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

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**Author contributions:** JS, AB, CW, SC, AW and IBL were responsible for the conception and design of study; AC and ASB provided technical advice on imaging and undertook all scoring; JS and LPT were responsible for supervision of the study and research governance; AFH provided technical training, supervision and audit of lung function data collection and analyses. LPT supervised CT imaging and audited data collection and analyses. Infants with CF were recruited by the paediatric respiratory consultants participating in the LCFC, including IBL, AB, CW, SC, RS, CP and GR. TDN, LPT and AFH recruited the healthy infants, undertook all lung function measurements and, together with JS and GD calculated and interpreted lung function results. NK performed inflammatory analyses. GD and AW performed statistical analyses; GD, LPT, JS, AW, AB, and IBL drafted the manuscript; all remaining authors revised and approved the manuscript for intellectual content before submission.

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Abstract
Background: With the widespread introduction of newborn screening for cystic fibrosis (CF), there has been considerable emphasis on the need to develop objective markers of lung health that can be used during infancy. We hypothesised that in a newborn screened (NBS) UK cohort, evidence of airway inflammation and infection at one year would be associated with adverse structural and functional outcomes at the same age.

Methods: Infants underwent lung function testing, chest CT scan and bronchoscopy with bronchoalveolar lavage (BAL) at 1 year of age when clinically well. Microbiology cultures were also available from routine cough swabs.

Results: 65 infants had lung function, CT and BAL. Mean (SD) lung clearance index and forced expiratory volume in 0.5 seconds z-scores were 0.9(1.2) and -0.6(1.1) respectively; median Brody II CF-CT air trapping score on chest CT =0 (interquartile range 0-1, maximum possible score 27). Infants isolating any significant pathogen by 1yr of age had higher LCI z-score (mean difference 0.9; 95%CI:0.4-1.4; p=0.001) and a trend towards higher air trapping scores on CT (p=0.06). BAL neutrophil elastase was detectable in 23% (10/43) infants in whom BAL supernatant was available. This did not relate to air trapping score on CT.

Conclusions: In this UK NBS cohort at one year of age, lung and airway damage is much milder and associations between inflammation, abnormal physiology and structural changes were at best weak, contrary to our hypothesis and previously published reports. Continued
follow-up will clarify longer term implications of these very mild structural, functional and inflammatory changes.
1. Introduction

With the widespread introduction of newborn screening (NBS) for cystic fibrosis (CF), there has been considerable emphasis on the need to develop objective markers of lung health that can be used during infancy, not only to detect early lung disease, but to inform potential intervention strategies and identify factors associated with increased risk. Having previously assessed the evolution of lung function in infants and young children diagnosed after clinical presentation[1-3], the London Cystic Fibrosis Collaboration (LCFC) commenced a longitudinal observational study of NBS infants with CF[4, 5]. Such cohorts can give important insight into markers of lung health. We have previously reported lung function and CT outcomes for the NBS LCFC cohort at one year (including the improvement in Forced Expiratory Volume in 0.5 seconds (FEV$_{0.5}$) from initial testing at 3 months of age, a mildly elevated (worse) lung clearance index (LCI) in comparison to healthy controls, and only minor changes on chest CT[4, 6]), but until now have not reported cross sectional associations between these outcomes nor their relationship with airway infection and inflammation. In the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF) study, the presence of neutrophil elastase (NE) in bronchoalveolar lavage (BAL) at 3 months of age was associated with the isolation of Staphylococcus aureus (SA) and Pseudomonas aeruginosa (PsA), and significantly lower lung function during the first 2 years of life[7]. Furthermore, free NE activity at 3 months was associated with bronchiectasis (strictly, airway dilatation which may or may not be reversible) from one year of age[8] suggesting that infections resulting in neutrophilic inflammation lead to adverse respiratory outcomes. Repeated detection of free NE has been shown to be associated with worse CT outcomes[9]. Although the presence of bronchiectasis showed no association with increased LCI during early life in the AREST-CF study[10], weak associations were observed between increased airway inflammation and an elevated LCI[11] and other indices of ventilation inhomogeneity[10], with isolation of PsA on BAL associated with higher LCI[11].

In keeping with the findings of AREST-CF, we hypothesised that in the LCFC newborn screened UK cohort, evidence of airway inflammation and infection at one year would be associated with adverse structural and functional outcomes at the same age.

