

Biomarkers in exhaled breath condensate indicate presence and severity of cystic fibrosis in children

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Chronic airway inflammation is present in cystic fibrosis (CF). Non-invasive inflammometry may be useful in disease management. The aim of the present cross-sectional study was to investigate: (i) the ability of fractional exhaled nitric oxide and inflammatory markers (IM) [exhaled breath condensate (EBC) acidity, nitrite, nitrate, hydrogen peroxide (H_2O_2), 8-isoprostane, Th1/Th2 cytokines] to indicate (exacerbations of) CF; and (ii) the ability of these non-invasive IM to indicate CF disease severity. In 98 children (48 CF/50 controls), exhaled nitric oxide was measured using the NIOX, and condensate was collected using a glass condenser. In CF interferon ($IFN-\gamma$) and nitrite concentrations were significantly higher, whereas exhaled nitric oxide levels were significantly lower compared with controls (3.3 ± 0.3 pg/ml, 2.2 ± 0.2 μ M, 10.0 ± 1.2 p.p.b. vs. 2.6 ± 0.2 pg/ml, 1.4 ± 0.1 μ M, 15.4 ± 1.4 p.p.b. respectively). Using multivariate logistic regression models, the presence of CF was best indicated by 8-isoprostane, nitrite and $IFN-\gamma$ [sensitivity 78%, specificity 83%; area under receiver operating characteristic curve (AUC) 0.906, $p < 0.001$]. An exacerbation of CF was best indicated by 8-isoprostane and nitrite (sensitivity 40%, specificity 97%, AUC curve 0.838, $p = 0.009$). Most indicative biomarkers of CF severity were exhaled nitric oxide, and condensate acidity (sensitivity 96%, specificity 67%; AUC curve 0.751, $p = 0.008$). In this cross-sectional study, the combination of different exhaled IM could indicate (exacerbations of) CF, and severity of the disease in children. Longitudinal data are necessary to further confirm the role of these markers for the management of CF in children.

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Cystic fibrosis (CF) is an autosomal recessive disorder, caused by genetic mutations in CF transmembrane conductance regulator (CFTR) protein. Defective CFTR causes impaired or absent transport of chloride through cell membranes, with an impaired mucociliary clearance and viscous mucus in the airways. Moreover, defective mucociliary action results in inability of the airways to clear bacteria, e.g. *Pseudomonas aeruginosa* (1, 2). Chronic airway infection,

inflammation and oxidative stress are the characteristics of CF disease, which are mainly due to neutrophilic immune responses (2, 3). Pulmonary disease is the leading cause of morbidity and mortality in CF patients (4).

Currently, monitoring of pulmonary disease in CF is based on clinical features and pulmonary function tests. However, these tests may not reflect the onset, origin or severity of airway inflammation (5). Assessment of inflammatory

markers (IM) in exhaled breath condensate (EBC) may be a promising tool in patients with chronic lung diseases (5–7). In CF, non-invasive IM may be helpful in the early detection of CF lung disease in infants, the prediction and follow-up of an exacerbation and the evaluation of treatment effects.

Several research groups detected altered IM in EBC of CF patients, compared with controls (8). However, study populations were small, and data on non-invasive markers in CF are limited and inconsistent. In addition, the relationships between IM and the severity or exacerbations of CF disease, are still unknown. Jöbsis et al. (9) showed high hydrogen peroxide (H_2O_2) concentrations during CF exacerbations, but it is unclear if H_2O_2 is a good indicator of CF exacerbations. Therefore, we assessed several important IM in EBC in almost 100 children with CF and controls, comparing the ability of these markers to indicate (exacerbations of) CF disease, and CF severity. Because of oxidative stress and airway inflammation, H_2O_2 , 8-isoprostane, nitrate/nitrite are upregulated in the airways of CF patients, and acidity is increased (10). In contrast, fractional exhaled nitric oxide (FeNO) levels have shown to be lower in CF. Recently, presence of interleukin-10 (IL-10), IL-4, tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) was demonstrated in EBC of CF patients (11).

