MULTIPLE-BREATH INERT GAS WASHOUT AND SPIROMETRY VS. STRUCTURAL LUNG DISEASE IN CYSTIC FIBROSIS

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KEY-WORDS: Cystic Fibrosis, High-Resolution CT, Lung Clearance Index, Spirometry, Ventilation inhomogeneity.

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ABSTRACT
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.

OBJECTIVES: 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: This is a retrospective study of 44 consecutive CF patients aged 5-19 yrs (mean 12 yrs). At an annual check-up inspiratory and expiratory HRCT scans, LCI and spirometry (FEV₁ and FEF75) were recorded. Abnormal structure was defined as a composite HRCT score >5 per cent, the presence of bronchiectasis, or air trapping >30 per cent. 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 ranged from 85-94% for LCI, 19-26% for FEV₁, and 62-75% for FEF75. Specificity ranged from 43-65% for LCI, 89-100% for FEV₁, and 75-88% for FEF75. LCI correlated better with HRCT scores (R = 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 findings of abnormal LCI in some patients with normal HRCT scans suggest that LCI may be even more sensitive than HRCT to lung involvement in CF.
INTRODUCTION

Cystic fibrosis (CF) lung disease is characterized 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 the recent decades the median survival age of CF patients 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 CF lung disease progression are therefore sought (1, 2, 6).

Spirometry (forced expiratory volume in one second; 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 range (10) or show a slow rate of progression (2, 10-12), even though they probably have lung disease that progresses faster than reflected in spirometric measurements. Apparently FEV₁ is not very sensitive to early CF lung disease (13-15). Second, reliable forced expiratory manoeuvres are difficult to obtain in children under the age of 5 years, with testing in the infant and pre-school age groups being largely confined to specialist laboratories (16-18). 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 computed tomography (HRCT) of the chest has repeatedly been shown to be more sensitive for both early disease detection (19-22) and to follow disease progression (23-25) in CF relative to FEV₁. Bronchiectasis, the hallmark of CF lung disease, can be identified early in the course of CF on HRCT (20) and is the HRCT abnormality that is most sensitive to the disease progression (6, 23-24). HRCT is the current gold standard to evaluate bronchiectasis (26). Air trapping, reflecting small airways disease, is another important feature of CF lung disease that can be demonstrated with expiratory HRCT scans (27, 28). Furthermore, mucus plugging and airway wall thickening can be seen on inspiratory scans in CF (29-34). The use of CT in CF is, however, restricted to examinations with a relatively long interval of one to two years. Despite substantial reduction of the radiation dose related to the chest CT examination in the last decade, its use should be limited to the absolute minimum with the aim to minimize radiation dose (35-37). Hence, CT scanning is not a feasible method to monitor CF lung disease over short time intervals. In addition, routine CT in young children is troublesome since sedation and a technically demanding procedure are required to obtain high quality images (38). Finally young children are thought to be particularly sensitive to the negative effects of radiation (39, 40).

Recently it has 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 (13-15, 18). The MBW method is not new but has been used for over 50 years (41), and pediatric studies were
published already during the 1960s (42) and the 1970s (43). Breath-by-breath studies in children using modern PC technology were reported by Wall (44) and Kraemer (45) more than 20 years ago. LCI has advantages over spirometry in that it can be measured easily in all age groups and that normative values are similar from early childhood to adulthood (15). It discriminates better between CF and healthy subjects than either FEV₁ or body plethysmography (residual volume /total lung capacity ratio) (15). The LCI has also been shown to be an earlier and stronger indicator of disease progression than spirometry (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 hypothesized that LCI would agree better with the presence of HRCT abnormalities than spirometry.

**METHODS**

**Subjects**

This is a retrospective study of spirometry, MBW and HRCT recordings obtained over a 30-month period in 44 consecutive CF patients aged less than 20 years. All patients underwent these investigations as part of their routine annual review at the Göteborg CF centre, where HRCT is performed routinely every third year in patients over 5 years of age. Chronic colonisation with 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 Göteborg.