2. Materials and Methods
2.1 Study design and participants
Screened infants with CF born between January 2009 and July 2011 under the care of the six LCFC specialist CF centres in London were eligible for enrolment in this longitudinal observational study, as described previously[5]. All infants were commenced on a standardised treatment protocol which at that time included prophylactic oral flucloxacillin[4, 5]. Exclusion criteria included <35 weeks gestation or coexisting congenital abnormalities.

Infant lung function testing, a chest CT scan and a bronchoscopy with lavage were undertaken at approximately 1 year of age when infants were clinically well and had been free of acute respiratory illness for at least 3 weeks. The study was approved by the North Thames Multi-Centre Research Ethics Committee (#09/HO71/314). Informed written consent was obtained from all parents.

2.2 Infant lung function testing
All infants were tested in the same centre during a period of clinical stability according to international standards as described previously[4, 5, 12]. LCI was measured by multiple breath washout (MBW) using a mass spectrometer and Sulphur hexafluoride as the tracer gas. Plethysmographic FRC (FRC_{pleth}) and FEV_{0.5}[13], using the raised volume technique (RVRTC) were also measured. Results were expressed as z-scores to adjust for body size, gender and age, using reference equations derived from healthy infants studied with identical equipment and protocols[14-16]. LCI was the primary lung function outcome for this study, due both to its greater discriminatory power at 1yr of age in this cohort[4] and its association with CT outcomes in older children[17].

2.3 Volume controlled chest CT scans
Thin section chest high resolution CT (HRCT) was performed under general anaesthesia within two weeks after lung function testing during a period of clinical stability. Inspiratory and expiratory HRCT scans were performed using a standardised protocol for volumetric CT image acquisition[6]. Scans were scored independently by two radiologists using the Brody-II CF-CT scoring system, with mean scores used[18]. Air trapping sub-score was the primary structural outcome, since this was the only reproducible measurement in our cohort[6], with a score >6 considered abnormal[17].
Flexible bronchoscopy was performed immediately after the CT scan under the same general anaesthetic. A 2.8 mm Olympus bronchoscope (Hamburg, Germany) was inserted via the endotracheal tube (with no suction above the vocal cords). Three aliquots of 1ml/kg room temperature 0.9% saline were instilled and aspirated from the right middle lobe with one further aliquot from the lingula[19]. The first recovered aliquot from each lobe was sent for routine bacterial and fungal culture, with viral detection by immunofluorescence. Subsequent samples from the right middle lobe were pooled, centrifuged and the supernatants frozen at -80°C. BAL was analysed using the Meso Scale Discovery® Multi-Array technology (MesoScale Discovery, Gaithersburg, MD, USA)[20] for the neutrophil chemoattractant IL-8; pro-inflammatory cytokines IL-1 IL-6, TNFα and chemoattractant Monocyte Chemoattractant Protein-1 (MCP-1); and the anti-inflammatory cytokine IL-10. Free NE activity was measured as described by the AREST-CF group[8, 21]. The lower limit of detection for the NE assay was 200 ng/ml. On the basis of results reported by AREST-CF, the primary outcome for inflammation was free NE.

Further microbiology cultures were available from cough swabs taken during each routine clinical review (minimum every 2–3 months) and at other times if the infant was symptomatic. Microbiology results (from cough swabs and/or BAL alone) were grouped into ‘any’ vs ‘no significant pathogens’ at any point (i.e. ‘ever’) by the time of testing at approximately 1 year of age. Significant pathogens included PsA, Methicillin-Sensitive or Resistant SA, Haemophilus influenzae, Stenotrophomonas maltophilia, Achromobacter xylosoxidans or Aspergillus fumigatus. Immunofluorescence for respiratory viruses was only performed on BAL samples.

