

Structural lung changes, lung function, and non-invasive inflammatory markers in cystic fibrosis

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Cystic fibrosis (CF) lung disease is characterized by chronic airway inflammation and recurrent infections, resulting in (ir)reversible structural lung changes and a progressive decline in lung function. The objective of this study was to investigate the relationship between non-invasive inflammatory markers (IM) in exhaled breath condensate (EBC), lung function indices and structural lung changes, visualized by high resolution computed tomography (HRCT) scans in CF. In 34 CF patients, lung function indices (forced expiratory volume in 1 s, forced vital capacity [FVC], residual volume, and total lung capacity [TLC]) and non-invasive IM (exhaled nitric oxide, and condensate acidity, nitrate, nitrite, 8-isoprostane, hydrogen peroxide, interferon-gamma) were assessed. HRCT scans were scored in a standardized and validated way, a composite score and component scores were calculated. In general, the correlations between non-invasive IM and structural lung changes, and between IM and lung function were low (correlation coefficients < 0.40). Patients with positive sputum *Pseudomonas* cultures had higher EBC nitrite levels and higher parenchymal HRCT subscores than patients with *Pseudomonas*-negative cultures ($p < 0.05$). Multiple linear regression models demonstrated that FVC was significantly predicted by hydrogen peroxide in EBC, and the scores of bronchiectasis and mosaic perfusion (Pearson correlation coefficient $R = 0.78$, $p < 0.001$). TLC was significantly predicted by 8-isoprostane, nitrate, hydrogen peroxide in EBC, and the mucous plugging subscore ($R = 0.92$, $p < 0.01$). Static and dynamic lung function indices in this CF group were predicted by the combination of non-invasive IM in EBC and structural lung changes on HRCT imaging. Future longitudinal studies should reveal whether non-invasive monitoring of airway inflammation in CF adds to better follow-up of patients.

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Cystic fibrosis (CF) is an autosomal recessive disorder, caused by genetic mutations in the CF transmembrane conductance regulator protein (1, 2). This results in impaired or absent transport of chloride through cell membranes, with impaired mucociliary clearance, viscous mucus in the airways, and an inability to effectively clear bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa* (1, 2). Therefore, chronic

airway infection, airway inflammation, and oxidative stress are the features of CF disease with mainly neutrophilic immune responses (3). In the course of time, structural lung changes will occur with diminishment of lung function.

Airway inflammation in patients with CF is already present early in life (1), and plays a major role in CF lung disease. Previous studies showed higher levels in sputum and broncho-alveolar

lavage of IFN- γ (2), IL-2 (2), IL-4 (2), IL-5 (3), nitrate (4), and nitrite (4, 5) in CF patients compared with controls. An increasing amount of studies emphasize the potential of non-invasive IM to reflect ongoing inflammation and infection (6–11). Such a new, non-invasive technique is exhaled breath condensate (EBC). Recently, higher EBC levels of 8-isoprostane (12), hydrogen peroxide (13), nitrite (6, 13, 14), IFN-gamma (15), and EBC acidity (16), but lower fractional exhaled nitric oxide (FeNO) levels were shown in CF compared with healthy subjects (15, 17). However, the relationship between these non-invasive IM and structural lung abnormalities in CF is unclear.

Structural lung changes in CF can be assessed by high resolution computed tomography (HRCT) imaging of the lungs, and include bronchiectasis, mucus plugging, peribronchial thickening, parenchymal abnormalities, and air trapping (16, 18–21). HRCT enables visualization of (small) areas with structural lung changes, even in an early stage (16, 22, 23). These structural abnormalities may be observed by HRCT imaging, even in the presence of normal lung function parameters and in the absence of pulmonary symptoms (16, 19–27). Imaging and pathology studies demonstrated that CF lung disease first develops in small peripheral airways, and, that early changes can be restricted to only local areas (28).

Lung function indices show the degree of restriction and obstruction of the overall airway status (29, 30), and thereby reflect the consequences of past and presently ongoing inflammatory processes. Lung function is related to morbidity and mortality in CF.

The relationship between lung function on the one hand, structural lung changes as visualized by HRCT scans, and non-invasive IM on the other is yet unclear in CF patients (31). We hypothesized that structural lung changes on HRCT imaging (as a result of long-term damage to the lungs by chronic airway infection and inflammation) together with ongoing airway inflammation (as assessed by IM in EBC) are important determinants of lung function (dynamic as well as static indices) in CF. Data on non-invasive IM in EBC, HRCT imaging, and static and dynamic lung function indices were analyzed in 34 CF patients.

