point at which no further increase in expiratory flow was observed despite further increases in applied jacket pressure.^{E18,19} The optimal P_j thus obtained was used for the raised volume maneuvers to obtain “full” or raised volume forced expiratory variables.

Airway function at raised lung volume was assessed as previously described.^{E20,21} Expiration was forced from an augmented lung volume using an inflation pressure of 30 cmH₂O (2.93kPa) and maneuvers repeated until three (minimum of two) technically acceptable and reproducible (sum of $FEV_{0.5}$ and FVC within 10% of each other) flow-volume curves were obtained. $FEV_{0.5}$, FVC and FEF_{75} and FEF_{25-75} were reported from the “best” curve, defined as the technically satisfactory maneuver with the highest sum of $FEV_{0.5}$ and FVC.^{E20} The criteria for technically acceptable forced expired flow-volume curves were: peak expiratory

flow achieved prior to 10% of expired volume, complete expiration towards residual volume (RV) (i.e., no evidence of early inspiration) and no marked flow transients or glottic closure.^{E20} In our laboratory, following quality control, the coefficient of variability for FEV_{0.5} during the first year of life is 3.6% (95% CI: 3.1%; 4.1%). Results were expressed as z-scores to account for sex, age and/or body length, adjusted for the equipment used (Jaeger Masterscreen BabyBody) as described previously.^{E22} Reduced airway function was defined as a z-score below -1.96 (<2.5th centile).

4. Lung Function Results

Table E2 summarises lung function results in absolute values and after adjustment for body weight at the time of tests for both the CF and control groups to allow for comparisons with the previous literature. For comparison, please refer to Table 4 in the main article, where results are presented more appropriately as z-scores, to adjust for sex, age and body size where necessary.

With the exception of a significantly lower FEV_{0.5} in those who had received any antibiotics for symptoms or positive cough swab (i.e., in addition to the routine prophylactic Flucloxacillin that was prescribed for all infants), there was no significant association between lung function outcomes and the infants' genotype, clinical status or treatment prior to the lung function tests at 3 months of age.

Table E3 compares mean z-scores between subgroups with and without homozygous $\Delta F508$, respiratory symptoms, positive culture on cough swab and use of additional antibiotic.

Differences are presented with 95% confidence intervals together with p-values from two sample *t*-tests. FEV_{0.5} z-score was significantly lower in those who had received additional antibiotics (difference: -0.7 (-1.29, -0.1), p=0.049). All other differences were non-

significant. However, note that some confidence intervals were quite wide and we could not exclude some differences of clinical importance. This sub-group analysis is exploratory.

Table E2: Comparison of lung function (absolute values and in relation to weight at test) in infants with CF and healthy controls

