Title: Early respiratory infection is associated with reduced spirometry in children with cystic fibrosis

Authors: Kathryn A Ramsey[1], Sarath Ranganathan[2,3,4], Judy Park[1], Billy Skoric[2,3], Anne-Marie Adams[2,3], Shannon J Simpson[1], Roy M Robins-Browne[5], Peter J Franklin[1], Nick H de Klerk[1], Peter D Sly[6], Steve M Stick[1,7], Graham L Hall[1,7*] on behalf of AREST CF[#]

Affiliations:

1. Telethon Kids Institute, University of Western Australia, Subiaco, Western Australia

2. Murdoch Childrens Research Institute, Parkville, Victoria

3. Department of Respiratory Medicine, Royal Children's Hospital, Parkville, Victoria

4. Department of Paediatrics, University of Melbourne, Grattan Street, Parkville, Victoria

5. Department of Microbiology and Immunology, University of Melbourne, Parkville, Victoria

6. Queensland Children's Medical Research Institute, The University of Queensland, Herston, Queensland

7. Department of Respiratory Medicine, Princess Margaret Hospital for Children, Western Australia

*__Corresponding author:__

Professor Graham Hall

Paediatric Respiratory Physiology, Telethon Kids Institute, University of Western Australia

100 Roberts Road, Subiaco, WA 6008 Australia

Graham.Hall@telethonkids.org.au

+618 9489 7816

The full membership of the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) is available at http://www.arestcf.org.

Author contributions: KAR, SR, PDS, SMS and GLH contributed to the literature search, study design, data collection, data analysis, data interpretation, figures and manuscript writing. BS, AA, SJS, PJF contributed to data collection, data analysis, data interpretation and manuscript writing. RMR contributed to study design, data interpretation and manuscript review. JP and NdK contributed to data analysis, data interpretation, figures and manuscript writing.

Funding: Funding for the AREST CF program was obtained from the Cystic Fibrosis Foundation Therapeutics (SLY04A0, STICK09A0) and the National Health and Medical Research Council of Australia (NHMRC; APP513730 and Centre of Research Excellence #1000896). G Hall is a NHMRC Fellow (APP1025550)

Running head: Early lung infection predicts spirometry in CF

Descriptor number: 9.17

Manuscript word count: 2,634

At a Glance Commentary

Scientific Knowledge on the Subject:

The preservation of lung function is a primary objective of interventional studies in school-aged children with cystic fibrosis (CF). Early signs of CF lung disease at infancy include pulmonary inflammation, detection of respiratory pathogens, structural abnormalities and

impairments to lung function. We aimed to determine which clinical factors of early CF lung disease are associated with persistent impairments to lung function at school-age.

What This Study Adds to the Field:

This study indicates that early infections with pathogens known to elicit a pro-inflammatory response modify disease progression and result in persistent impairments to respiratory function. Early pathogen surveillance programs in the first two years of life are required to adequately detect and eradicate these pathogens in order to maintain respiratory function.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

Abstract

Rationale: Pulmonary inflammation, infection and structural lung disease occur early in life in children with cystic fibrosis.

Objectives: We hypothesised that the presence of these markers of cystic fibrosis lung disease in the first two years of life would be associated with reduced lung function in childhood.

Methods: Lung function ($FEV_{0.75}$, FVC) was assessed in individuals with cystic fibrosis diagnosed following newborn screening and healthy subjects during infancy (0 – 2 years) and again at early school-age (4 – 8 years). Individuals with cystic fibrosis had annual bronchoalveolar lavage fluid, and chest computed tomography. We examined which clinical outcomes (pulmonary inflammation, infection, structural lung disease, respiratory hospitalisations, antibiotic prophylaxis) measured in the first two years of life were associated with reduced lung function in infants and young children with cystic fibrosis using a mixed effects model.

Measurements and Main Results: Children with cystic fibrosis (n=56) had 8.3% (95% confidence interval (CI) -15·9, -6·6; p = 0·04) lower $FEV_{0.75}$ compared with healthy subjects (n=18). Detection of pro-inflammatory bacterial pathogens (*Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae, Aspergillus species, Streptococcus pneumoniae*) in BAL fluid was associated with clinically significant reductions in $FEV_{0.75}$ (ranging between 11.3% and 15.6%).

Conclusions: The onset of lung disease in infancy, specifically the occurrence of lower respiratory tract infection is associated with low lung function in young children with cystic

fibrosis. Deficits in lung function measured in infancy persist into childhood emphasising the need for targeted therapeutic interventions in infancy to maximise functional outcomes later in life.

