

**Lung clearance index is a sensitive, repeatable and practical measure
of airways disease in adults with cystic fibrosis**

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ABSTRACT

Background: Lung clearance index (LCI) is a sensitive marker of early lung disease in children, but has not been assessed in adults. Measurement is hindered by the complexity of the equipment required. The aims of this study were to assess performance of a novel gas analyser (InnocorTM), and to use it as a clinical tool for the measurement of LCI in cystic fibrosis (CF).

Methods: LCI was measured in 48 healthy adults, 12 healthy school-age children, and 33 adults with CF, by performing an inert gas washout from 0.2% sulphur hexafluoride (SF₆). SF₆ signal:noise ratio and 10-90% rise time of Innocor were compared to a mass spectrometer used in similar studies in children.

Results: Compared to the mass spectrometer, Innocor has a superior signal:noise ratio, but a slower rise time (150 ms v 60 ms) which may limit its use in very young children. Mean (SD) LCI in healthy adults was significantly different from that in CF patients: 6.7 (0.4) v 13.1 (3.8), $p < 0.0001$. Ten of the CF patients had FEV₁ \geq 80% predicted but only one had a normal LCI. LCI repeats were reproducible in all three groups of subjects (mean intra-visit coefficient of variation ranged from 3.6 to 5.4%).

Conclusions: Innocor can be adapted to measure LCI and affords a simpler alternative to a mass spectrometer. LCI is raised in CF adults with normal spirometry, and may prove to be a more sensitive marker of effects of therapy in this group.

INTRODUCTION

As part of a programme aimed at measuring response to gene therapy in cystic fibrosis (CF) we are interested in developing more sensitive measures of changes in CF airway function and structure. In the US, the only marker of lung function currently recognised as a primary endpoint in cystic fibrosis (CF) trials is the FEV₁¹. In early disease, this reflects total airways resistance and is insensitive to changes in small airways, which contribute less than 10% of the overall resistance in healthy adult subjects². Significant structural airway damage can be demonstrated on CT in the presence of a normal FEV₁³.

In early lung disease, ventilation heterogeneity results from regional differences in small airway calibre (those beyond division 8)^{4,5}. This can be demonstrated both in computer models of the human lung⁶ and by looking *in vivo* at MR images⁷ or radio-labelled tracer gas distribution^{7,8}. Inert gas washout is an alternative technique, which involves measuring the elimination of a non-absorbed gas as it is exhaled during tidal breathing. The gas can either be resident nitrogen washed out by breathing 100% oxygen, or an exogenous tracer gas which has first been breathed in to equilibrium. Lung clearance index (LCI), a simple marker of deranged ventilation, can be calculated from the washout curves⁹. Past studies using a variety of methods have demonstrated that LCI is reproducible and more sensitive than FEV₁ at identifying early lung disease in children⁹⁻¹³. In addition, Aurora *et al.* showed that LCI is further raised in children infected with *Pseudomonas aeruginosa*¹⁰ and Kraemer *et al.* showed LCI to be an early predictor of deteriorating lung function in children¹³.

Although an old technique¹⁴, measurement of LCI has always relied upon complex and bulky equipment, usually assembled from separate components by the investigators themselves, and has largely been restricted to a research setting. The current best method involves using a

mass spectrometer to measure the washout of the inert tracer gas sulphur hexafluoride (SF_6). Although these are simpler than nitrogen washouts, mass spectrometers are expensive to purchase and maintain.

The purpose of this study was to investigate LCI in healthy subjects and CF adults using a modified InnocorTM device (Innovision, Odense, Denmark). Innocor is a compact gas analyser and flow sensor, originally designed to measure cardiac output by inert gas re-breathing. The gas analyser uses photoacoustic spectroscopy to measure several gases including low concentrations of the inert tracer SF_6 , making it a suitable device for ventilation distribution measurements. More information on the gas analyser is given in the online supplement (pages 2-5).