2.5 Statistical analysis
The sample size was opportunistic. Data were analysed using SPSS (PASW Statistics v.24, Chicago, IL, US) and GraphPad Prism (version 7; GraphPad software, CA, US). CT air trapping and inflammatory markers were non-normally distributed and expressed as median (interquartile range(IQR)). Differences between two groups (e.g. according to airway infection status) were compared using 2-sample t-tests or Mann Whitney U-tests, and between three groups (e.g. according to PsA status) using ANOVA with Tukey multiple
comparison test. Chi squared tests were used to compare proportions of infants with detectable NE with other parameters e.g. significant airway pathogens. Associations between lung function, air trapping, infection and inflammation were examined using Spearman rank correlations and plotted in correlation matrices in R software (R Foundation for Statistical Computing, version 3.3.1.; Institute for Statistics and Mathematics, Vienna, Austria; www.R-project.org).
3. Results

3.1 Patient details
Details of subject recruitment and follow-up are shown in OLS Figure E1, with reasons for parental decline, exclusion and withdrawal documented previously[4-6]. Of the 76 NBS CF infants with lung function at 1yr, 65 also underwent CT and bronchoscopy. The characteristics of these 65 infants (Table 1) were very similar to those from the entire group with 1yr lung function[4].
Table 1: Characteristics of the 65 NBS CF infants in whom lung function, CT and BAL were assessed at one year of age.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Male (%)</td>
<td>31 (48%)</td>
</tr>
<tr>
<td>Postnatal age at diagnosis (median, IQR weeks)</td>
<td>3.4 (3.0 - 4.6)</td>
</tr>
<tr>
<td>Meconium ileus presentation</td>
<td>7 (11%)</td>
</tr>
<tr>
<td>CF Genotype</td>
<td></td>
</tr>
<tr>
<td>p.Phe508del homozygous</td>
<td>39 (60%)</td>
</tr>
<tr>
<td>p.Phe508del heterozygous</td>
<td>19 (29%)</td>
</tr>
<tr>
<td>Others</td>
<td>7 (11%)</td>
</tr>
<tr>
<td>Pancreatic insufficient</td>
<td>61 (94%)</td>
</tr>
<tr>
<td>Age at infant lung function test (weeks)</td>
<td>51.6 (4.6)</td>
</tr>
<tr>
<td>Weight z-score$^#$</td>
<td>0.34 (0.90)</td>
</tr>
<tr>
<td>Height z-score$^#$</td>
<td>0.49 (0.96)</td>
</tr>
<tr>
<td>Wheeze and/or crackles ever (physician diagnosed)</td>
<td>22 (34%)</td>
</tr>
<tr>
<td>Intravenous antibiotics†</td>
<td>17 (26%)</td>
</tr>
<tr>
<td>Dornase alfa</td>
<td>7 (11%)</td>
</tr>
<tr>
<td>Gastro-oesophageal reflux therapy (ever)</td>
<td>32 (49%)</td>
</tr>
</tbody>
</table>

Results presented as n(%) or mean(SD) unless otherwise stated. $^#$Weight and height normal ranges from Cole et al[22]. †9/17 received one course, 4/17 two courses, and 4/17 three courses.
Table 2: Summary of objective markers of lung health at 1 year of age

<table>
<thead>
<tr>
<th>Airway pathogens ever on cough swab ± BAL n (%)</th>
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<tbody>
<tr>
<td><em>Pseudomonas aeruginosa (PsA) (+/- other organisms)</em></td>
<td>21 (32%)</td>
</tr>
<tr>
<td>Growth of pathogens other than <em>PsA</em> #</td>
<td>19 (29%)</td>
</tr>
<tr>
<td>No growth #</td>
<td>25 (38%)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>BAL microbiology n(%)</th>
<th></th>
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<tbody>
<tr>
<td><em>PsA (+/- other organisms)</em></td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Growth of pathogens other than <em>PsA</em></td>
<td>13 (20%)</td>
</tr>
<tr>
<td>Other #</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>No growth</td>
<td>48 (74%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BAL virology (n(%))</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Positive # #</td>
<td>5 (8%)</td>
</tr>
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<thead>
<tr>
<th>BAL inflammation (n(%) or median [IQR])</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Detectable free neutrophil elastase activity, ng/ml (n=43) $\triangledown$</td>
<td>10 (23%)</td>
</tr>
<tr>
<td>IL-8, pg/ml (n=45)</td>
<td>286 [82-1209]</td>
</tr>
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<tr>
<th>Lung function: mean(SD) z-scores</th>
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<tbody>
<tr>
<td>LCI (n=64)</td>
<td>0.86 (1.21)</td>
</tr>
<tr>
<td>FRC&lt;sub&gt;pleth&lt;/sub&gt; (n=62)</td>
<td>0.81 (1.20)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.5&lt;/sub&gt; (n=62)</td>
<td>−0.57 (1.07)</td>
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<tr>
<th>Brody II scores on chest CT (median [IQR])</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Air trapping (n=65)</td>
<td>0 [0-1]</td>
</tr>
</tbody>
</table>