Abstract word count: 249 words

Keywords: longitudinal, lung function, infant, child, pulmonary infection, bronchoalveolar lavage

Introduction

Morbidity and mortality from cystic fibrosis (CF) is primarily due to progressive lung disease resulting in structural lung damage and lung function decline (1). Data from the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) which utilises annual bronchoalveolar lavage (BAL) and chest computed tomography (CT) from diagnosis has provided evidence that lung disease starts early in life in infants and young children with CF (2, 3). The AREST CF has reported that neutrophilic pulmonary inflammation, lower respiratory tract infections and early structural changes in the lung are common in the first two years of life in children with CF, despite a low prevalence of respiratory symptoms (2-4).

In CF lung disease, the presence of neutrophilic inflammation and respiratory infections in the first months of life are associated with the development of structural lung disease and early lung function decline (2-5). Pulmonary inflammation and infection are independently associated with the progression of bronchiectasis in young children with CF (3, 6) and the presence of free neutrophil elastase (NE) in the BAL at three months of age is a significant risk factor for the development of persistent bronchiectasis at 3 years (7). Early pulmonary inflammation and infection are likely to impair postnatal lung development and significantly impact lung function later in life.

Lung function outcomes can be tracked from infancy using forced expiratory manoeuvres as induced by the raised-volume rapid thoracoabdominal compression technique (RVRTC). Studies reporting RVRTC data have shown that a significant proportion of infants with CF diagnosed either clinically (8-11) or by newborn screening (4, 5, 12) have abnormal lung function in the first two years of life. Prior longitudinal studies have shown that forced expiratory flows and volumes track from infancy to school-age in children diagnosed

clinically with CF, and are lower in those children with recent symptoms and respiratory infection (13-15). As school-aged FEV_1 is a strong predictor of lung function decline, and both respiratory morbidity and mortality later in life (16), we aimed to determine the early life risk factors of lung function outcomes in children with CF diagnosed following newborn screening. We hypothesised that the presence of pulmonary inflammation and infection in the first two years of life would be predictive of spirometry ($FEV_{0.75}$, FVC) outcomes at early school-age. Some of the results of these studies have been previously reported in the form of abstracts (17, 18)

Methods

A detailed version of the methods is provided in the online data supplement.

Study population

Infants diagnosed with CF following newborn screening were recruited from the CF clinics of Princess Margaret Hospital for Children, Perth, and Royal Children's Hospital, Melbourne between 2002 and 2007 and enrolled in the AREST CF early surveillance program. Healthy infants without CF were recruited from the local population in Perth between 1996 and 2003 (19). Infant lung function in the healthy and CF cohorts have been reported previously (5). These children were later followed up with school age lung function between the ages of four to eight years. The study was approved by the ethics committee of each institution and parents consented to each aspect of the study separately.

Lung function testing and clinical data

The RVRTC technique was performed in infants as described previously (5). Spirometry was performed in school aged children according to current ATS/ERS guidelines (Vmax,

CareFusion, California, USA) (20). Outcomes measured included forced expiratory volume (FEV) in the first half second ($FEV_{0.5}$), FEV in the first three quarters of a second ($FEV_{0.75}$), FEV in the first second (FEV_1) and forced vital capacity (FVC). Parent and clinician reported symptoms and current medication use were obtained on the morning of the BAL in infants with CF. The number of days spent in hospital for a respiratory illness was determined using clinical notes.

Chest CT, bronchoscopy and BAL collection

A chest CT followed by a bronchoscopy was performed in infants with CF while under general anaesthetic at 3 months, 1 year and 2 years of age. A volume controlled, limited slice chest CT scan was obtained at end inspiration (P_{rs} = 25cm H_20) and end expiration (P_{rs} = 0cm H_2O) as described previously(2, 3). CT images were scored for the presence of structural lung disease using a simplified CF CT scoring method (2, 3). During the bronchoscopy BAL fluid was collected for the detection of pathogens and quantification of inflammation. Pulmonary infection was defined as colony counts for a specific organism (excluding mixed oral flora) of greater than 10^4 colony-forming units per ml. Bacterial pathogens known to elicit a pro-inflammatory response when isolated from the lower respiratory tract (*Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumonia,* and *Aspergillus* species) were described as pro-inflammatory pathogens(21).