The aim of the present cross-sectional study was to investigate: (i) the ability of FeNO and IM in EBC (pH, nitrite, nitrate, H_2O_2 , 8-isoprostane and Th1/Th2 cytokines), to indicate (exacerbations of) CF; and (ii) the ability of these non-invasive IM to indicate CF disease severity.

Methods

Study subjects

Forty-eight Children with CF, and 50 control children without a history of lung disease or atopy were included. Informed consent was obtained from all subjects. The study was approved by the Medical Ethics Committee of the University Hospital of Maastricht.

CF population. Children known with CF were recruited from the outpatient clinics. CF disease was defined as a combination of typical clinical features (e.g. persistent pulmonary problems, meconium ileus, failure to thrive, steatorrhoe) and an abnormal sweat test (chloride > 60 mm). An exacerbation of CF was diagnosed by the paediatric pulmonologist based on a significant

increase in CF related symptoms, and/or a decrease of at least 10% in forced expiratory volume in 1 s (FEV_1) or forced vital capacity (FVC) compared to previous measurements (12). Height and weight were expressed as Z-scores (age and gender specific standard deviation scores). CF severity was assessed using the Shwachman–Kulczycki score.

Control population. Control children without lung disease were recruited from the outpatient clinic of the University Hospital Maastricht. The reasons of consultation were constipation and enuresis nocturna. All children completed the 'International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire to exclude children with a (history of) airway or allergy complaints or asthmatic disease (13).

Exclusion criteria. Exclusion criteria were: (i) diseases that may interfere with the results of the study (e.g. upper airway infection, 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: papaverin, sodium nitroprusside, angiotensin-converting enzyme inhibitors, oxymetazoline, L-arginine or nitric oxide synthase (NOS) inhibitors.

Design and measurements

The design of this study was cross-sectional. At first, FeNO was measured. Subsequently, EBC was collected, and lung function was measured by means of dynamic spirometry. All tests were performed within 1 h.

NO in exhaled air. Fractional exhaled nitric oxide was measured using a NIOX[®] chemiluminescence analyser (Aerocrine, Solna, Sweden). Measurements were assessed according to the recommendations of the American Thoracic Society/European Respiratory Society for children (ATS/ERS) (7). The mean NO value of the plateau reached during the last 3 s of exhalation, was used to obtain the FeNO value of that measurement (7). The mean FeNO of three measurements was used for analysis.

In 14 CF patients, FeNO was measured using the offline method, because there was not a NIOX[®] analyser available in these clinics. The first 4 s of exhaled air were discarded and during the next 4 s, exhaled air was collected in an inert

balloon. FeNO levels were analysed on the NIOX[®] chemiluminescence analyser within 8 h (14). The exhalation flow rate during these measurements was 50 ml/s.

Exhaled breath condensate. Exhaled breath condensate was collected using a home-made 50 cm double-wall borosilicate glass condenser, continuously cooled by circulating ice water (1°C), as described previously (6, 11, 15). Subjects were asked to breath tidally for 30 min through a mouthpiece, while wearing a nose-clip. The mouthpiece was connected to a two-way non-rebreathing valve (Hans Rudolph Inc, series 1420, Kansas City, KS, USA), which also served as a saliva trap. In addition, children were allowed to swallow saliva during EBC collection. The acidity of the EBC was measured immediately after collection (Radiometer[®], type PHM201; Radiometer Nederland BV, Zoetermeer, the Netherlands). Samples were not deaerated (15). Subsequently, the EBC samples were frozen and stored at -80°C in 1 ml Eppendorf[®] tubes.

In EBC, nitrite and nitrate concentrations were assessed using a fluorimetric assay (Cayman Chemical, Ann Arbor, MI, USA) (16). To measure 8-isoprostane, an EIA-kit was used (Cayman Chemical), which was modified to lower the detection limit from 5.0 pg/ml (80% B/B₀) to 0.5 pg/ml. Standard curves, patient samples and quality control (QC) samples of 2.5 and 10 pg/ml, were assayed in triplicate. The coefficients of variation (%) of the absorption signal were assessed. Intra-assay variation of standard curves, patient samples and QC samples should be less than 15% otherwise all samples measured in that assay were excluded and re-analysed. Finally, in all accepted samples, 8-isoprostane concentrations were determined from mean absorption signal intensities.