In preparation for its use as an endpoint in clinical trials, the aims of this study were:

1. To compare the performance (assessed by response time and signal:noise ratio) of the Innocor gas analyser to that of the current inert gas washout standard (mass spectrometer).
2. To adapt the Innocor device, and analysis software, into a clinical system for measurement of functional residual capacity (FRC) and lung clearance index (LCI).
3. To assess how LCI changes with age of subject in healthy volunteers.
4. To assess the intra and inter-visit reproducibility of LCI in healthy volunteers.
5. To use the adapted Innocor to measure FRC and LCI in normal adult subjects and patients with CF and to compare LCI with spirometry.

METHODS

Equipment

To measure LCI, a mouthpiece fitted with a flowmeter and gas sampling port is required (Figure 1). This is connected to a detachable flowpast tube which is used to supply tracer gas during the wash-in, and then removed at the start of washout. A more detailed Methods section is available in the online supplement, and the modifications to the standard Innocor patient interface are described in detail on pages 5-7.

Spirometry was measured according to American Thoracic Society / European Respiratory Society guidelines¹⁵; predicted values for FEV₁ are those provided by the European Community for Coal and Steel (adults \geq 17yrs)¹⁶ and Rosenthal *et al.* (children \leq 16yrs)¹⁷.

Performance of Innocor gas analyser

The signal:noise ratio at the start and end of a washout and the rise time of the gas analyser in response to a step change in SF₆ concentration were assessed as described in the online supplement (page 8). Performance was compared to that of a mass spectrometer used routinely for LCI measurements. The ability of the complete modified system to integrate flow and gas signals accurately was assessed using a gas calibration syringe which can be set to deliver different volumes (Hans Rudolph, Missouri, USA). This was filled with 0.2% SF₆ in air (BOC, Guildford, UK) to a range of different starting volumes and a washout performed by incomplete filling and emptying of the syringe around this starting point. The syringe volume derived from the calculated “expired” volume of SF₆ was then compared with the known starting volume.

Flow and SF₆ data were exported for analysis on custom built software. FRC was derived from the total expired SF₆ volume, calculated by integration of flow and SF₆ signals. LCI is defined as the number of lung turnovers (i.e. multiples of FRC) required to reduce end tidal marker gas concentration to 1/40th of the starting value (as described in the online supplement, page 11-12).

Subjects

Forty nine healthy non-smokers (less than 10 pack years smoking history) with no active lung disease and on no regular respiratory medications were recruited as normal adult volunteers (age range 19-58yrs). Thirteen healthy child volunteers (age range 6-16yrs) were recruited if they had no previous diagnosis of recurrent wheeze or asthma and were taking no current inhaled medication. There was no history of significant respiratory disease requiring hospitalisation (e.g. pneumonia, pertussis, TB), no prematurity (<34 weeks gestation) and no significant co-morbidity. Thirty three CF patients (age range 17-49yrs) were recruited from the Scottish Adult CF Service, the diagnosis being based upon a combination of clinical presentation and sweat testing and confirmed by genotyping. All volunteers, patients and (where relevant) parents provided informed consent. Paediatric volunteers provided assent where appropriate. This study was approved by the Lothian Research and Ethics committee.

Washout test

Subjects were seated and suitably distracted by watching television. A noseclip was applied and tidal breathing established whilst the subject was connected to the flowpast circuit containing 0.2% SF₆ in air. This was supplied from a compressed gas cylinder with the flow rate adjusted to ensure that rebreathing did not occur. This wash-in phase continued for at least 5 minutes in adults or 4 minutes in children under 16 yrs, and in all cases until

inspiratory and expiratory SF₆ concentrations differed by less than 0.004% (absolute difference in SF₆ concentration). The flowpast circuit was then detached during expiration and the washout measured until the end tidal SF₆ had fallen to less than 1/40th of the starting concentration (i.e. <0.005%). In healthy children (<16 yrs), an identical gas analyser and protocol were employed at a separate research site, but a smaller filter was used to reduce the pre-capillary deadspace (36 ml, v 46 ml in adults).

