Journal of Cystic Fibrosis xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Original Article

Key inflammatory markers in bronchoalveolar lavage predict bronchiectasis progression in young children with CF

Hamed Horati [a], Camilla Margaroli [b], Joshua D. Chandler [b], Matthew B. Kilgore [b], Badies Manai [a], Eleni-Rosalina Andrinopoulou [d], Limin Peng [e], Lokesh Guglani [b], Harm A.M.W. Tiddens [a,f,g], Daan Caudri [a], Bob J. Scholte [a,c,1], Rabindra Tirouvanziam [b], HettieM. Janssens [a,*]

[a] Department of Pediatrics, Division of Respiratory Medicine and Allergology, Erasmus MC-Sophia Children's Hospital, University Hospital Rotterdam, I-BALL program, office Sp3456 Dr. Molewaterplein 40, 3015 GD Rotterdam, Postal address: Box 2060, Rotterdam 3000 CB, The Netherlands
[b] Department of Pediatrics, Emory University School of Medicine & Center for CF and Airways Disease Research, Children's Healthcare of Atlanta, Atlanta, GA, USA
[c] Department of Cell Biology, Erasmus MC, University Hospital Rotterdam, Rotterdam, The Netherlands
[d] Department of Biostatistics and Bioinformatics, Erasmus MC, University Hospital Rotterdam, Rotterdam, The Netherlands
[e] Department of Biostatistics and Bioinformatics, Emory University School of Public Health, Atlanta, GA, USA
[f] Department of radiology, Erasmus MC, University Hospital Rotterdam, Rotterdam, The Netherlands
[g] Thirona, Nijmegen, The Netherlands

ARTICLE INFO

Keywords:
Cystic fibrosis
Children
Bronchiectasis
Bronchoalveolar lavage
Inflammatory markers

ABSTRACT

Introduction: Inflammation appears early in cystic fibrosis (CF) pathogenesis, with specific elevated inflammatory markers in bronchoalveolar lavage fluid (BALF) correlating with structural lung disease.
Our aim was to identify markers of airway inflammation able to predict bronchiectasis progression over two years with high sensitivity and specificity.
Methods: Children with CF with two chest computed tomography (CT) scans and bronchoscopies at a two-year interval were included (n= 10 at 1 and 3 years and n= 27 at 3 and 5 years). Chest CTs were scored for increase in bronchiectasis (Δ%Bx), using the PRAGMA-CF score. BALF collected with the first CT scan were analyzed for neutrophil% (n= 36), myeloperoxidase (MPO) (n= 25), neutrophil elastase (NE) (n= 26), and with a protein array for inflammatory and fibrotic markers (n= 26).
Results: MPO, neutrophil%, and inducible T-cell costimulator ligand (ICOSLG), but not clinical characteristics, correlated significantly with Δ%Bx. Evaluation of neutrophil%, NE, MPO, interleukin-8 (IL-8), ICOSLG, and hepatocyte growth factor (HGF), for predicting an increase of > 0.5% of Δ%Bx in two years, showed that IL-8 had the best sensitivity (82%) and specificity (73%). Neutrophil%, ICOSLG and HGF had sensitivities of 85, 82, and 82% and specificities of 59, 67 and 60%, respectively. The odds ratio for risk of >0.5% Δ%Bx was higher for IL-8 (12.4) than for neutrophil%, ICOSLG, and HGF (5.9, 5.3, and 6.7, respectively). Sensitivity and specificity were lower for NE and MPO).
Conclusions: High levels of IL-8, neutrophil%, ICOSGL and HGF in BALF may be good predictors for progression of bronchiectasis in young children with CF.

1. Introduction

Progression of structural lung disease over time is an important cause of mortality in people with cystic fibrosis (CF). A large proportion of children with CF have already developed bronchiectasis by the age of 5 years [1]. Inflammation plays an important role in the pathogenesis of

Abbreviations: BALF, bronchoalveolar lavage fluid; Bx, bronchiectasis; CF, cystic fibrosis; CT, computed tomography; Dis, disease; HGF, hepatocyte growth factor; ICOSLG, inducible T–cell costimulator ligand; IL-8, interleukin-8; MPO, myeloperoxidase; NE, neutrophil elastase; NPX, normalized protein expression; OR, odds ratio; PRAGMA-CF, Perth-Rotterdam Annotated Grid Morphometric Analysis for CF; RML, right middle lobe; ROC, receiver operating characteristics; SE, standard error.

* Corresponding author.
E-mail address: h.janssens@erasmusmc.nl (HettieM. Janssens).

[1] L Bob Scholte passed away in July 2021.

https://doi.org/10.1016/j.jcf.2024.01.002
Received 14 September 2023; Received in revised form 2 January 2024; Accepted 5 January 2024

lung disease in early stages of CF. The lungs of children with CF contain large numbers of neutrophils and increased concentrations of pro-inflammatory cytokines and neutrophil effector proteins [2]. Prior longitudinal studies of young children with CF have shown that specific inflammatory markers correlate with structural lung disease and can predict an increased risk for development of bronchiectasis [3–5].

Early identification of risk factors linked to development of bronchiectasis is important to limit lung damage and to improve long-term survival. However, there are currently few options available for monitoring CF lung disease in preschool children. Preschool children cannot perform spirometry or expectorate sputum, and treating physicians often need to rely on parental report of symptoms. Although lung clearance index (LCI) measured by the multiple-breath washout technique is promising it requires special equipment and trained personnel, and the information on inflammation in the airways that it provides is limited [6]. While chest computed tomography (CT) scans enable quantification of structural lung damage such as bronchiectasis, biomarkers predicting such damage are needed preferably before it develops.