**Footnote:** Results are presented as mean (SD), n(%) or median [Interquartile range; IQR]. Abbreviations: LCI: lung clearance index; FRC<sub>pleth</sub>: plethysmographic functional residual capacity; FEV<sub>0.5</sub>: forced expired volume in 0.5 seconds. Lung function outcomes expressed as z-scores; corresponding mean (SD) absolute LCI 7.41(0.66) units. *One infant isolated *Mycobacterium Tuberculosis* on BAL culture and was excluded from further analysis. **Viruses detected included rhinovirus (n=3), parainfluenza 3 (n=1), and cytomegalovirus (n=1). Range in air trapping score on chest CT 0-15.5; maximum possible score = 27. $\triangledown$NE analysis for 43/45 infants as supernatant sufficient for cytokines only.

### 3.2 Infection
**PsA** was isolated on at least one occasion in 21 (32%) infants during the first year from cough swabs (Table 2); three of whom also isolated **PsA** in BAL at 1yr of age. Only one infant had chronic **PsA** infection as defined by the Leeds criteria[23]. Growth of pathogens other than **PsA** was identified in 19/65(29%) infants. Of the 65 BAL samples at 1yr, 12 (18%) had positive significant bacterial growth (see OLS for details). Five infants (8%) had a virus detected on BAL immunofluorescence.

### 3.3 Inflammation

Although lavage fluid was obtained from all 65 infants, regrettably 20 samples were frozen prior to centrifugation, precluding extraction of supernatant. Results for NE and the neutrophil chemoattractant IL-8 are summarised in Table 2 (IL-6, IL10, MCP-1 and TNFα are presented in the OLS table E1). NE was detectable in 10/43 (23%). Of these, 7 had levels that were only just above the 200ng/ml lower detection limit for the assay. Airway infection and inflammation data are summarised in Fig 2 and Fig E3 OLS.

### 3.4 Lung function and structure

There were mild elevations in LCI and FRC_pleth and a reduction in FEV_{0.5} at 1yr of age in CF NBS infants (Table 2). Changes on chest HRCT were generally trivial. The median air trapping score was zero (range 0-15.5) out of a maximum total score of 27 (Table 2). Although total CT score and bronchial dilatation sub-score were not reproducible in our cohort [6], to illustrate the minimal changes seen these results are summarised in the OLS (Table E1). There was a significant, albeit weak, association between LCI z-score and air trapping (r = 0.44 (95% CI 0.22 to 0.62), p<0.001)(Fig 1). However, an elevated LCI (>1.96 z-scores) discriminated poorly between those with and without evidence of air trapping.

### 3.5 Relationship between BAL inflammation, infection, and lung function

Infants isolating a significant pathogen in the first year of life had, on average, an LCI at 1yr of age almost 1 z-score greater than those with no such isolates (mean zLCI 1.2 vs 0.3 respectively; 95% CI of difference: 0.4 to 1.4; p=0.001, Fig 3). By contrast there was no significant difference in zFEV_{0.5} or zFRC_pleth between the two groups (Fig 3). Infants with **PsA** by their 1yr test had a significantly higher zFRC_pleth, but not LCI, 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
significant differences were observed between categories for zFEV_{0.5}. A similar magnitude of difference in zLCI (1 z-score) according to significant pathogens was seen when microbiology comparisons were restricted to those only obtained at BAL (Fig E5).

Detectable NE was not associated with either a higher zLCI or zFRC_{pleth}, nor a lower zFEV_{0.5}. Associations between cytokines and LCI, and for FEV_{0.5} and FRC_{pleth} are summarised in Fig E6.

3.6 Relationship between BAL inflammation, infection, and CT changes
There was no significant difference in median air trapping scores between those with or without airway infection. However although the difference in median air trapping scores between those with or without airway infection did not reach statistical significance (p=0.06), the trend was towards higher scores in those with infection (Fig E7). There was no significant difference in the median air trapping score between infants with and without detectable NE in BAL (Fig E8).
4. Discussion

In this UK newborn screened cohort of infants with CF at one year of age who were clinically well at the time of testing, contrary to our hypothesis and previous reports, we found that evidence of inflammation and current or prior infection was associated with only mild abnormalities on chest CT and lung function. We found that significant airway pathogens were associated with a worse LCI (of around 1 z-score), but little evidence of association between inflammation and lung function or air trapping on chest CT at 1yr of age. These findings may reflect the mild nature of these changes.