Material and methods

Study subjects

Thirty-four patients with CF (19 male), capable of performing lung function tests and EBC manoeu-

vres, were selected from the outpatient clinic of the CF centers of the Maastricht and Nijmegen University Medical Center. The age (mean $\pm$ s.d.) of the CF population was 12.6 ± 4.4 yr. Symptoms, medication use, and the results of physical examination were documented. Standard deviations of weight and height were calculated based on Dutch caucasian population corrected for age and gender (32). Informed consent was obtained from parents and children, from the age of 12 yr, participating in this study. When children were younger than 12, informed consent was obtained from parents only. The study was approved by the Medical Ethics Committee of the University Hospital Maastricht.

Exclusion criteria

Cystic fibrosis disease was defined as a combination of characteristic clinical features (e.g., persistent pulmonary problems, meconium ileus, failure to thrive, steatorrhoe) and an abnormal sweat test (chloride > 60 mM) (33). Exclusion criteria were: (i) Diseases that may interfere with the results of the study (e.g., upper airway infection, congenital heart disease, anatomic abnormalities of the airways and other chronic inflammatory diseases, such as Crohn's disease and rheumatoid arthritis), (ii) mental retardation, (iii) inability to perform the EBC collection procedure, (iv) active smoking, and (v) use of the following medication: sodium nitroprusside, angiotensin-converting enzyme inhibitors, oxymetazoline, L-arginine, or nitric oxide synthase inhibitors.

HRCT scanning

High resolution computed tomography scans were performed in supine position from lung apex to base. In each hospital, a sequential scanning technique was used with 1-mm thick CT slices at intervals ranging from 6 to 10 mm. Scans were obtained at full inspiration in all subjects and in expiration in a subset. Exposure time ranged from 0.75 to 1.1 s. Beam current ranged from 90 to 200 mA depending on the patients' age. The peak of beam potential ranged from 120 to 140 kV. All scans were reconstructed using a high-resolution algorithm and displayed at lung settings (window width -400 to -500 hounsfield units and window level 1400–1600 hounsfield units. Different CT scanners were used: a two-slice spiral CT scanner (Elscent MX Twin; Philips Medical Systems presently, Haifa, Israel), a 16 detector row CT scanner (Siemens sensation 16; Siemens Medical Systems,

Erlangen, Germany), a 64 detector row CT scanner (Siemens Somatom 64; Siemens Medical Systems), and a four-slice spiral CT scanner (Toshiba Aquillion; Toshiba Medical Systems, Tokyo, Japan).

HRCT scoring

All inspiratory HRCT-scans were scored using a scoring system described by Brody et al. (18) by two blinded and independent observers (27, 34). The scoring system evaluates the five lobes. The lingual of the left lung was scored as a sixth lobe. The items scored were presence and severity of bronchiectasis, mucous plugging, peribronchial thickening, parenchymal abnormalities and air trapping. Because expiration scans were not available in all patients, mosaic perfusion was scored instead of air trapping in all patients and expiratory images were not used. Component scores and composite scores were calculated as previously reported and expressed on a scale 0–100 (16). The higher the score, the more a specific item is found in the lungs.

Clinical scores

To determine the general clinical condition of the patient we determined the Shwachman–Kulczycki score for each subject.

Nitric oxide in exhaled breath

Fractional exhaled nitric oxide was measured using a NIOX[®] chemiluminescence analyzer (Aerocrine, Solna, Sweden). Measurements were assessed according to the recommendations of the American Thoracic Society/European Respiratory Society for children (ATS/ERS) (35, 36).

Exhaled breath condensate

Exhaled breath condensate was collected using a glass condenser system (11, 12). Samples were deep frozen (−80°C) immediately after collection. The acidity of the EBC was measured immediately after collection (Radiometer[®], type PHM201, Radiometer Nederland BV, Zoetermeer, the Netherlands). Samples were not deaerated. In EBC, nitrate and nitrite concentrations were assessed using a fluorimetric assay (Cayman Chemical, Ann Arbor, USA) (36). To measure 8-isoprostane, an EIA-kit was used (Cayman Chemical, Ann Arbor, USA), which was modified in order to lower the detection limit from 5.0 pg/ml (80% B/B₀) to 0.5 pg/ml, as described previously (8). H₂O₂ measurements were based

on a method previously described (37). Briefly, EBC was mixed with 0.4 M citrate buffer, HRP solution (50 U/ml) and tetramethylbenzidine (TMB, 20 mM) and incubated for 20 min at room temperature. Then, the sample was mixed with sulphuric acid (2 M). The TMB oxidation product as a measure of the amount of H₂O₂ was determined spectrometrically at 450 nm, using a Perkin Elmer UV-VIS Spectrometer Lambda 10 (Norwalk, CT, USA). Cytokine IFN- γ level was assessed with flow cytometry (CBA, BD Bioscience[®], San Diego, CA, USA), as described previously (8).

