ORIGINAL ARTICLE

Evaluation of pulmonary disease using static lung volumes in primary ciliary dyskinesia

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ABSTRACT

Background In primary ciliary dyskinesia (PCD) lung damage is usually evaluated by high-resolution CT (HRCT).

Objective To evaluate whether HRCT abnormalities and Pseudomonas aeruginosa infection were better predicted by spirometry or plethysmography.

Methods A cross-sectional study performed in consecutive patients with PCD who underwent sputum culture, spirometry, plethysmography and HRCT within 48 h. Principal component analysis and soft computing were used for data evaluation.

Results Fifty patients (26 children) were studied. P aeruginosa infection was found in 40% of the patients and bronchiectasis in 88%. There was a correlation between infection with P aeruginosa and extent of bronchiectasis (p = 0.009; r = 0.367) and air-trapping (p = 0.03; r = 0.315). Moreover, there was an association between infection with P aeruginosa and residual volume (RV) values >150% (p = 0.04) and RV/total lung capacity (TLC) ratio >140% (p = 0.001), but not between infection with P aeruginosa and forced expiratory volume in 1 s (FEV1) <80%, or forced expiratory flow between 25% and 75% of forced vital capacity (FVC) (FEF25–75% <70% or FEV1/FVC <70% (<80% in children). Severity of the total lung impairment on chest HRCT directly correlated with RV when expressed as per cent predicted (p = 0.003; r = 0.423), and RV/TLC (p <0.001; r = 0.513) or when expressed as z scores (p = 0.002, r = 0.451 and p <0.001, r = 0.536 respectively). Principal component analysis on plethysmographic but not on spirometry data allowed recognition of different severities of focal air trapping, atelectasis and extent of bronchiectasis.

Conclusions Plethysmography better predicts HRCT abnormalities than spirometry. Whether it might be a useful test to define populations of patients with PCD who should or should not have HRCT scans requires further longitudinal studies.

INTRODUCTION

In primary ciliary dyskinesia (PCD), abnormal motility of respiratory cilia and impaired mucociliary clearance result in recurrent infections of the airways, eventually causing permanent parenchymal damage, and progressive decline in lung function.1

Chest high-resolution CT (HRCT) has become the method of choice to evaluate structural airway changes,2 and is used in a number of chronic respiratory diseases including PCD.3, 4

However, there is no evidence that regular CT scans affect outcome in PCD5 and the potential lifetime cumulative radiation exposure should be considered.6, 7

PCD guidelines suggest that lung function should be evaluated at every visit in cooperative children1 5 8 since it has been shown that the severity of structural abnormalities may correlate with impairment in forced expiratory volume in 1 s (FEV1).3, 4 However, a decreased FEV1 is not indicative of the site of airway obstruction7 and, as in cystic fibrosis,10 there are preliminary suggestions that PCD is characterised by marked peripheral airway dysfunction.11 Since small airways obstruction may lead to air trapping and to a consequent increase in residual volume (RV) and RV/total lung capacity (TLC) ratio,9 we hypothesised that raised functional residual capacity (FRC) made by plethysmography (FRCpleth), and RV and/or RV/TLC ratio, indirect calculated estimates of pulmonary hyperinflation and lung restriction respectively, may be more sensitive than spirometry to structural changes as shown by HRCT in PCD. The aim of the study was to determine the
correlations between HRCT abnormalities and dynamic or static lung function parameters in patients with PCD to assess their potential use as clinical monitoring tools in PCD.  

**MATERIALS AND METHODS**

**Subjects**

Between March 2008 and May 2010, we enrolled all consecutively newly diagnosed patients with PCD aged ≥6 years followed up in the Department of Paediatrics of the University of Pisa. PCD was diagnosed on standard criteria. We obtained sputum, cough swab or pharyngeal aspirate cultures, spirometry, body plethysmography and a HRCT of the chest. A sputum culture was collected, preferably by expectoration. Pharyngeal aspirate was obtained in uncooperative children after an overnight fast using a disposable catheter connected to a mucus extractor inserted into the mouth to a depth of 7–10 cm and drawn back while applying gentle suction.

Informed consent was obtained from adult patients or from the parents of children. The study protocol was approved by the local Hospital Ethical Committee.

