Minimal change in structural, functional and inflammatory markers of lung disease in newborn screened infants with cystic fibrosis at one year

*Gwyneth Davies, *Lena P Thia, Janet Stocks, Andrew Bush, Ah-Fong Hoo, Angie Wade , The Thanh Diem Nguyen, Alan S Brody, Alistair Calder, Nigel J Klein, Siobhán B Carr, Colin Wallis, Ranjan Suri, Caroline S Pao, Gary Ruiz, Ian M Balfour-Lynn; on behalf of London Cystic Fibrosis Collaboration (LCFC)

***Joint first authors**

Data Supplement

This online supplement presents additional details and results, and should be read in conjunction with the main manuscript

Methods

Further details of Volume controlled chest CT scans

Thin section chest high resolution CT (HRCT) was performed no more than two weeks after infant lung function testing during a period of clinical stability. Measurements were obtained under general anaesthesia (GA) immediately before bronchoscopy, in one of three participating centres. A standardised protocol for volumetric inspiratory and expiratory image acquisition and GA was undertaken [1]. Scans were scored using the Brody-II CF-CT scoring system by two independent radiologists (ASB - the score's developer, and AC)[2]. For the purposes of this study, air trapping sub-score was used as the primary outcome, since this was the only reproducible measurement [1]. However results for bronchial dilatation sub-scores and Total CT score are also presented. Bronchial dilatation was assessed both in the central and peripheral lung, and rated from 0-3 for both severity and extent [2]. A broncho-arterial ratio (BAR) >1 specified in Brody II was used to define bronchial dilatation, using luminal bronchial diameter. For each sub-score or Total CT score, results represent mean values across the two scorers. A total CT score >5% of the maximum possible score of 243

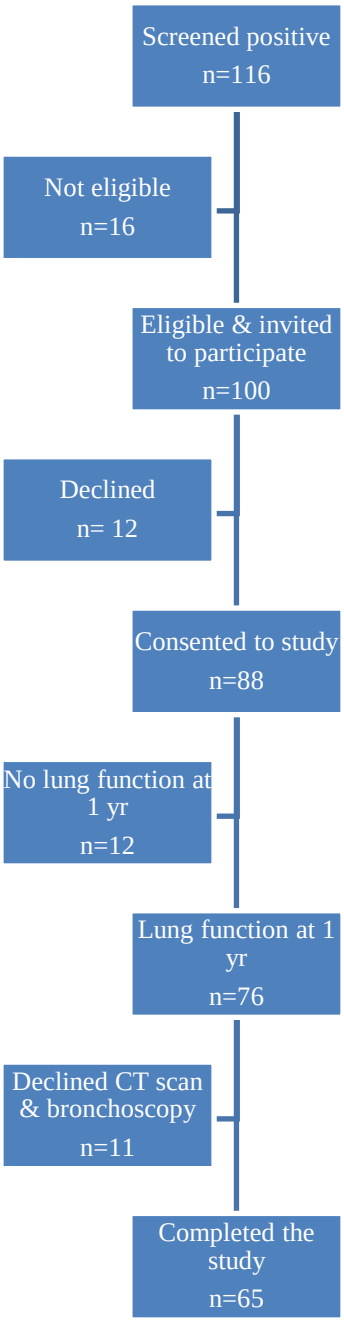
(i.e. ≥12), air trapping score >6 (max possible 27), or any evidence of bronchial dilatation (maximum score 72) was considered abnormal[1, 3].

Results

Patient details

Figure E1: Consort diagram for the study

Reasons for ineligibility were social (n=10), co-morbidity (n=4), prematurity (n=1) and sudden infant death (n=1). Reasons for non-testing of lung function at one year were parent withdrawal (n=6), and inability to be tested either before 15 months of age or within 2 weeks prior to the CT/BAL while clinically stable (n=6).



Technical success of Multiple Breath Washout in infants

Technical success in measurements of LCI at 1yr for this cohort have been published in Nguyen et al[4], in their online supplement table E1, with success rates for MBW at 1yr of 99% in CF and 100% in healthy controls. We explored intra-test variability over the first two years of life and found that this was greater in CF than in the healthy control group[5]. However, this current manuscript describes cross-sectional outcomes at 1yr rather than longitudinal changes over time and details of intra-test variability have therefore not been included in this manuscript.