**Measurement of lung structure**

Lung structure was evaluated using HRCT scans. The scanning protocol has previously been reported (24) and is described in more detail in the on-line supplement. Briefly, images were obtained from lung apex to base at 15 mm (children) and 10 mm (adults, ≥18 yrs) 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 scored in random order by an experienced observer (21, 24, 25) using an adapted scoring system developed by Brody et al (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 per cent of the maximal score (24, 47). A composite CT score greater than 5 per cent was set as abnormal based on findings in 15 normal individuals that were scored intermixed with CF patients. All normal individuals had a CT score below 5% (unpublished data). Air trapping involving more than 30% the lung was also defined as abnormal. It is known that air trapping can be seen in normal individuals (48), and in other studies in the bronchiolitis obliterans syndrome a 32% cut-off has been used (49).
Measurement of lung function

The lung function measurements are described in detail in the on-line supplement. Briefly, spirometric measurements included FEV\(_1\), forced vital capacity (FVC) and the maximal expiratory flow when 75% of FVC was expired (FEF\(_{75}\)). They were related to normative values for Swedish children (7-18 years) (50) or adults (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 sulphur hexafluoride (SF\(_6\)) washout was performed using a mass spectrometer for gas analysis, as previously described in detail (15). The LCI was calculated as the number of lung volume turnovers (i.e. the cumulative expired volume divided by the functional residual capacity) needed to lower the end-tidal tracer gas concentration to 1/40\(^{th}\) of the starting concentration (15). 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 (15).

Statistical analysis

Abnormal structure was defined as a composite HRCT-score >5%, presence of bronchiectasis, or air trapping >30%. 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\(_1\) or FEF\(_{75}\) below -1.96 z-scores. Proportions of patients with normal or abnormal FEV\(_1\) or LCI results in relation to HRCT classifications (cross tabulations) were compared using the Yates corrected \(\chi^2\)-test. The sensitivity and specificity were determined for LCI, FEV\(_1\), and FEF\(_{75}\) with respect to abnormal HRCT composite score, the presence of bronchiectasis, and the presence of abnormal air trapping, as diagnosed by HRCT. 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 around the sensitivity and specificity findings were calculated as follows: 95CI for \(p = p +/- 1.96*SE\); 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 HRCT composite score, bronchiectasis, mucus plugging, airway wall thickness, parenchyma and air trapping. A p-value <0.05 was accepted as statistically significant. Statistica 6.0 (StatSoft, Tulsa, OK, USA) was used for the statistical analyses.

RESULTS

The study group included 27 males and 17 females aged 5.4-19.6 yrs (mean and median age 12.2 years). Twenty-one patients (48%) were homozygotes and 19 (43%) were heterozygotes for \(\Delta F508\). Eleven patients were chronically colonized with \textit{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 vs. 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. Out of the 27 patients with a HRCT score above 5%, 25 had an abnormally elevated LCI, whilst 17 showed abnormal FEF75 results and seven had reduced FEV1. Seventeen patients had normal HRCT scores (≤5%). Six of these had elevated LCI, two had abnormal FEF75, and all had FEV1 within normal limits.

LCI and FEF75 showed closer agreement with findings of structural lung abnormalities than did FEV1 (Table 1 A, B and C).

| Table 1 A. Agreement between LCI and structural lung changes classified as abnormal or normal. (LCI is the lung clearance index. HRCT score >5% is defined as abnormal structure. More than 30% air trapping is defined as abnormal. P-values refer to the Yates corrected χ²-test.) |
|-----------------------------------------------|-------------------|-----------------|-----------------|
|                                | Bronchiectasis    | HRCT score      | Air trapping    |
|                                | Yes   | No   | >5% | ≤5% | >30% | ≤30% |
| Abnormal                       | 22    | 9    | 25  | 6   | 15   | 16   |
| LCI Normal                     | 4     | 9    | 2   | 11  | 1    | 12   |
|                               | p=0.033 | p<0.001 | p=0.027 |

| Table 1 B. Agreement between FEV1 and structural lung changes classified as abnormal or normal. |
|-----------------------------------------------|-------------------|-----------------|-----------------|
|                                | Bronchiectasis    | HRCT score      | Air trapping    |
|                                | Yes   | No   | >5% | ≤5% | >30% | ≤30% |
| Abnormal                       | 5     | 2    | 7   | 0   | 4    | 3    |
| FEV1 Normal                    | 21    | 16   | 20  | 17  | 12   | 25   |
|                               | p=0.761 | p=0.062 | p=0.413 |