Subjects completed three sets of wash-ins and washouts. A washout was discarded if the resulting calculated FRC differed by more than 10% from both the other two repeats¹⁸.

Statistical Analysis

Data were analysed using Prism (GraphPad Software Inc, CA, USA). Results are quoted as mean (SD) unless otherwise stated. Within test repeatability for LCI was determined by calculating the coefficient of variation (CV) as $100 \times \text{SD} \times \text{mean}^{-1}$. Inter-visit reproducibility was assessed using the Bland-Altman technique. Correlation with age and height were assessed by multiple linear regression analysis. Numerical values for LCI and FEV₁% predicted were compared using a Mann-Whitney U test. The 95% limits of normality for LCI were calculated as mean \pm 1.96 x residual standard deviations. A *p* value of below 0.05 was considered as statistically significant.

RESULTS

Technical Validation of Innocor device

Signal: Noise ratio of Innocor and MS

The Innocor device has a lower gas concentration operating range than the MS. Signal quality is therefore given at the starting and finishing concentrations of a washout, which are different for the two devices (Table 1). For both devices, there is a fall in signal:noise ratio as the gas concentration falls, but the Innocor signal quality remains superior throughout, despite much lower SF₆ concentrations.

	SF ₆ Concentration (%)		Signal:Noise ratio	
	Start	End	Start	End
Mass spectrometer	4.0	0.1	200	13
Innocor	0.2	0.005	944	53

Table 1: Signal:noise ratios of Innocor and mass spectrometer at gas concentrations encountered at start and end of washout. The signal:noise is calculated as the ratio of mean to standard deviation of a stable gas signal over ten seconds.

Rise time and delay of gas signal.

The mean (SD) SF₆ 10-90% rise time for Innocor was measured as 154 (5) ms. The mass spectrometer rise time was measured at 64 (5) ms, $p < 0.00001$ compared to Innocor. The longer rise time of the Innocor gas analyser was allowed for by offsetting gas and flow signals during analysis by an additional 50 ms. This corresponds to the 50-75% rise time of the gas signal, and has the effect of speeding response time by realigning the flow signal with

the 75% response fraction of the gas signal ¹⁹. The accuracy of this adjustment was then confirmed by integration of known volumes of SF₆ from a calibration syringe.

Validation of FRC measurements

Sixteen washouts were performed using a calibration syringe with the starting volume varied between 1.5 and 3L. There was good agreement between the measured and actual syringe volumes (see Figure 5, page 17 in online supplement I). The mean (SEM) error between measured and actual syringe volume was 16.3 (2.4) ml or 1.1 (0.2)%.

In vivo LCI measurement

LCI was assessed successfully in 12 healthy children, 48 adult healthy volunteers, and 33 CF adults. Demographics are given in Table 2. Data from two additional healthy volunteers (one adult, one child) could not be analysed because of technical difficulties (see below).

	Healthy Volunteers		CF patients
	Children (age≤16yrs)	Adults (age≥17yrs)	
Number	12	48	33
Male / Female	7 / 5	19 / 29	21 / 12
Mean Age [range] yrs	11 [6-16]	33 [19-58]	30 [17-49]
FEV₁ % predicted (SD)	95 (11)	102 (12)	66 (23)*
Mean LCI (SD)	6.3 (0.5)	6.7 (0.4)	13.1 (3.8)*
[Range]	[5.6 – 7.1]	[6.0-7.8]	[6.3-20.4]
Mean CV% LCI (SD)	5.4 (3.8)	3.6 (2.1)	4.5 (2.7)

Table 2: Demographics, spirometry and lung clearance index of healthy volunteers and cystic fibrosis patients.

LCI = lung clearance index

CV% = Coefficient of variation (%) for intra-visit repeats.