Hydrogen peroxide measurements were based on a method previously described by Dekhuijzen et al. (17). Briefly, EBC was mixed with 0.4 M citrate buffer, horseradish peroxidase (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 (Perkin Elmer Inc., Norwalk, CT, USA). Cytokines (TNF- α , IFN- γ , IL-2, IL-4, IL-5, IL-10) were assessed with flow cytometry (CBA; BD Bioscience[®], San Diego, CA, USA), as described previously (11).

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 spirometry was performed by means of the Flowscreen[®] (Jaeger[®], Wuerzburg, Germany). The lung function parameters were assessed according to ERS standards. The highest values of one of three correct performed manoeuvres were used for analysis. Recorded parameters were: FEV₁ and FVC. In addition, in the CF population, residual volume, total lung capacity and intrathoracic gas volume, were assessed by body plethysmography (Jaeger[®], Würzburg, Germany). 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.

Statistical analysis

Statistical calculations were performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean $\pm$ s.e.m., or as median (interquartile range) values for normal and not normal distributed continuous variables respectively. Comparison between groups was performed using the chi-square for categorical variables, the Student's *t*-tests for normal distributed parameters, and Mann-Whitney *U*-tests were used when the distribution of the parameters was considered not normal. *p*-values < 0.05 were considered statistically significant.

At first, the influence of separate IM was tested in univariate logistic regression analyses. Thereafter, the different IM were combined in multivariate logistic regression models with CF disease, CF exacerbation and the CF severity as dependent binary variables. Age, height, weight, FEV₁% of predicted value and the FEV₁/FVC% were introduced in the model as covariates, but were removed if *p*-values exceeded 0.10, to keep the degrees of freedom as high as possible. The stepwise backward method was applied to reduce the number of independent variables and to assess which IM were most predictive: only IM with a *p*-value < 0.05 were kept in the final models. Based on these models, receiver operating characteristic curves were obtained. EBC samples with negative detections were not considered as missings, because they actually inform us that the measurements of IM were below the lower detection limit. Therefore, the negative detections were given an arbitrary value between the lower detection limit and zero.

Power analysis

The standard error of the sensitivity and the specificity of an IM will be <5%, given a population of 50 children with CF and 50 controls, and an assumed sensitivity of 70%. This was considered sufficiently accurate for the purpose of this study.

Results

Population characteristics

Two of the 48 CF patients were older than 18 (21 and 25 yr). Children with CF (n = 48) were older, smaller, weighed less and had more airway obstruction compared to controls (n = 50) (Table 1; p < 0.05). Thirteen per cent of the

Table 1. Clinical characteristics of the study population†

	Cystic Fibrosis (n = 48)	Controls (n = 50)
Age (years)	13.0 ± 0.6*	10.0 ± 0.4
Height (cm)	148 ± 3	142 ± 2
Z-score height‡	-1.0 ± 0.2*	0.1 ± 0.2
Z-score weight§	-1.0 ± 0.1*	0.5 ± 0.2
Sex (male/female)	27 / 21	27 / 23
FEV ₁ % predicted	76 ± 4*	102 ± 2
FEV ₁ /FVC (%)	77 ± 2*	88 ± 1
TLC % predicted	104 ± 2	NA
RV % predicted	174 ± 10	NA
ITVG % predicted	123 ± 4	NA
Reversibility¶	23%	NA
CF exacerbation	6 (13)	NA
Atopy**	17 (36)	NA
Positive sputum cultures		
<i>Pseudomonas aeruginosa</i>	23 (58)	NA
<i>Staphylococcus aureus</i>	29 (60)	NA
<i>Haemophilus influenzae</i>	14 (29)	NA
<i>Haemophilus parainfluenzae</i>	3 (6)	NA
<i>Aspergillus fumigatus</i>	17 (35)	NA
<i>Candida albicans</i>	6 (13)	NA
Hemolytic <i>Streptococcus</i> group A or B	8 (17)	NA
Therapy		
DNase	25 (52)	NA
Antacids	24 (50)	NA
Corticosteroid	13 (27)	NA
Therapeutic antibiotics	7 (15)	NA
Prophylactic antibiotics	24 (50)	NA

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; ITGV, intrathoracic gas volume; RV, residual volume; TLC, total lung capacity; NA, not applicable.