		Infants with CF n=71		Healthy controls n=54	Δ (95% CI) CF- controls	p values
Weight at test		5.32 (0.84)		6.05 (0.78)	-0.73 (-1.02; -0.45)	<0.001
Weight z-score		-0.59 (1.1)		0.25 (1.04)	-0.84 (-1.22; -0.46)	<0.001
Lung Function results						
Multiple breath washout	n		n			
LCI	70	7.89 (0.92)	51	7.46 (0.55)	0.46 (0.19; 0.72)	0.002
FRC _{MBW} , mL	70	105.7 (22.0)	51	106.7 (19.8)	-1.2 (-8.8; 6.5)	0.800
FRC _{MBW} , mL/kg	70	20.0 (3.4)	51	18.0 (3.5)	2.0 (0.8; 3.3)	0.002
Tidal breathing						
Respiratory rate, per min	71	37.8 (7.6)	54	36.5 (6.5)	1.3 (-1.2; 3.8)	0.297
Tidal volume, mL	71	51.4 (8.1)	54	54.6 (7.0)	-3.2 (-5.9; -0.6)	0.018
Tidal volume, mL/kg	71	9.7 (1.3)	54	9.1 (1.2)	0.6 (0.2; 1.1)	0.007
t _{PTEF} /t _E , %	71	28.7 (10.1)	54	30.3 (9.4)	-1.6 (-5.1; 1.8)	0.356
Minute ventilation, mL/min	71	1913 (342)	54	1968 (285)	-55 (-166; 57)	0.332
Minute ventilation, mL/min/kg	71	363 (61.1)	54	329 (47.9)	35 (15; 54)	0.001
Passive mechanics						
C _{rs} , mL/kPa	47	67.3 (13.5)	41	73.9 (10.7)	-6.7 (-11.8; -1.5)	0.011
C _{rs} , mL/kPa/kg	47	12.7 (1.9)	41	12.3 (1.8)	0.38 (-0.40; 1.16)	0.331
R _{rs} , kPa/L/s	47	5.50 (1.41)	41	4.83 (1.28)	0.63 (0.06; 1.20)	0.031
Plethysmography						
FRC _{pleth} , mL	56	111.3 (24.7)	47	104.4 (21.2)	7.0 (-2.0; 15.9)	0.127
FRC _{pleth} , mL/kg	56	20.9 (4.0)	47	17.3 (3.5)	3.6 (2.1; 5.0)	<0.001
Δ FRC (pleth-MBW), mL/kg	55	1.1 (4.1)	45	-0.4 (3.2)	1.5 (0.05; 2.9)	0.043
Raised volume technique						
FEV _{0.5} , mL	68	146.2 (27.1)	52	177.3 (25.2)	-31.0 (-40.5; -21.5)	<0.001
FVC, mL	68	172.8 (34.3)	52	208.5 (31.0)	-35.7 (-47.6; -23.9)	<0.001
FEF ₂₅₋₇₅ , mL/s	68	316.0 (87.0)	52	380.2 (85.5)	-64.2 (-95.7; -32.8)	<0.001
FEF ₇₅ , mL/s	68	169.6 (55.5)	52	207.1 (57.2)	-37.5 (-58.2; -16.8)	<0.001

Data expressed as mean (SD)

Δ = mean difference between groups; CI = confidence interval of the difference.

Table E3. Associations between potential clinical determinants and lung function outcomes at 3 months of age in infants with CF

Predictor	Outcome			
	Homozygous Δ F508 (n=42/71)	Respiratory symptoms (cough and/or wheeze), ever [†] (n=43/71)	Positive growth on cough swab, ever [†] (n= 16/71)	Received additional antibiotics, included nebulised & intravenous [†] (n=52/71)
LCI z-score	0.04 (-0.71; 0.78) p = 0.921	0.02 (-1.65; 0.68) p = 0.964	-0.40 (-1.22; 0.42) p = 0.335	0.37 (-0.33; 1.06) p = 0.092
FEV _{0.5} z-score	-0.14 (-0.80; 0.53) p = 0.683	-0.52 (-1.09; 0.54) p = 0.075	-0.06 (-0.76; 0.65) p = 0.878	-0.70 (-1.29; -0.10) p = 0.049
FEF ₂₅₋₇₅ z-score	-0.03 (-0.79; 0.74) p = 0.947	-0.25 (-0.92; 0.42) p = 0.458	-0.32 (-1.15; 0.51) p = 0.444	-0.61 (-1.30; 0.09) p = 0.306
FRC _{pleth} z-score	0.27 (-0.43; 0.97) p = 0.443	-0.14 (-0.76; 0.47) p = 0.640	0.08 (-0.68; 0.85) p = 0.829	0.06 (-0.60; 0.71) p = 0.860

Footnote: See Figures 1 and 2 in main article for further details.