Lung inflammation and structure

Table E1: Summary of objective markers of lung health at 1 year of age: inflammation and CT scores

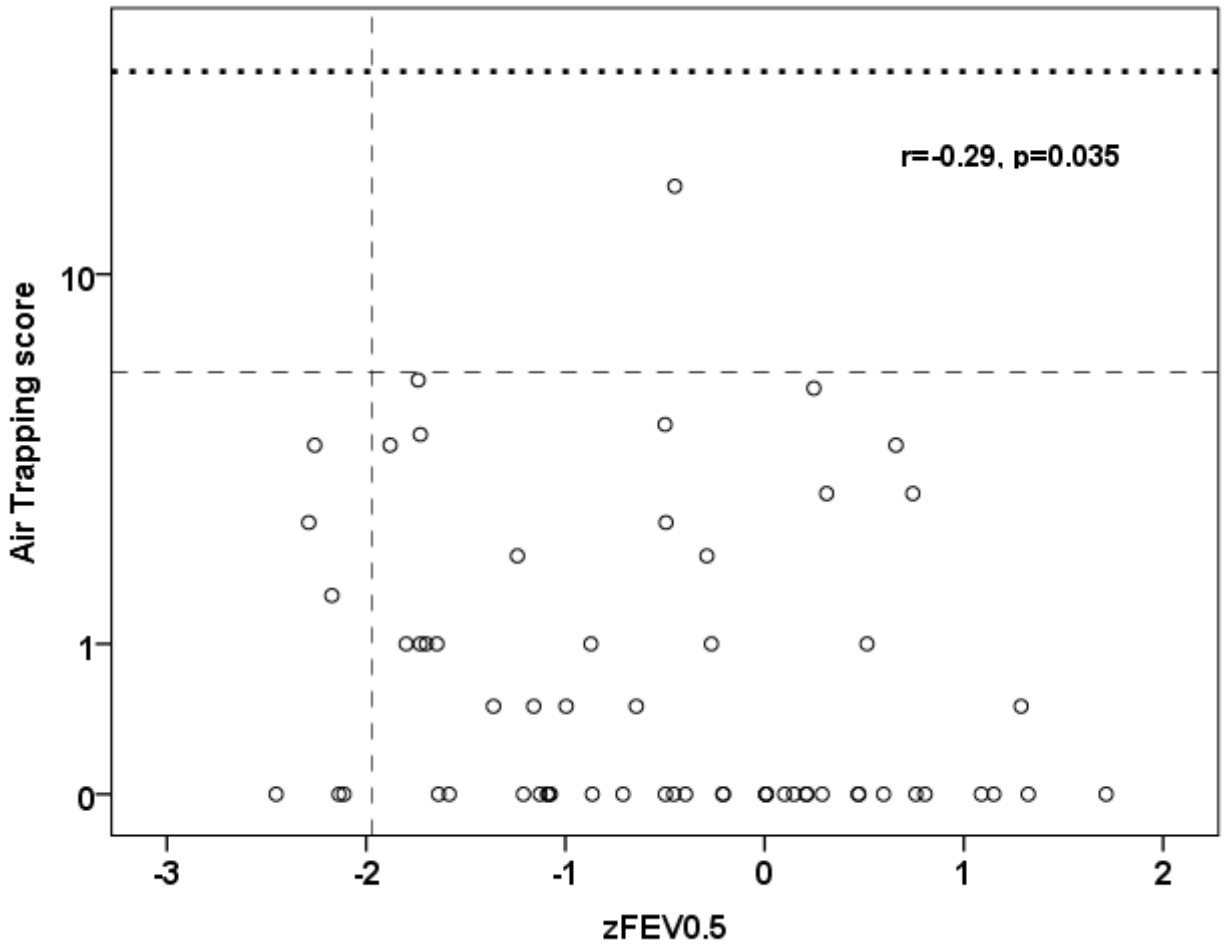
BAL inflammation (n (%) or median (IQR))		
Detectable free neutrophil elastase activity (n=43)[#]		10 (23%)
IL-8 (n=45)		286 (82-1209) pg/ml
IL-6 (n=45)		34.2 (13-118) pg/ml
IL-10 (n=45)		2.0 (0.6-6) pg/ml
TNFα (n=45)		2 (1.0-6.1) pg/ml
MCP-1 (n=45)		158 (67-450) pg/ml
Brody II scores chest CT (median (IQR))		
Total CT score[$] (n=65)	[max score 243]	2 (1-4)
Air trapping (n=65)	[max score 27]	0 (0-1)
Bronchial dilatation[$] (n=65)	[max score 72]	0 (0-0.5)

Footnote: Results are presented as mean (SD), n(%) or median (Interquartile range (IQR). CT scores represent the mean of two scorers. [$]Although Air Trapping was the only reproducible score in our cohort[1], Total CT score, and Air Trapping and Bronchial dilatation sub-scores are presented here to illustrate mild changes overall. [#] NE analysis for 43/45 infants as supernatant sufficient for cytokines only.