*Significantly different from controls (p < 0.05).

†Data are shown as mean ± SEM n (%).

‡Z-score height: standard deviation of height corrected for age and sex according to the Dutch standard values.

§Z-score weight: standard deviation of weight corrected for age and sex according to the Dutch standard values.

¶Reversibility is defined as an increase in FEV₁ after 400 µg salbutamol of 9% of predicted or more.

**A child was considered atopic when the total IgE level exceeded 20 kU/l and/or the Phadiatop was positive and/or the radioallergosorbent test was positive.

CF patients had an exacerbation. Twenty-four patients were treated with prophylactic antibiotics. All of these patients were receiving antibiotics orally (trimethoprim/sulphamethoxazol 17%, azitromycin 27%, amoxicillin/clavulanic acid 4% and flucloxacillin 2%). In addition, 11 patients received inhaled antibiotics [eight (17%) tobramycin and six (13%) colistin]. Therapeutic antibiotic treatment was given to the six patients experiencing an exacerbation [oral: azitromycin 2 (4%), ciprofloxacin 2 (4%), amoxicillin/clavulanic acid 1 (2%) and intravenously: tobramycin 3 (6%), ceftazidime 1 (2%), piperacillin/tazobactam 1 (2%)].

Collection of EBC and FeNO measurements

The mean EBC volume collected was 3.1 ± 0.2 ml and 3.0 ± 0.2 ml in the CF population and control population respectively. No side-effects were reported during or following FeNO measurements and EBC collection.

Detection of inflammatory markers

Detections of IM in EBC are shown in Table 2. Nitrite, nitrate, H₂O₂ and 8-isoprostane were measured in all EBC samples. Cytokines were not detected in some EBC samples. IFN-γ and IL-4 in EBC were detected significantly more often in samples of CF patients (42% and 13% respectively), compared to samples originating from controls (20% and 2% respectively; chi-squared test, p < 0.05).

Table 2. Concentrations and number of positive detection of inflammatory markers in exhaled breath condensate

IM	Detection limit	Cystic fibrosis (n = 48)		Control (n = 50)	
		n %	Mean ± s.e.m.	n %	Mean ± s.e.m.
FeNO	p.p.b.	90	10.0 ± 1.2**	100	15.4 ± 1.4
pH		100	6.3 ± 0.1	100	6.3 ± 0.0
Nitrite	µm 0.05	100	2.2 ± 0.2**	100	1.4 ± 0.1
Nitrate	µm 0.05	100	14.4 ± 2.0	100	16.5 ± 1.4
H ₂ O ₂	µm 0.1	100	2.4 ± 0.2	100	2.2 ± 0.3
8-isoprostane	pg/ml 0.5	100	4.3 ± 0.5	100	4.7 ± 0.6
IFN-γ	pg/ml 1.5	42	3.3 ± 0.3*,**	20	2.6 ± 0.2
TNF-α	pg/ml 1.2	15	1.6 ± 0.1	15	1.6 ± 0.1
IL-2	pg/ml 1.2	2	1.2	0	ND
IL-4	pg/ml 1.4	13	1.8 ± 0.1*	2	2.0
IL-5	pg/ml 1.1	0	ND	0	ND
IL-10	pg/ml 1.1	10	1.3 ± 0.1	6	1.4 ± 0.2

n%, number of positive detection: data are given as percentage of samples above detection limit; ND, not detectable.

*p < 0.05 positive detections of cystic fibrosis vs. controls.

