Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis

P M Gustafsson,1 P A De Jong,2,3,4 H A W M Tiddens,2 A Lindblad1

See related editorial page 96

ABSTRACT

Background: A sensitive and valid non-invasive marker of early cystic fibrosis (CF) lung disease is sought. The lung clearance index (LCI) from multiple-breath washout (MBW) is known to detect abnormal lung function more readily than spirometry in children and teenagers with CF, but its relationship to structural lung abnormalities is unknown. A study was undertaken to determine the agreements between LCI and spirometry, respectively, with structural lung disease as measured by high-resolution computed tomography (HRCT) in children and teenagers with CF.

Methods: A retrospective study was performed in 44 consecutive patients with CF aged 5–19 years (mean 12 years). At an annual check-up inspiratory and expiratory HRCT scans, LCI and spirometric parameters (forced expiratory volume in 1 s (FEV1) and maximal expiratory flow when 75% of forced vital capacity was expired (FEF75)) were recorded. Abnormal structure was defined as a composite HRCT score of >5%, the presence of bronchiectasis or air trapping >30%. Abnormal lung function was defined as LCI above the predicted mean +1.96 residual standard deviations (RSD), or FEV1 or FEF75 below the predicted mean −1.96 RSD. Sensitivity/specificity assessments and correlation analyses were done.

Results: The sensitivity to detect abnormal lung structure was 85–94% for LCI, 19–26% for FEV1 and 62–75% for FEF75. Specificity was 43–65% for LCI, 89–100% for FEV1 and 75–88% for FEF75. LCI correlated better with HRCT scores (Rs+0.85) than FEV1 (−0.62) or FEF75 (−0.66).

Conclusions: LCI is a more sensitive indicator than FEV1 or FEF75 for detecting structural lung disease in CF, and a normal LCI almost excludes HRCT abnormalities. The finding of an abnormal LCI in some patients with normal HRCT scans suggests that LCI may be more sensitive than HRCT scanning for detecting lung involvement in CF.

Cystic fibrosis (CF) lung disease is characterised by persistent infection and inflammation from early in life,1 leading to chronic airway disease and a progressive decline in lung function. Airway abnormalities are thought to start in the peripheral airways, but eventually lead also to the destruction of the larger airways (bronchiectasis). Over recent decades the median age of survival of patients with CF has improved dramatically to 35 years in 2004, with most morbidity and mortality caused by CF lung disease.1 Intensive clinical monitoring of the airway disease and early intervention are needed to delay lung disease progression.2–5 Sensitive markers of early lung involvement and sensitive methods to monitor progression of CF lung disease are therefore sought.1 2 6

Spirometry (forced expiratory volume in 1 s (FEV1)) is still the most widely used method for clinical monitoring of CF lung disease, and it is believed to be a good predictor of outcome in patients with moderate to severe CF lung disease.7–9 Over the last two decades there has been a shift towards closer monitoring and more aggressive treatment of early CF lung disease. As a consequence, two major disadvantages of the use of FEV1 in children with CF have become apparent. First, many school-age children with CF now have FEV1 within the normal range8 or show a slow rate of progression,2 10–12 even though they probably have lung disease that progresses faster than is reflected in spirometric measurements. Apparently FEV1 is not very sensitive to early CF lung disease.10–15 Second, reliable forced expiratory manoeuvres are difficult to obtain in children under the age of 3 years, with testing in the infant and preschool age groups being largely confined to specialist laboratories.10–12 As interest in monitoring younger patients increases, so does the need for alternative more sensitive measures of CF lung disease that can be obtained in children of all ages.