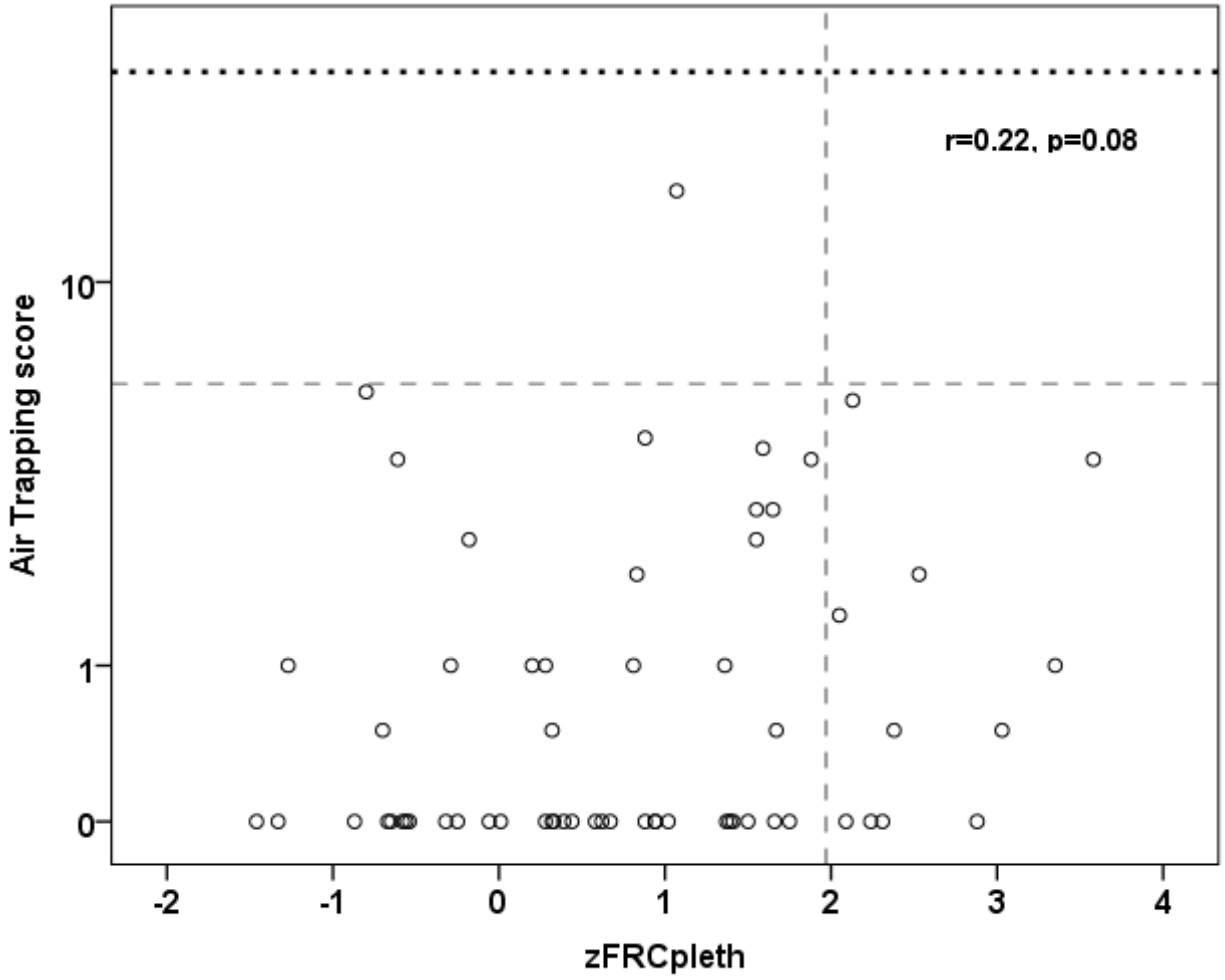
Relationship between lung function and structure

Air trapping sub-score was the primary structural outcome, since this was the only reproducible measurement in our cohort[1], with a score >6 considered abnormal. $zFEV_{0.5}$ was weakly associated with air trapping on chest CT at 1 year of age (Fig E2). There was no association between zFRCpleth and air trapping (Fig E2).

Figure E2. Association between lung function ($FEV_{0.5}$ and FRC_{pleth}) and structure at 1 year of age in NBS infants with CF



Air Trapping and FRC_{pleth}



Footnote: Forced expiratory volume ($FEV_{0.5}$) and plethysmographic functional residual capacity (FRC_{pleth}) z-score vs. air trapping Brody II sub-score. CT scores are presented as the mean of the two scorers, and plotted on a Log10 axis to demonstrate the potential range of CT scores possible. The maximum possible score for Air trapping of 27 is shown by dotted horizontal lines, with results considered abnormal (>6 for air trapping) indicated by the dashed horizontal lines. The upper and lower Limits of Normality for FRC_{pleth} and $FEV_{0.5}$ are marked by the thin dashed vertical lines at +1.96 and -1.96 z-scores respectively, with the majority of infants having results within the normal range. CT changes were very mild, with only one infant having an air trapping score >6. Spearman rank correlation coefficients and p values shown.