**Ciliary motion analysis and ultrastructural studies**

Samples were obtained from the inferior turbinate using a cytology brush (Microvasive, Milford, Massachusetts, USA) for immediate light microscopy, transmission electron microscopy and cell cultures. Ciliary morphology, motion pattern, beat frequency, ultrastructural studies and ciliary activity after ciliogenesis in culture were evaluated.

Ciliary motion analysis, ultrastructural assessment and cultures were performed by different operators each one blind to the results obtained by the others.

**Lung function**

All lung function testing (Master Screen Body equipment; Jaeger, Wuerzburg, Germany) was performed by experienced doctors (MF, MD) utilising standard American Thoracic Society methodology. 

For immediate light microscopy, transmission electron microscopy and cell cultures, the results obtained by the others.

The principal component analysis (PCA) methodology was applied to display data. PCA is a mathematical linear transformation aimed at reducing the dimensionality of (number of variables in) a dataset to a manageable level while retaining as much as possible of the variance present in the original dataset.

Data from correlated groups of variables are used to calculate a smaller number of uncorrelated variables (principal components) to simplify further analyses. We used the eigenvalue decomposition to obtain the first three principal components in our dataset (denoted Y1, Y2 and Y3) which are associated with 99% of the variance.

Thus, a severity class (from 1 to 3) for total lung impairment was obtained (class of severity 1 for total score of 0–6, class 2 for total score of 7–12, class 3 for total score of 13–18).

**Soft computing analysis of data**

Data were analysed with soft computing methodologies. Basic elements of soft computing and the application of intelligent control have been recently introduced. The soft computing-based modelling approach was applied to either flow-volume data or body plethysmography or both to develop a model predictive of chest HRCT scores. The model was identified by means of a self-organising artificial neural network (ANN). ANNs are mathematical models in which distributed adaptable parameters are modified through a learning process according to real data. Kohonen self-organising map (KSOM) predictive models were identified to classify chest HRCT scores, (total lung impairment, severity of bronchiectasis, extent of bronchiectasis, peribronchial thickening, mucous plugging, atelectasis, focal air-trapping), starting from flow-volume parameters (FEV1, FVC, FEV1/FVC, FEF25–75%), body plethysmography data (FRCPleth, RV, TLC, RV/TLC) and parameters of airway mechanics (Raw, sRaw, sReff). To check the generalisation capability of the neural network, a 10-fold cross-validation process was carried out. In this work, we fixed a 5×5 neurons KSOM with the parameters α(T) = 0.8 and a training of 5000 epochs, which allowed us to obtain the best performance of the model. Further details on KSOM ANNs are reported in the supplementary material online.

**Statistical analyses**

The principal component analysis (PCA) methodology was applied to display data. PCA is a mathematical linear transformation aimed at reducing the dimensionality of (number of variables in) a dataset to a manageable level while retaining as much as possible of the variance present in the original dataset. 

Data from correlated groups of variables are used to calculate a smaller number of uncorrelated variables (principal components) to simplify further analyses. We used the eigenvalue decomposition to obtain the first three principal components in our dataset (denoted Y1, Y2 and Y3) which are associated with 99% of the variance.

There are no data in the published literature to inform a power calculation, so the sample size was opportunistic. Baseline variables were expressed as group mean ± SD or as median and IQR when the variables were non-normally distributed. Moreover, to allow comparison of observations from different normal distributions, all lung function data were expressed as SD (z) scores. Z-score transformation was carried out by using the equation $z = (x - \mu)/\sigma$, where $x$ is the raw score or observation to be standardised, $\mu$ is the mean of the reference population, and $\sigma$ is the SD of the reference population. Differences between means and distributions were evaluated by the two-tailed Student t test.

Correlations between continuous non-normally distributed variables were assessed using Spearman’s rank correlation coefficients. One-way analysis of variance (ANOVA) comparing the values of plethysmography indexes between the severity class for total lung impairment at HRCT was also applied. The $\chi^2$ test was used to evaluate the association between *Pseudomonas aeruginosa* (alone or with other bacterial infection) and RV >150%, RV/TLC ratio >140% and FEV1 <80% or FEV1/FVC <70% of predicted. The association between *P aeruginosa* and FEV1/FVC <80% predicted for children and <70% predicted for adults was also evaluated. These cut-offs were selected because...
they were previously demonstrated to correlate with air-trapping on HRCT in children. 