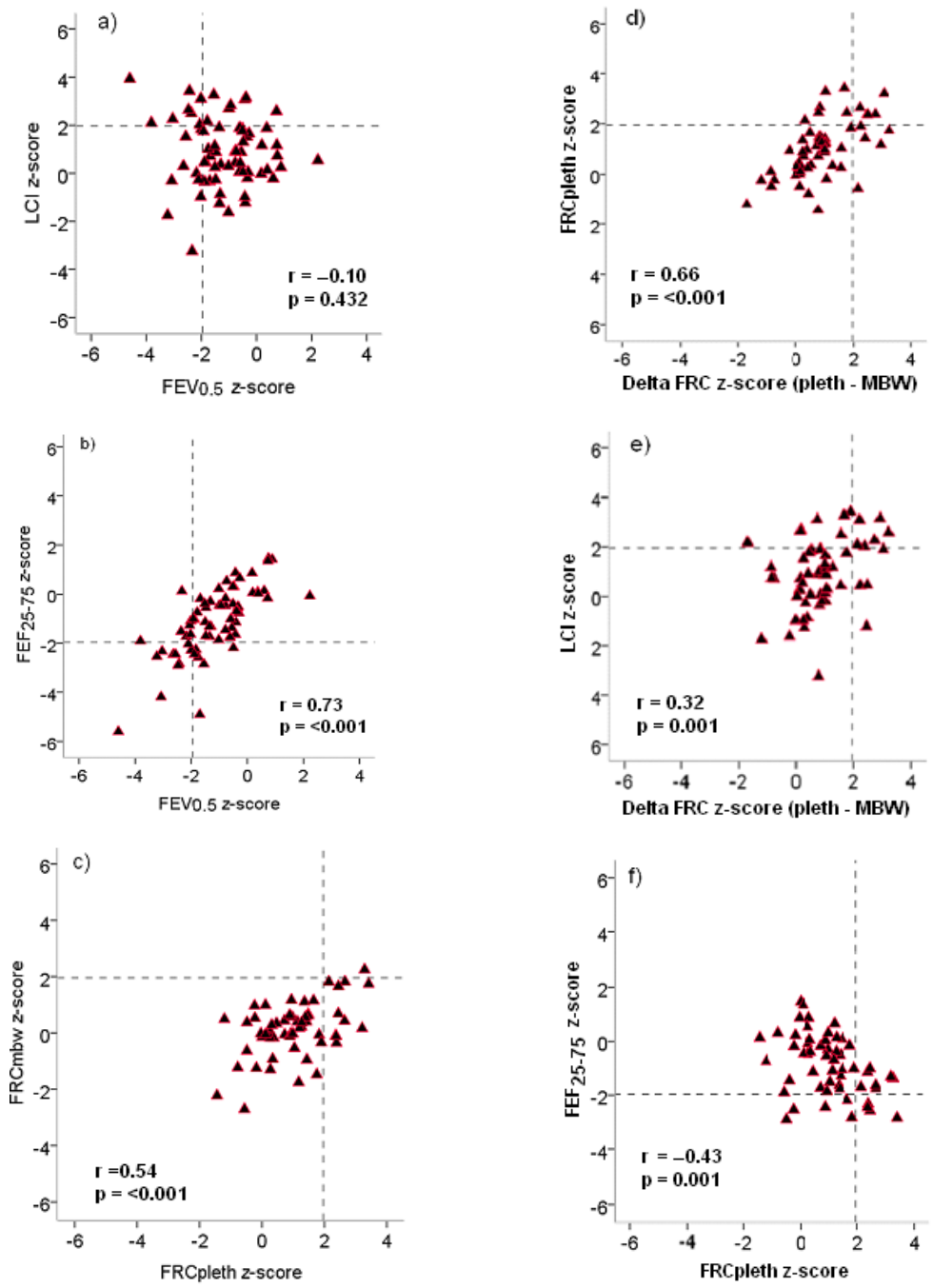
LCI: lung clearance index; FEV_{0.5}: forced expired volume in 0.5s; FEF₂₅₋₇₅: forced expired flow between 25–75% of forced vital capacity; FRC_{pleth}: plethysmographic functional residual capacity.