Microbiology

As summarised in Table 2 (main manuscript), *Pseudomonas aeruginosa (PsA)* was isolated on at least one occasion in 21 (32%) infants during the first year from cough swabs; three of whom also isolated *PsA* in BAL at 1yr of age (see below). All underwent eradication therapy. Six infants with *PsA* also grew *Staphylococcus aureus (SA)* on at least one occasion, two of whom also isolated *Haemophilus influenzae (HI).* Four infants isolated both *PsA* and *HI.* One infant had chronic *PsA* infection as defined by the Leeds criteria[6]. Pathogens other than *PsA* were identified in 19/65(29%) infants (Table 2).

Of the 65 BAL samples at a year of age, 12 (18%) had a positive significant bacterial growth: six with *HI* (one of whom co-isolated *S.pneumoniae);* three (5%) with *PsA* (two of whom co-isolated *SA*), one with *SA* alone, and one with *Xanthomonas* species. Unexpectedly, one infant had *Mycobacterium tuberculosis* isolated on BAL culture, was treated for tuberculosis and excluded from further analysis. Five infants (8%) isolated *Aspergillus fumigatus*; all these had negative bacterial cultures. Five infants (8%) had a virus detected on BAL immunofluorescence (rhinovirus (n=3), parainfluenzae (n=1) and cytomegalovirus (n=1)), three of whom had negative bacterial BAL cultures.

Relationship between BAL infection and inflammation

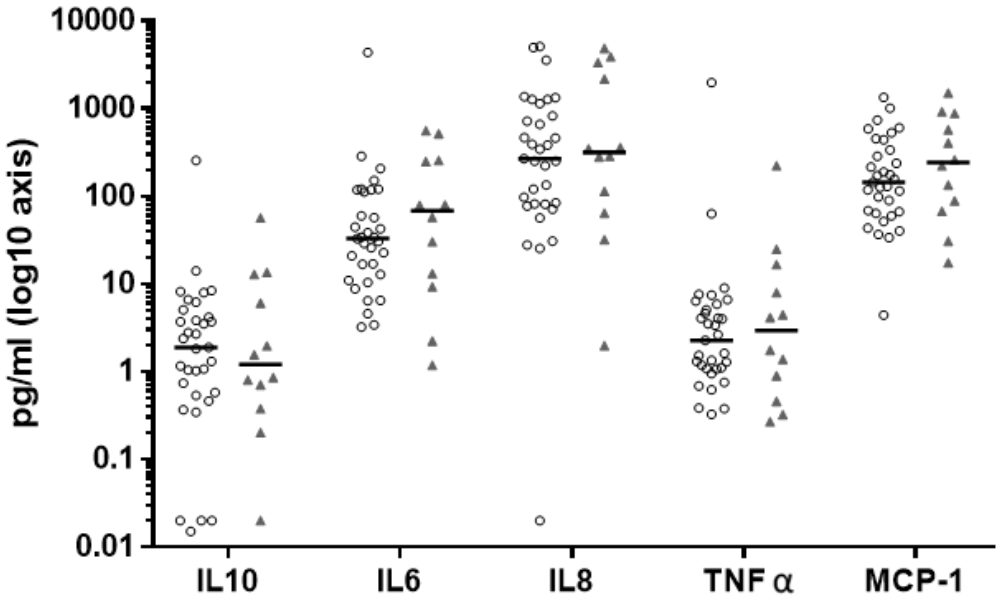
There was no difference between the proportion of infants with detectable free neutrophil elastase (NE) according to whether they had ever isolated any significant pathogens in the first year of life up to and including their bronchoscopy at ~1 year of age (7 of 27 infants that had isolated significant pathogens had detectable free NE, in comparison to 3 of 16 infants with no history of any significant pathogens having detectable free NE; Fisher's exact test p=0.72). Similarly, there was no difference in IL8 concentration between infants in these infection categories (Fig 2 main manuscript). Although MCP-1 was higher in those with significant pathogens, this was not statistically significant when multiple comparisons were taken into account.

Similarly, on the 1yr BAL culture alone, there were no significant differences for either detectable NE nor IL-8 and infection categories (any vs. none) (Fig E3). For NE however, this may reflect study power. Although numbers are limited, those infants with a positive

viral immunofluorescence on BAL fluid were observed, in general, to have higher levels of both pro- and anti-inflammatory cytokines.

