Airway infection, systemic inflammation and lung clearance index in children and adults with cystic fibrosis

To the Editor:

The 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 forced expiratory volume in 1 s (FEV_1) 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 are few data on how LCI relates to markers of infection and inflammation in children >6 years and adults.

Although 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 and 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% SF_6 and a modified Innocor device (Innovision, Glamsbjerg, Denmark). The LCI was calculated from two or more valid and repeatable 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 $p<0.05$ indicating statistical significance. This study was approved by the Office for Research Ethics Committee Northern Ireland (ORECNI); reference number 10/NIR01/41.

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LCI is a sensitive marker of infection and inflammation in clinically stable children and adults with cystic fibrosis http://ow.ly/vumZ30hzHL0

Cite this article as: O'Neill K, Bradley JM, Reid A, *et al*. Airway infection, systemic inflammation and lung clearance index in children and adults with cystic fibrosis. *Eur Respir J* 2018; 51: 1701704 [https://doi.org/10.1183/13993003.01704-2017].

A total of 110 CF subjects were recruited, with data collected at a stable visit. The mean±SD age (range) of subjects was 23.8±13.9 (6–67) years, with a mean±SD FEV_1 % predicted of 77.0±20.0% and median (interquartile range; range) LCI of 8.8 (3.8; 5.4–17.2) turnovers.

A sputum or throat swab sample was successfully collected from 106 out of 110 subjects (n=68 sputum, n=38 throat swabs). Four subjects were unable to provide a sample. *Pseudomonas aeruginosa* was detected in 40 out of 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 out of 106 (37%) subjects (55% sputum, 45% throat swabs) (non-*P. aeruginosa* infection group). No significant growth was detected in 26 out of 106 (25%) subjects (54% sputum, 46% throat swabs).

LCI was significantly elevated in *P. aeruginosa* and non-*P. aeruginosa* infection groups compared with those with no significant growth (figure 1a). FEV_1 % pred was significantly lower in those subjects with *P. aeruginosa* compared with those with no significant growth (figure 1b). When only subjects with abnormal FEV_1 % pred (<80% predicted) (n=57) were considered, the significant difference in LCI across groups (*P. aeruginosa versus* non-*P. aeruginosa* infection *versus* no significant growth) remained ($p<0.001$), whereas there was no significant difference in FEV_1 % pred 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 years; non-*P. aeruginosa* 22.8±16.7 years; no significant growth 23.3±15.8; $p=0.07$).

A blood sample was successfully collected from 83 out of 110 patients. Sufficient sample for CRP analysis was available for 71 out of 83 (85%) patients. Mean±SD WCC was $8.2\pm3.2\times10^9$ L^{-1} and median (range) CRP was 142.7 (10.0–6842) $\mu g \cdot dL^{-1}$.

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 FEV_1 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 ($r=0.15$; $p=0.16$).

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 with those subjects with no significant growth, whereas FEV_1 % pred 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 FEV_1 % pred.

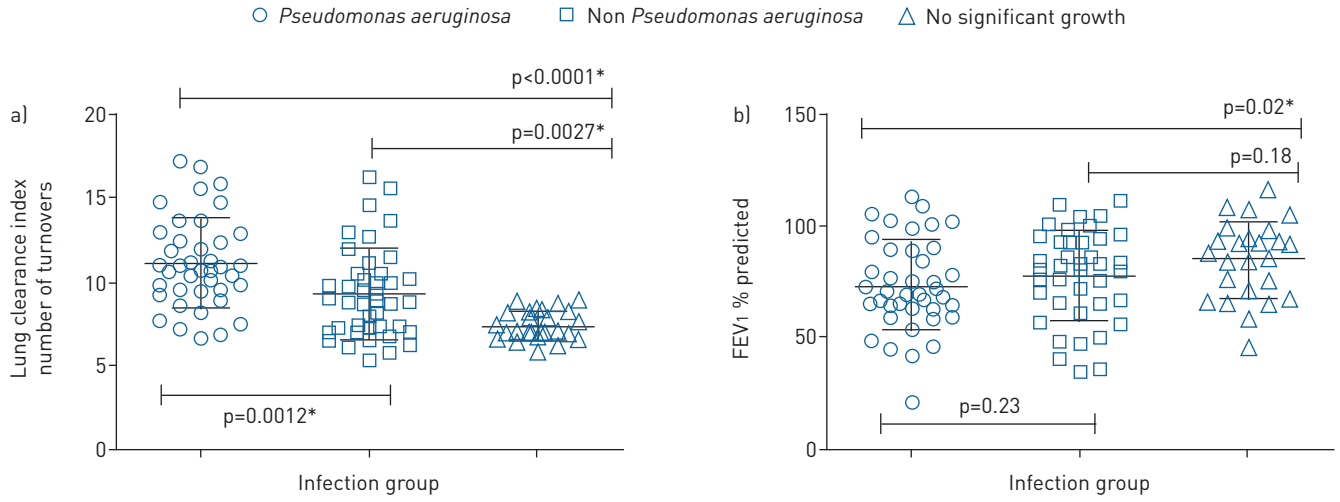

FIGURE 1 a) Lung clearance index across infection groups (n=106). b) Forced expiratory volume in 1 s (FEV_1) % predicted across infection groups (n=106). Scatter dots represent each subject and lines represent mean±SD. *: $p<0.05$.

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, these data show that LCI is elevated in individuals with infection with other pathogens including *S. aureus, S. maltophilia* and *H. influenzae,* whereas FEV_1 % pred showed no difference. LCI provides most useful information in subjects with mild disease (normal FEV_1 % pred). However, this study also found a significant difference in LCI between infection groups when considering subjects with abnormal FEV_1 % pred only, indicating that LCI may also have clinical utility in subjects with more advanced disease.

There are 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.* [12] did not find any association between LCI, IL-8 and neutrophil elastase in infants. A study including adults investigated the relationship between LCI and a range of systemic markers of inflammation at the beginning of a pulmonary exacerbation. Although 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 suggest 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. Although throat swabs may not be representative of lower airway colonisation, it remains unclear whether sampling methods significantly affect the outcome of analysis. Furthermore, although CRP and WCC are two of the most clinically meaningful blood biomarkers in CF [15], systemic inflammation may link to both pulmonary and nonpulmonary 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 FEV_1 % pred, 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].

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

Katherine O'Neill[1], Judy M. Bradley[2], Alastair Reid[3], Damian G. Downey[1,3], Jacqueline Rendall[3], John McCaughan[3], John E. Moore[3], Michael M. Tunney[4] and J. Stuart Elborn[1]
[1]Centre for Experimental Medicine, Queen's University Belfast, Belfast, UK. [2]Northern Ireland Clinical Research Facility, Queen's University Belfast, Belfast, UK. [3]Belfast Health and Social Care Trust, Belfast, UK. [4]School of Pharmacy, Queen's University Belfast, Belfast, UK.

Correspondence: Katherine O'Neill, Queen's University of Belfast, Centre for Infection and Immnuity, Medical Biology Centre, Lisburn Road, Belfast, BT9 7AB, UK. E-mail: k.oneill@qub.ac.uk

Received: Aug 21 2017 | Accepted after revision: Nov 17 2017

Support statement: This work was funded by the Health and Social Care Research and Development Division, Public Health Agency, Northern Ireland and the Medical Research Council through a US-Ireland Partnership Grant. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: Disclosures can be found alongside this article at erj.ersjournals.com

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