[†] duration between diagnosis of CF to time of lung function tests

4.1. Associations between different lung function outcomes

For clarity, the following paragraph is reproduced here from the main article in order to explain the graphs in Figure E2, which shows the relationship between selected pulmonary function outcomes in infants with CF. As can be seen, Figure E2a illustrates that there was no significant relationship between the two primary outcome measures, FEV_{0.5} and LCI ($r = -0.16$, $p=0.09$), suggesting that they reflect different aspects of underlying pathophysiology. The RV forced expiratory technique is likely to be most sensitive in early life when the chest wall is highly compliant and airway closure more likely to occur even in the tidal range. Airflow limitation is thus more likely to occur in the presence of relatively mild lung disease than in later life. This situation changes by the preschool years, when spirometry becomes less discriminative in CF lung disease than during infancy despite overall disease progression,^{E23} such that by 4 years of age the LCI is by far the most discriminative test.^{E24} During early infancy, mild airway obstruction in CF may be associated with less uneven distribution of ventilation than in later life, and hence less impact on LCI. In addition, the increased LCI found among younger subjects due to developmental differences^{E9} may make LCI less discriminatory during early life. 21% infants had an LCI above 1.96 z-scores, whereas 25% had an FEV_{0.5} below -1.96 z-scores. If using either test, 35% (24/68) would be identified with abnormal results, whereas only 12% (8/68) had abnormalities detected by both these tests. Both FEV_{0.5} and FEF₂₅₋₇₅ ($r = 0.73$, $p<0.0001$) detected a similar proportion of infants outside the normal range (25% and 24% respectively), whereas FEF₇₅ was less discriminative (only detecting abnormalities in 15% infants) (Figure E2b). FRC_{pleth} z-score and delta FRC were highly correlated ($r = 0.66$, $P<0.001$) (Figure E2d) and both detected a similar proportion of infants with abnormally elevated results (18% and 20%, respectively). Forty four percent (31 /71) NBS CF infants had at least one abnormal result if based on MBW, plethysmography or the raised volume technique.

Figure E2. Association between selected pulmonary function outcomes in infants with CF



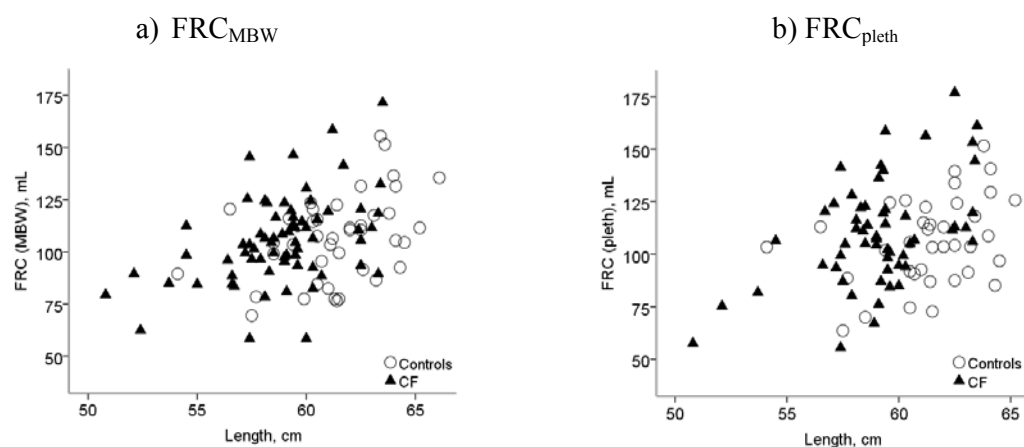
Legend: The horizontal dashed line denotes *either* the upper limit of normality for LCI and FRC (1.96 z-score) *or* the lower limit of normality (-1.96 z-score) for FEF₂₅₋₇₅; the vertical dashed line represents *either* the upper limit of normality for FRC (1.96 z-score) *or* the lower limit of normality (-1.96 z-score) for FEV_{0.5}.

4.2. Impact of CF on lung function

4.2.1. Lung volumes

The relationship between FRC_{MBW} and FRC_{pleth} and body length in infants with CF and healthy controls is shown in Figure E3. It can be seen that despite being of similar age, CF infants tended to be shorter at time of test, and that despite much overlap, FRC_{MBW} was slightly higher at any given length in those with CF (Figure E3a, Table E2) as reflected by the small, but significant increase in FRC_{MBW} , once differences in length are adjusted for by expressing results as z-scores (Table 3, main article). This difference would, however, have been over-estimated had results simply been expressed as a ratio of body weight (Table E2), due to the relative growth restriction in those with CF by 3 months.