As part of the Australian Respiratory Early Surveillance Team for CF (AREST-CF) research program, regular bronchoscopies and chest CT are done in children with CF following their diagnosis by neonatal screening [7]. Within that cohort, it was previously shown that in bronchoalveolar lavage fluid (BALF) of 3-month-old infants with CF, the presence of detectable neutrophil elastase (NE) was associated with an increased risk of developing bronchiectasis by 12 and 36 months of age [3]. The surveillance program I-BALL (**I**nflammatory markers in **B**roncho-**A**lveolar **L**avage to predict early CF **L**ung disease) in Rotterdam paralleling the AREST-CF program, was started in 2014, a few years after CF newborn screening was introduced in the Netherlands. Within the I-BALL-cohort, we reported previously that measurements in BALF of NE exocytosis by neutrophils [8], myeloperoxidase activity (MPO) [4], interleukin-8 (IL-8) and lipid profiles [5,9] positively correlated with early CF lung disease on chest CT scans [4,5,8]. Increased markers of oxidative stress, arginine metabolism, protein catabolism and lipid metabolism in the airways were also observed in BALF of young CF children with bronchiectasis [10]. Finally, in a cross-sectional analysis of the I-BALL cohort, signaling proteins involved in inflammation and tissue remodeling, including hepatocyte growth factor (HGF), inducible T cell costimulator ligand (ICOSLG,), IL-8, adenosine deaminase, arginase 1, and TNF receptor superfamily members 9 and 14, measured by the Olink protein array platform, were increased in BALF and correlated positively with structural lung disease(9).

Considering the critical role of inflammation in early CF lung disease development, it is important to identify markers that could be used, as there are no clinical tests yet, to predict which children are at risk of developing later structural lung damage. The primary aim of this study was to identify candidate markers of inflammation in BALF that can be used with high sensitivity and specificity in young children with CF that are at risk for development and progression of structural lung disease over two years.

2. Methods

2.1. Subjects

Children diagnosed with CF by newborn screening were included in a prospective, longitudinal early CF monitoring program (I-BALL study; Inflammatory markers in Broncho-Alveolar Lavage to predict early CF Lung disease) at the CF Center of the Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands. A bronchoscopy and chest CT scan were performed at the age of 1, 3 and 5 years as part of their routine annual check. Preschool children were included in this analysis when at least two serial chest CT scans were available. Clinical data were collected from patient's medical records. All bronchoscopies and chest CTs were performed in the period from 2014 to 2019. None of the children were on CFTR modulators at that time. The Institutional Review Board of the Erasmus MC approved the study, and all parents signed informed consent. The I-BALL study is registered on Clinicaltrial.gov (Identifier: NCT02907788).

2.2. Bronchoscopy and BALF collection

Children were clinically stable at the time of the bronchoscopy. If they displayed symptoms of fever or a cold, the bronchoscopy was postponed. Bronchoscopy was performed under general anesthesia and with a laryngeal mask. During bronchoscopy, BALF was collected as follows: three aliquots of normal saline (1 ml/kg body weight, to a maximum of 20 ml/aliquot) were lavaged in the right middle lobe (RML), and one in either the lingula or the most affected lobe, as determined on the chest CT scan before bronchoscopy by the pediatric pulmonologist performing the bronchoscopy. Pooled equal parts of each aliquot were analyzed for microbiology and cell count. The remainder from the second and third aliquots of BALF from the RML were pooled and immediately put on ice, spun at 800 *g* to separate supernatant and cells, and stored at −80 °C until analysis for inflammatory markers.

2.3. Chest CT scans

Free-breathing chest high-resolution CT scans were performed using a Siemens SOMATOM® Force ultra-fast scanner, with a low-dose protocol. The chest CT scan was performed before bronchoscopy, with a maximum of one week in between. Structural CF lung disease was scored using the Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF) method [11]. The PRAGMA-CF score includes the total percentage of disease in the lungs (%Dis), which is the sum of percent bronchiectasis (%Bx) and other abnormalities (mucus plugging and airway wall thickening) as a portion of the whole lung after excluding areas of atelectasis. Intra- and inter-class correlation coefficients were assessed. The intraclass correlation coefficient was 0.95 and interclass correlation coefficient was 0.73 (see supplemental Methods and Table 3 for the validation of PRAGMA scoring) [12].

2.4. Measurement of inflammatory markers

Cell counts were done by counting 300 cells from the pooled BALF, and neutrophil% was calculated. NE, MPO, and proteomics analyses were performed in the stored supernatant of the BALF sample from the RML. NE activity was measured using a fluorometric assay (Cat #600610, Cayman Chemicals, Ann Arbor, Michigan, USA). MPO activity and protein abundance were analyzed by a serial fluorometric activity and ELISA method [4]. For the protein array, we used a Fluidigm-based protein array (Olink, Sweden) with a panel of 92 pro-inflammatory protein markers, as previously described [9]. Olink provided no absolute concentrations, only normalized protein values in log2 ratio as normalized protein expression (NPX) (Supplementary methods) [9]. Out of 92 protein markers acquired, ICOSLG, HGF, and IL-8 were the best candidates for correlation with %Dis and %Bx in this expanded cohort (Supplementary Table 4 and Supplementary Methods). We used these markers in this study for correlation with structural lung disease progression.

2.5. Statistical analysis

The primary aim was to identify the inflammatory marker with the best sensitivity and specificity to predict an increase in the PRAGMA-CF score two years later. Progression of structural lung disease was defined as the change in PRAGMA-CF score between chest-CT at baseline and 2 years later (Δ%Dis and Δ%Bx).

First, univariate correlations between different clinical variables and the outcomes Δ%Dis and Δ%Bx were assessed using Spearman correlation tests, and potential predictive biomarkers were selected based on

their unadjusted *p*-value.