High-resolution CT (HRCT) scanning of the chest has repeatedly been shown to be more sensitive than FEV1 both for early detection of CF lung disease19–22 and for following disease progression.23–25 Bronchiectasis, the hallmark of CF lung disease, can be identified early in the course of CF on HRCT scanning26 and is the HRCT abnormality that is most sensitive to disease progression.6 25–24 HRCT is the current gold standard to evaluate bronchiectasis.26 Air trapping—reflecting small airways disease—is another important feature of CF lung disease, can be demonstrated with expiratory HRCT scans.27–28 Furthermore, mucus plugging and airway wall thickening can be seen on inspiratory scans in CF.29–31 The use of CT scanning in CF is, however, restricted to examinations with a relatively long interval of 1–2 years. Despite a substantial reduction in the dose of radiation used in CT examinations of the chest in the last decade, its use should be limited to the absolute minimum.32–34 Hence, CT scanning is not a feasible method for monitoring CF lung disease over short time intervals. In addition, routine CT scanning in young children is troublesome because sedation and a technically demanding procedure are required to obtain high quality images.35 In addition, young children are thought to be particularly sensitive to the negative effects of radiation.36–38

It has recently been suggested that the lung clearance index (LCI), an index of uneven ventilation distribution measured by multiple breath...
washout (MBW) of an inert marker gas, could be a sensitive early marker of CF lung disease.\textsuperscript{15–18} The MBW method is not new but has been used for over 50 years,\textsuperscript{40} and paediatric studies were published during the 1960s\textsuperscript{42} and 1970s.\textsuperscript{43} Breath-by-breath studies in children using modern PC technology were reported by Wall\textsuperscript{44} and Kraemer\textsuperscript{45} more than 20 years ago. LCI has advantages over spirometry in that it can be measured easily in all age groups and normative values are similar from early childhood to adulthood.\textsuperscript{15} It discriminates between patients with CF and healthy subjects better than either FEV\textsubscript{1} or body plethysmography (residual volume/total lung capacity ratio).\textsuperscript{15} The LCI has also been shown to be an earlier and stronger indicator of disease progression than spirometry.\textsuperscript{46} Finally, the MBW test is a non-invasive functional measurement which involves no risk or hazardous exposures. The relationship of LCI to structural lung abnormalities in CF has, however, not been reported previously.

The present study aimed to assess the correlations between LCI and spirometry and structural lung abnormalities in CF, and to determine their sensitivity and specificity in the detection of bronchiectasis and other structural lung abnormalities as measured by HRCT. It was hypothesised that LCI would agree better with the presence of HRCT abnormalities than spirometry.

METHODS
Subjects
A retrospective study of spirometry, MBW and HRCT recordings was performed over a 30-month period in 44 consecutive patients with CF aged <20 years. All patients underwent these investigations as part of their routine annual review at the Goteborg CF centre where HRCT is performed routinely every third year in patients over 5 years of age. Chronic colonisation by Pseudomonas aeruginosa was defined as three or more positive cultures over a 6-month period. This retrospective analysis was approved by the ethics committee for human research at the University of Goteborg.

Measurement of lung structure
Lung structure was evaluated using HRCT scans. The scanning protocol has previously been reported \textsuperscript{24} and is described in more detail in the online supplement. Briefly, images were obtained from lung apex to base at 15 mm (children) and 10 mm (adults >18 years) intervals using 1.25 mm thick slices in inspiration. In addition, three expiratory HRCT images were obtained through the upper, middle and lower lung zones.

All scans were blinded to date and patient identification and were scored in random order by an experienced observer \textsuperscript{21, 24, 25} using an adapted scoring system developed by Brody \textit{et al}.\textsuperscript{22, 47} The scoring system evaluates the severity and extent of central and peripheral bronchiectasis; extent of central and peripheral mucus plugging; severity and extent of central and peripheral airway wall thickening; parenchymal abnormalities (extent of opacities, ground glass pattern and cysts and bullae); and gas trapping. Composite HRCT score and component HRCT scores were expressed as a percentage of the maximal score.\textsuperscript{47}

A composite CT score >5% was set as abnormal based on findings in 15 normal individuals that were scored intermixed with patients with CF. All normal individuals had a CT score below 5% (unpublished data). Air trapping involving more than 50% of the lung was also defined as abnormal. It is known that air trapping can be seen in normal individuals,\textsuperscript{49} and in other studies in the bronchiolitis obliterans syndrome a 52% cut-off has been used.\textsuperscript{9}