In our study, pro-inflammatory cytokine concentrations in BAL were in the same order of magnitude to those previously reported in young children with CF [24]. Pillarisetti reported BAL measures of infection and inflammation for the AREST study (including IL-1β and IL-8 concentration, neutrophil cell counts and NE), detecting NE in 8/31 (29.6%) infants at 1yr, [7]. Sly et al reported a prevalence of infection (any; excluding mixed oral flora) in BAL cultures in NBS CF infants who were ‘clinically stable’ at the time of testing of 21.1% (23/109 infants) at 1yr, with 8.3% and 5.5% isolating PsA and SA respectively and 18.1% infants positive for NE activity at 1yr[8]. These results are very similar to the BAL microbiology results in our cohort. The prevalence of PsA infection ‘ever’ in our cohort was similar to that reported for all infants in London and the South East of England following the introduction of CF NBS[25]. Detection of viruses (particularly rhinovirus) in the respiratory tract of infants and young children with CF is common[26, 27], and the role of such viruses in exacerbations is increasingly recognised[28], although our infants were clinically well with no reported recent respiratory illness.

Direct comparison of disease severity between the AREST-CF and LCFC cohorts is hampered by differences in infant lung function equipment, the reference ranges used to interpret lung function results, and methods of CT scoring. There are also differences in environment and climate, and treatment regimens between the UK and Australia. Nevertheless, results from both cohorts are crucial to better understand the natural history of CF, and identify potential predictors of significant later morbidity. The prevalence of bronchiectasis reported by the AREST-CF group at 1yr of age has varied according to the CT scanning protocol and scoring system employed. While initial reports from AREST-CF described bronchiectasis in 29% of NBS infants just 10 weeks old, and 47% infants with bronchial dilatation at some point during
the first year[8], more recent reports from this group suggest milder changes in infancy, these only becoming more marked beyond the 3\textsuperscript{rd} year of life[29]. Ramsey et al[29] reported a prevalence of 20\% for bronchiectasis and 58\% for air trapping using PRAGMA-CT scoring at mean age of 0.94yrs (range 3mth-2yrs), but the mean extent of bronchiectasis was extremely small at only 0.2\% of the lung. They found no association between LCI and any structural disease extent scores, concluding that LCI had a low sensitivity and positive predictive value for bronchiectasis in infants with CF. In an earlier publication reporting CT changes at 1yr of age which pre-dated their use of the PRAGMA scoring system, Sly et al[8] reported a point prevalence of 31.5\% for bronchiectasis and 68.5\% for air trapping.

Although bronchial dilatation was reported in 26\% and air trapping in 42\% respectively of our LCFC NBS cohort at one year of age, the changes observed on volumetric chest CT scan at this age were extremely mild, such that, with the exception of air-trapping, scoring was poorly reproducible[6]. We do not routinely perform chest CT in infants, due to the radiation burden and low likelihood of a finding that alters management.

In our cohort, we demonstrated that although lung function was abnormal shortly after diagnosis when compared with contemporaneous controls[5], NBS CF infants had significantly better lung function by one year of age[4] than previously reported either by the LCFC for clinically diagnosed infants[1] or for NBS infants in the Australian cohort[7, 30]. In contrast to the dramatic decline in FEV\textsubscript{0.5} reported by AREST-CF[7], no such deterioration was seen during the first or second year of life in the London cohort[4, 31]. We have previously shown that the LCI is a reasonable predictor of CT scan abnormalities in older children with CF[17], but this may not be true in NBS infants with much milder functional changes[4]. Among the LCFC cohort, relatively few infants had abnormal LCI at either 3 months[5] or 1 year of age[4]. Neither ourselves nor AREST-CF[11] have found LCI to be useful in predicting abnormal CT air trapping in this age group. LCI at 1 year of age may be more useful as a potential marker of infection and inflammation[11, 29, 32] and may be transiently elevated rather than reflecting a permanent deterioration of lung function in later childhood, although we also acknowledge the lack of association between LCI and NE in our cohort. While it would be beneficial if an alternative test could reliably reflect evidence of early structural CF lung disease in NBS infants, our one year results do not support using LCI for this purpose, however this may reflect the sensitivity of the CT scoring system used.
Strengths of this study include the comprehensive range of infant lung function tests undertaken in a single centre using standardised equipment, protocols and quality control, and conversion of results to z-scores using appropriate reference equations[14-16]. Infants across all the LCFC centres were managed according to the same protocols. The 1yr assessments were performed when infants were clinically well, with lung function measurements predating chest CT by a maximum of two weeks. A standardised anaesthetic and imaging protocol for CT was adhered to in the three centres where imaging took place, with one investigator (LT) present to ensure quality control, and with scoring undertaken independently by two experienced radiologists.