*p<0.0001 compared to adult healthy volunteers (Mann-Whitney U test).

Effect of age, height and gender on LCI in non-CF subjects

Figure 2 shows the relationship between LCI and age (min 6 yrs, max 58 yrs). In those >16yrs there was no relationship between LCI and age. When the two cohorts were combined, there was a weak but statistically significant correlation with age (Pearson $r^2=0.16$, $p<0.002$). The small dependence of LCI on age is best summarised by a normal range (95% limits of normality) in adults of 5.9-7.5 and in children (≤16yrs) of 5.3-7.3. A weak

relationship between height and LCI in the combined cohorts disappeared on multiple regression analysis. LCI was unrelated to gender of subject. By contrast, FEV₁ varied between 76 and 133% predicted in the same group of 60 healthy adults and children.

LCI in non-CF and CF adults

The group mean (SD) [range] for LCI in adult healthy controls was 6.7 (0.4) [6.0-7.8], with 95% limits of normality calculated as 5.9-7.5. The group mean (SD) [range] for CF patients was 12.8 (3.6) [6.3-20.4], $p<0.0001$ compared to healthy controls. The mean (SD) FEV₁ was also significantly different between the two groups, being 102 (12) % predicted in healthy controls v 68 (23) % predicted in CF patients, $p<0.0001$.

Figure 3 shows the relationship between FEV₁ % predicted and LCI for healthy control and CF adults. In controls LCI was restricted to a narrow range but in CF patients LCI increased with reducing FEV₁ % predicted ($r^2=0.69$, $p<0.0001$).

There were 10 CF patients with FEV₁ \geq 80% predicted; all but one had LCI greater than the upper limit of normal. By contrast LCI was marginally elevated in only two healthy adults (measuring 7.7 and 7.8). The sensitivity of LCI for detecting CF was 97%, v 70% for FEV₁.

Repeatability of washout at same visit

A washout test was excluded if the measured FRC differed by >10% from both of the other two washouts. In adult subjects, this resulted in the exclusion of a total of 7 tests, representing less than 3% of the total number of repeats from both healthy volunteers and CF patients. Three tests were excluded from the paediatric cohort, representing 9% of the total number of

washout repeats. All three washout repeats from an additional single adult healthy volunteer could not be analysed because they were unable to achieve a regular and reproducible breathing pattern. All three repeats from an additional healthy child (age 8) were also excluded because of evidence of air leak.

After exclusion of these repeats, the mean (SD) intra-subject coefficient of variation (CV) for FRC derived from repeat washout manoeuvres on the same visit was 3.2 (1.9) % for adult healthy volunteers, 3.9 (2.1) % in healthy children and 3.5 (2.3) % for CF patients. The mean (SD) CV for LCI was 3.6 (2.1) % for healthy adults, 5.4 (3.8) % for healthy children, and 4.4 (2.8) % for CF patients. There was no significant correlation between the LCI CV and FEV₁ % predicted.

Inter-visit reproducibility of LCI in healthy adults

Repeat measurements of LCI were performed in triplicate on 16 healthy volunteers after an average (SEM) of 36 (10) days. A Bland-Altman plot²⁰ of the difference between repeat measures v the mean of the measurements is shown for LCI in Figure 4. For FRC, the 95% limits of agreement between the two measurements were -0.43 to 0.45 L. For LCI, the 95% limits of agreement for the two measurements were -0.78 to 0.46. Thus the inter-visit reproducibility of the FRC measurement was around 400 ml and that of the LCI measurement was 0.6.

DISCUSSION

In this paper we have demonstrated that clinical measurement of inert gas washout is practical using equipment that is cheaper, more portable and has more sensitive gas signal resolution than the current mass spectrometer standard. We have also shown for the first time that in adults with CF, a simple measure of ventilation heterogeneity is more sensitive than FEV₁ in detecting lung function abnormalities. Finally, we have shown that this measurement is reproducible both within and between visits, and that there is little change over a wide range of subject height and age.