Measurement of lung function
The lung function measurements are described in detail in the online supplement. Briefly, spirometric measurements included FEV\textsubscript{1}, forced vital capacity (FVC) and the maximal expiratory flow when 75% of FVC was expired (FEF\textsubscript{75}). They were related to normative values for Swedish children (7–18 years)\textsuperscript{50} or adults.\textsuperscript{51, 52} The lower limit of normality (LLN) was defined as the predicted mean minus 1.96 residual standard deviations (RSD). In addition, tidal breathing multiple-breath sulfur hexafluoride (SF\textsubscript{6}) washout was performed using a mass spectrometer for gas analysis, as previously described in detail elsewhere.\textsuperscript{15} The LCI was calculated as the number of lung volume turnovers (ie, the cumulative expired volume divided by the functional residual capacity) needed to lower the end-tidal tracer gas concentration to 1/40th of the starting concentration.\textsuperscript{14} A high value of LCI thus indicates abnormal ventilation distribution. The mean LCI result from three MBWs in each subject was used for analysis. In a previous study including healthy subjects, the mean, RSD and upper limit of normality (ULN; mean plus 1.96 RSD) for LCI were 6.33, 0.43 and 7.17, respectively.\textsuperscript{15}

Statistical analysis
Abnormal structure was defined as a composite HRCT score of >5%, the presence of bronchiectasis or air trapping >50%. Lung function was expressed as z-scores which were calculated as (measured value – predicted value)/RSD from the reference population. Abnormal lung function was defined as LCI above +1.96 z-scores or FEV\textsubscript{1} or FEF\textsubscript{75} below −1.96 z-scores. The proportions of patients with normal or abnormal FEV\textsubscript{1} or LCI results in relation to HRCT classifications (cross tabulations) were compared using the Yates corrected χ\textsuperscript{2} test. The sensitivity and specificity were determined for LCI, FEV\textsubscript{1} and FEF\textsubscript{75} with respect to an abnormal HRCT composite score, the presence of bronchiectasis and the presence of abnormal air trapping, as diagnosed by HRCT scanning. Sensitivity was calculated as the proportion of the study population with abnormal HRCT findings that showed abnormal lung function results. Specificity was calculated as the proportion of the study population with
normal HRCT finding that had normal lung function findings. The 95% confidence intervals (CI) around the sensitivity and specificity findings were calculated as follows: 95% CI for $p = p \pm 1.96 \times SE$; SE for $p = (p(1-p)/n)^{1/2}$ where $p$ denotes the sensitivity or specificity expressed as a ratio.

Spearman rank correlation coefficients ($R_s$) were calculated for FEV$_1$, LCI and FEF$_{75}$ with respect to the HRCT composite score, bronchiectasis, mucus plugging, airway wall thickness, parenchyma and air trapping. A $p$ value of $0.05$ was accepted as statistically significant. Statistica 6.0 (StatSoft, Tulsa, Oklahoma, USA) was used for the statistical analyses.

RESULTS

The study group included 27 males and 17 females aged 5.4–19.6 years (mean and median age 12.2 years). Twenty-one patients (48%) were homozygotes and 19 (43%) were heterozygotes for dF508. Eleven patients were chronically colonised with *Pseudomonas aeruginosa* and 37 were pancreatic insufficient. FEV$_1$ ranged from 44% to 127% of predicted values (mean and median 95%).

Bronchiectasis was diagnosed in 26 patients (59%), an abnormal composite HRCT score (>5%) was found in 27 patients (61%), and pathological gas trapping was present in 16 (36%). Figure 1 gives lung function findings in z-scores versus age. Age was evenly distributed and there was no significant correlation between any lung function parameter and age. Figure 2 shows lung function in relation to HRCT scores. Of the 27 patients with an HRCT score >5%, 25 had an abnormally elevated LCI, 17 had abnormal FEF$_{75}$ results and 7 had reduced FEV$_1$. Seventeen patients had normal HRCT scores (<5%); 6 of these had elevated LCI, 2 had abnormal FEF$_{75}$ and all had FEV$_1$ within normal limits.