Statistical analysis

Longitudinal analysis of spirometric lung function outcomes was performed using absolute data from the RVRTC technique in infancy and spirometry at school-age. A mixed effects model using natural log-transformed lung function data was used to assess differences between groups. All models were adjusted for height, sex, age, test-type and testing centre

with random slopes and intercepts for each participant. Model 1 examined the extent to which a diagnosis of CF was independently associated with lung function outcomes using both healthy and CF data. Model 2, using data from children with CF only, examined which clinical outcomes measured in the first two years of life were independently associated with lung function outcomes. The clinical outcomes significantly associated with lung function ($p < 0{\cdot}05$) in Model 2 were then placed into the multivariate analysis for each specific lung function outcome (Model 3).

Results

Infant lung function data were available in 68 infants with CF, 56 of whom returned for at least one school-age visit (Table 1). Forty-eight infants with no history of lung disease (healthy) had acceptable infant lung function data, 18 of whom returned for school-age spirometry. Infants and children with CF had lower weight z-scores, BMI and BMI z-scores compared with healthy controls (Table 2).

Of the 56 children with CF with longitudinal lung function measurements, 51 (91%) were pancreatic insufficient, 28 (50%) had respiratory symptoms and 34 (61%) had spent at least one night in hospital for a respiratory illness in the first two years of life (Table 3). Twenty-one (38%) of these children had free NE detected and 40 (71%) had a bacterial infection at $>10^4$ cfu/ml in the BAL at any time in the first two years. The most common pathogen was *S. aureus* (36%), followed by *P. aeruginosa* (18%), *H. influenzae* (13%) and *Aspergillus sp* (13%). Forty-two of these children had a chest CT scan in the first two years of life. Bronchiectasis and air trapping was detected in 18 (43%) and 39 (93%) of these children respectively.

On grouped data, children with CF had 8·3% (95% confidence interval (CI) -15·9, -6·6; p = 0·04) lower $FEV_{0.75}$ and 7·7% (95% CI -12·4, -2·7; p < 0·01) lower $FEV_{0.75}$/FVC compared with healthy children. There were no differences in FVC between children with CF and healthy children. We then examined which early life factors were predictive of lower lung function in children with CF. Children with CF who were pancreatic insufficient had 11·4% (95% CI -20·6, -1·1; p = 0.03) lower $FEV_{0.75}$ and 11·6% (95% CI -20·9, -1·3; p = 0.03) lower FVC compared with pancreatic sufficient children with CF (Table 3). Children with CF who took antibiotic prophylaxis in the first two years of life had 8·6% (95% CI 0·2, 17·6; p = 0.04) higher $FEV_{0.75}$ and 10·2% (95% CI 1·6, 19·4; p = 0·02) higher FVC compared with children with CF who did not take prophylactic antibiotics. There was no evidence to suggest that this group had less infection (p = 0·32) or lower levels of NE (p = 0·24) during infancy. The presence of respiratory symptoms, number of respiratory hospitalisation days and the presence of bronchiectasis or air trapping on chest CT in the first two years of life were not associated with the tracking of lung function into early school-age (Table 3).

The presence of free neutrophil elastase activity in the BAL fluid within the first two years of life was independently associated with an 8·7% (95% CI -15·8, -1·1; p = 0·03) reduction in $FEV_{0.75}$ (Table 3). There was no effect of the presence of IL-8 in the BAL or inflammatory response score on lung function. Infection with a pathogen known to elicit a pro-inflammatory response including *Aspergillus sp, S. aureus, H. influenzae, P. aeruginosa* or *S. pneumoniae* in the first two years of life was associated with 11·3 – 15·6% lower $FEV_{0.75}$ compared with those who were uninfected (Table 3; Figure 1). Infection with *H. influenzae* was also associated with a 14·2% decrease in FVC. Infection with pathogens not known to

elicit a pro-inflammatory response (non-pro-inflammatory pathogen) was not associated with reductions in lung function (Table 3).

The early life predictors of lung function identified in the univariate analyses (prophylactic antibiotic use, the presence of free neutrophil elastase activity and presence of pro-inflammatory pathogens) were then placed into a multivariate analysis along with height, sex, age, test-type and centre (Table 4). In this analysis the presence of pro-inflammatory pathogens in the BAL fluid remained the only significant variable associated with lower lung function.

Discussion

The primary objective of this analysis was to examine which clinical factors in the first two years of life were associated with reduced lung function in young children with CF diagnosed following newborn screening. Children with CF in this cohort had reduced lung function throughout early childhood from infancy until the early school years. The detection of a pro-inflammatory bacterial pathogen in the BAL fluid over the first two years of life was associated with reductions in lung function in children with CF and highlights the importance of early lower respiratory infection in CF and its influence on future disease status.