Fig E3. BAL inflammation and infection at ~1year of age

A. BAL cytokine concentration according to the detection of significant pathogens in BAL



Footnote: Infants were divided into two groups according to the detection of significant pathogens (bacterial or fungal) on BAL culture. Infants with no significant pathogens are represented by open circles, and those with any significant pathogen by solid grey triangles. Horizontal lines are medians.

B. Significant pathogens in BAL vs Neutrophil Elastase status

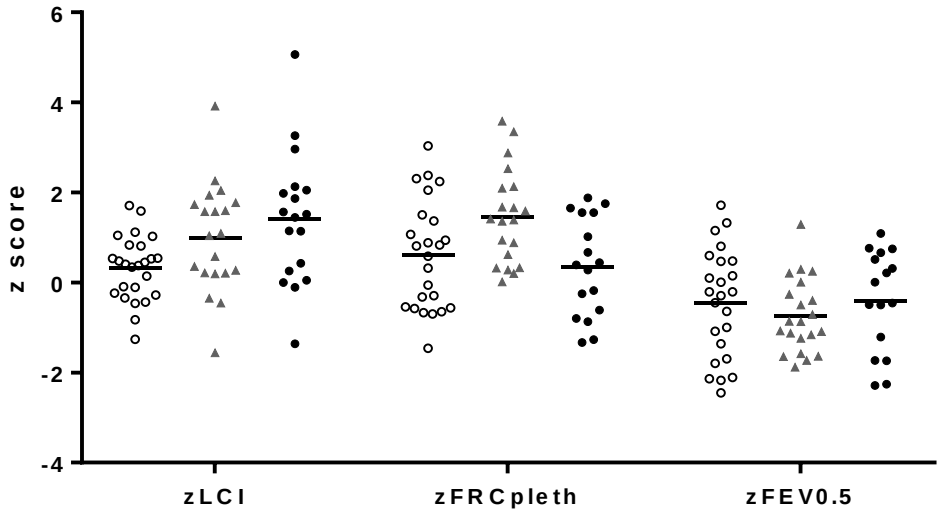
	Neutrophil Elastase below limit of detection	Detectable Neutophil Elastase	Total
BAL culture negative	27	5	32
BAL significant pathogens	6	5	11
	33	10	43

Footnote: Number of subjects according to neutrophil elastase status and detection of significant bacterial or fungal pathogens on BAL at ~1year of age. Fisher's exact test p=0.09.

Relationship between BAL inflammation, infection, and lung function

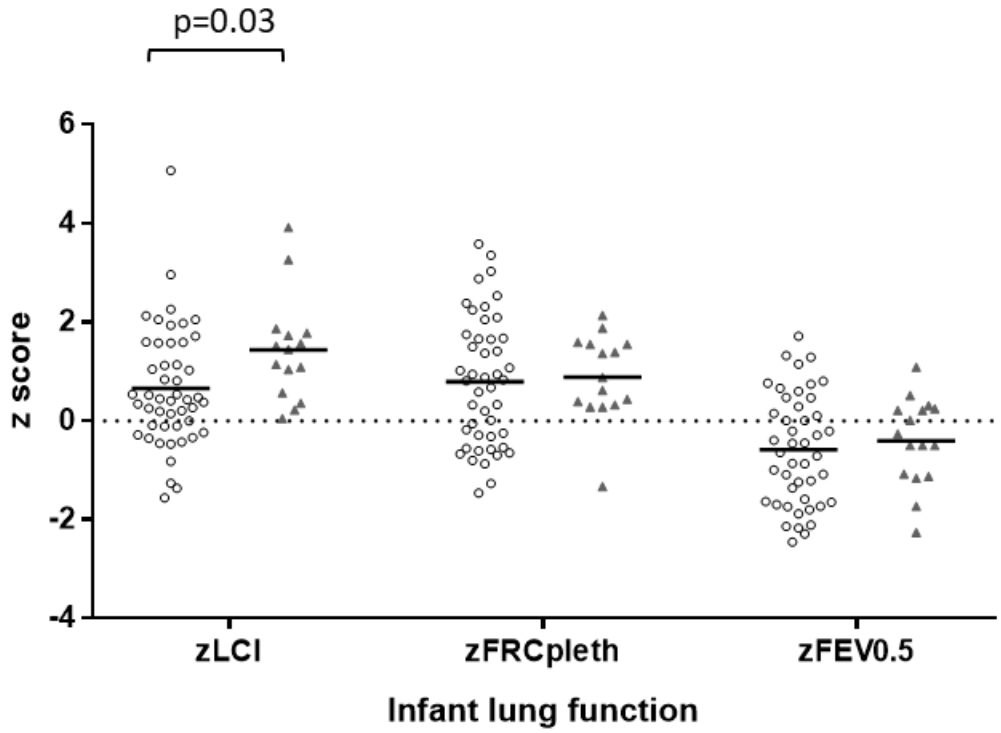
There was no difference in zLCI between infants with *PsA*, and those without significant pathogens (mean difference 0.68, 95% CI -0.13 to 1.50, p=0.12) or only pathogens other than PsA (mean difference -0.41, 95% CI -1.29 to 0.47, p=0.51) by their tests at 1 year (Fig E4). However, infants with pathogens other than *PsA* had a significantly raised zLCI when compared to those with none (mean difference 1.09, 95% CI 0.25 to 1.94, p=0.008). In comparison, infants with *PsA* by their 1yr test had a significantly higher zFRCpleth than those with no significant pathogens (mean difference 0.86, 95% CI 0.03 to 1.68, p=0.04), and those with pathogens other than *PsA* (mean difference 1.11, 95% CI 0.22 to 2.01, p=0.01)(Fig E4). No differences were observed between categories for $zFEV_{0.5}$.