**p < 0.05 concentrations of inflammatory markers in cystic fibrosis vs. controls.

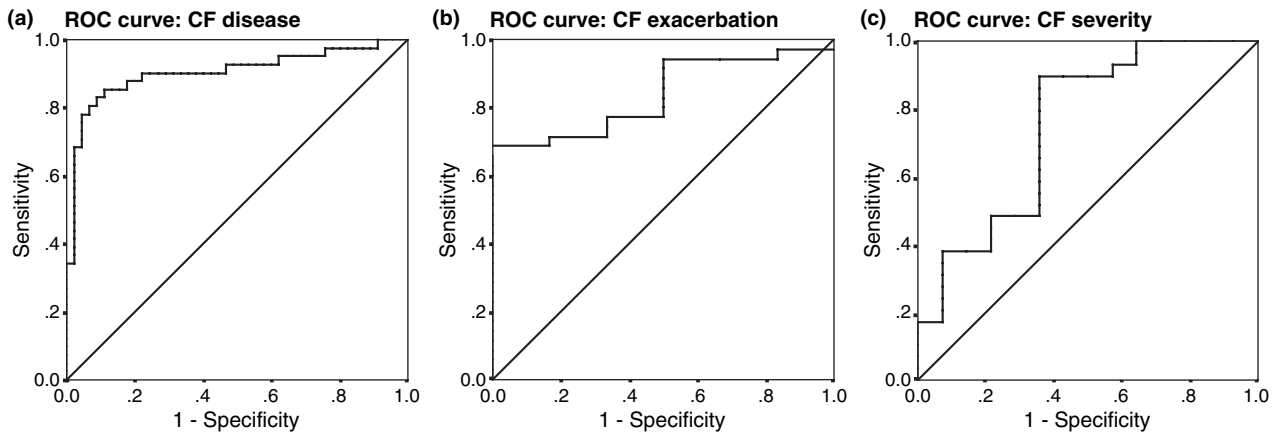


Fig. 1. (a) Receiver operating characteristic curve of the multivariate backward logistic regression model of 8-isoprostane, nitrite and IFN- γ , to indicate the presence of CF disease. 0 = controls; 1 = cystic fibrosis. The area under the curve is 0.906. The maximum sensitivity is 78% with a specificity 83%. (b) Receiver operating characteristic curve of the multivariate backward logistic regression model of 8-isoprostane and nitrite, to indicate CF exacerbation. 0 = stable CF; 1 = CF exacerbation. The area under the curve is 0.838. The maximum sensitivity is 40% with a specificity 97%. (c) Receiver operating characteristic curve of the multivariate backward logistic regression model of FeNO, and EBC acidity, to indicate CF severity. 0 = not excellent CF status; 1 = excellent CF status. CF severity was classified using the Shwachman-Kulczycki score. The area under the curve is 0.751. The maximum sensitivity is 96% with a specificity 67%.

Concentration of inflammatory markers

Mean concentrations of IFN- γ and nitrite were significantly higher in the CF group (3.3 ± 0.3 pg/ml and 2.2 ± 0.2 μ M respectively) compared to controls (2.6 ± 0.2 pg/ml and 1.4 ± 0.1 μ M respectively; Student's *t*-test, $p < 0.05$) (Table 2). In contrast, FeNO was significantly lower in children with CF (10.0 ± 1.2 p.p.b.) compared to control children (15.4 ± 1.4 p.p.b.; $p < 0.05$). No influence was present of inhaled corticosteroids, use of antibiotics or presence of atopy.

CF disease

Multivariate logistic regression models showed that EBC 8-isoprostane, nitrite and IFN- γ and FEV₁% of predicted value, were all significant indicators for CF disease (Table 3). The model including exhaled markers only had a sensitivity

of 78%, a specificity of 92%, and an area under the receiver operating characteristic (AUC) curve of 0.906 (Fig. 1a).

CF exacerbation

Six (13%) of the CF population had an exacerbation, based on an increase in symptoms, and/or decrease in lung function parameters. In the sputum of these patients, the following micro-organisms were cultured: *Pseudomonas aeruginosa* (100%), *Staphylococcus aureus* (67%), *Haemophilus influenzae* (33%), *Aspergillus fumigatus* (83%), *Candida albicans* (17%), Hemolytic *Streptococcus* group A or B (17%). In a multivariate analysis, 8-isoprostane and nitrite indicated a CF exacerbation (Table 4). However, sensitivity was only 40%, with a corresponding specificity of 97% (AUC = 0.838, $p = 0.009$), (Fig. 1b). Correction for age, gender, presence of atopy, lung function parameters or use of antibiotics, did not influence the results.