Our study investigated the long-term impact of clinical findings during the first two years of life on the tracking of lung function into school-age. In this longitudinal study the most important early clinical finding was that bacterial pathogens in the BAL previously shown to elicit a pro-inflammatory response in the lower airway (21), such as *P. aeruginosa, S. aureus,*

H. influenzae, P. pneumonia and *Aspergillus* sp., were each associated with reductions in $FEV_{0.75}$ greater than 10%. Respiratory infections in infancy have been associated in cross-sectional analyses with lower $FEV_{0.5}$ (22) and longitudinal analyses with greater decline in $FEV_{0.5}$ (4) compared with uninfected children in the first two years of life. Our study suggests these early reductions in lung function are clinically important and persist into later life. Long term diminished lung function is of clinical importance and this study points to the importance of new approaches to early intervention to preserve lung function.

There have been a small number of longitudinal studies performed in children with CF that have tracked lung function from infancy to preschool or early school age. Significant associations between forced expiratory volumes and flows from RVRTC at infancy and spirometry outcomes at preschool (14, 15, 23) and school age (24) have been described. These studies were conducted in children diagnosed with CF clinically and the relevance of these findings to populations diagnosed with CF following newborn screening is unclear. In addition, only Kozlowska *et al* has performed longitudinal lung function measurements in both children with CF and healthy control subjects (15). The degree of lung function impairment in children with CF compared with controls in our newborn screened cohort (8·3% lower $FEV_{0.75}$) was similar to the clinically diagnosed cohort (7·5% lower $FEV_{0.75}$) reported by Kozlowska(15). These data indicate that, despite newborn screening, the lung function trajectory into school age appears similar in each of these populations. One plausible explanation is that there are no specific interventions included in modern CF care specifically targeting prevention of structural lung damage or other mechanisms of airflow limitation..

The presence of free NE activity in the lower respiratory tract at any point in the first two years was independently associated with reduced lung function throughout infancy and

school age, but did not remain a significant variable after adjusting for the presence of pro-inflammatory pathogens. Free NE activity in the BAL is an indicator of the amount of NE that has exceeded the anti-protease binding capacity of the lung (25) and is the primary cause for the structural destruction of the airways and parenchyma of CF lungs (26). We have previously shown that free NE activity in BAL fluid at 3 months of age is a risk factor for the development of bronchiectasis at 12 months and 3 years of age (7). While free NE activity in the BAL has been associated with lower $FEV_{0.5}$ and FVC in infancy (4), the present study suggests that respiratory infections are the crucial early driver for lung function progression into school-age.

The presence of pro-inflammatory pathogens in the BAL in the first two years of life was the strongest predictor of lung function in infants and school aged children with CF. We have previously shown that the presence of *P. aeruginosa* or *S. aureus* in the BAL results in a greater rate of decline in lung function in infancy (4). The present study provides evidence that these early life respiratory infections result in deficits in lung function that persevere to the early school years. In addition, early life respiratory tract infections with other pro-inflammatory pathogens including *H. influenzae*, *S. pneumoniae* and *Aspergillus sp*, also appear to result in persistent reductions in $FEV_{0.75}$. These data indicate that early respiratory tract infections are crucial modifiers of early CF lung disease progression. Early surveillance combined with new and improved interventional strategies are required to prevent, eradicate and manage these infections to prevent disease establishment.

In the present study, children with CF who took bacterial prophylaxis in the first two years of life had 8·6% higher lung function than those not taking prophylaxis. However, we do not have any objective measures of adherence to antibiotic prophylaxis so these observations

much be treated with caution. We found no associations between CT or clinicometric outcomes (respiratory symptoms or number of days spent in hospital for respiratory illness in the first two years) and lung function in children with CF. Strengths of our study include the use of BAL to detect lower respiratory tract inflammation and infection, chest CT to quantify lung disease, serial lifetime sampling, the inclusion of contemporary controls and newborn screened children with CF. Weaknesses of the study include the poor follow-up of healthy control children to school age and the use of annual surveillance meant it was unknown if children were infected with other pathogens in the time between annual BAL visits. Also, as BAL were performed when subjects were well rather than at times of exacerbations we are unable to comment on the potential role of viruses in the development and persistence of diminished lung function.