The technical limitation whereby supernatant was not available for all infants resulted in a reduction of the number of samples available for inflammatory markers. An assay with a lower limit of detection of 200ng/ml for free NE activity may have been too crude to detect lung disease in a cohort that is generally doing well. It is also possible that stronger correlations may have been shown with other inflammatory parameters than those included within our study, including alternative methodologies for measuring free extracellular NE activity [33]. Interpretation of the CT scans was potentially limited by the CT scoring method. Although the Brody scoring system is a validated scoring system for CF lung disease, it was not designed to score signs of very early lung disease. The limited agreement between the two radiologists primarily reflected discrepancies when scoring the very subtle changes observed on chest CT [6]. The PRAGMA scoring system [34] (developed after our study was underway), as utilised by the AREST-CF group, may go some way to addressing this as it aims to quantify the extent of early structural abnormalities in early CF lung disease, and has been shown to be more sensitive for detecting early changes in CF lung disease than the CF-CT score with higher agreement between observers [34]. We have reported our results with the Brody scores in line with our pre-specified protocol, for consistency with prior publications from the LCFC cohort and following reflection that, irrespective of which method is used for assessment, changes on CT were minimal in our cohort. Novel methodologies for quantifying bronchiectasis (such as counting airway segments [35]), or automated approaches to quantitative measurement of air trapping may also ultimately help better understand structure-function relationships. While it is possible that with alternative, more sensitive outcome measures we may have detected more minor abnormalities, these could prove to be as transient as the changes in lung function during the first two years of life that we have previously reported [31].
Conclusions

Despite evidence of airway inflammation and infection in UK NBS infants with CF at one year of age being similar to that reported by an Australian NBS cohort, contrary to our hypothesis we observed only mild changes in structural and functional outcomes. We show that there is a relationship (albeit weak) between CT air trapping and the minor elevation in zLCI. However neither inflammatory measures, lung function nor CT findings were sufficiently abnormal to be a useful measure of severity of disease.

In our cohort, significant airway pathogens were associated with a worse LCI. Importantly, the association of significant bacterial infection with worse lung function confirms the need for prevention including cohorting, and prompt detection and treatment of infection. The identification of outcome measures (for either clinical practice or trials) in CF NBS infants remains challenging, particularly in terms of whether mild changes reflect mild disease or insensitivity of an outcome measure to detect significant pathology. Whether these measures can inform prognosis remains to be determined by continued follow-up of our cohort.

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Figures

Figure 1. Association between lung clearance index and CT air-trapping sub-scores at 1 year of age

Footnote: Lung Clearance Index (LCI) z-score vs. Brody II sub-score for air trapping on chest CT. Air trapping is plotted on a Log10 axis to demonstrate the potential range possible (maximum possible score is 27, shown by dotted horizontal line), with results considered abnormal (>6) indicated by a thin dashed horizontal line. The upper Limit of Normality for LCI is marked by the thin dashed vertical line at +1.96 z-scores. Spearman rank correlation coefficient and p value shown.
Figure 2. BAL inflammation vs airway infection status up to 1 year of age from cough swabs and bronchoscopy

Footnote: Infants were divided into two groups according to airway microbiology status ‘ever’ by the ~1year tests (and therefore include both cough swabs and bronchoalveolar lavage (BAL) results). Infants with no significant pathogens are represented by open circles, and those in whom at least one significant pathogen had been isolated by solid grey triangles. Cytokine concentrations are plotted on a Log10 axis. Horizontal lines are medians. There were no statistically significant differences between groups for IL10, IL6, IL8, TNFα or MCP-1 when taking multiple comparisons into account.
Figure 3. Lung function and airway microbiology (BAL and cough swabs) at ~1year of age

Footnote: Infants were divided into two groups according to airway microbiology 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, and those in whom at least one significant pathogen had been isolated by ~1year tests by 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.
References


