To the editor,

Lung clearance index (LCI) is a measure of ventilation distribution derived from multiple breath washout (MBW). It is a promising measure for monitoring early lung disease in cystic fibrosis (CF) (1-4) and is increasingly being used as a surrogate efficacy endpoint in CF clinical trials (5,6). LCI is reliable and more sensitive than FEV₁ in detecting lung disease in infants, children and adults with CF (7-9), tracks early disease progression and symptoms (3) and predicts the onset of pulmonary exacerbations (10). LCI has been shown to be elevated in infants and younger children with respiratory infection and correlates with airway inflammation, measured using a range of biomarkers in bronchoalveolar lavage (11-13). However, there is little data on how LCI relates to markers of infection and inflammation, in children > 6 years and adults.

Whilst it is widely recognised that pulmonary infection results in an excessive inflammatory response in the CF airways, the potential role of inflammation as an independent contributor to CF pathophysiology is also established. Systemic inflammatory biomarkers, such as C-reactive protein (CRP), consistently correlate with worse clinical disease (14,15). An important step in further validating LCI is to assess the relationship between airway infection and inflammation across the age range in CF thereby determining if LCI is a useful tool to detect the presence of infection and inflammation.
The aim of this study was to investigate the relationship between airway infection as measured by routine culture, systemic inflammation (CRP, White Cell Count [WCC]) and LCI in clinically stable CF child and adult patients.

Clinically stable patients with CF from the paediatric & adult Northern Ireland CF centres, aged ≥ 6 years old were recruited to assess the clinimetric properties of LCI (9). Written informed consent/assent was obtained. Participants completed a MBW test using 0.2% SF6 and a modified Innocor™ device. LCI was calculated from ≥2 valid MBW tests. Spirometry was performed to ATS/ERS standards.

An expectorated sputum sample or throat swab (if unable to expectorate) was collected for routine culture analysis. Samples were analysed in the Belfast Health and Social Care Trust (BHSCT) clinical microbiology laboratory by routine diagnostic culture. The presence or absence of target pathogens was recorded. Where no pathogens were detected but where the clinical specimen grew normal commensal flora, a report was issued detailing "no significant growth" (16).

Venous blood samples were collected and analysed for WCC (biochemistry laboratories, BHSCT) and CRP (measured by turbidimetric immunoassay, Queen’s University Belfast).

Data were analysed using PASW Statistics and Prism packages. CRP values were log transformed for analysis. Spearman rank correlation coefficient, Mann-Whitney U, Kruskal Wallis and multiple regression statistics were used with a $p$-value <0.05 indicating statistical significance. This study was approved by the Office for Research Ethics Committee Northern Ireland (ORECNI); reference number 10/NIR01/41.

Results

One hundred and ten CF subjects were recruited, with data collected at a stable visit. The mean (SD) age [range] (years) of subjects was 23.8 (13.9) [6 - 67] with a mean (SD) FEV1 % predicted of 77.0 (20.0) and median (IQR) LCI [range] (no. turnovers) of 8.8 (3.8) [5.4 – 17.2].

A sputum or throat swab sample was successfully collected from 106/110 subjects (n=68 sputum, n=38 throat swabs). Four subjects were unable to provide a sample. *Pseudomonas aeruginosa* was detected in 40/106 (37%) subjects (82% sputum, 18% throat swabs). Other organisms including *Staphylococcus aureus, Stenotrophomonas maltophilia, Haemophilus influenzae, Achromobacter* species, *Haemophilus parainfluenzae* and *Pantoea* species (*Enterobacteriaceae*) were detected in 40/106 (37%) subjects (55% sputum, 45% throat swabs).
swabs). No significant growth was detected in 26/106 (25%) subjects (54% sputum, 46% throat swabs).

LCI was significantly elevated in *P. aeruginosa* and non *P. aeruginosa* infection groups compared to those with no significant growth (Figure 1a). FEV1% predicted was significantly lower in those subjects with *P. aeruginosa* compared with those with no significant growth (Figure 1b). When only subjects with abnormal FEV1% predicted (<80% predicted) (n=57) were considered, the significant difference in LCI across groups (*P. aeruginosa* vs. non *P. aeruginosa* infection vs. no significant growth) remained (p<0.001), whereas there was no significant difference in FEV1% predicted across groups (p=0.60). There was a significant relationship between age and LCI in the total group (n=110; r=0.50; p<0.001). However, there was no difference in mean (SD) age between the different infection groups (*P. aeruginosa*=25.2 [9.7]; non *P. aeruginosa*= 22.8 [16.7]; no significant growth=23.3 [15.8]; p=0.07).