In children with CF, there is already increasing evidence that LCI is a more sensitive measure of early lung disease than FEV₁^{9-13,21}. LCI has also been shown to correlate better with scores of airway damage on HRCT than spirometry²². As such it may fill an important gap in our ability to monitor airway function and disease progression non-invasively. Since only tidal breathing is required, it is particularly suitable for airways assessment in subjects who find complex respiratory manoeuvres difficult.

The potential of multiple breath washout measurements, however, has been hampered by the lack of a convenient method of performing them^{12,21,23}. The original method for assessing lung clearance was the nitrogen washout. Although this avoids the need for a wash-in first, sufficient time must be allowed between tests for the end tidal nitrogen concentration to return to normal¹⁸. The use of a mass spectrometer (MS) to measure LCI by following changes in exogenous SF₆ is now well described in children^{12,21}, and is probably the accepted gold standard technique in this population. The MS offers the additional advantage that it can measure a wider range of different gases, which is a useful option when measuring vital capacity single breath washouts²⁴. However, the MS is an expensive, temperamental and

bulky piece of equipment that cannot readily be taken out of the laboratory. In contrast, Innocor contains both the gas analyzer and the pneumotachograph in a single unit that is both portable and robust²⁵. A supply of SF₆ is required for both systems, but the concentration required for Innocor is 1/20th of that used in the MS washouts, which reduces gas wastage and the potential environmental (greenhouse) effects of SF₆.

The ideal comparison would be to compare performance of both systems simultaneously, as has been done for other gas analysers²⁶. However washouts would have to be performed at the operating range of the Innocor gas analyser, since the response is not linear above 0.2% SF₆, but the signal resolution of the mass spectrometer shows excessive noise at this level. Accepting this as a limitation of the current comparison, we have shown that the gas analyser is suitable for use in a multiple breath washout apparatus. The characteristics of the two analysers are summarised in Table 2 of the online supplement (page 18). Our technical validation demonstrates that the device with our modifications is capable of measuring gas volume by dilution with high accuracy. Despite operating at a much lower SF₆ concentration it produces washouts with superior signal:noise ratio compared to a MS. Our comparison has also identified the possible limitations of the device imposed by the slower signal rise and fall time. The system is able to integrate flow and SF₆ concentration accurately at a physiological breathing rate of 10-30 breaths/minute, and should therefore be suitable for use in school-age children and adults. The response time may however limit use of the method in neonates and pre-school children with faster respiratory rates^{21,27}. Further *in vitro* assessment is required before using the analyser in this age group.

To date, we have used the modified Innocor to measure LCI in over 100 patients and volunteers. From the data presented here, over 85% of subjects are able to complete all three

washout manoeuvres without difficulty and generate reproducible measurements of FRC and LCI. Even for CF patients, the whole process (wash-in and wash-out) usually takes little more than 10 minutes, and considerably less in children. Despite the relatively uncontrolled conditions, the mean CV for repeat FRC is similar to that described in the literature, which varies from 3.5-6.7% for plethysmography and 4.9-10.4% for helium dilution²⁸. The mean CV for LCI is also better than that described in children¹¹. Repeat measurements of LCI at a separate time point, in a cohort of the adult healthy volunteers, also demonstrate good inter-visit reproducibility.

It has been shown that LCI may be influenced by large changes in tidal volume, respiratory rate or FRC^{29,30}. We used tidal volume feedback to control tidal volume and respiratory rate within a range which should not affect the result. Since LCI is a ratio of cumulative expired volume and FRC, it is also independent of small changes in FRC over the physiological range. This is supported by the reproducibility of LCI and the narrow range of LCI in healthy controls found in the current study. Furthermore because it is normalised for FRC the normal range of LCI is largely unaffected by age, height or gender of subject. There was a weak, albeit statistically significant, rise in LCI with age. The clinical significance of this is unclear, since the magnitude of the difference (over a 52 year age range) remains very small and is less than inter-visit reproducibility. Serial deadspace is known to affect LCI in infants and neonates³¹. However, the change in normal values of LCI was in the opposite direction to that which would be caused by a greater dead space/tidal volume ratio (as found in infants). It is possible therefore that this represents a true effect of age on lung elasticity and hence gas mixing. By contrast, there is a wide range of “normal” for FEV₁ % predicted, which is influenced by the choice and accuracy of the normal range selected³².