LCI and FEF$_{75}$ showed closer agreement with findings of structural lung abnormalities than did FEV$_1$ (table 1). The LCI had the best sensitivity (85–94%) for detecting structural lung abnormalities (bronchiectasis, composite HTCT score and air trapping) (table 2). The sensitivity for FEV$_1$ ranged from 19% to 26% and for FEF$_{75}$ from 62% to 75%. The specificity in the detection of structural lung disease was, however, higher for FEV$_1$ (89–100%) and FEF$_{75}$ (75–88%) than for LCI (43–65%).

The LCI had a markedly stronger correlation with all six structural abnormality scores ($R_s = 0.65$ to 0.85) than did the FEV$_1$ ($R_s = 0.35$ to 0.62) or FEF$_{75}$ ($R_s = -0.44$ to 0.66) (table 3).

The proportion of patients with normal or abnormal FEF$_{75}$ showed better agreement with LCI classification than did the FEV$_1$ ($R_s = 0.66$, $p = 0.001$; FEV$_1$: $R_s = -0.63$, $p < 0.001$).

DISCUSSION

This study aimed to determine the sensitivity of spirometry and MBW for detecting bronchiectasis and other structural lung changes as measured by HRCT scanning in children and teenagers with CF, and to compare the correlations between LCI and spirometry and structural lung abnormalities. The hypothesis was that the LCI would be a more sensitive test for detecting structural lung disease than FEV$_1$ or FEF$_{75}$, and that LCI would correlate better than spirometry with structural lung changes.

![Figure 2: Lung function (lung clearance index (LCI), forced expiratory volume in 1 s (FEV$_1$) and maximal expiratory flow when 75% of forced vital capacity was expired (FEF$_{75}$)) expressed as z-scores plotted against HRCT (composite scores) in 44 children and teenagers with CF. Black filled circles denote LCI, open circles FEV$_1$, and grey circles FEF$_{75}$. Vertical hatched line denotes upper limit of normality (ULN) for HRCT score and horizontal hatched lines denote upper and lower limits of normality (LLN) for the lung function variables.](http://thorax.bmj.com/)

![Table 1: Agreement between LCI, FEV$_1$ and FEF$_{75}$ and structural lung changes classified as abnormal or normal](http://thorax.bmj.com/)

<table>
<thead>
<tr>
<th></th>
<th>Bronchiectasis</th>
<th>HRCT score</th>
<th>Air trapping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>&gt;5%</td>
</tr>
<tr>
<td><strong>LCI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>22</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>$p = 0.033$</td>
<td>$p &lt; 0.001$</td>
<td>$p = 0.027$</td>
</tr>
</tbody>
</table>

|                |     |    |     |     |      |      |
| **FEV$_1$**    |     |    |     |     |      |      |
| Abnormal       | 5   | 2  | 7   | 0   | 4    | 3    |
| Normal         | 21  | 16 | 20  | 17  | 12   | 25   |
|                | $p = 0.761$ | $p = 0.062$ | $p = 0.413$ |

|                |     |    |     |     |      |      |
| **FEF$_{75}$** |     |    |     |     |      |      |
| Abnormal       | 16  | 3  | 17  | 2   | 12   | 7    |
| Normal         | 10  | 15 | 10  | 15  | 4    | 21   |
|                | $p = 0.008$ | $p = 0.003$ | $p = 0.004$ |

LCI, lung clearance index; FEV$_1$, forced expiratory volume in 1 s; FEF$_{75}$, maximal expiratory flow when 75% of forced vital capacity was expired; HRCT, high-resolution CT.

HRCT score >5% is defined as abnormal and >30% air trapping is defined as abnormal.