A p value <0.05 was considered statistically significant. All statistical calculations were performed using SPSS V18.0 for Windows (XP/Vista/7).

RESULTS

Fifty patients were studied. Twenty-six were children (19 boys and seven girls, age range 6–17 years, median 11 years; IQR 5.25) and 24 adults (nine men and 15 women, age range 18–47 years, median 30.5 years; IQR 9.5).

Infection with *P. aeruginosa* (with or without other bacteria) was found in 20 (40.0%) patients, seven of whom were children (27% infected). Other organisms (*Serratia marcescens*, *Rhodococcus equi*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *Alcaligenes xylosoxidans*) were found in 12 (24.0%) subjects.

Results of spirometry and plethysmography expressed as per cent of predicted and as z scores are reported in table 1.

HRCT Bhalla score was 0 in 5 subjects, 1 in 12, 2 in 27, and 3 in the remaining 8 subjects. Bronchiectasis was documented in 44 (88%) patients, 21 of whom were children. Bronchiectasis was present in multiple lobes in 36/44 (82%) subjects. The distribution of bronchiectasis is detailed in table 2.

Patient age was inversely correlated with per cent predicted FEV<sub>1</sub> (p = 0.038; r = −0.294), with FEV<sub>1</sub>/FVC (p = 0.008; r = −0.572) and directly correlated with FVC (p = 0.001; r = 0.451), with FRCPleth (p < 0.001; r = 0.599), RV (p < 0.001; r = 0.623), TLC (p < 0.001; r = 0.646) and RV/TLC (p = 0.034; r = 0.313). There was no correlation between patient age and Raw, sRaw or sReff per cent predicted.

Considering z scores, patient age was inversely correlated with FEV<sub>1</sub> (p = 0.02; r = −0.322), FVC (p = 0.007; r = −0.576), but not with FEV<sub>25</sub>–75% or FEV<sub>1</sub>/FVC, and directly correlated with RV (p = 0.003; r = 0.423) (figure 1A) and RV/TLC (p < 0.001; r = 0.513) (figure 2A) per cent predicted, but not with TLC. This was confirmed with lung function z scores; severity of the total lung impairment on chest HRCT was inversely correlated with FEV<sub>1</sub> (p = 0.02; r = −0.322), FVC (p = 0.007; r = −0.576), but not with FEV<sub>25</sub>–75% or FEV<sub>1</sub>/FVC, and directly correlated with RV (p = 0.003; r = 0.423) (figure 1B) and RV/TLC (p < 0.001; r = 0.513) (figure 2B) and FRCPleth (p = 0.039; r = 0.305), but not with TLC. The correlations between the different HRCT parameters (severity and extent of bronchiectasis, peribronchial thickening, extent of mucus plugging, consolidation or atelectasis and air trapping) and lung function results are reported in tables 3 and 4. As can be seen there were some negative correlations between different HRCT parameters and some per cent predicted spirometry values (table 3) which disappeared when z scores were analysed (table 4). By contrast the positive correlations between severity and extent of bronchiectasis and air trapping with RV and RV/TLC were dependent on whether per cent predicted (table 3) or z scores (table 4) were used.

Moreover, plethysmography indices in patients with different HRCT class severity were found to be significantly different according to one-way ANOVA (p = 0.026 for FRCPleth, p = 0.007 for RV and p = 0.001 for RV/TLC).
Only weak or no correlations were found between the different HRCT parameters and Raw, sRaw and sReff depending on whether per cent predicted values (table 5) or z-scores (table 4) were considered.

Moreover, there was a direct correlation between the age of patients and severity of total lung impairment on chest HRCT \((p=0.002; r=0.425)\), and severity \((p=0.004; r=0.404)\) and extent of bronchiectasis \((p<0.001; r=0.510)\), but not with focal air-trapping scores or other parameters.

PCA performed using the body plethysmography data is depicted in figure 3, which shows three-dimensional scatter plots of the first four principal components evaluated after processing the original body plethysmography variables \(\text{FRCpleth, RV and RV/TLC ratio per cent predicted}^\text{a}\) and TLC ratio z scores and total lung impairment severity score at chest HRCT.

Regarding the extent of bronchiectasis, peribronchial thickening and mucous plugging to be discriminated. The PCA topological study of flow-volume data and of airway mechanics parameters \((\text{Raw, sRaw and sReff})\) does not permit any discrimination of chest HRCT scores. The PCA topological study does not identify patients with \(P.\ aeruginosa\) infection. Further results obtained by KSOM models are reported in the supplementary material online.