The results of this longitudinal study are consistent with previous AREST CF studies which have shown that the presence of neutrophil elastase and respiratory pathogens in BAL fluid are associated with the development of structural and physiological lung disease in young children with CF (2-4, 7). Our study has shown that infection with a pro-inflammatory pathogen in the first two years modifies disease progression resulting in persistent impairments to respiratory function. Early surveillance and interventions need to focus on preventing, identifying and eradicating infections with a range of respiratory pathogens (not just with *P. aeruginosa*) until modifying agents are available. We speculate that interventions that prevent infection in the first two years of life are required in order to improve later lung function, minimise structural lung disease and maximise survival.

Acknowledgements:

We would like to thank Barry Linnane, Naveen Pillarisetti and Vaska Stavreska who contributed to lung function data collection, the respiratory fellows who performed the bronchoscopies, and laboratory staff members who processed and analysed the samples. We would also like to thank the participants and their families who contribute to the AREST CF program.

References

1. de Boer K, Vandemheen KL, Tullis E, Doucette S, Fergusson D, Freitag A, Paterson N, Jackson M, Lougheed MD, Kumar V, Aaron SD. Exacerbation frequency and clinical outcomes in adult patients with cystic fibrosis. *Thorax* 2011; 66: 680-685.
2. Sly PD, Brennan S, Gangell C, de Klerk N, Murray C, Mott L, Stick SM, Robinson PJ, Robertson CF, Ranganathan SC. Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. *Am J Respir Crit Care Med* 2009; 180: 146-152.
3. Stick SM, Brennan S, Murray C, Douglas T, von Ungern-Sternberg BS, Garratt LW, Gangell CL, De Klerk N, Linnane B, Ranganathan S, Robinson P, Robertson C, Sly PD. Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. *J Pediatr* 2009; 155: 623-628 e621.
4. Pillarisetti N, Williamson E, Linnane B, Skoric B, Robertson CF, Robinson P, Massie J, Hall GL, Sly P, Stick S, Ranganathan S. Infection, inflammation, and lung function decline in infants with cystic fibrosis. *Am J Respir Crit Care Med* 2011; 184: 75-81.
5. Linnane BM, Hall GL, Nolan G, Brennan S, Stick SM, Sly PD, Robertson CF, Robinson PJ, Franklin PJ, Turner SW, Ranganathan SC. Lung function in infants with cystic fibrosis diagnosed by newborn screening. *Am J Respir Crit Care Med* 2008; 178: 1238-1244.
6. Mott LS, Park J, Murray CP, Gangell CL, de Klerk NH, Robinson PJ, Robertson CF, Ranganathan SC, Sly PD, Stick SM. Progression of early structural lung disease in young children with cystic fibrosis assessed using CT. *Thorax* 2012; 67: 509-516.
7. Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, Mott LS, Murray CP, Stick SM. Risk factors for bronchiectasis in children with cystic fibrosis. *N Engl J Med* 2013; 368: 1963-1970.

8. Davis SD, Rosenfeld M, Kerby GS, Brumback L, Kloster MH, Acton JD, Colin AA, Conrad CK, Hart MA, Hiatt PW, Mogayzel PJ, Johnson RC, Wilcox SL, Castile RG. Multicenter evaluation of infant lung function tests as cystic fibrosis clinical trial endpoints. *Am J Respir Crit Care Med* 2010; 182: 1387-1397.

9. Lum S, Gustafsson P, Ljungberg H, Hulskamp G, Bush A, Carr SB, Castle R, Hoo AF, Price J, Ranganathan S, Stroobant J, Wade A, Wallis C, Wyatt H, Stocks J. Early detection of cystic fibrosis lung disease: multiple-breath washout versus raised volume tests. *Thorax* 2007; 62: 341-347.

10. Ranganathan SC, Dezateux C, Bush A, Carr SB, Castle RA, Madge S, Price J, Stroobant J, Wade A, Wallis C, Stocks J. Airway function in infants newly diagnosed with cystic fibrosis. *Lancet* 2001; 358: 1964-1965.

11. Ranganathan SC, Stocks J, Dezateux C, Bush A, Wade A, Carr S, Castle R, Dinwiddie R, Hoo AF, Lum S, Price J, Stroobant J, Wallis C. The evolution of airway function in early childhood following clinical diagnosis of cystic fibrosis. *Am J Respir Crit Care Med* 2004; 169: 928-933.

12. Hoo AF, Thia LP, Nguyen TT, Bush A, Chudleigh J, Lum S, Ahmed D, Balfour Lynn I, Carr SB, Chavasse RJ, Costeloe KL, Price J, Shankar A, Wallis C, Wyatt HA, Wade A, Stocks J. Lung function is abnormal in 3-month-old infants with cystic fibrosis diagnosed by newborn screening. *Thorax* 2012; 67: 874-881.