The mean (SD) LCI determined here is very similar to that reported in the literature in children and adolescents. In preschool children (mean age 4) this has been reported as 6.9 (0.4)¹⁰, and in school age children (mean age 11 yrs) as 6.5 (0.5)¹¹ and 6.3 (0.4)⁹ in two different populations from UK and Sweden respectively. This supports our observation that LCI changes little with the age of the subject (>6 yrs). This may be especially useful during long term follow up studies.

These are the first data on LCI in CF adults; previous studies have only reported measurements in subjects up to 19 years. Even in adult patients with normal spirometry, the LCI may be markedly elevated, indicating significant “silent” lung damage. Some of the patients with normal FEV₁ gave no symptoms and were on no treatment, the diagnosis of CF having been made incidentally. Yet despite this, there was abnormal gas mixing in almost all cases. There is a risk that the extent of lung disease in such patients will be underestimated and hence under-treated.

Whilst FEV₁ is the currently accepted gold standard to monitor trials of new treatments for CF, the rate of decline in this parameter has steadily reduced over the last decade³³. Power calculations show that many hundreds of patients would need to be treated over a year, or more, to see any beneficial effect of a novel therapeutic agent aimed at the basic defect³⁴. We have, therefore, instituted a large programme to assess novel biomarkers which could act as surrogates for FEV₁. Ventilation heterogeneity is thought to be altered by small airways dysfunction^{4,5} and measurements of this should therefore reflect the earliest pathology in CF - as has already been shown in children⁹⁻¹¹. This is also the region of the lungs which is likely to be a key target for gene therapy. The choice of which subject to recruit into trials of gene therapy represents a conflict between patients with sufficiently clear airways that the gene

therapy complex can be delivered into the lungs, and selecting patients with sufficient abnormality in lung function so that any improvement can be measured. LCI offers the ability to measure dysfunction in the airways of interest, and also to extend the range of patients suitable for these assessments.

We have demonstrated that there is the possibility of a robust and compact apparatus to measure LCI that can be used in multi-centre studies after relatively straightforward modification. This will permit us to assess LCI routinely in patients to obtain longitudinal data, and in particular it may serve as a more sensitive measure of lung function after changes in therapy. The value of this technology may however extend beyond just CF, and we anticipate that it may provide valuable information about the development and treatment of airways disease in other conditions. In particular, it may be useful in conditions that initially affect the small airways such as asthma, COPD and obliterative bronchiolitis.

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COMPETING INTERESTS

No authors have any competing interests

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Figure Legends

Figure 1: Patient-interface used for inert gas washout with Innocor gas analyser.

Figure 2: Effect of age on lung clearance index (LCI) in healthy volunteers. LCI remains within a narrow band of normal over an age range of 52 years. The dotted line is the regression line, showing the extent of age related increase in LCI over this range.

Figure 3: Lung clearance index (LCI) v FEV₁ % predicted for adult healthy volunteers (circles) and patients with cystic fibrosis (diamonds). The horizontal line represents the mean and the horizontal dotted lines the 95% limits of normality of the LCI, calculated from the healthy adult population. The vertical dotted line represents the lower limit of normal for % predicted FEV₁.

Figure 4: Bland-Altman plot of difference between lung clearance index (LCI) measured on two separate occasions (quoted as mean of triplicate repeats), and mean of the two measurements of LCI.

T-piece and Flowpast