Figure E3. FRC measured using the MBW and plethysmograph respectively as a function of crown-heel length at time of test



When FRC_{pleth} was plotted versus body length, despite considerable overlap between the groups, relatively higher values were observed in infants with CF when compared with controls of similar body size. Thus, although there was no significant difference between the groups when results were expressed in absolute terms (Table E2), after adjusting for length by

expressing results as z-scores, FRC_{pleth} was significantly higher in those with CF (Table 3 and Figure 3c in main article). Again, had results simply been expressed as a ratio of body weight (Table E2), this difference would have been over-estimated.

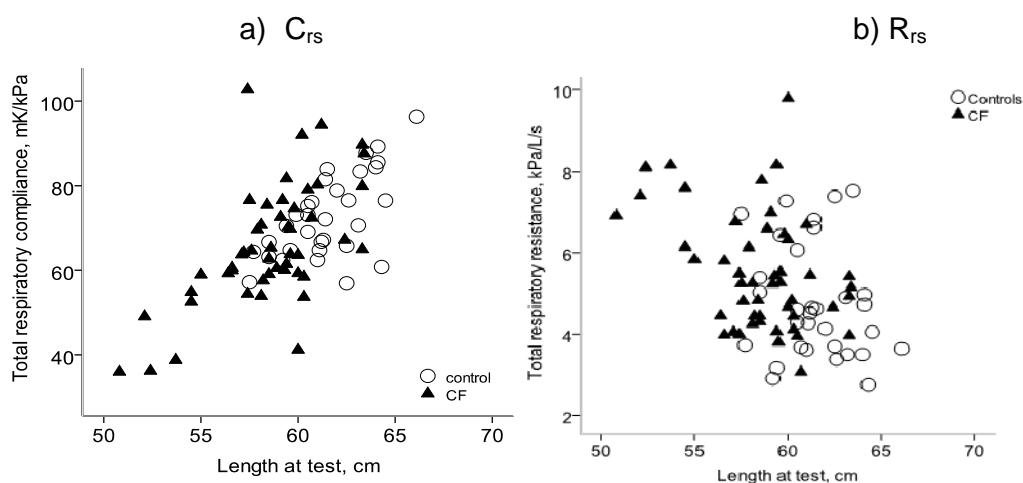
4.2.2. Effect of CF on tidal breathing variables

There was no difference in respiratory rate or $t_{\text{PTEF}}/t_{\text{E}}$ between infants with CF and healthy controls, whether results were expressed in absolute terms or as z-scores (Table E2, and Table 3 in main article). While tidal volume appeared lower in those with CF when expressed in absolute terms (Table E2), once expressed as z-scores to correct for body size and age (section 3.4.1), tidal volume was significantly higher in those with CF (Table 3, main article). Tidal volume was also increased in CF if results were expressed in mL/kg body weight, although this approach is not recommended as it will tend to over-estimate differences in growth restricted infants.^{E17}

4.2.3. Effect of CF on Respiratory mechanics

The relationship between respiratory mechanics and length according to diagnostic group is illustrated in Figure E4. It can be seen that while values of C_{rs} were lower and R_{rs} higher in those with CF when expressed as absolute values (Table E2), this was largely related to body size at time of test; C_{rs} increasing and R_{rs} decreasing with somatic growth. After adjusting for length and/or age by expressing results as z-scores,^{E17} no significant difference was observed between the groups with respect to either C_{rs} or R_{rs} (Table 3 and Figure 3b in main article).

Figure E4. Comparison of: a) C_{rs} and b) R_{rs} in NBS CF infants and healthy controls at 3 months of age



4.2.4. Effect of CF on forced expired flows and volume

As can be seen from Table E2, there were highly significant reductions in FVC, forced expired volume and flows among infants with CF at 3 months when results were expressed in absolute terms ($p < 0.001$ for all). However, given the shorter length for age amongst those with CF, a more accurate estimation of this reduction is obtained once results are expressed as z-scores to adjust for sex and body size, as presented in Table 3 of the main article.