| Table 1 C. Agreement between FEF75 and structural lung changes classified as abnormal or normal. |
|-----------------------------------------------|-------------------|-----------------|-----------------|
|                                | Bronchiectasis    | HRCT score      | Air trapping    |
|                                | Yes   | No   | >5% | ≤5% | >30% | ≤30% |
| Abnormal                       | 16    | 3    | 17  | 2   | 12   | 7    |
| FEF75 Normal                   | 10    | 15   | 10  | 15  | 4    | 21   |
|                               | p=0.008 | p=0.003 | p=0.004 |
The LCI had the best sensitivity (85 to 94%) to detect structural lung abnormalities (bronchiectasis, composite HTCT score, and air trapping) (Table 2). For FEV₁, sensitivity ranged from 19 to 26%, and for FEF75 from 62 to 75%. The specificity in the detection of structural lung disease was, however, higher for the FEV₁ (89-100%) and for FEF75 (75-88%), than for the LCI (43-65%).

<table>
<thead>
<tr>
<th></th>
<th>Bronchiectasis</th>
<th>HRCT score</th>
<th>Air trapping</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>85% (71; 98)</td>
<td>93% (83; 100)</td>
<td>94% (82; 100)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>50% (27; 73)</td>
<td>65% (42; 87)</td>
<td>43% (25;61)</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>19% (4; 34)</td>
<td>26% (9; 42)</td>
<td>25% (4; 46)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>89% (74; 100)</td>
<td>100% (100; 100)</td>
<td>89% (78; 100)</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>62% (43; 80)</td>
<td>63% (45; 81)</td>
<td>75% (54; 96)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>83% (66; 100)</td>
<td>88% (73; 100)</td>
<td>75% (59; 91)</td>
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</table>
The LCI showed a markedly stronger correlation to all six structural abnormality scores ($R_s$ 0.65 to 0.85) than the FEV$_1$ ($R_s$ -0.35 to -0.62) or FEF$_{75}$ ($R_s$ -0.44 to -0.66) did (Table 3).

**Table 3.** Correlation between LCI, FEV$_1$, FEF$_{75}$ (standard deviation scores) and structural abnormalities. (LCI is the lung clearance index. Data given are Spearman rank correlation coefficients ($R_s$) * $p<0.05$ ** $p<0.01$, and *** $p<0.001$.)

<table>
<thead>
<tr>
<th>HRCT score</th>
<th>Bronchiectasis</th>
<th>Mucus plugs</th>
<th>Airway wall thickness</th>
<th>Parenchyma</th>
<th>Air trapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>0.85***</td>
<td>0.64***</td>
<td>0.73***</td>
<td>0.76***</td>
<td>0.79***</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>-0.62***</td>
<td>-0.35*</td>
<td>-0.45**</td>
<td>-0.55***</td>
<td>-0.58***</td>
</tr>
<tr>
<td>FEF$_{75}$</td>
<td>-0.66***</td>
<td>-0.44**</td>
<td>-0.49***</td>
<td>-0.56***</td>
<td>-0.65***</td>
</tr>
</tbody>
</table>

The proportion of patients with normal or abnormal FEF$_{75}$ showed better agreement with LCI classification than the FEV$_1$ did (Table 4), but the overall correlation with LCI was similar (FEF$_{75}$: $R_s$ = -0.66; $p<0.001$, and FEV$_1$: $R_s$ = -0.63; $p<0.001$).

**Table 4.** Agreement between LCI and spirometry classified as abnormal or normal. (LCI is the lung clearance index. P-values refer to the Yates corrected $\chi^2$-test.)

<table>
<thead>
<tr>
<th></th>
<th>FEV$_1$</th>
<th></th>
<th>FEF$_{75}$</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Abnormal</td>
<td>Normal</td>
<td>Abnormal</td>
<td>Normal</td>
</tr>
<tr>
<td>LCI</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>
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$p=0.065$ $p=0.006$
DISCUSSION

This study aimed to determine the sensitivity of spirometry and MBW to detect bronchiectasis and other structural lung changes as measured by HRCT 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 relative to FEV\textsubscript{1} or FEF\textsubscript{75}, and that LCI would correlate better than spirometry with structural lung changes.