DISCUSSION

Statement of principal findings

To our knowledge this is the largest dataset of contemporaneous spirometry, plethysmography and HRCT in patients with PCD. HRCT was performed in all patients aged 6 years or older because it is known that from this age many patients already have some degree of bronchiectasis\(^b\) and from this age patients can both cooperate with the procedure and be able to perform reproducible lung function testing.\(^c\)

We show that soft computing based on plethysmographic lung volumes, but not spirometry, is sensitive to a range of HRCT abnormalities. Reduction in lung function associated with bronchiectasis has been previously reported in patients with PCD.\(^d\)\(^e\)\(^f\)\(^g\) However, we report for the first time that FRCpleth, RV and RV/TLC ratio is associated with the severity of air trapping, atelectasis and extent of bronchiectasis and with \(P.\ aeruginosa\) infection. Hence follow-up of PCD patients may be preferable with plethysmography rather than simple spirometry.

Strengths and weaknesses of the study

Our study has some strengths. First the completeness of ascertainment, since all patients performed all the tests, obviating selection bias. Second all the evaluations were done within 48 h. Third the objective analytical techniques using soft computing greatly reduces the chance of investigator bias.

Strengths and weaknesses in relation to other studies

In our study group the vast majority of adults and 80\% of the children had bronchiectasis and, as previously reported, half of the adults\(^d\)\(^e\) and approximately one-third of the children had chronic \(P.\ aeruginosa\) infection. The latter was associated with air-trapping, as previously documented in young children with cystic fibrosis.\(^h\) Focal air-trapping, which indicates small airways disease, probably starts early and our results document that static lung volume evaluation can detect this even in younger patients. This finding lends further support to the hypothesis that peripheral lung damage may be an early event in PCD.\(^i\)

It has recently been suggested that even the youngest patients already have irreversible lung damage\(^j\) and that bronchiectasis,

### Table 3: Correlation between chest high-resolution CT and functional parameters expressed as per cent of predicted normal

<table>
<thead>
<tr>
<th>HRCT parameters</th>
<th>FEV(_1)</th>
<th>FVC</th>
<th>FEV(_{25-75})</th>
<th>FEV(_{1}/\text{FVC})</th>
<th>Raw</th>
<th>sRaw</th>
<th>sReff</th>
<th>FRCpleth</th>
<th>RV</th>
<th>TLC</th>
<th>RV/TLC</th>
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<tbody>
<tr>
<td><strong>Severity of bronchiectasis</strong></td>
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<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p=0.01</td>
<td>p=0.005</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p=0.02</td>
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<td>p=0.005</td>
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<td>(r=-0.350)</td>
<td>(r=-0.393)</td>
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<td></td>
<td>(r=0.331)</td>
<td>(r=0.408)</td>
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<td><strong>Extent of bronchiectasis</strong></td>
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<td>NS</td>
<td>NS</td>
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<td>NS</td>
<td>p=0.01</td>
<td>p=0.02</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p=0.02</td>
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<td>p=0.001</td>
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<tr>
<td>(r=-0.346)</td>
<td>(r=-0.356)</td>
<td>(r=-0.316)</td>
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<td>(r=0.336)</td>
<td>(r=0.459)</td>
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<td><strong>Peribronchial thickening</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p=0.035</td>
<td>p=0.044</td>
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<td>r=0.315</td>
<td>r=0.301</td>
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<tr>
<td><strong>Extent of mucus plugging</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p=0.04</td>
<td>p=0.035</td>
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<td>(r=0.315)</td>
<td>(r=0.301)</td>
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<tr>
<td><strong>Consolidation or atelectasis</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p=0.027</td>
<td>p=0.023</td>
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<td>(r=0.327)</td>
<td>(r=0.334)</td>
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<tr>
<td><strong>Air-trapping</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p=0.002</td>
<td>p=0.001</td>
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<td>(r=0.438)</td>
<td>(r=0.389)</td>
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</table>

\(\text{FEV}_{25-75}\), forced expiratory flow between 25\% and 75\% of forced vital capacity; \(\text{FEV}_{1}\), forced expiratory volume in 1 s; FRCpleth, functional residual capacity by plethysmography; FVC, forced vital capacity; HRCT, high-resolution CT; NS, non-significant; Raw, airway resistance; RV, residual volume; sRaw, specific airway resistance; sReff, effective specific resistance; TLC, total lung capacity.
A reduction in lung flows and increased RV and RV/TLC can be present in patients with PCD as young as <3 years. We were not able to determine this because we could not study very young children with PCD.