Fig E4. Lung function and *Pseudomonas aeruginosa* status at ~1year of age



Footnote: Infants were divided into three groups according to *PsA* status 'ever' by the ~1year tests (and therefore include both cough swabs and BAL results). Infants with no significant pathogens are represented by open circles. Those isolating *PsA* on at least one occasion by ~1 year are in solid grey triangles, and those in whom pathogens other than *PsA* had been isolated on at least one occasion, but never *PsA*, in black circles. Horizontal lines are means. Lung function outcomes are presented as z-scores (z). LCI= lung clearance index, FRCpleth= plethysmographic functional residual capacity, $FEV_{0.5}$=forced expiratory volume in 0.5 seconds.

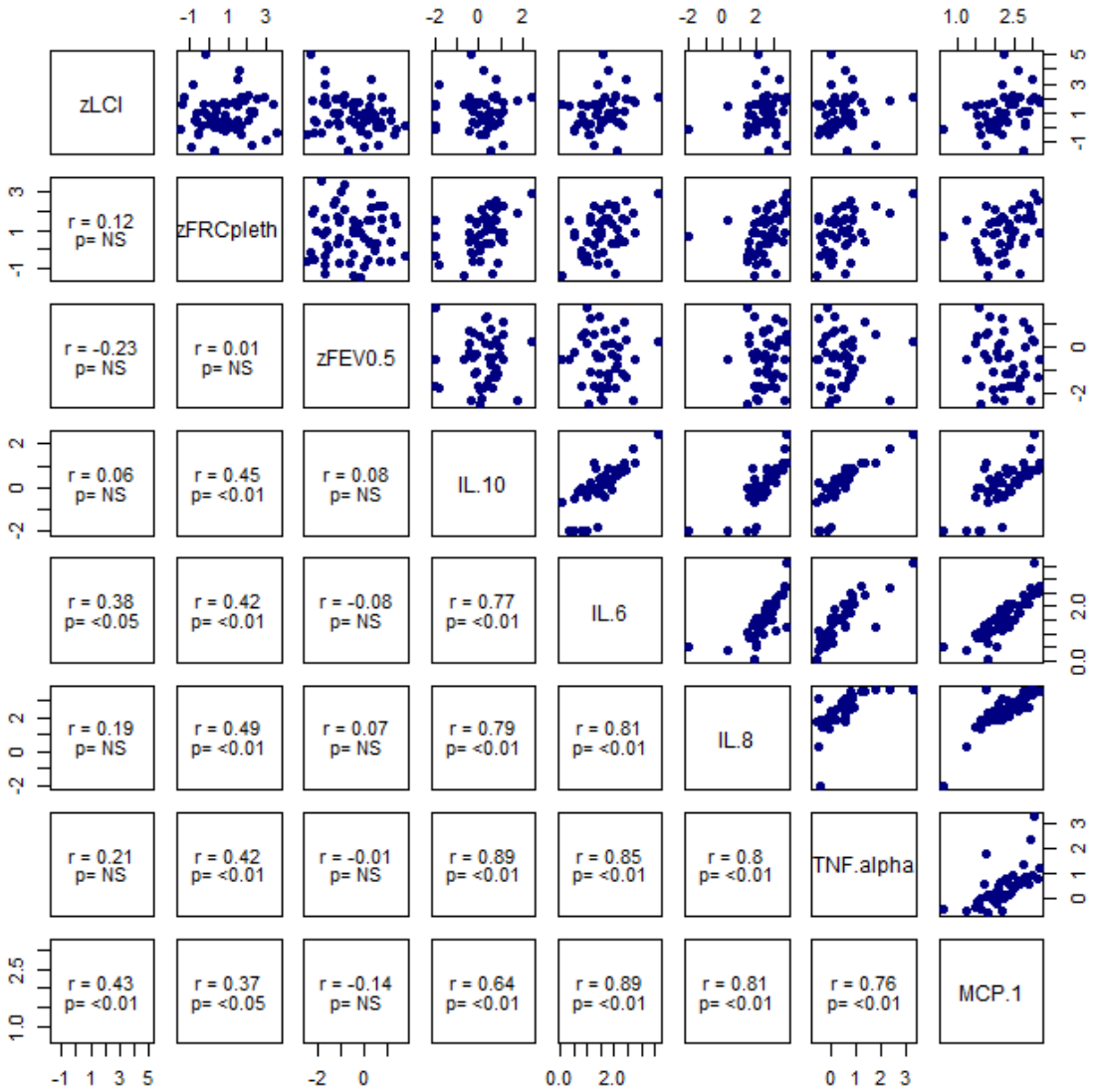
Fig E5. Lung function and significant pathogens on BAL at ~1yr of age