CF severity

In this part of the analysis, the CF severity classes were reduced to two groups, according to the Shwachman-Kulczycki score: group 1 ($n = 15$) consisted of patients with no excellent severity status, whereas group 2 ($n = 33$) consisted of children with an excellent status. In the multivariate logistic regression model, FeNO, EBC acidity and FEV₁/FVC% were significant markers to indicate CF severity (Table 5). The

Table 3. Multivariate logistic regression model to indicate the presence of CF disease

	p(-2LL)	$\beta \pm$ s.e.	OR (CI 95%)
8-Isoprostane	0.02	-0.08 ± 0.76	0.92 (0.88–0.98)
Nitrite	0.03	0.75 ± 0.37	2.11 (1.35–3.25)
IFN- γ	0.02	0.74 ± 0.35	2.10 (1.17–2.54)
FEV ₁ % predicted	<0.01	-0.07 ± 0.02	0.93 (0.89–0.97)

p(-2LL), significance of change in -2 log likelihood; CF, cystic fibrosis; s.e., standard error; OR, odds ratio; CI, confidence interval; FEV₁, forced expiratory volume in 1 s.

Table 4. Multivariate logistic regression model to indicate a CF exacerbation

	p(−2LL)	$\beta \pm \text{s.e.}$	OR (CI 95%)
8-Isoprostane	0.03	-0.42 ± 0.21	0.70 (0.44–0.99)
Nitrite	0.01	-1.34 ± 0.65	0.26 (0.07–0.93)

p(−2LL), significance of change in −2 log likelihood; CF, cystic fibrosis; s.e., standard error; OR, odds ratio; CI, confidence interval.

The definition of poor CF lung disease control was based on presence or severity of symptoms, and decrease in lung function parameters.

Table 5. Multivariate logistic regression model to indicate CF severity

	p(−2LL)	$\beta \pm \text{s.e.}$	OR (CI 95%)
FeNO	0.01	-0.19 ± 0.09	0.83 (0.70–0.98)
pH	0.01	-6.04 ± 2.66	0.02 (0.00–0.44)
FEV ₁ /FVC %	<0.01	0.19 ± 0.08	1.21 (1.03–1.41)

p(−2LL), significance of change in −2 log likelihood; CF, cystic fibrosis; s.e., standard error; OR, odds ratio; CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity. CF severity was classified using the Shwachman–Kulczycki score.

model including exhaled markers only, had a sensitivity of 96% and a specificity of 67% (AUC = 0.751, $p = 0.008$), (Fig. 1c).

Discussion

This study shows that different non-invasive IM in exhaled air (condensate) are able to reflect different aspects of CF disease with considerably good sensitivity and specificity: (i) 8-isoprostane, nitrite and IFN- γ concentrations, were able to indicate CF disease; (ii) 8-isoprostane and nitrite indicated CF exacerbations and (iii) CF severity was assessed by FeNO and EBC pH. Therefore, this study indicates that non-invasive IM can be helpful in the management of the disease. Longitudinal studies have to confirm that these markers – for instance – are able to indicate the early onset of CF lung disease in young CF children, can detect exacerbations in an early stage, are helpful in the follow-up of exacerbations, and can evaluate treatment effects.

The advantages of this study are: (ii) a relatively large study population of about 100 children (48 CF); (ii) the simultaneous assessment of several non-invasive inflammatory biomarkers which allows a comparison between them; and (iii) the possibility to assess relationships with CF severity and CF exacerbation, which has not been attempted before.

To our knowledge, this is the first study, which demonstrates that profiles of IM in EBC and FeNO, can indicate a CF diagnosis, and,

are able to assess exacerbation and severity of CF lung disease. The limitation of this study is that no CF patients were actually categorized in the severe category. Therefore, results cannot be generalized to these types of patients. FeNO levels in the CF population were reduced compared to the control population. This is in accordance to available data in literature (9, 18–26).