13. Beardsmore CS. Lung function from infancy to school age in cystic fibrosis. *Arch Dis Child* 1995; 73: 519-523.

14. Brumback LC, Davis SD, Kerby GS, Kloster M, Johnson R, Castile R, Hiatt PW, Hart M, Rosenfeld M. Lung function from infancy to preschool in a cohort of children with cystic fibrosis. *Eur Respir J* 2013; 41: 60-66.

15. Kozlowska WJ, Bush A, Wade A, Aurora P, Carr SB, Castle RA, Hoo AF, Lum S, Price J, Ranganathan S, Saunders C, Stanojevic S, Stroobant J, Wallis C, Stocks J. Lung function from infancy to the preschool years after clinical diagnosis of cystic fibrosis. *Am J Respir Crit Care Med* 2008; 178: 42-49.

16. Kerem E, Reisman J, Corey M, Canny GJ, Levison H. Prediction of mortality in patients with cystic fibrosis. *N Engl J Med* 1992; 326: 1187-1191.

17. Ramsey K, Ranganathan S, Park J, Skoric B, Linnane B, Franklin P, De Klerk NH, Sly P, Stick S, Hall G. Pulmonary inflammation and infection in infants with cystic fibrosis (CF) leads to reduced spirometry at school-age. Pediatr Pulmonol; 2013. p. 353.

18. Ramsey K, Ranganathan S, Skoric B, Linnane B, Franklin P, Sly P, Stick S, Hall G. Structural lung disease, pulmonary infection and inflammation in infancy is associated with reduced spirometry at school-age in children with cystic fibrosis (CF). *Eur Respir J* 2013; 42: 5054.

19. Franklin PJ, Hall GL, Moeller A, Horak F, Jr., Brennan S, Stick SM. Exhaled nitric oxide is not reduced in infants with cystic fibrosis. *Eur Respir J* 2006; 27: 350-353.

20. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319-338.

21. Gangell C, Gard S, Douglas T, Park J, de Klerk N, Keil T, Brennan S, Ranganathan S, Robins-Browne R, Sly PD. Inflammatory responses to individual microorganisms in the lungs of children with cystic fibrosis. *Clin Infect Dis* 2011; 53: 425-432.

22. Nixon GM, Armstrong DS, Carzino R, Carlin JB, Olinsky A, Robertson CF, Grimwood K, Wainwright C. Early airway infection, inflammation, and lung function in cystic fibrosis. *Arch Dis Child* 2002; 87: 306-311.

23. Marostica PJ, Weist AD, Eigen H, Angelicchio C, Christoph K, Savage J, Grant D, Tepper RS. Spirometry in 3- to 6-year-old children with cystic fibrosis. *Am J Respir Crit Care Med* 2002; 166: 67-71.

24. Harrison AN, Regelmann WE, Zirbes JM, Milla CE. Longitudinal assessment of lung function from infancy to childhood in patients with cystic fibrosis. *Pediatr Pulmonol* 2009; 44: 330-339.

25. Griese M, Essl R, Schmidt R, Rietschel E, Ratjen F, Ballmann M, Paul K. Pulmonary surfactant, lung function, and endobronchial inflammation in cystic fibrosis. *Am J Respir Crit Care Med* 2004; 170: 1000-1005.

26. Downey DG, Bell SC, Elborn JS. Neutrophils in cystic fibrosis. *Thorax* 2009; 64: 81-88.

27. de Onis M, Onyango A, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* 2007; 85: 661-668.

28. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. *Acta Pædiatrica Suppl* 2006; 450: 76-85.

1

Figure legends:

Figure 1: Association of $FEV_{0.75}$ with height in children with CF based on predicted values from Model 2. The figure illustrates the predicted values for a child with CF who did not have a lower respiratory tract infection in the first two years of life (uninfected: black), who was infected with *Aspergillus sp (blue), S. aureus (orange), H. influenza (red) or P. aeurginosa* (green) in the first two years of life. The insert provides a close up of the infection groups at heights during infancy.