It was shown that LCI was markedly more sensitive than either FEV\textsubscript{1} or FEF\textsubscript{75} with respect to structural lung abnormalities (bronchiectasis, air trapping and combined abnormalities). The sensitivity of LCI ranged between 85 and 94%. Out 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 CF subject 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, including very small airways close to or within the gas exchange zone (53). Geometric alterations in very small airways due to airway wall thickening or mucus accumulation can result in uneven ventilation distribution, but such alterations may not be detected by HRCT. Thus, the LCI may be abnormally elevated in subjects with CF lung disease but lack HRCT evidence of structural lung changes. Another explanation for the somewhat low specificity of the LCI could be that our HRCT protocol might have missed some structural aberrations. Only three expiratory images were obtained which may limit the ability to detect the full extent of air trapping. In addition, for the inspiratory CT scans there are relatively large gaps of 15 mm between the CT images, which potentially result in underestimation of the structural abnormalities (36). For the detection of bronchiectasis it has recently been shown that bronchiectasis can be missed using an incremental HRCT protocol compared to a full-lung HRCT scan (36, 54). Conventional HRCT is thus no longer the ideal method and is therefore more and more replaced by full-lung CT scans.

We set an abnormal composite CT score as a score greater than 5 per cent. In 15 normal individuals that were scored intermixed with CF patients, all normal individuals had a CT score below 5% (unpublished data). We defined an abnormal amount of air trapping when more than 30% of the lung was involved. It is known that air trapping can be seen in normal individuals (48), and in other studies in the bronchiolitis obliterans syndrome a 32% cut-off has been used (49).

As expected, the FEV\textsubscript{1} had a low sensitivity making it of little value in early disease detection in CF. This corresponds to previous studies showing structural disease in about 30% of the patients with normal spirometry (21, 24, 25). The better specificity of FEV\textsubscript{1} compared to the LCI means that when FEV\textsubscript{1} starts to decline, major bronchiectasis or other structural damage is likely to be present. On the other hand, many patients in the present study who had normal FEV\textsubscript{1} or FEF\textsubscript{75} results showed evidence of quite advanced structural lung damage. The present study suggests that the FEF\textsubscript{75} is considerably more sensitive than FEV\textsubscript{1} to detect structural alterations. This reflects the closer agreement shown between abnormal LCI and FEF\textsubscript{75} compared to FEV\textsubscript{1}, and supports the common view that FEF\textsubscript{75} is more sensitive than FEV\textsubscript{1} to small airway disease. Nevertheless, the high frequency of
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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 the present study is cross-sectional it can only be speculated that the LCI will rise in parallel with advancing structural lung alterations in CF patients 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 to verify disease progression is probably of limited value. 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 given. It is acknowledged that both HRCT and LCI should be viewed as potential surrogate outcome markers in CF at this point in time. Their relationships to 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. Furthermore, patient identity, demographic information or lung function findings were not available to the observer scoring the images. Ideally, spirometry, MBW and HRCT should have been performed on the same day, in a randomized fashion. All investigations were done on 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 CF patients attending the CF center in Göteborg. This sample constituted all consecutive patients, over a 30-month period, aged less than 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 in contrast to 52% in the entire CF population. The study involved only children and teenagers who could perform spirometry and who were aged >5.0 years because the routine schedule includes HRCT 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 demonstrates that the LCI is more sensitive than spirometry (FEV₁ or FEF₇₅) to detect structural lung alterations in CF, and that a normal LCI indicates the absence of structural lung damage detectable by HRCT. The usefulness of LCI to detect progression of CF lung disease compared to HRCT needs to be assessed in longitudinal studies.

CONFLICTS OF INTERESTS
The authors have no conflicting interests to declare.
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FIGURE LEGENDS

Figure 1. Lung function (LCI, FEV₁ and FEF₇₅) expressed as z-scores plotted vs. age in 44 children and teenagers with CF. Black filled circles denote LCI (lung clearance index), open circles FEV₁, and grey filled circles FEF₇₅. The horizontal hatched lines denote the upper and lower limits of normality (LLN) for the lung function variables.