**Meaning of the study**

Our findings suggest that lung disease in PCD begins early and that it is better identified at least in school-aged children and adults by the finding of air-trapping or HRCT, rather than...
spirometry. In previous studies of adults and children with PCD, total lung abnormalities and bronchiectasis severity on HRCT were shown to be related to FEV₁ and FVC.5 6 Our results do not confirm this in a larger series of PCD, even when analysed using a soft computing-based modelling approach, but instead suggest that plethysmography is a much more sensitive test.

CT scanning of the chest has become a popular tool to monitor patients with cystic fibrosis,32 a disease that has similarities with PCD. However, no study has evaluated the impact of such monitoring on health outcomes or on clinical decision-making in patients with PCD or cystic fibrosis. Moreover, there are concerns on the uncritical use of high-tech medical imaging and on the potential harm of repeated radiological exposures, particularly in children with lifelong diseases.6 7 Consequently, careful thought is needed before requesting repeated chest CT scans. By contrast a detailed evaluation of lung volumes is only limited by the availability of the equipment, and by the ability of the technician to obtain patients’ collaboration, but this is usually not a problem in the specialist centres that should care for these patients.

Unanswered questions and future research

Even though we have not studied very young subjects, from our results it is tempting to speculate that accumulation of secretions, in the early stages of disease, may induce primarily an obstruction in terminal bronchioles which may go undetected for a long period during alveolar growth of the lung. The consequent focal air-trapping during this phase of alveolar growth might lead to impaired septation and decreased elastic recoil as has been observed in animal models.35 Early structural changes in the distal airways could explain why we found a lack of correlation between per cent predicted RV and RV/TLC with age, unlike for spirometry, which may reflect slower progressive, age-related damage to large airways. To confirm this hypothesis, data are required from a longitudinal study in patients with PCD from the newborn period, using age-appropriate physiological techniques.

Although our findings show the superior sensitivity of plethysmography over spirometry in detecting HRCT changes in PCD, the findings need to be validated in a second cohort of patients. It would be valuable to compare plethysmography with sophisticated assessments of distal airway function, such as lung clearance index34 and distal airway production of exhaled nitric oxide in PCD.35–37

Longitudinal data are needed to determine which technique is more sensitive to a change in the patient’s condition. Finally, comparisons of plethysmography and spirometry in other airway diseases and in longitudinal studies would be of interest.

In conclusion, in this group who have a high pretest probability of lung damage,3,5 and given the different correlations between the various lung function parameters and HRCT-derived lung disease scores we suggest that measurement of lung volumes rather than spirometry may be preferable in the routine clinical management of PCD. Furthermore, this may reduce the number of scans used to monitor disease progression, although this needs testing prospectively. As with all investigations, HRCT should be requested only when the results are going to change the therapeutic strategy in the individual patient, or as part of an ethically approved, focused-research protocol.6 Our data represent training results and further work would be required to establish predictive validity, in particular in a second cohort studied longitudinally.

Acknowledgements We wish to thank the Editor and the reviewers for their help in the evaluation of our data.

Contributors M Pifferi: contributed to study design, data evaluation and drafting and revising the submitted manuscript. He had full access to all of the data in the study and he takes full responsibility for the integrity of all of the data and the accuracy of the data analysis. A Bush: contributed to data evaluation and drafting and revising the submitted manuscript. G Poggia: contributed to data analysis and drafting the submitted manuscript. D Caramella: contributed to data analysis and drafting the submitted manuscript. G Tartarisco: contributed to data analysis and drafting the submitted manuscript. M Di Cicco: contributed to collecting data. M Zangani: contributed to collecting data. I Chinellato: contributed to study design, data evaluation and drafting and revising the submitted manuscript.

Funding This research was supported by the Fondazione Carlo Laviosa, Italy.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics approval was provided by Hospital Ethical Committee of Pisa.

Provenance and peer review Not commissioned; externally peer reviewed.

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Thorax published online July 7, 2012

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