Table 1: Clinical characteristics from first infant visit of the children with cystic fibrosis who did and did not have paired infant and school age data

Cystic Fibrosis	With Paired Results	No Paired Results
Total number	56	12
Male	28 (50)	8 (66·7)
Age diagnosis, wk	6·57 ± 1·94	5·15 ± 0·61
Genotype		
Homozygous Phe508del/Phe508del	31 (55·4)	4 (33·3)
Heterozygous Phe508del/other	22 (39·3)	8 (66·7)
Other/Other	3 (5·4)	0 (0)
Mode of presentation		
Newborn screening	41 (73·2)	9 (75)
Meconium ileus	7 (12·5)	1 (8·3)
Failure to thrive	4 (7·1)	0 (0)
Recurrent respiratory infections	0 (0)	1 (8.3)
Family history	3 (5·4)	0 (0)
Other	1 (1·8)	1 (8·3)
Data from first infant visit		
Age, y	0·98 ± 0·09	0·94 ± 0·17
Height, z score	-0·27 ± 0·23	0·41 ± 0·29
Weight, z score	-0·54 ± 0·16	- 0·28 ± 0·26
BMI, z score	-0·52 ± 0·16	-0·74 ± 0·26
$FEV_{0.5,}$ z-score	-0·58 ± 0·14	-0·94 ± 0·33
FVC, z-score	-0·28 ± 0·20	-0·38 ± 0·32

Data are presented as n (%) or mean ± standard deviation. $FEV_{0.5}$: forced expiratory volume in 0·5 seconds. FVC: forced vital capacity. BMI: body mass index. Children with paired results had at least one acceptable infant lung function (raised volume rapid thoracoabdominal compression technique) test and one acceptable school age spirometry test. Height, weight and BMI z-scores were calculated using WHO growth standards (27, 28)

Table 2: Characteristics of study participants with paired lung function at infancy and school-age

Demographics	**Infancy**			**School-age**		
	CF	**Healthy**	**p value**	**CF**	**Healthy**	**p value**
Number	68	48	-	56	18	-
Male, n %	36 (53)	19 (40)	0·21	28 (50)	7 (39)	0·41
Age at test, yr	0.97 ± 0·10	0·77 ± 0·44	0·08	6·4 ± 1·67	7·2 ± 0·61	**<0·001**
Height, z-score	-0·15 ± 0·24	0·30 ± 1·0	0·16	-0·39 ± 1·04	0·45 ± 0·97	**0·001**
Weight, z-score	-0·49 ± 0·18	0·24 ± 1·0	**0·001**	-0·21 ± 0·95	0·66 ± 0·66	**<0·001**
BMI, z-score	-0·56 ± 0·18	0·05 ± 1·1	**<0·01**	-0·05 ± 0·89	0·55 ± 0·77	**<0·01**

Data are presented as n (%) or mean ± standard deviation. BMI: body mass index. Children in this study had at least one acceptable infant lung function (raised volume rapid thoracoabdominal compression technique) test and one acceptable school age spirometry test. Height, weight and BMI z-scores were calculated using WHO growth standards (27, 28)

Table 3: Independent analysis of early life predictors of lung function (Model 2)