A blood sample was successfully collected from 83/110 patients. Sufficient sample for CRP analysis was available for 71/83 (85%) patients. Mean (SD) WCC (10^9/L) was 8.2 (3.2) and median (range) CRP (ug/dL) was 142.7 (10.0-6842).

LCI correlated with both WCC (r=0.41; p=0.0001) and CRP (r=0.45; p<0.0001) indicating that increased systemic inflammation was associated with an elevated LCI. Similarly, there was a significant correlation between FEV1 and WCC (r=−0.47; p<0.0001) and CRP (r=−0.39; p<0.0001). As there was a significant relationship between CRP and age (r=0.38; p=0.001), a multiple regression was carried out to assess which variable was the best predictor of CRP; age or LCI. LCI had a significant contribution to explaining CRP (beta=0.27; p=0.04) whilst age did not (beta=0.21; p=0.11). There was no relationship between WCC and age (r=0.15; p=0.16).

**Discussion**

In this study, we demonstrated that LCI was significantly elevated in *P. aeruginosa* positive subjects compared with *P. aeruginosa* negative subjects, indicating that it may be a sensitive marker of *P. aeruginosa* infection in CF children and adults. LCI was significantly elevated in subjects with non *P. aeruginosa* infection compared to those subjects with no significant growth, whereas FEV1% predicted showed no difference. Furthermore, this is the first study to show that increased WCC, as a potential marker of infection, and increased CRP as a marker of systemic inflammation in CF are associated with an elevated LCI. The moderate strength of correlations with LCI were similar to that observed with FEV1% predicted.
The results from this study including older children and adults are in agreement with studies in infants and younger children reporting a significant relationship between elevated LCI and the presence of lower respiratory tract infection (12,13) and specifically between LCI and *P. aeruginosa* infection (11,17,18). Furthermore, this data shows that LCI is elevated in individuals with infection with other pathogens including *S. aureus*, *S. maltophilia* and *H. influenzae*, whereas FEV₁% predicted showed no difference. LCI provides most useful information in subjects with mild disease (normal FEV₁% predicted). However, this study also found a significant difference in LCI between infection groups when considering subjects with abnormal FEV₁% predicted only, indicating that LCI may also have clinical utility in subjects with more advanced disease.

There is conflicting results in the literature on the relationship between LCI and biomarkers of inflammation. Previous studies in infants and young children have found that LCI correlated with airway inflammation as measured by IL-8 and neutrophil count in bronchoalveolar lavage fluid samples (11,13). Conversely Simpson et al did not find any association between LCI, IL-8 and neutrophil elastase in infants (12). A study including adults investigated the relationship between LCI and a range of systemic markers of inflammation at the beginning of a pulmonary exacerbation. Whilst there was no correlation between LCI and CRP, the study reported a moderate relationship between LCI and serum calprotectin ($n=37$; $r=-0.338$, $p<0.05$) and serum TNFα ($r=-0.358$, $p<0.05$). In addition, the change in LCI from start to end of treatment for pulmonary exacerbation correlated with WCC ($n=28$; $r=-0.417$; $p<0.05$) (19). Data from our study suggests that LCI is also sensitive to systemic inflammation during clinical stability.

This study is limited as data are from a single cross sectional visit and further longitudinal data would be required to determine if LCI can track inflammation in this older age group. As both age and pulmonary infection are factors associated with lung function decline, a future study should account for age as a potential confounder. However, in this study we did not identify age as an important confounder of the relationship between the presence of inflammation and LCI.

Two different methods were used to obtain a sample for culture. Whilst throat swabs may not be representative of lower airway colonization, it remains unclear whether sampling methods significantly affect the outcome of analysis. Furthermore, whilst CRP and WCC are two of the most clinically meaningful blood biomarkers in CF (15), systemic inflammation may link to both pulmonary and non-pulmonary comorbidities.
The results of this study demonstrate that LCI is a sensitive marker of infection and inflammation in older children and adults when clinically stable, providing additional information to FEV1% predicted, even in subjects with more advanced disease. These results further validate LCI as a useful outcome measure in this age range.

In previous studies, it has been shown that children with normal spirometry are the patient subgroup where LCI can yield most useful clinical information (1). However, LCI can also provide additional clinically relevant information in older age groups with more advanced disease and our findings support this (20-23).

LCI requires set-up and training in the use of specialist equipment, age and equipment specific protocols and ongoing data quality control. Therefore, LCI may best utilised in sites with onsite special interest and expertise in this test method or with links and ongoing communication to an expert site.

References


(22) Amin R, Stanojevic S, Kane M, Webster H, Ratjen F. A randomized controlled trial to evaluate the lung clearance index as an outcome measure for early phase studies in patients with cystic fibrosis. Respir Med 2016 Mar;112:59-64.