Lung function tests

Bronchodilator medication was stopped prior to lung function testing: short-acting bronchodilators for at least 8 h, and long-acting bronchodilators at least for 36 h. Dynamic and static spirometry were performed by means of the Flowscreen[®] (Viasys[®], Hoechst, Germany), and by body plethysmography (Viasys[®]). The lung function parameters were assessed according to ERS standards (38). The highest values of three correctly performed manoeuvres were used for analysis. Recorded parameters were: forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), FEV₁/VC and the intrathoracic gas volume. The reversibility on a bronchodilator was determined 15 min after inhalation of 400- μ g salbutamol. Reversibility was defined as an increase in measured FEV₁ of at least 9% of predicted value (39). The static indices total lung capacity (TLC) and residual volume (RV) were determined.

Statistical analysis

Intra-observer variability for HRCT scoring (composite score and component scores) was tested with the intra-class correlation coefficient. An intra-class correlation coefficients >0.8 represented good agreement between observers. The relationship among HRCT scores (composite and component), lung function indices and non-invasive inflammatory markers (IM) were tested using Pearson correlation coefficients. Spearman's rank test was used when distribution of parameters were considered not normal. The mean HRCT scores of both observers were used to calculate Pearson correlation coefficients. For IFN- γ samples with concentrations below the detection limit, arbitrary values between 0 and the detection limit were ascribed. Stepwise, backward multiple regression linear analysis was used to analyze the predicting ability of IM

and HRCT subscores to predict lung function indices (dependent variable). Described tests were considered statistically significant, when $p < 0.05$.

Power analysis

Twenty-five subjects with CF are necessary to assess clinically relevant correlation coefficients of ≥ 0.6 between for instance IM and HRCT changes with an alpha of 0.05, and a power of 90%. Thirty-four subjects were selected for this study.

Results

Population characteristics

Of the 34 CF patients, 19 patients (56%) had a weight 1 s.d. below the mean, and a height 1 s.d. below the mean (Table 1). Six (18%) and seven (21%) of the patients had a weight and height below -2 s.d., respectively. Lung function indices showed a mild to moderate impairment with a mixed restrictive and obstructive component. Expiratory volumes and flow rates were decreased, while residual volumes were increased. DNase and corticosteroids (inhaled or oral) were used frequently (62% and 29%, respectively).

Table 1. Clinical characteristics of the cystic fibrosis (CF) study population (n = 34)

Characteristics	Mean $\pm$ s.e.
Age (yr)	12.6 $\pm$ 0.7
Height (cm)	146 $\pm$ 3.3
s.d. height*	-1.1 $\pm$ 0.2
Weight (kg)	36 $\pm$ 2.2
s.d. weight*	-1.2 $\pm$ 0.2
Gender (male/female)	19/15
Positive sputum bacterial cultures (nr - %)	28/34 (82)
Positive sputum <i>Pseudomonas</i> cultures (nr - %)	14/34 (41)
Total SK score, median (range)	65 (55-70)
Dynamic lung function indices:	
FEV ₁ (% pred)	72 $\pm$ 4.4
FVC (% pred)	75 $\pm$ 3.6
FEV ₁ /VC (%)	76 $\pm$ 2.2
Reversibility† (%)	15
Statistic lung function indices:	
TLC (% pred)	103 $\pm$ 2.2
RV (% pred)	181 $\pm$ 12.4
ITGV (% pred)	120 $\pm$ 4.6

Data are given as mean $\pm$ s.e., unless indicated otherwise.

The SK score has a range of 0-75.

s.e., standard error of the mean; SK-score, CF severity score according to Shwachman-Kulczycki; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; VC, vital capacity; TLC, total lung capacity; RV, residual volume; ITGV, intrathoracic gas volume.

*Values were based on a Dutch caucasian population corrected for age and gender.

†Reversibility is defined as the increase in FEV₁% predicted of 9% or more after use of a bronchodilator.

Antibiotics were used prophylactically in 41% (inhaled 18%, oral 3%), and therapeutically in 12% (inhaled 6%, oral 6%, intravenous 9%).

FeNO and non-invasive IM in EBC

Nitrate, nitrite, H₂O₂, and 8-isoprostane were detected in all EBC samples. IFN- γ was detected in 38% of the EBC samples. The mean $\pm$ s.e. of the IM were: FeNO 9.0 $\pm$ 1.5 p.p.b.; pH 6.3 $\pm$ 0.1; nitrite 2.0 $\pm$ 1.1 μ M; nitrate 17.7 $\pm$ 2.6 μ M; H₂O₂ 2.4 $\pm$ 0.3 μ M; 8-isoprostane 4.2 $\pm$ 0.5 pg/ml and IFN- γ (range) 0.7-3.5 pg/ml.

Between observer agreement of HRCT scoring

The intra-class correlation coefficient was 0.98 (95% confidence interval 0.97-0.99, $p < 0.01$), for the composite score. The intra-class correlation coefficients for the subscores (with a 95% confidence interval) were: bronchiectasis 0.91 (0.81-0.95, $p < 0.01$); airway wall thickening 0.92 (0.85-0.96, $p < 0.01$); mucous plugging 0.93 (0.85-0.96, $p < 0.01$); parenchymal abnormalities 0.84 (0.68-0.92, $p < 0.01$); mosaic perfusion 0.98 (0.95-0.99, $p < 0.01$).