Second, a mixed model was used to investigate the progression of %Dis score, %Bx score and neutrophil% in BALF with age. As the population average progression of Δ%Dis score or Δ% Bx score is unknown, to avoid aggressive classification of progressors, we conservatively define the cutoff for a progressor as the upper bound of the 95% confidence interval for the true population mean of Δ%Dis score or Δ% Bx score. We labeled each child as either *stable* when the Δ%Dis or Δ%Bx was below the total mean + two standard errors of the whole study group, or as *progressor* when it was above the mean + two standard errors. After this, the prognostic value of the different markers for being in the *progressor* group was analyzed using the receiver operating characteristics (ROC) method. Finally, the cut-off value of the markers with optimal sensitivity and specificity was estimated using Youdens Index. R-studio analytics software version 3.6.1 was used for statistical analysis. We performed logistic regression to estimate the odds ratio (OR) of each marker dichotomized based on these cut-off values for whether a child belongs to the progressor group or the corresponding 95% CI, using NLME package version 3.1. The Youdens Index and ROC curve analyses used the ROCR package version 1.0-7.

3. Results

3.1. Baseline characteristics

A total of 37 children with CF were included of which paired samples were available of 10 children aged 1 and 3 years old, and 27 aged 3 and 5 years old. Table 1 shows the clinical characteristics of the study group. In the supplement we show the data for the two age groups separately (supp Table 1).

Table 1
Clinical characteristics study group. Clinical data were acquired from hospital records. The study consists of 37 children with CF with a mean age of 2.8 years. Percentages of the total number included are shown for gender, Pancreatic insufficiency, mutation, hospitalization and positive cultures for *Pseudomonas aeruginosa* and *Aspergillus fumigatus*, For the number of positive culture and oral antibiotic courses, the mean and range is shown. The mean, standard deviation of length, weight, and BMI is based on the Dutch population.

		Study cohort
Number of children with CF		10
Age 1 and 3		27
Age 3 and 5		
Age	Mean	2.8[1–5]
Gender	Male	17 (46 %)
	Female	20 (54%)
PRAGMA-CF Δ%Dis	Stable	29 (78%)
	Increase in %Dis	8 (22%)
PRAGMA-CF Δ%Bx	Stable	22 (59%)
	Increase in %Bx	15 (41%)
Pancreatic insufficient	Yes	30 (81%)
	No	7 (19%)
Mutation	Homozygote F508Del	12 (32%)
	Heterozygote F508Del	21 (57%)
	Other mutations	4 (11%)
positive BAL culture	Yes	12 (32%)
	No	25 (68%)
Number of positive microbiology airway* cultures per child in 2 yrs		5.5 [021]
Number of children with a positive Pseudomonas aeruginosa culture in 2 yrs		7 (19%)
Number of children with a positive Aspergillus fumigatus culture in 2 yrs		3 (8%)
Number of children hospitalized for pulmonary exacerbations in 2 yrs		5 (13%)
Mean number of oral antibiotic courses per child in two years		6.4 [014]

*: airway cultures: nasopharyngeal swab, throat swab, sputum cultures, BAL. Positive microbiology airway cultures: defined as at least one micro-organism cultured in the microbiology lab: microorganisms found were: Pseudomonas aeruginosa, Staphylococcus aureus, Moraxella catarrhalis, Haemophilus influenzae, Escherichia coli and Streptococcus pneumoniae.

3.2. Correlation of inflammatory markers in BALF with progression of early CF lung disease

Values for PRAGMA-CF scores and inflammatory mediators at the different time points are outlined in Table 2 and Supplementary Table 2. Data were not normally distributed, therefore Spearman's correlation tests were used. There was no significant correlation between clinical parameters and change in PRAGMA-CF score (Δ%Dis and Δ%Bx). Clinical parameters tested were: CFTR mutation, pancreatic status, microbiology culture results, hospitalizations, number of positive cultures, oral antibiotic courses prescribed, length, weight and BMI. PRAGMA-CF %Dis and %Bx scores at baseline did not correlate with Δ% Dis or Δ%Bx. There was a significant correlation between PRAGMA-CF %Dis and %Bx and age, but no significant correlation between the inflammatory markers and age (Table 2). The PRAGMA-CF Δ%BE is larger in group 3–5 yrs compared to age group 1–3 yrs, although this a not statistically significant difference.

Correlation was found between change in PRAGMA-CF score Δ%Dis and Δ%Bx and several inflammatory markers using Spearman correlations as shown in Table 3 and Supplementary Table 4. There was significant correlation between ICOSLG and HGF at first bronchoscopy and Δ%Dis ($p < 0.05$), while other inflammatory markers did not show significant correlations. Δ%Bx was significantly correlated with MPO, neutrophil%, ICOSLG, IL-8 and HGF, but not with NE activity.

3.3. Sensitivity and specificity of inflammatory markers in BALF to predict development and progression of bronchiectasis over two years

Children with CF were grouped into either a stable group or a group with more than average Δ%Dis and Δ%Bx defined as follows: the mean Δ%Dis in two years was 0.62 % in this cohort, with a standard error (SE) of 0.17 %. Children with CF with Δ%Dis value that was greater than the mean Δ%Dis plus two SE (= 0.96 %) over two years were grouped as "increase in %Dis" (Supplementary Methods). Analyses were done with 0.96 % and 1 % Δ%Dis, which did not differ from each other. As 1 % is a more practical clinical measure, we continued with a 1 % increase in Δ% Dis. The same method was used for Δ%Bx (mean = 0.28 %, SE = 0.07 %, = 0.42 % increase in %Bx) and grouped as an "increase in %Bx" (Table 1). Calculations were done for 0.42 % and 0.5 %, which did not differ. So we chose to continue with a 0.5 % increase in Δ%Bx, as this is a more practical clinical measure to apply.