5. Validation of parental report of smoking exposure

In this study, the reported incidence of maternal smoking during pregnancy and postnatally were relatively low ($\leq 13\%$; Table 1, main article), when compared with values from infants in London reported a decade ago.^{E25} Table E4 summarises the level of cotinine concentrations (a metabolite of nicotine) for infant urine and maternal saliva, collected from those whose mothers reported no smoking during pregnancy and postnatally. The results were well below the reported optimum cut-off values to distinguish non-smokers from smokers: i.e., 50 ng/mL

for urine and 12 ng/mL for salivary samples,^{E26} inferring that parents in this cohort were honest when reporting their smoking habit, thus suggesting that passive smoke exposure is not likely to bias interpretation of infant lung function in this study.

Table E4. Urine and salivary cotinine results

	n	Infants with CF	n	Healthy controls	Δ (95% CI) CF- controls
non-smoking mothers	63		50		
Infant urine cotinine, ng/mL	45	1.1 (0.4) (range: 0.1 –3.0)	26	2.8 (5.4) (range: 0.9–24.5)	-1.8 (-4.0; 0.4) p =0.148
Maternal saliva cotinine, ng/mL	8	0.1 (0.01) (range: 0.1 – 0.1)	11	0.9 (2.2) (range: 0.1 – 7.6)	-0.8 (-2.3; 0.7) p =0.276

Data were expressed as mean (SD) unless otherwise stated.

CF= cystic fibrosis; CI=confidence interval.

Δ = mean difference between groups.

6. Standardised treatment for newborn infants with CF

Prior to commencement of this study, a standardised protocol was developed and agreed upon by all participating consultants. This was adhered to throughout the duration of the study.

Following diagnosis, all infants commenced on multivitamins, pancreatic supplements (if pancreatic insufficient) and prophylactic flucloxacillin (25mg/kg twice daily).^{E27} The extent to which protocol was adhered to was checked both by regular review of prospectively completed Case Record Forms (CRF) and by discussions at collaborative meetings of the LCFC. There was no evidence of deviations from this protocol prior to or at the time of the 3 month lung function tests. Cough swabs were taken routinely at clinic visits (minimum every 2–3 monthly) and additionally when the infant is symptomatic. A standardised protocol for collection, storage and analysis of samples was used.^{E28}

Within the UK, all centres encourage daily chest physiotherapy to infants and children with CF. Within the London CF Collaboration, parents/carers of CF infants and children are educated about the importance of physical activity and its benefits on the respiratory system, and they are taught an appropriate airway clearance technique. Physiotherapy is carried out as appropriate to the child's condition and reviewed frequently in conjunction with medical treatment.

6.1. Infection with *Pseudomonas aeruginosa* (PsA)

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

- **Well infant (based on clinical judgement)**
 - Home therapy with 3 weeks of oral ciprofloxacin (15mg/kg twice daily) and
 - 3 months of nebulised Colistin (Colomycin: 1 million units twice daily).^{E29}
- **Unwell infant (based on clinical judgement)**
 - Hospital admission for 2 weeks of intravenous (IV) antibiotics. The choice of antibiotics was guided by results from culture and sensitivity;

- Intravenous Ceftazidime (50mg/kg three times daily) and intravenous Tobramycin (10mg/kg once daily);
- Also started on 3 months of nebulised Colistin, initiated whilst in hospital.

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

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

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

- **intravenous antibiotics**, and either
 - a further 3 months of nebulised Colistin, or
 - monthly alternating nebulised Colistin and Tobramycin (300mg twice daily).

6.2. Infection with *Staphylococcal aureus* (SA)

(a) First growth

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

(b) Regrowth less than 6 months from first growth

- Oral flucloxacillin 50mg/kg for 28 days.

(c) Further regrowth within 6 months

- Two oral anti-staphylococcal antibiotics for 4 weeks.

6.3. Infection with *Haemophilus influenzae* (HI)

(a) First growth

- **Well infant**

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

- **Unwell infant**

- Hospital admission for 2 weeks of intravenous antibiotics

(b) Regrowth less than 6 months from first growth

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

(c) Further regrowth within 6 months

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

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