HRCT scores

The mean $\pm$ s.e. of the separate HRCT scores were: bronchiectasis 15 $\pm$ 2.4%; airway wall thickening 19 $\pm$ 2.0%; mucous plugging 19 $\pm$ 2.0%; parenchymal abnormalities 7 $\pm$ 1.1%; mosaic perfusion 24 $\pm$ 4.3%, and total score 17 $\pm$ 1.9%, indicating moderate structural lung changes. The mean $\pm$ s.e. time span between HRCT imaging and determination of IM and lung function was -2.0 ± 1.9 months.

Relationships between IM and HRCT scores

Overall, the association between IM and HRCT scores was not very strong with correlation coefficients < 0.4 . The only significant correlation was found between the mucous plugging subscore and H₂O₂ ($R = -0.39$, $p = 0.03$), Fig. 1. The correlation between H₂O₂ and airway wall thickening showed a trend towards significance ($R = -0.32$, $p = 0.07$).

Relationships among sputum cultures, non-invasive IM and HRCT scores

Patients with positive sputum *Pseudomonas aeruginosa* cultures had higher EBC nitrite levels and parenchymal HRCT subscores

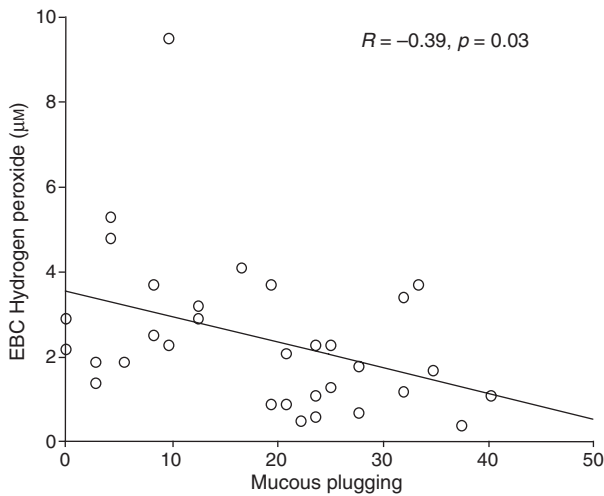


Fig. 1. The correlation between hydrogen peroxide in exhaled breath condensate (EBC) and the mucous plugging high resolution computed tomography (HRCT) subscore in 34 cystic fibrosis patients.

(mean $\pm$ s.e.; $2.37 \pm 0.27 \mu\text{M}$ and $10.3 \pm 1.7\%$) compared with CF patients with negative *Pseudomonas aeruginosa* cultures ($1.55 \pm 0.25 \mu\text{M}$ and $4.23 \pm 1.37\%$, $p = 0.04$ and $p = 0.01$, respectively). In patients with positive sputum cultures of any pathologic micro-organism, higher scores were given to the bronchiectasis HRCT subscore (mean $\pm$ s.e.; 17.5 ± 2.7 , $p < 0.01$), implicating more extensively distributed and more severe bronchiectasis on HRCT scans than patients with no positive sputum cultures ($4.7 \pm 2.7\%$, $p = 0.04$).

Relationships between IM and lung function indices

There were significant correlations between FVC and H_2O_2 ($R = 0.39$, $p = 0.03$), and between TLC and nitrate ($R = -0.64$, $p < 0.001$) (Figs 2 and 3, respectively).

Relationships among HRCT scores, lung function indices, and SK score

Lung function indices (FEV_1 , FVC, and RV) strongly correlated with HRCT scores (Table 2). In general, a stronger correlation was found between the total HRCT score and lung function parameters, than with the HRCT composite and component scores. There were no correlations between TLC % predicted value and any HRCT composite and component scores.

Results of multivariate backward linear regression models

Multivariate backward linear regression analyses with TLC % of predicted as dependent variable,

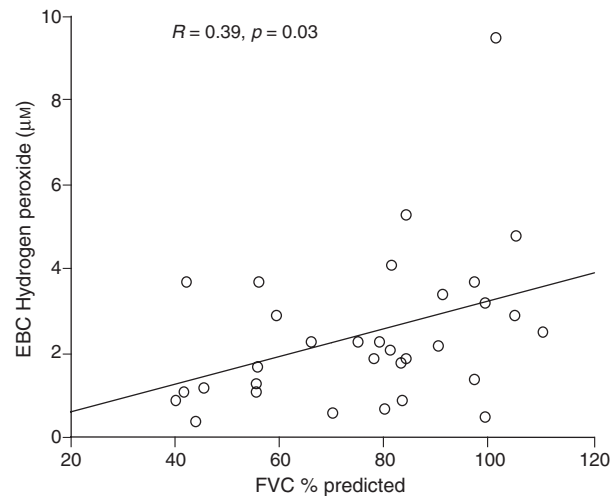


Fig. 2. The correlation between hydrogen peroxide in exhaled breath condensate (EBC) and the forced vital capacity in 34 cystic fibrosis patients.