To assess which inflammatory markers can be used to predict the development and progression of lung disease, the optimal threshold, defined as the highest sensitivity/specificity calculated with the Youdens Index on the ROC curve [13], was determined (Supplementary Table 5) (Fig. 1). The level of IL-8 >12.2 NPX yields the highest AUC as a predictor for more than 0.5 % of Δ%Bx in two years with the best combination of sensitivity (85 %) and specificity (73 %) AUCs of ICOSLG, Neutrophil%, MPO and HGF were all greater than 0.70. However, their specificity was generally quite lower (Fig. 1 and Supplementary Table 5).

The OR (CI) for risk of more than 0.5 % of Δ%Bx in two years was highest for IL-8 (12.4 (2]–109). For neutrophil%, ICOSLG, and HGF, the ORsOR (CI) were 5.9 (1.8–23.7), 5.3 (1.0–33.9), and 6.7 (1.2–55.7), respectively. OR's for NE and MPO were not statistically significant. Also, OR's for more than 1 % increase in PRAGMA-CF %Dis score were not significant (Fig. 2 and Supplementary Table 5).

4. Discussion

In this study, we found that high levels of ICOSLG, IL-8, HGF, and neutrophil% had high sensitivity at the highest Youdens index for predicting a more than average increase in %Bx over two years in young children with CF. We showed a 5 to 12 times higher risk of developing a

Table 2

CF BALF measurement. Reported in this table are sample size (n), median, and Q25-Q75 range of the measurements. Chest-CT scans were scored using the PRAGMA-CF scoring method. The percentage of disease (%Dis) and bronchiectasis (%Bx) were used as outcomes. NE, MPO, Neutrophil%, ICOSLG, IL-8, and HGF were measured in BALF.

Age (years)	1			3			5		
	n	Median	Q25–Q75	*n*	Median	Q25–Q75	*n*	Median	Q25–Q75
PRAGMA-CF %Dis	10	1.07	0.61–1.45	37	1.8	0.91–2.90	27	2.12	1.23–3.53
PRAGMA-CF %Bx	10	0	0–0.11	37	0.17	0–0.37	27	0.44	0.26–0.91
PRAGMA-CF Δ%Dis	X	X	X	36	0.65	-0.34–1.58	27	-0.23	-0.64–1.7
PRAGMA-CF Δ%Bx	X	X	X	36	0.05	0–0.28	27	0.27	0.05–0.67
NE (μg/mL)	10	5.7	4.4–8.4	20	6.9	5.0–9.5	5	7.8	3.8–14.7
MPO (μg/mL)	8	0.14	0.1–0.4	24	0.42	0.15–1.58	15	1.06	0.30–2.78
Neutrophil percentage (%)	10	14	8–24	36	30	18–51	24	33	19-46
ICOSLG (NPX)	10	0.6	0.3–1.0	20	0.5	0.2–1.3	15	1.3	11.1–13.8
IL-8 (NPX)	10	11.9	10.1–12.8	20	11.7	9.7–13.3	15	13.2	0.3–1.9
HGF (NPX)	10	4.9	4.5–6.0	20	5.3	4.1–6.6	15	6.7	4.9–7.0

Table 3

Correlation analysis of inflammation with change in PRAGMA-CF score. Spearman correlation test was used for the analysis of markers with both change in PRAGMA-CF scores in two years and the yearly increase of PRAGMA-CF score due to differences in age between children with CF. Shown: number of children with CF (*n*)Spearman Rho (Rho), and unadjusted *p*-value (< 0.05 as significance value)

Δ PRAGMA-CF scores within two years		PRAGMA-CF Δ%Dis		PRAGMA-CF Δ% Bx	
	N	Rho	*P*-value	Rho	*P*-value
Baseline PRAGMA-CF %Dis	37	0.73	<0.01	0.26	0.03
Baseline PRAGMA-CF %Bx	37	0.51	<0.01	0.33	<0.01
Neutrophil Elastase	26	0.06	0.77	0.14	0.49
MPO	25	0.25	0.23	**0.57**	**<0.01**
Percentage Neutrophil*	37	0.04	0.79	**0.42**	**<0.01**
ICOSLG	26	**0.48**	**0.01**	**0.53**	**<0.01**
IL-8	26	0.32	0.11	**0.44**	**0.02**
HGF	26	**0.39**	**0.05**	**0.39**	**0.05**

more than average increase in bronchiectasis when either neutrophil%, ICOSLG, IL-8, or HGF were above certain cut-off levels. Furthermore, we found that MPO, neutrophil%, and ICOSLG, IL-8 and HGF correlated positively with Δ%Bx and ICOSLG and HGF with Δ%Dis.

The results show the involvement of several inflammatory processes, which are already present in an early stage of CF lung disease and are a prelude to the development of bronchiectasis. Apart from neutrophilic inflammation, reflected by the increased levels of IL-8, MPO and neutrophil%, there are signs of T-cell activation (ICOSLG), as well as tissue repair and macrophage anti-inflammatory signaling (HGF) [14–16]. Assessment of markers of airway inflammation in early life can help to identify those who are more at risk for developing bronchiectasis.

IL-8 was found to have the best properties as predictive marker for bronchiectasis, in terms of sensitivity and specificity in this cohort. The correlation between IL-8 and structural lung damage has been shown previously in CF ([5,8]). Here we show that IL-8 levels in BALF also have a predictive value in preschool children. IL-8 is released by neutrophils and bronchial epithelial cells and acts as a neutrophil chemotactic and activating factor. Increased levels of IL-8 have also been shown to predict pulmonary exacerbations in children with CF [17]. Therefore, IL-8 seems to be a good candidate to use in clinical practice.

Neutrophil% in BALF has been shown to be one out of five important factors in a prediction model for development of bronchiectasis at the age of 3 years in the AREST-CF cohort [18]. Here we confirm that an increased neutrophil% in BALF itself is important for progression or development of bronchiectasis in the subsequent 2 years. This may be explained by excretion of cytokines and proteases such as NE by neutrophils in a state of hyperexocytosis, as shown in a previous study [8]. Continued neutrophilic inflammation causes tissue damage in the long term [8].