Footnote: Infants were divided into two groups according to the detection of significant pathogens (bacterial or fungal) on BAL. Infants with no significant pathogens are represented by the open circles, those with any significant pathogen in solid grey triangles. Horizontal lines are means. Lung function outcomes are presented as z-scores (z). LCI= lung clearance index, FRCpleth= plethysmographic functional residual capacity, $FEV_{0.5}$=forced expiratory volume in 0.5 seconds.

There was a positive significant association between BAL IL-8 and $zFRC_{pleth}$ ($r=0.49$, $p<0.05$), but not for zLCI or $zFEV_{0.5.}$ $zFRC_{pleth}$ was also significantly associated with IL-10, IL-6, TNFα and MCP-1 (Fig E6). zLCI was weakly associated with IL6 and moderately with MCP-1, while $zFEV_{0.5}$ was not associated with any of these inflammatory markers. However apparent associations between LCI and Il-6 were influenced by positive viral immunofluorescence (in whom both zLCI and IL-6 tended to be higher). By contrast, no such relationship with viruses was observed for $zFRC_{pleth}$ and any of the inflammatory markers.

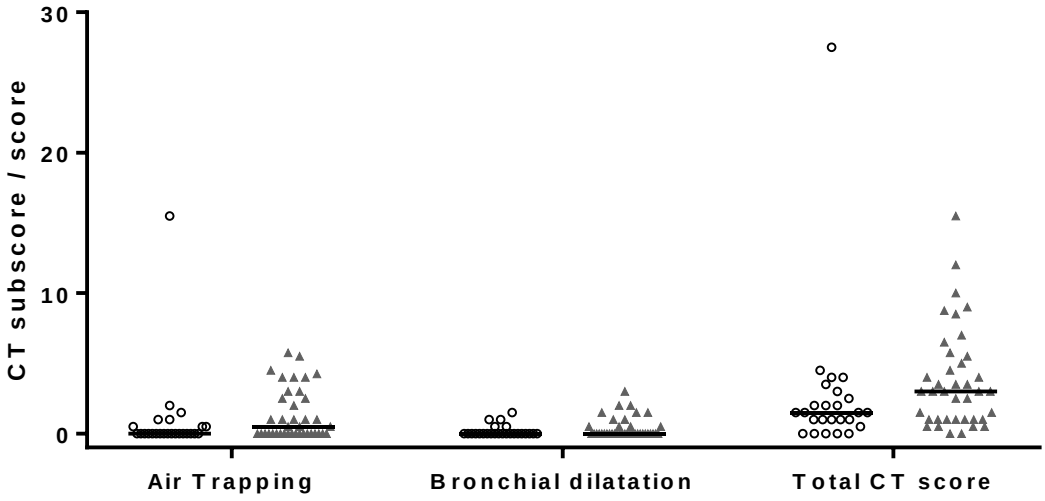
Fig E6. Spearman correlation matrix of lung function and inflammatory cytokines



Footnote: Bronchoalveolar lavage (BAL) cytokine results and lung function at ~1 year of age in clinically well NBS infants with CF. BAL cytokines (IL10, IL6, IL8, TNFα, Monocyte chemoattractant protein-1) were measured in 45 infants. Lung function outcomes and inflammatory cytokines are listed diagonally in the correlation matrix. For each pairing (e.g. zLCI and IL-10), a graphical plot is shown in the upper panel at the location where a horizontal and vertical co-ordinate for the pairing would meet. All potential pairings of variables are plotted. The corresponding Spearman rank correlation coefficient (r) and p values are given in the lower panel, again according to where a horizontal and vertical co-ordinate for the pairing would meet. For example, for zLCI and IL-10, r=0.06, p=NS (non-significant). Lung function outcomes are presented as z-scores (z). LCI= lung clearance index, FRCpleth= plethysmographic functional residual capacity, $FEV_{0.5}$=forced expiratory volume in 0.5 seconds. BAL cytokines (pg/ml) are plotted on a Log 10 axis.