	n	$FEV_{0.75}$	$FEV_{0.75}$/FVC	FVC
Pancreatic Insufficiency	51/56	**-11·4 (-20·6, -1·1); p = 0·03**	1·3 (-4·7, 7·8); p = 0·67	**-11·6 (-20·9, -1·3); p = 0·03**
Prophylactic antibiotic use	43/56	**8·6 (0·2, 17·6); p = 0·04**	0·5 (-4·8, 4·0); p = 0·83	**10·2 (1·6, 19·4); p = 0·02**
Respiratory symptoms	28/56	-4·2 (-10·9, 3·1); p = 0·25	-1·8 (-5·8, 2·3); p = 0·38	-2·8 (-10·1, 4·9); p = 0·47
Respiratory hospitalisation days	34/56	-0·8 (-21·1, 0·5); p = 0·23	0·1 (-0·6, 0·8); p = 0·85	-0·8 (-2·1, 0·6); p = 0·25
Neutrophil elastase (NE) any time	21/56	-6·7 (-13·2, 0·2); p = 0·06	-0·2 (-4·2, 3·9); p = 0·91	-6·4 (-13·0, 0·7); p = 0·08
NE present (1 time point)	14/56	**-8·7 (-15·8, -1·1); p = 0·03**	-2·3 (-6·5, 2·1); p = 0·31	-7·8 (-15·1, 0·0); p = 0·05
NE present (> 1 time point)	7/56	-2·4 (-12·3, 8·6); p = 0·66	4·2 (-1·8, 10·6); p = 0·17	-2·9 (-13·4, 8·8); p = 0·61
Max IL-8 count	56/56	1·2 (-0·7, 3·1); p = 0·21	0·9 (0·0, 1·9); p = 0·06	0.4 (-1·4, 2·3); p = 0·66
Inflammatory response score	56/56	-0·02 (-2·0, 2·0); p = 0·98	0·3 (-0·8, 1·4); p = 0·56	-0·3 (-2·3, 1·7); p = 0·75
Any bacterial pathogen	40/56	**-13·3 (-20·2, -5·8); p = 0·001**	-4·2 (-8·9, 0·7); p = 0·09	-8·2 (-17·3, 2·0); p = 0·11
Pro-inflammatory pathogen	30/56	**-14·5 (-21·6, -6·9); p < 0·001**	-3·5 (-8·7, 2·0); p = 0·21	-10·4 (-19·9, 0·17); p = 0·54
1 pro-inflammatory pathogen	19/56	**-14·6 (-22·0, -6·4); p = 0·001**	-3·3 (-8·8, 2·6); p = 0·26	-10·6 (-20·6, 0·72); p = 0·06
> 1 pro-inflammatory pathogen	11/56	**-14·5 (-22·8, -5·2); p < 0·01**	-3·9 (-10·1, 2·7); p = 0·24	-10·1 (-21·6, 3·0); p = 0·13
P. aeruginosa	10/56	**-15·6 (-23·1, -7·3); p < 0·001**	-2·6 (-7·9, 2·9); p = 0·34	-8·6 (-20·4, 4·9); p = 0·20
H. influenzae	7/56	**-15·3 (-25·5, -3·8); p = 0·01**	-4·1 (-10·8, 3·0); p = 0·25	**-14·2 (-24·5, -2·4); p = 0·02**
S. aureus	20/56	**-12·5 (-19·7, -4·6); p < 0·01**	-2·7 (-8·2, 3·1); p = 0·35	-8·6 (-17·5, 1·3); p = 0·09
Aspergillus sp.	7/56	**-11·3 (-18·9, -3·1); p < 0·01**	0·3 (-4·0, 4·8); p = 0·88	-9·2 (-18·1, 0·8); p = 0·07
Non-inflammatory pathogens	10/56	-0·5 (-9·0, 8·9); p = 0·92	-3·6 (-8·0, 1·0); p = 0·12	3·6 (-7·1, 15·6); p = 0·52
Bronchiectasis	18/42	2·2 (-4·5, 9·5); p = 0·53	3·1 (-0·9, 7·2); p = 0·13	-1·6 (-9·0, 6·4); p = 0·68
Air trapping	39/42	-2·5 (-14·9, 11·7); p = 0·71	0·0 (-7·8, 8·4); p = 0·99	0·7 (-13·9, 17·8); p = 0·93

All clinical predictors of lung function are from the first two years of life. Results are expressed as mean (95% confidence interval; p value) percent change compared to CF children not described by the variable (e.g. Pancreatic insufficient children compared with pancreatic sufficient children). Respiratory symptoms included any parentally reported cough, upper respiratory tract infection or sputum production in the last month, or any clinician reported wheeze, respiratory tract infection or crackles on the day of the bronchoscopy. All models were adjusted for height, sex, age, test-type (RVRTC or spirometry) and testing centre. Statistically significant ($p < 0{\cdot}05$) changes are shown in bold.

Table 4: Multivariate analysis of early life predictors of lung function (Model 3)

	n	$FEV_{0.75}$	$FEV_{0.75}$/FVC	FVC
Prophylactic antibiotic use	43/56	5·9 (-4·2, 17·0); p = 0·26	-2·4 (-8·0, 3·5); p = 0·42	12·6 (-0·07, 27·6); p = 0·06
NE present (1 time point)	14/56	-4·3 (-12·6, 4·8); p = 0·34	-4·0 (-9·3, 1·6); p = 0·16	0·4 (-10·3, 12·4); p = 0·95
NE present (> 1 time point)	7/56	3·3 (-8·0, 16·0); p = 0·58	3·8 (-3·3, 11·5); p = 0·30	6·5 (-8·8, 24·4); p = 0·43
Pro-inflammatory pathogens	30/56	**-13·3 (-20·8, -5·1); p < 0·01**	-3·8 (-9·1, 1·9); p = 0·19	-8.9 (-18·6, 1·9); p = 0·10

All clinical predictors of lung function are from the first two years of life. All results are expressed as mean (95% confidence interval; p value) percent change compared to CF children not described by the variable. Variables associated with a significant change ($p < 0·05$) in lung function outcomes in Model 2 were included in the multivariate Model 3. All models were adjusted for height, sex, age, test-type (RVRTC or spirometry) and testing centre. Statistically significant ($p < 0·05$) changes are shown in bold.