In contrast with former studies, we found that in our cohort soluble NE activity was not predictive of an increase in %Dis and %Bx. Although it has been shown that preschool children with NE activity above a detection threshold at the age of 3 months had almost four times higher odds of having bronchiectasis at the age of 3 yrs [3]. These divergent findings may be due to the fact that we used a different assay to detect NE activity, which is more sensitive. In the AREST-CF study, NE was used as a dichotomous parameter with all levels above a 200 ng/ml threshold being considered positive, but in our study, we measured the levels of NE in a continuous scale, where the lowest detectable level was 0.4 mU/mL (0.14 ng/ml). All samples used in our study had detectable levels of NE. The difference observed could also be due to our smaller sample size and the difference in age of collection of BALF. The importance of NE in development of lung damage in CF has been shown in other studies as well [19–21].

Interestingly, we found that another neutrophil effector protein, MPO, is a possible candidate for predicting Δ%Bx (but not Δ%Dis). MPO is also released by neutrophils from the same granules as NE [22] and is present in large amounts in the airways of children with CF [23–25]. Not only is MPO higher in children with CF compared to non-CF children [23–25], but MPO also correlates with pulmonary symptoms and lung function in older children with CF [24,26]. In addition, people with CF with high MPO have more structural lung disease as [5] measured by chest CT [4,23]. In our study, relatively high levels of MPO correlated with development and progression of bronchiectasis in the following two years, but the sensitivity and specificity for its predictive value for an increase in bronchiectasis were found to be lower than those for ICOSLG, IL-8, and HGF. The same was true for neutrophil%, but not for NE activity.

We previously showed enhanced BALF lipid peroxidation, lysolipid and sphingosine levels are correlated with neutrophil%, NE and MPO [5]. We observed a significant correlation between the lipid markers and progression of %Dis, but not with %Bx. This study confirms in a larger cohort that presence of early inflammation is important for the risk to development of bronchiectasis. Recently IL-8, NE and neutrophil % in BAL have been shown to predict pulmonary exacerbation in children adding yet another dimension to inflammatory markers as monitoring tool [17]. In the COMBAT CF study in preschool children, azithromycin showed decrease in IL-8, NE, number of pulmonary exacerbation, IV antibiotics and inhaled antibiotic treatments compared to placebo. However, they did not see a difference in %Dis and %Bx [27]. Despite evidence for the predictive value of these markers they are not routinely measured in clinical practice. This is due to the fact that bronchoscopy is not a routine examination in most clinics, and is not frequently done because of the invasive character. Our explorative study provides us with potential targets for new clinical laboratory test to measure the degree of inflammation. More research into these markers is needed to acquire data past the age of 5. Also further research is needed to compare the markers with healthy subjects, although this is quite