Table 2. Relationships among high resolution computed tomography scores, general clinical (SK) score, and lung function indices

	B	AWT	Mu	P	Mo	Total
FEV_1 (% pred)						
R	-0.64	-0.62	-0.54	-0.48	-0.64	-0.77
p-value	0.00	0.00	0.00	0.00	0.00	0.00
FEV_1/VC						
R	-0.26	-0.44	-0.41	-0.16	-0.46	-0.43
p-value	0.13	0.01	0.02	0.37	0.01	0.01
FVC (% pred)						
R	-0.59	-0.55	-0.47	-0.56	-0.62	-0.73
p-value	0.00	0.00	0.01	0.00	0.00	0.00
TLC (% pred)						
R	0.10	0.22	0.37	-0.29	-0.16	0.04
p-value	0.64	0.28	0.07	0.17	0.46	0.84
RV (% pred)						
R	0.69	0.63	0.64	0.37	0.56	0.73
p-value	0.00	0.00	0.00	0.07	0.00	0.00
ITGV (% pred)						
R	0.61	0.56	0.52	0.26	0.39	0.59
p-value	0.00	0.00	0.01	0.21	0.06	0.00
SK score						
R	-0.44	-0.25	-0.40	-0.37	-0.47	-0.50
p-value	0.01	0.16	0.02	0.03	0.01	0.00

Data were analyzed using Pearson correlation tests.

B, bronchiectasis; AWT, airway wall thickening; Mu, mucous plugging; P, Parenchymal abnormalities; Mo, Mosaic perfusion; Total, accumulative score; SK-score, CF severity score according to Shwachman-Kulczycki; FEV_1 , forced expiratory volume in 1 s; VC, vital capacity; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; ITGV, intrathoracic gas volume; R, correlation coefficient.

and, measured non-invasive IM and HRCT subscores (bronchiectasis, peribronchial thickening, mucous plugging, and mosaic perfusion) as independent variables showed that TLC % of predicted value was significantly predicted by 8-isoprostane, nitrate, H_2O_2 , and mucous plugging

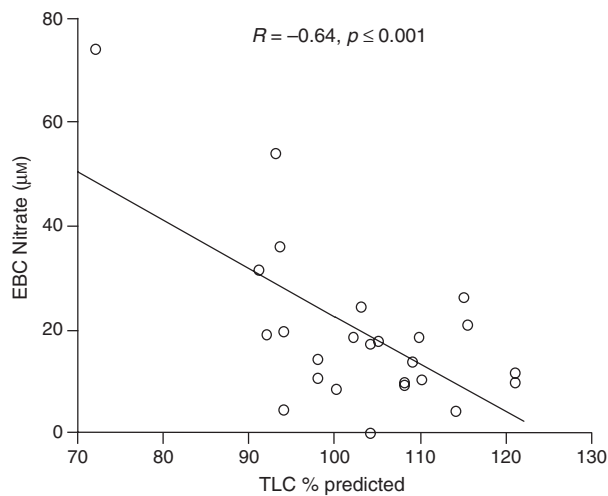


Fig. 3. The correlation between nitrate in exhaled breath condensate (EBC) and total lung capacity in 34 cystic fibrosis patients.

Table 3a. Backward multiple linear regression model of IM in EBC and HRCT scores on (a) total lung capacity (TLC)* (dependent variable) and (b) on forced vital capacity (FVC)† (dependent variable)

	$\beta \pm \text{s.e.}$	p
(a) Constant	104.10 $\pm$ 4.02	
8-IP	-1.92 $\pm$ 4.02	0.006
Nitrate	-0.52 $\pm$ 0.09	0.000
H ₂ O ₂	2.38 $\pm$ 0.77	0.010
Mucous plugging	0.56 $\pm$ 0.15	0.003
(b) Constant	83.00 $\pm$ 7.40	
H ₂ O ₂	3.75 $\pm$ 1.69	0.037
Bronchiectasis	-0.62 $\pm$ 0.25	0.019
Mosaic perfusion	-0.39 $\pm$ 0.13	0.005

8-IP, 8-isoprostane; H₂O₂, hydrogen peroxide; s.e., standard error; IM, inflammatory markers; EBC, exhaled breath condensate; HRCT, high resolution computed tomography.

*TLC was significantly predicted by EBC 8-isoprostane, nitrate, hydrogen peroxide, and the mucous plugging subscore ($R = 0.92$, $p < 0.01$).