Furthermore, we found significantly higher concentrations of nitrite in EBC originating from children with CF, compared to controls. These results are similar to the results of Formanek et al. and Cunningham et al., as well as Ho, Ojoo and Tate et al. in young adults with CF (18, 19, 27–29). Lower FeNO levels and higher nitrite concentrations in the CF population can be explained by several hypotheses (18, 30). At first, the viscous mucus in CF airways may impair diffusion of gaseous NO into the airway lumen (19). This can result in conversion of NO in nitrite, which is consistent with the higher levels of nitrite in CF than in controls. Secondly, decreased expression of inducible NOS II in epithelial cells may result in low FeNO levels in children with CF (26, 31–34).

More positive detections of IFN- γ and IL-4 were found in EBC samples originating from children with CF, and, IFN- γ concentrations were higher in this group compared to controls. This is in accordance to the study of Wojnarowski et al. (35), which showed transcription of IFN- γ and IL-4 in bronchial biopsies from children with CF, whereas normal controls did not express IFN- γ and IL-4. Immunoregulatory effects of IFN- γ on various cells, are extensive and diverse (36, 37). IFN- γ and IL-4 upregulate class II molecules on macrophages and dendritic cells. Thereby, the antigen presenting capacity of epithelial and endothelial cells, among others, is being induced (37). Additionally, defensive mechanisms to antigens in CF are impaired. By consequence, additional mechanisms need to be addressed to resolve ongoing inflammatory processes and infections (38).

Interleukin-5 was not detected in any sample of the populations, which resembles the findings of Armstrong et al. (39) They could not detect IL-5 in bronchoalveolar lavage fluid. Also cytokine concentrations were relatively low and close to the detection limits of the assays. Previously, we reported, that IFN- γ , IL-2, IL-4, IL-10 were detected in 8–25% of the samples originating from children with CF whereas TNF- α and IL-5 were not detected (11).

In addition, the EBC pH and concentrations of 8-isoprostane and H_2O_2 did not differ between the CF and control populations. This result of H_2O_2 is in accordance to the findings of Jöbsis et al. and Ho et al (9, 40). However, various research groups demonstrated lower EBC pH levels and higher 8-isoprostane concentrations in children with CF (41–43). This may be explained by the mild-to-moderate CF severity in this study, as 8-isoprostane was a significant predictor of CF disease and CF exacerbation in the multivariate models, whereas EBC pH contributed significantly to the indication model of CF severity.

We acknowledge limitations in the use of non-invasive measurements of inflammatory biomarkers. At first, sensitive detection techniques are necessary for the measurements of inflammatory biomarkers in EBC, as the concentration of markers is often low. We were not able to detect all cytokines in all subjects. The lack to detect cytokines probably does not mean cytokines are not present. There is an overall agreement that cytokines are involved in the pathophysiology of CF. A sample with negative detection was not considered as a missing value, because it actually informed us that the measurement of an IM was below the lower detection limit. Secondly the value of IM to predict CF severity and CF exacerbation should be confirmed in a longitudinal study design.

Conclusions

This study showed that several non-invasive IM were able to indicate a (exacerbation of) CF, and can assess CF severity with good sensitivity and specificity. These findings emphasize the importance of evaluating several IM reflecting different aspects of the disease, instead of evaluating one individual IM which may not be able to reflect an evolving chronic inflammatory process appropriately. This study showed that FeNO, and EBC IFN- γ , nitrite, 8-isoprostane and acidity were most important IM to distinguish between CF and non-CF children, and to indicate the severity of exacerbations in CF children. Currently, management and treatment is mainly based on symptoms and deterioration of lung function parameters, which may not reflect ongoing inflammatory processes. In the future, IM in EBC may be a valuable additional tool to monitor inflammatory activity in patients with CF. This may result in earlier detection of a pulmonary CF exacerbation and earlier treatment, which may improve quality of life in children with CF.

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