Figure 2. Lung function (LCI, FEV₁ and FEF₇₅) expressed as z-scores plotted vs. HRCT (composite scores) in 44 children and teenagers with CF. Black filled circles denote LCI (lung clearance index), open circles FEV₁, and grey filled circles FEF₇₅. 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.

REFERENCES
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Figure 1

Lung function (z-scores)
Figure 2

Lung function (z-scores)

HRCT score (%)
ON-LINE SUPPLEMENT

MULTIPLE-BREATH INERT GAS WASHOUT AND SPIROMETRY VS. STRUCTURAL LUNG DISEASE IN CYSTIC FIBROSIS

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METHODS

Subjects

This is a retrospective study of spirometry, MBW and HRCT recordings obtained over a 30-month period in 44 consecutive CF patients aged less than 20 years. All patients underwent these investigations as part of their routine annual review at the Göteborg CF center, where HRCT is done routinely every third year in patients over 5 years of age. Chronic colonisation with 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 Göteborg.

Measurement of lung structure

Lung structure was evaluated using HRCT scans. For children a multi detector CT scanner (General Electric light speed ultra, 8 rows of detectors, GE Medical Systems, Milwaukee, WI) was used. Scans were obtained using a beam current of 120 mA, an exposure time of 0.5 s (60 mAs) and a beam potential of 120 kV from lung apex to base at 15 mm 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. For adults a PQ 6000 scanner (Picker International Inc., Highland Heights, OH) was used. Scans were obtained using a beam current of 160 mA, a 1.0 s exposure time (160 mAs) and a beam potential of 120 kV from lung apex to lung base at 10 mm intervals using 1.5 mm thick slices in inspiration. In addition three expiratory HRCT images were obtained through the upper, middle and lower lung zones.

All scans were reconstructed with a high-spatial frequency algorithm (bone), blinded to date and patient identification, and scored in random order by an experienced observer (E1-4) using an adapted scoring system developed by Brody et al (E5). The scoring system evaluates the five lung lobes and the lingula as a sixth lobe on the inspiratory HRCT scans for: 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; and parenchymal abnormalities (extent of opacities, ground glass pattern and cysts and bullae). On the expiratory HRCT scans hyperinflation (gas trapping) was scored in right and left middle, upper and lower lung zones.

We set an abnormal composite CT score as a score greater than 5 per cent. In 15 normal individuals that were scored intermixed with CF patients, all normal individuals had a CT score below 5% (unpublished data). We defined an abnormal amount of air trapping when more than 30% of the lung was involved. It is known that air trapping can be seen in normal individuals (E6), and in other studies in the bronchiolitis obliterans syndrome a 32% cut-off has been used (E7).
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The maximum possible composite HRCT score is 207 (E5). Component HRCT scores were calculated by adding the component scores from the six lobes. For statistical analysis the composite HRCT score and component HRCT scores were expressed on a 0-100 scale (per cent) (E5).

Measurement of lung function

Spirometry: Forced expiratory maneuvers were measured using the Jaeger Masterslab equipment (Erich Jaeger AG, Wurzburg, Germany). A minimum of three technically acceptable maximal forced expiratory maneuvers were performed. Patients were encouraged to exhale for at least three seconds and were required to produce two reproducible FEV\textsubscript{1} results (lower being within 5% of the higher). The best forced vital capacity (FVC) and FEV\textsubscript{1} results were noted. The recording with the highest sum of FEV\textsubscript{1} and FVC was used to obtain the maximal expiratory flow when 75% of FVC was expired (FEF\textsubscript{75}). Spirometry findings were related to Swedish normative data for children (6-18 years) (E8) and adults (≥20.0 yrs of age) (E9, E10), respectively. The lower limits of normality (LLN) were defined as the predicted mean minus 1.96 residual standard deviations (RSD).