$p$ Values refer to the Yates corrected $\chi^2$ test.
It was shown that LCI was markedly more sensitive than either FEV1 or FEF75 with respect to structural lung abnormalities (bronchiectasis, air trapping and combined abnormalities). The sensitivity of LCI ranged between 85% and 94%. Of 27 patients with abnormal HRCT scores, 25 had elevated LCI and 22/26 patients with evidence of bronchiectasis had abnormal LCI, which suggests that a normal LCI in a subject with CF almost excludes structural lung damage. The specificity of LCI was modest, however, because LCI was abnormal in one-third of the patients with normal HRCT scores, half of those without evidence of bronchiectasis, and in more than half of those without diagnosed air trapping.

The LCI is a measure of overall ventilation inhomogeneity in the lungs, which occurs at branch points in large or small airways, and is thought to represent small airways disease. Geometric alterations in very small airways of the exchange zone.53 Geometric alterations in very small airways of the lung, which occurs at branch points in large or small airways, and is thought to represent small airways disease. The present study suggests that the FEF75 is considerably more sensitive than FEV1 for detecting structural alterations. This reflects the closer agreement shown between abnormal LCI and FEF75 than FEV1, and supports the common view that FEF75 is more sensitive than FEV1 to small airway disease. Nevertheless, the high frequency of abnormal LCI in CF and the strong correlation between LCI and HRCT abnormalities demonstrate that the LCI is a better indicator of structural lung abnormalities than spirometry.

Because this study was cross-sectional, we can only speculate that the LCI will rise in parallel with advancing structural lung alterations in patients with CF who are followed over several years. This important question needs to be addressed in future longitudinal studies. Based on the present findings, it may nevertheless be argued that the MBW test should be used to monitor lung disease in young children with CF. As long as the LCI remains normal, the presence of structural lung abnormalities is unlikely and HRCT scanning is probably of limited value to verify disease progression. Only when the LCI starts to rise may an HRCT scan be needed to confirm disease progression. This could be a dose-saving strategy of particular importance for young children who are more sensitive to radiation. Further investigations are needed, however, before firm clinical recommendations regarding such a strategy can be established.

### Table 2

<table>
<thead>
<tr>
<th>LCI</th>
<th>Bronchiectasis</th>
<th>HRCT score</th>
<th>Air trapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>85 (71 to 98)</td>
<td>93 (83 to 100)</td>
<td>94 (82 to 100)</td>
</tr>
<tr>
<td>Specificity</td>
<td>50 (27 to 73)</td>
<td>65 (42 to 87)</td>
<td>43 (25 to 61)</td>
</tr>
<tr>
<td>FEV1</td>
<td>Sensitivity</td>
<td>19 (4 to 34)</td>
<td>26 (9 to 42)</td>
</tr>
<tr>
<td>Specificity</td>
<td>89 (74 to 100)</td>
<td>100 (100 to 100)</td>
<td>89 (78 to 100)</td>
</tr>
<tr>
<td>FEF75</td>
<td>Sensitivity</td>
<td>62 (43 to 80)</td>
<td>63 (45 to 81)</td>
</tr>
<tr>
<td>Specificity</td>
<td>83 (66 to 100)</td>
<td>88 (73 to 100)</td>
<td>75 (59 to 91)</td>
</tr>
</tbody>
</table>

LCI, lung clearance index; FEV1, forced expiratory volume in 1 s; FEF75, maximal expiratory flow when 75% of forced vital capacity was expired; HRCT, high-resolution CT.

Data shown as Spearman rank correlation coefficients (R^s^).

*p* < 0.05; **p** < 0.01; ***p** < 0.001.
Table 4  Agreement between LCI and spirometry classified as abnormal or normal

<table>
<thead>
<tr>
<th></th>
<th>FEV1 Abnormal</th>
<th>FEV1 Normal</th>
<th>FEF25 Abnormal</th>
<th>FEF25 Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>7</td>
<td>24</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>13</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>p</td>
<td>0.065</td>
<td></td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

LCI, lung clearance index; FEV1, forced expiratory volume in 1 s; FEF25, maximal expiratory flow when 75% of forced vital capacity was expired. p Values refer to the Yates corrected χ2 test.