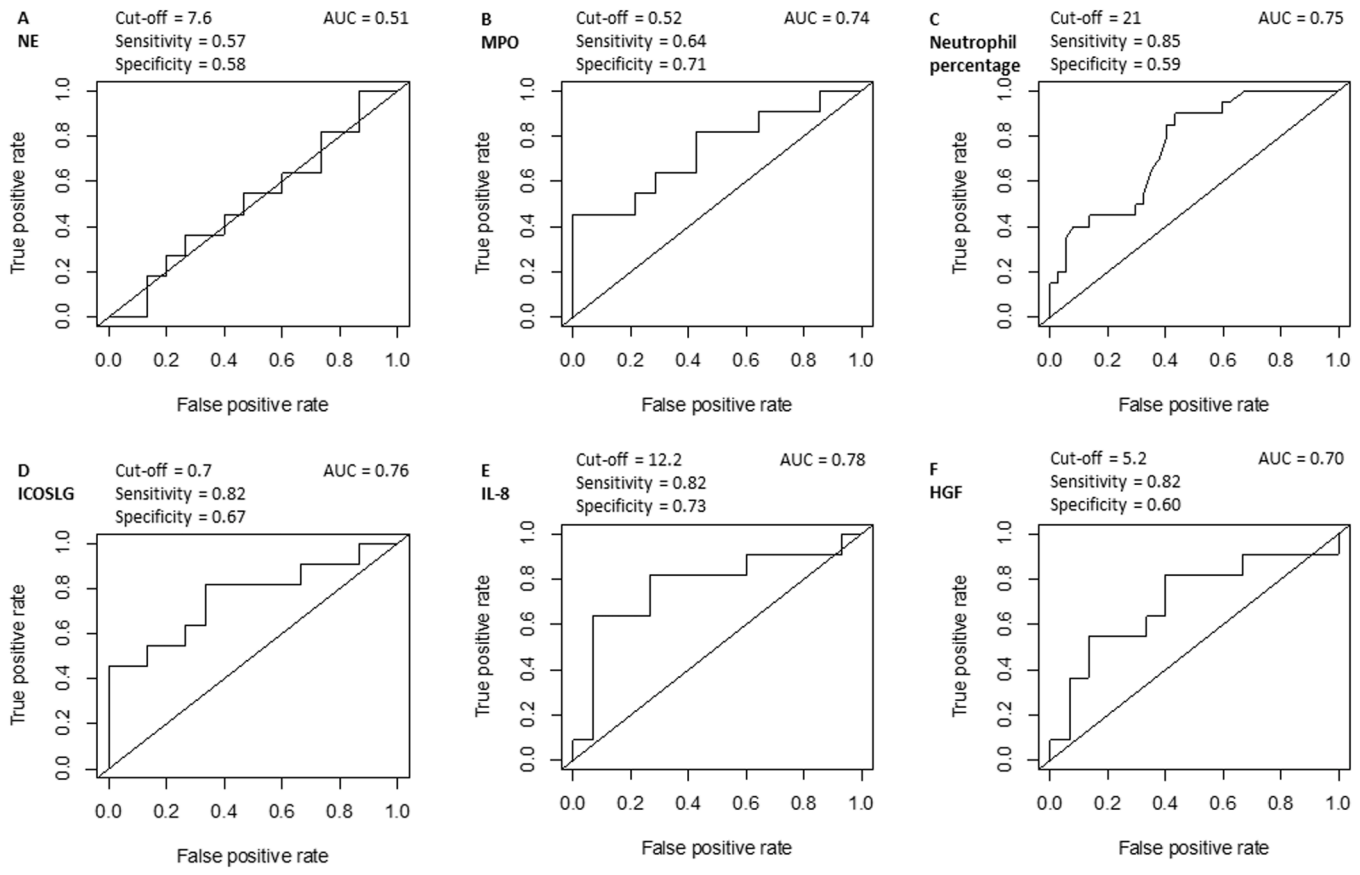

Fig. 1. *ROC curve analysis of inflammatory markers with a 0.5 % increase in percent bronchiectasis.* Receiver Operating Characteristics (ROC) curve were analysed, and the optimal threshold for optimal Youdens Index was determined. Bronchiectasis of > 0.5% in 2 years was categorized as progressive bronchiectasis. The cut-off, sensitivity, specificity and area under the curve (AUC) are shown for NE (A), MPO (B), neutrophil%% (C), ICOSLG (D), IL-8 (E) and HGF (F).

difficult material to achieve for this age group.

Non-invasive methods of monitoring markers of inflammation in infants with CF should be explored. The current era of CFTR modulators will change the course of CF lung disease. Although lung function and exacerbation rate may be improved, there are conflicting data on the level of improvement of lung inflammation. While some clinical studies of CFTR modulators show no significant change in inflammatory markers in sputum, whereas others show beneficial effects on inflammation [28–31]. Recent *in vitro* studies show that current CFTR modulators do not antagonize the up-regulation of CF airway epithelial cytokine production, as IL-8, resulting from exposure to the infectious and inflammatory CF airway milieu and, thus, do not exhibit anti-inflammatory properties [32]. Another study in young children showed that there was no significant difference in NE positivity, IL-8, or absolute neutrophil count in BAL in the years before or in the year after initiation of ivacaftor [33]. With the introduction of highly effective modulators such as elexacaftor/tezcaftor/ivacaftor the issue of inflammation may seem to be not important anymore. However, studies have shown, that although inflammation does decrease, there are still several inflammatory markers that do not improve [34,35]. Furthermore, in most countries young children with CF below 2 years of age, CFTR modulators cannot be started yet. Therefore, inflammation is still relevant to monitor in CF.

There are several limitations to this study. First, the cohort had a relatively small sample size, which included children diagnosed with CF in the Rotterdam CF center over a time span of 5 years. This emphasizes the need for similar data from additional CF centers to provide cross-validation. No multivariate analysis or compound analysis was performed because of the small sample size. Due to the small sample size we also did not correct for multiple testing because of the explorative nature of the study. Second, this study used free-breathing CT scans instead of pressure controlled CT scans. While motion artifacts may be a concern with free-breathing, this was not an issue in our chest CT scans as an ultra-fast scanner was used. This allows to make high quality chest CT's without anaesthesia. Oudraad et al showed that no significant differences where seen in %Bx and %Dis when comparing functional residual capacity (FRC) free breathing CT scans with FRC pressure controlled CT scans [36]. Lastly, the relatively short time interval of 2 years between study visits could cause changes in %Dis and %Bx to be rather small and variable, complicating the search for relevant biological correlations [37]. The findings of our study are in line with what was found in another cohort in our hospital showing that an increase of 1% in %Dis at the preschool age (2–6 yrs), resulted in an increase of 1.18% ($p<0.001$) in %Bx at school age (>7 yrs). In both study we see the %Dis and %Bx skewed to the right and the differences become bigger between patients further emphasizing the important of early markers [38]. Still, having predictive markers for a short-term worsening of CF lung disease may also increase the chance to be able to change the course of the disease.

Despite these limitations, we found several markers to be strong and significant predictors of development and progression of structural lung disease over 2 years in this cohort of CF preschool children. The current study helps improve our understanding of CF lung disease in young children, specifically in relation to inflammatory biomarkers in BALF. Neutrophil activity, T-cell and macrophage function are important in the early stages of CF lung disease. Overall, we have shown that increased levels of IL-8, ICOSLG, and HGF in young children have good sensitivity and reasonable specificity in identifying those at risk for the development and progression of bronchiectasis.