†The FVC was significantly predicted by EBC hydrogen peroxide and by the bronchiectasis and mosaic perfusion subscores ($R = 0.78$, $p < 0.001$).

subscore with a high level of explained variance ($R = 0.92$, $p < 0.01$) (Table 3a). In addition, another significant prediction model was possible for the FVC (as dependent variable), which was significantly predicted by H₂O₂, and the scores of bronchiectasis and mosaic perfusion ($R = 0.78$, $p < 0.001$) (Table 3b).

Discussion

This study supports the hypothesis that lung function in CF (both static and dynamic indices) is determined by the combination of structural lung changes on HRCT imaging and airway inflammation, as assessed by non-invasive bio-

markers in EBC: the FVC was indicated to a large degree by mosaic perfusion, bronchiectasis and hydrogen peroxide levels, whereas TLC was strongly predicted by mucous plugging, 8-isoprostane, nitrate, and hydrogen peroxide. In general, the correlation between HRCT changes and non-invasive IM in EBC was low: only hydrogen peroxide correlated significantly with mucous plugging. In patients with positive *Pseudomonas aeruginosa* sputum cultures, significant higher EBC nitrite levels and more parenchymal abnormalities were found compared with patients with *Pseudomonas* negative cultures. In general, high correlations were demonstrated between lung function indices and structural lung changes on HRCT imaging.

To our knowledge, this is one of the first studies investigating the relationship among non-invasive IM, structural lung changes, assessed by HRCT scans, and lung function in CF patients. One study by Dakin et al. (40), investigated the relationship between IM in induced sputum and HRCT images in 34 CF patients (mean age 12.3 yr; mean FEV_{1%} of predicted 67%). The time span among assessments of lung function, HRCT scan, and a sputum sample was maximally 1 month. They found no correlation between induced sputum IL-8 concentrations and the Bhalla HRCT score (40), which is consistent with the low correlations between EBC markers and HRCT in our study. Hilliard et al. studied the relationship between structural lung changes as assessed by endobronchial biopsies, and airway inflammation (IM in bronchoalveolar lavage) in a recent cross-sectional study of 43 children with CF (aged 0.3–16.8 yr) and 40 control children (37). The reticular basement membrane thickness was greater in the CF group than in the controls, and correlated positively with levels of transforming growth factor-beta 1, although not with other IM or pulmonary function. This is consistent with the generally low correlation between IM in EBC and structural lung changes in our study.

In addition, our study examined the relationship between lung function parameters and structural lung changes. FEV₁, FEV₁/FVC, FVC, and RV strongly correlated with HRCT scores. In general, lung function parameters correlated better with the total HRCT score, than with the HRCT subscores, which confirms that lung function parameters indeed reflect the overall status of lung changes. This is in accordance to other data in the literature (16, 19, 27).

How can we explain the results of this study?

In general, the low correlations between non-invasive IM and structural lung changes indicate

that structural lung changes are the consequence of a long-term process of infection and inflammation, and are not adequately described by one discrete assessment of IM. Another explanation is that the relationship between airway inflammation and structural lung changes is modified by an individual (genetic) susceptibility to develop damage. There are several genetic CF components that may influence this susceptibility (41, 42). Recently, the importance of two modifier genes (mannose-binding lectin 2 and transforming growth factor beta 1) for the levels of lung function in CF was demonstrated in a cohort of 1019 Canadian CF patients (41). CF patients with mannose-binding lectin 2 deficiency had the more rapid decline in lung function, most significantly in those carrying the high-producing transforming growth factor beta 1 genotype. This last finding points to an important gene-gene interaction. We found that lung function in our group of CF patients was very well predicted by the combination of non-invasive IM in EBC and of structural lung changes on HRCT imaging.

The strengths of our study were: (i) we included a relative large study population of 34 CF patients; (ii) two observers scored the HRCT scans with an excellent intra-class correlation coefficient; (iii) the simultaneous assessment of several relevant non-invasive IM in CF, with detection rates of 38–100%.

A limitation of this study may be the time interval between the HRCT imaging and the assessment of lung function and EBC, which on average was 2 months. It is unlikely that the relationships between IM and CT findings was significantly influenced by this time interval as correlation between lung function and HRCT imaging appeared to be very high. This study included patients with mild to moderate CF. In more severe CF, results might be different.

Some correlations in this study were different from expected: decreasing H_2O_2 levels were significantly related to a decrease in FVC % of predicted, whereas lower nitrate values were related to an increase in TLC % of predicted. This may be the consequence of mucous entrapment of IM. A comparable mechanism had been suggested as one of the explanations for the diminished FeNO values in CF (43, 44). The significant correlation between the extent of mucous plugs and EBC H_2O_2 levels in this study demonstrates that this hypothesis of mucus entrapment of IM is probably correct.