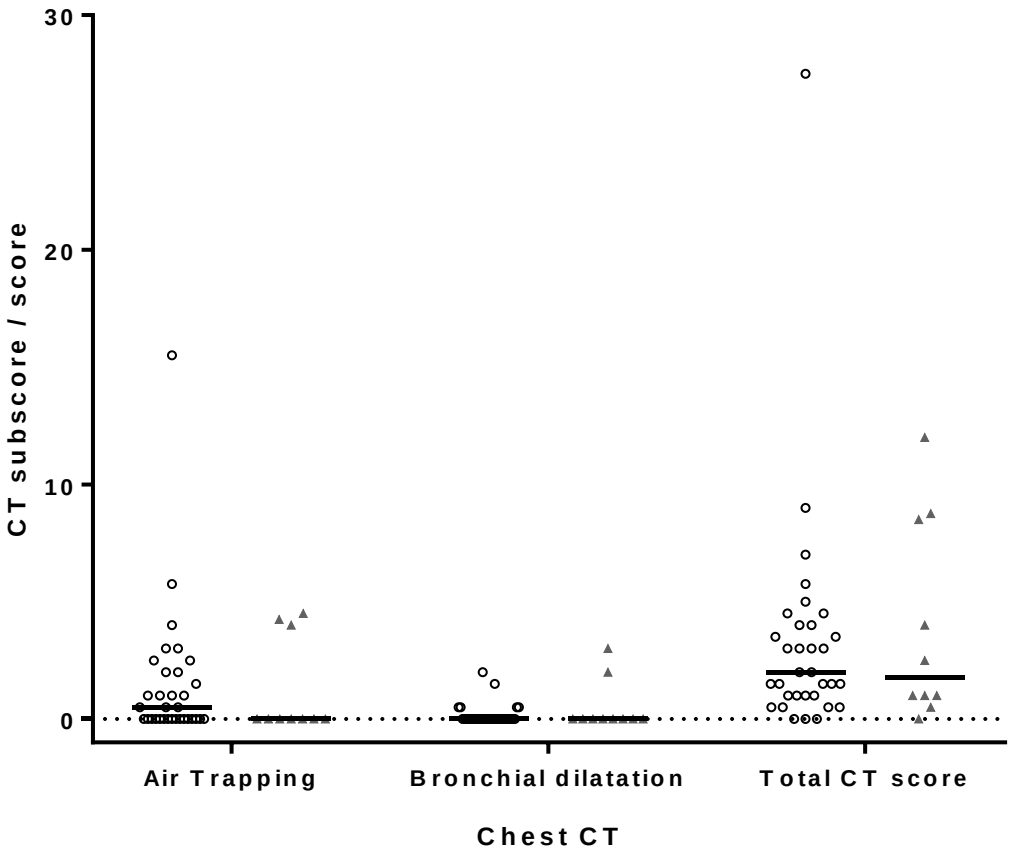
Infection, BAL inflammation and Chest CT

Fig E7. Chest CT and airway microbiology status (BAL and cough swabs) at ~1yr of age



Footnote: Infants were divided into two groups according to airway microbiology status 'ever' by at the ~1year tests (and therefore include both cough swabs and BAL results). Infants with no significant pathogens are represented by open circles, and and those in whom at least one significant pathogen has been isolated by solid grey triangles. Horizontal lines are medians. CT subscores (mean of 2 scorers) for Air Trapping, Bronchial dilatation and Total CT score are presented to illustrate how mild the changes were, although Air Trapping was the only reproducible outcome between the two CT scorers. Similar results were observed when analysis was restricted to pathogens on BAL alone. Range in air trapping score on chest CT 0-15.5; maximum possible score = 27. Maximum possible score for bronchial dilatation and total CT score 72 and 243 respectively.

Fig E8. Chest CT and Neutrophil Elastase status in BAL at ~1yr of age.



Footnote: Infants were divided into two groups according to BAL neutrophil elastase status. Infants with NE below the limit of detection are represented by open circles, and and those with detectable NE as grey triangles. Horizontal line are medians. CT subscores (mean of 2 scorers) for Air Trapping,

References for OLS

1. Thia LP, Calder A, Stocks J, Bush A, Owens CM, Wallis C, Young C, Sullivan Y, Wade A, McEwan A, Brody AS, London Cystic Fibrosis Collaboration. Is chest CT useful in newborn screened infants with cystic fibrosis at 1 year of age? Thorax. 2014;69:320-7.
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6. Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. J Cyst Fibros. 2003;2:29-34.