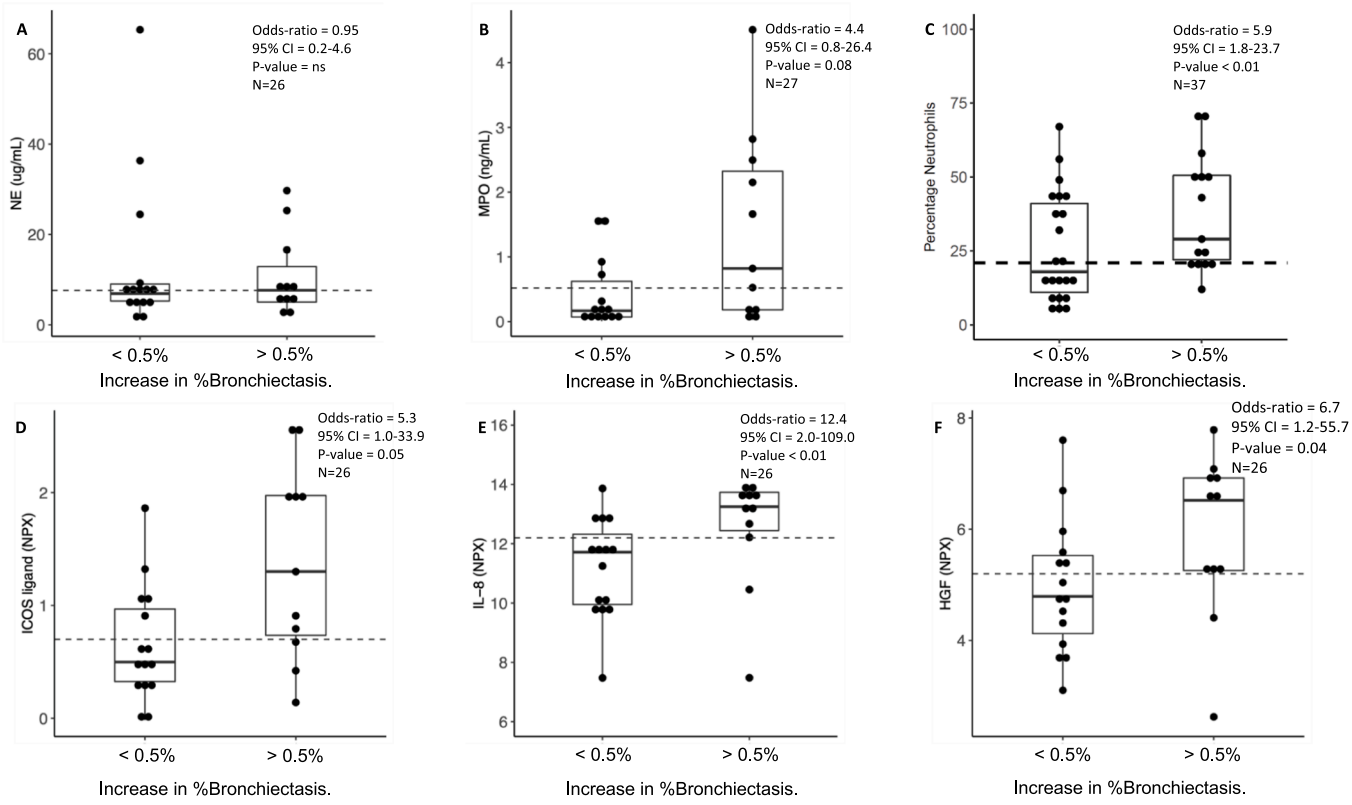

Fig. 2. *Comparison of BALF inflammatory markers among children with CF above or below 0.5% increase in bronchiectasis over two years.* Boxplot figures showing to demonstrate the difference between the stable group (< 0,5% increase in %Bx) and the progressor group (>0,5 % increase in Bx group) for NE (A), MPO (B), neutrophil% (C), ICOSLG (D), IL-8 (E) and HGF (F). The dotted line represents the cut-off point calculated in the ROC curve analysis shown in Fig. 1 and Suppl. Table 5. The odds ratio, 95% Conficence interval (CI), *p*-value were obtained from logistic regression with the marker dichotomized at the cut-off point indicated by the horizontal dashed lines. We reported the sample size (*N*). Not significant differences are reported as ns.

CRediT authorship contribution statement

Hamed Horati: Writing – original draft, Formal analysis, Data curation. **Camilla Margaroli:** Writing – original draft, Data curation. **Joshua D. Chandler:** Writing – original draft, Formal analysis, Data curation. **Matthew B. Kilgore:** Data curation. **Badies Manai:** Data curation. **Eleni-Rosalina Andrinopoulou:** Writing – original draft, Formal analysis, Data curation. **Limin Peng:** Writing – original draft, Formal analysis. **Lokesh Guglani:** Writing – original draft, Formal analysis. **Harm A.M.W. Tiddens:** Writing – original draft, Data curation. **Daan Caudri:** Writing – original draft, Formal analysis. **Bob J. Scholte:** Conceptualization, Funding acquisition, Formal analysis, Data curation, Writing – original draft. **Rabindra Tirouvanziam:** Conceptualization, Funding acquisition, Formal analysis. **HettieM. Janssens:** Conceptualization, Funding acquisition, Writing – original draft, Formal analysis, Data curation.

Declaration of competing interest

There is no conflict of interest associated with this paper.

Supported by

NIH R01HL126603, NCFS HIT-CF 1, Sophia Foundation (S19-06-WO).

Acknowledgments

We would like to acknowledge Drs. Pijnenburg, Duijts, Kloosterman and van der Beukel-Bakker of the Sophia Children'sHospital for diligently performing the bronchoscopies in a standardized way, and for their assistance in collecting the samples. We sincerely thank the children with CF and their parents who participated, without whom this study would not have been possible.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2024.01.002.

References

[1] Stick SM, Brennan S, Murray C, Douglas T, von Ungern-Sternberg BS, Garratt LW, et al. Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. J Pediatr 2009;155(5):623–8. e1.
[2] Roesch EA, Nichols DP, Chmiel JF. Inflammation in cystic fibrosis: an update. Pediatr Pulmonol 2018;53(S3):S30–50.
[3] Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, Mott LS, et al. Risk factors for bronchiectasis in children with cystic fibrosis. N Engl J Med 2013;368(21):1963–70.
[4] Chandler JD, Margaroli C, Horati H, Kilgore MB, Veltman M, Liu HK, et al. Myeloperoxidase oxidation of methionine associates with early cystic fibrosis lung disease. Eur Respir J 2018;52(4):1801118.
[5] Scholte BJ, Horati H, Veltman M, Vreeken RJ, Garratt LW, Tiddens HA, et al. Oxidative stress and abnormal bioactive lipids in early cystic fibrosis lung disease. J Cyst Fibros 2019;18(6):781–9.
[6] Ramsey KA, Foong RE, Grdosic J, Harper A, Skoric B, Clem C, et al. Multiple-breath washout outcomes are sensitive to inflammation and infection in children with cystic fibrosis. Ann Am Thor Soc 2017;14(9):1436–42.
[7] Sly PD, Brennan S, Gangell C, de Klerk N, Murray C, Mott L, et al. Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. Am J Respir Crit Care Med 2009;180(2):146–52.
[8] Margaroli C, Garratt LW, Horati H, Dittrich AS, Rosenow T, Montgomery ST, et al. Elastase exocytosis by airway neutrophils is associated with early lung damage in children with cystic fibrosis. Am J Respir Crit Care Med 2019;199(7):873–81.