How should we monitor CF patients? Our cross-sectional study design is not suitable to answer this question. However, the impression

from this study is that chronic airway inflammation is an important determinant of structural lung changes and also of lung function. It is possible that monitoring of airway inflammation will add to better follow-up of CF patients. One of the tools for monitoring of airway inflammation in CF may be non-invasive assessment of IM in EBC. However, longitudinal data are necessary to investigate this. HRCT imaging is an interesting technique as it is able to assess early lung changes, but there is concern about the long-term harmful effects of particularly repeated scans and radiation exposures (44). In summary, this study demonstrated that non-invasive IM in EBC in combination with structural lung changes on HRCT imaging significantly predicted static and dynamic lung function indices in this group of CF patients. Future longitudinal studies should reveal whether non-invasive monitoring of airway inflammation in CF adds to better follow-up of patients.

References

1. KHAN TZ, WAGENER JS, BOST T, MARTINEZ J, ACCURSO FJ, RICHES DW. Early pulmonary inflammation in infants with cystic fibrosis. *Am J Respir Crit Care Med* 1995; 151: 1075–82.
2. HARTL D, GRIESE M, KAPPLER M, et al. Pulmonary T(H)2 response in *Pseudomonas aeruginosa*-infected patients with cystic fibrosis. *J Allergy Clin Immunol* 2006; 117: 204–11.
3. KOLLER DY, NETHING I, OTTO J, URBANEK R, EICHLER I. Cytokine concentrations in sputum from patients with cystic fibrosis and their relation to eosinophil activity. *Am J Respir Crit Care Med* 1997; 155: 1050–4.
4. LINNANE SJ, KEATINGS VM, COSTELLO CM, et al. Total sputum nitrate plus nitrite is raised during acute pulmonary infection in cystic fibrosis. *Am J Respir Crit Care Med* 1998; 158: 207–12.
5. CETIN I, OZCELIK U, GOCMEN A, KIPER N, DOGRU D, YALCIN E. BALF nitrite as an indicator of inflammation in children with cystic fibrosis. *Respiration* 2004; 71: 625–9.
6. CARPAGNANO GE, BARNES PJ, GEDDES DM, HODSON ME, KHARITONOV SA. Increased leukotriene B4 and interleukin-6 in exhaled breath condensate in cystic fibrosis. *Am J Respir Crit Care Med* 2003; 167: 1109–12.
7. KHARITONOV SA, BARNES PJ. Exhaled biomarkers. *Chest* 2006; 130: 1541–6.
8. ROBROEKS CMHHT, JÖBSIS Q, DAMOISEAUX JGMC, et al. Cytokines in exhaled breath condensate of children with asthma and cystic fibrosis. *Ann Allergy Asthma Immunol* 2006; 96: 349–55.
9. CARPAGNANO GE, BARNES PJ, FRANCIS J, WILSON N, BUSH A, KHARITONOV SA. Breath condensate pH in children with cystic fibrosis and asthma: a new noninvasive marker of airway inflammation? *Chest* 2004; 125: 2005–10.
10. RAHMAN I, KELLY F. Biomarkers in breath condensate: a promising new non-invasive technique in free radical research. *Free Radic Res* 2003; 37: 1253–66.