It is acknowledged that both HRCT and LCI should be viewed as potential surrogate outcome markers in CF at this time. Their relationships to the long-term outcome of CF lung disease—as reflected in widely accepted outcome markers such as FEV1 or mortality—are not fully known. Nevertheless, when an aggressive approach to early CF lung disease is evaluated, more sensitive outcomes than FEV1 and mortality are definitely needed.

It is unlikely that a bias has influenced the general outcome of the study. The MBW parameters were determined without having information on the CT scan. Furthermore, patient identity, demographic information or lung function findings were not available to the observer scoring the images. Ideally, spirometry, MBW and HRCT scans should have been performed on the same day in a randomised fashion. All investigations were carried out in clinically stable patients during their annual review at the CF centre. They were undertaken on the same day in 26 of the patients (59%), and in all remaining patients but one there was only one day between any of the tests. The patient group included a sample of approximately one-third of all patients with CF attending the CF Center in Göteborg. This sample constituted all consecutive patients over a 30-month period aged <20 years who were investigated with spirometry and MBW as part of their annual review and, in addition, HRCT which is done routinely every third year in patients older than 5 years. There was a slight over-representation of males (61%) in the study population compared with 52% in the entire CF population. The study involved only children and teenagers who could perform spirometric tests and who were aged >5 years because the routine schedule includes HRCT scans only above that age. The relationships between LCI and HRCT abnormalities in younger and older patients therefore remain to be determined.

In conclusion, this cross-sectional analysis shows that the LCI is more sensitive than spirometry (FEV1 or FEF25) for detecting structural lung alterations in CF, and that a normal LCI indicates the absence of structural lung damage detectable by HRCT. The usefulness of LCI for detecting progression of CF lung disease compared with HRCT needs to be assessed in longitudinal studies.

Funding: Pim de Jong was supported by a British Columbia Lung Association Fellowship in Respiratory Medicine and a Canadian Institute of Health Research Michael Smith Foundation Transplant Research Training Award.

Competing interests: None.

REFERENCES

Cystic fibrosis


Lung alert

A new treatment proposed for lung transplant rejection

Despite intensive immunosuppressant therapy following lung transplantation, acute rejection occurs in over 50% of recipients within the first 6 months. Bronchiolitis obliterans syndrome (BOS) is related to acute rejection and independently contributes to mortality. It is thought that T cell depleting agents might reduce the incidence of these complications. Alemtuzumab is a humanised monoclonal antibody to the CD52 antigen which is expressed on T cells, B cells, monocytes, macrophages and platelets and causes lymphocyte depletion. The authors studied the effects of this agent on 12 patients with refractory acute rejection (RAR; ie, with biopsy proven rejection over at least 4 weeks) and 10 patients with BOS.

All patients had failed to respond to corticosteroids and antithymocyte globulin (ATG). The efficacy of alemtuzumab for the treatment of RAR was compared with ATG using the rejection grades on consecutive biopsies immediately before and after treatment. In patients with RAR, a significant and sustained reduction was observed in A and B grade biopsy rejection after alemtuzumab treatment (p<0.001). Treatment resulted in a significant reduction in the severity of RAR immediately, while there was no change in the mean severity after treatment with ATG. There was no significant change in mean forced expiratory volume in 1 s in BOS after treatment, but there was an improvement in the BOS scores. One-year graft survival for RAR after alemtuzumab treatment was 86%. Survival with BOS at 1 and 2 years was 69%.

Alemtuzumab is potentially useful in the treatment of RAR or BOS in lung transplant recipients who have failed prior conventional therapy, but this study is limited by the lack of a control arm. The authors point out that a randomised controlled study is required before recommending this treatment over existing therapies.


Ghassan El-Sayed

Correspondence to: G El-Sayed, ST1 in Respiratory Medicine, Homerton University Hospital, London, UK; ghassanovii@yahoo.co.uk