Multiple-breath sulphur hexafluoride washout (MBW): The patients were investigated in the sitting position. During the MBW tests the younger patients (<10 yrs) watched a video while the older patients watched a tidal volume trace on a computer screen and were instructed to breathe regularly with a tidal volume between 10 and 15 mL*kg\textsuperscript{-1} body weight. All participants used a nose clip and breathed through a Fleisch no.1 pneumotachometer (PNT) (Metabo SA, Lausanne, Switzerland) via a mouthpiece. A sampling tube from a mass spectrometer was introduced into the middle of the air stream between the mouthpiece and the PNT through a short connecting piece. The external dead space was 15 mL. The PNT was connected to a differential pressure transducer (MP 45-14-87; Validyne Corp., CA, USA;+2 cmH\textsubscript{2}O) and the flow signal was demodulated and amplified (CD12 C-2A; Validyne Corp.). Gas concentrations were measured using a respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark). The PNT was calibrated with separate calibration constants for inspiratory and expiratory flows using a precision syringe. Recorded inspiratory and expiratory flows and volumes were converted to body temperature, ambient pressure, and saturated water vapor conditions. Gas samples and flow signals were aligned in time. The sample flow of the mass spectrometer was approximately 20 mL*min\textsuperscript{-1} and the gas concentration signals were updated at a rate of 33.3 Hz. All signals were recorded at 100 Hz by a computer through a 16-channel AD-conversion board (DAS-1602; Keithley Metrabyte, Taunton, MA, USA). The software corrected the flow signal sample-by-sample for changes in dynamic viscosity caused by the variations in gas composition. One of the two inert tracer gases (SF\textsubscript{6}) was used for the evaluations presented in this paper. Helium was included for other assessments of ventilation distribution not presented here.

Each test consisted of two phases: a washin phase during which a dry gas mixture containing 4% SF\textsubscript{6} (sulphur hexafluoride), 4% He (helium) 21% O\textsubscript{2} (oxygen), and balance N\textsubscript{2} (nitrogen) was administered using a bias flow applied via a T-piece on the external opening of the PNT. Washin was continued until the inspiratory and expiratory SF\textsubscript{6} concentrations were stable and equal, plus another 30 s. At this moment the bias flow was stopped during expiration by disconnecting the T-piece and washout was started. The
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patients breathed room air during the washout phase. The washout phase continued until the end-tidal SF\textsubscript{6} concentration was <0.1% (i.e. 1/40th of the starting concentration) for at least three breaths. The functional residual capacity (FRC) was determined from the cumulative exhaled volume of marker gas (SF\textsubscript{6}) divided by the difference in end-tidal SF\textsubscript{6} concentration at the start of the washout and end-tidal SF\textsubscript{6} concentration at completion of the washout. The number of lung volume turnovers, i.e, the cumulative expired volume of gas (sum of tidal volumes) divided by the FRC, was calculated for each subsequent breath. The cumulative expired volume was corrected for the external dead space in each breath. The lung clearance index (LCI) was calculated as the number of turnovers needed to lower the end-tidal tracer gas concentration to 1/40\textsuperscript{th} of the starting concentration. 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 (E11).

HRCT, MBW and spirometry were performed on the same day in 26 patients, and in all remaining patients but one, there was only one day between any of the tests.

Statistical analysis

Abnormal structure was defined as a composite HRCT-score >5%, presence of bronchiectasis, or air trapping >30%. 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. 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 $\chi^2$-test. The sensitivity and specificity were determined for LCI, FEV\textsubscript{1}, and FEF\textsubscript{75} with respect to abnormal HRCT composite score, the presence of bronchiectasis, and the presence of abnormal air trapping, as diagnosed by HRCT. 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 around the sensitivity and specificity findings were calculated as follows: 95CI for $p = p +/- 1.96*SE$; $SE$ 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\textsubscript{1}, LCI and FEF\textsubscript{75} with respect to HRCT composite score, bronchiectasis, mucus plugging, airway wall thickness, parenchyma and air trapping. A p-value <0.05 was accepted as statistically significant. Statistica 6.0 (StatSoft, Tulsa, OK, USA) was used for the statistical analyses.

REFERENCES

E3. de Jong PA, Ottink MD, Robben SG, Lequin MH, Hop WC, Hendriks JJ, Pare PD, Tiddens HA. Pulmonary disease assessment in cystic fibrosis: comparison of CT scoring...
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