11. ROSIAS P, DEN HARTOG G, ROBROEKS C, et al. Free radicals in exhaled breath condensate in cystic fibrosis and healthy subjects. *Free Radic Res* 2006; 40: 901–9.
12. MONTUSCHI P, KHARITONOV SA, CIABATTONI G, et al. Exhaled 8-isoprostane as a new non-invasive biomarker of oxidative stress in cystic fibrosis. *Thorax* 2000; 55: 205–9.
13. HO LP, INNES JA, GREENING AP. Nitrite levels in breath condensate of patients with cystic fibrosis is elevated in contrast to exhaled nitric oxide. *Thorax* 1998; 53: 680–4.
14. FORMANEK W, INCI D, LAUENER RP, WILDHABER JH, FREY U, HALL GL. Elevated nitrite in breath condensates of children with respiratory disease. *Eur Respir J* 2002; 19: 487–91.
15. THOMAS SR, KHARITONOV SA, SCOTT SF, HODSON ME, BARNES PJ. Nasal and exhaled nitric oxide is reduced in adult patients with cystic fibrosis and does not correlate with cystic fibrosis genotype. *Chest* 2000; 117: 1085–9.
16. BRODY AS, KLEIN JS, MOLINA PL, QUAN J, BEAN JA, WILMOTT RW. High-resolution computed tomography in young patients with cystic fibrosis: distribution of abnormalities and correlation with pulmonary function tests. *J Pediatr* 2004; 145: 32–8.
17. GASEMANN H, MICHLER E, WALLOT M, RATJEN F. Decreased concentration of exhaled nitric oxide (NO) in patients with cystic fibrosis. *Pediatr Pulmonol* 1997; 24: 173–7.
18. BRODY AS, MOLINA PL, KLEIN JS, ROTHMAN BS, RAMAGOPAL M, SWARTZ DR. High-resolution computed tomography of the chest in children with cystic fibrosis: support for use as an outcome surrogate. *Pediatr Radiol* 1999; 29: 731–5.
19. HELBICH TH, HEINZ-PEER G, EICHLER I, et al. Cystic fibrosis: CT assessment of lung involvement in children and adults. *Radiology* 1999; 213: 537–44.
20. DE JONG PA, NAKANO Y, LEQUIN MH, et al. Progressive damage on high resolution computed tomography despite stable lung function in cystic fibrosis. *Eur Respir J* 2004; 23: 93–7.
21. MAFFESSANTI M, CANDUSSO M, BRIZZI F, PIOVESANA F. Cystic fibrosis in children: HRCT findings and distribution of disease. *J Thorac Imaging* 1996; 11: 27–38.
22. BHALLA M, TURCIOS N, APONTE V, et al. Cystic fibrosis: scoring system with thin-section CT. *Radiology* 1991; 179: 783–8.
23. TIDDENS HA, DE JONG PA. Update on the application of chest computed tomography scanning to cystic fibrosis. *Curr Opin Pulm Med* 2006; 12: 433–9.
24. TIDDENS HA. Detecting early structural lung damage in cystic fibrosis. *Pediatr Pulmonol* 2002; 34: 228–31.
25. JUDGE EP, DODD JD, MASTERSON JB, GALLAGHER CG. Pulmonary abnormalities on high-resolution CT demonstrate more rapid decline than FEV1 in adults with cystic fibrosis. *Chest* 2006; 130: 1424–32.
26. ROBINSON TE. High-resolution CT scanning: potential outcome measure. *Curr Opin Pulm Med* 2004; 10: 537–41.
27. DE JONG PA, OTTINK MD, ROBBEN SG, et al. Pulmonary disease assessment in cystic fibrosis: comparison of CT scoring systems and value of bronchial and arterial dimension measurements. *Radiology* 2004; 231: 434–9.
28. DAVIS SD, FORDHAM LA, BRODY AS, et al. Computed tomography reflects lower airway inflammation and tracks changes in early cystic fibrosis. *Am J Respir Crit Care Med* 2007; 175: 943–50.
29. GIBSON RL, BURNS JL, RAMSEY BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003; 168: 918–51.
30. RATJEN F, DORING G. Cystic fibrosis. *Lancet* 2003; 361: 681–9.
31. GURAN T, ERSU R, KARADAG B, et al. Association between inflammatory markers in induced sputum and clinical characteristics in children with non-cystic fibrosis bronchiectasis. *Pediatr Pulmonol* 2007; 42: 362–9.
32. GERVER W, DE BRUIN R. Paediatric morphometrics. A Reference Manual. 2nd extended edn. Maastricht, the Netherlands: University, 2001.
33. ROSENSTEIN BJ, CUTTING GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr* 1998; 132: 589–95.
34. BRODY AS, KOSOROK MR, LI Z, et al. Reproducibility of a scoring system for computed tomography scanning in cystic fibrosis. *J Thorac Imaging* 2006; 21: 14–21.
35. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. *Am J Respir Crit Care Med* 2005; 171: 912–30.
36. MISKO TP, SCHILLING RJ, SALVEMINI D, MOORE WM, CURRIE MG. A fluorometric assay for the measurement of nitrite in biological samples. *Anal Biochem* 1993; 214: 11–6.
37. HILLIARD TN, REGAMEY N, SHUTE JK, et al. Airway remodelling in children with cystic fibrosis. *Thorax* 2007; 62: 1074–80.
38. MILLER MR, HANKINSON J, BRUSASCO V, et al. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–38.
39. DALES RE, SPITZER WO, TOUSIGNANT P, SCHECHTER M, SUISSA S. Clinical interpretation of airway response to a bronchodilator. Epidemiologic considerations. *Am Rev Respir Dis* 1988; 138: 317–20.
40. DAKIN CJ, PEREIRA JK, HENRY RL, WANG H, MORTON JR. Relationship between sputum inflammatory markers, lung function, and lung pathology on high-resolution computed tomography in children with cystic fibrosis. *Pediatr Pulmonol* 2002; 33: 475–82.
41. ZIELENSKI J, TSUI LC. Cystic fibrosis: genotypic and phenotypic variations. *Annu Rev Genet* 1995; 29: 777–807.
42. FERRARI M, CREMONESI L. Genotype-phenotype correlation in cystic fibrosis patients. *Ann Biol Clin (Paris)* 1996; 54: 235–41.
43. BALFOUR-LYNN IM, LAVERTY A, DINWIDDIE R. Reduced upper airway nitric oxide in cystic fibrosis. *Arch Dis Child* 1996; 75: 319–22.
44. GASTON B, RATJEN F, VAUGHAN JW, et al. Nitrogen redox balance in the cystic fibrosis airway: effects of antipseudomonal therapy. *Am J Respir Crit Care Med* 2002; 165: 387–90.