[9] Horati H, Janssens HM, Margaroli C, Veltman M, Stolarczyk M, Kilgore MB, et al. Airway profile of bioactive lipids predicts early progression of lung disease in cystic fibrosis. J Cyst Fibros 2020;19(6):902–9.
[10] Esther CR, Turkovic L, Rosenow T, Muhlebach MS, Boucher RC, Ranganathan S, et al. Metabolomic biomarkers predictive of early structural lung disease in cystic fibrosis. Eur Respir J 2016;48(6):1612–21.
[11] Rosenow T, Oudraad MC, Murray CP, Turkovic L, Kuo W, de Bruijne M, et al. PRAGMA-CF. A quantitative structural lung disease computed tomography outcome in young children with cystic fibrosis. Am J Respir Crit Care Med 2015; 191(10):1158–65.
[12] Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med 2016;15(2):155–63.
[13] Schisterman EF, Perkins NJ, Liu A, Bondell H. Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology 2005;16(1):73–81.
[14] Felder M, Trueeb B, Stucki AO, Borcard S, Stucki JD, Schnyder B, et al. Impaired wound healing of alveolar lung epithelial cells in a breathing lung-on-a-chip. Front Bioeng Biotechnol 2019;7:3.
[15] Przybylski G, Chorostowska-Wynimko J, Dyczek A, Wędrowska E, Jankowski M, Szpechciński A, et al. Studies of hepatocyte growth factor in bronchoalveolar lavage fluid in chronic interstitial lung diseases. Polskie Archiwum Medycyny Wewnętrznej 2015;125(4):260–71.
[16] Zhang HY, Ruan LB, Li Y, Yang TR, Liu WJ, Jiang YX, et al. ICOS/ICOSL upregulation mediates inflammatory response and endothelial dysfunction in type 2 diabetes mellitus. Eur Rev Med Pharmacol Sci 2018;22(24):8898–908.
[17] Ishak A, Stick SM, Turkovic L, Ranganathan SC, King L, Harrison J, et al. BAL inflammatory markers can predict pulmonary exacerbations in children with cystic fibrosis. Chest 2020;158(6):2314–22.
[18] Caudri D, Turkovic L, de Klerk NH, Rosenow T, Murray CP, Steyerberg EW, et al. A screening tool to identify risk for bronchiectasis progression in children with cystic fibrosis. Pediatr Pulmonol 2022;57(1):122–31.
[19] Dittrich AS, Kühbandner I, Gehrig S, Rickert-Zacharias V, Twigg M, Wege S, et al. Elastase activity on sputum neutrophils correlates with severity of lung disease in cystic fibrosis. Eur Respir J 2018;51(3):1701910.
[20] Mayer-Hamblett N, Aitken ML, Accurso FJ, Kronmal RA, Konstan MW, Burns JL, et al. Association between pulmonary function and sputum biomarkers in cystic fibrosis. Am J Respir Crit Care Med 2007;175(8):822–8.
[21] Pillarisetti N, Williamson E, Linnane B, Skoric B, Robertson CF, Robinson P, et al. Infection, inflammation, and lung function decline in infants with cystic fibrosis. Am J Respir Crit Care Med 2011;184(1):75–81.
[22] Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol 2005;77(5):598–625.
[23] Dickerhof N, Pearson JF, Hoskin TS, Berry LJ, Turner R, Sly PD, et al. Oxidative stress in early cystic fibrosis lung disease is exacerbated by airway glutathione deficiency. Free Radic Biol Med 2017;113:236–43.
[24] Thomson E, Brennan S, Senthilmohan R, Gangell CL, Chapman AL, Sly PD, et al. Identifying peroxidases and their oxidants in the early pathology of cystic fibrosis. Free Rad Biol Med 2010;49(9):1354–60.
[25] Van der Vliet A, Nguyen MN, Shigenaga MK, Eiserich JP, Marelich GP, Cross CE. Myeloperoxidase and protein oxidation in cystic fibrosis. Am J Physiol Lung Cell Mol Physiol 2000;279(3):L537–LL46.
[26] Regelmann WE, Siefferman CM, Herron JM, Elliott GR, Clawson CC, Gray BH. Sputum peroxidase activity correlates with the severity of lung disease in cystic fibrosis. Pediatr Pulmonol 1995;19(1):1–9.
[27] Stick SM, Foti A, Ware RS, Tiddens H, Clements BS, Armstrong DS, et al. The effect of azithromycin on structural lung disease in infants with cystic fibrosis (COMBAT CF): a phase 3, randomised, double-blind, placebo-controlled clinical trial. Lancet Respir Med 2022;10(8):776–84.
[28] Graeber SY, Boutin S, Wielpütz MO, Joachim C, Frey DL, Wege S, et al. Effects of lumacaftor-ivacaftor on lung clearance index, magnetic resonance imaging, and airway microbiome in Phe508del homozygous patients with cystic fibrosis. Ann Am Thorac Soc 2021;18(6):971–80.
[29] Harris JK, Wagner BD, Zemanick ET, Robertson CE, Stevens MJ, Heltshe SL, et al. Changes in airway microbiome and inflammation with ivacaftor treatment in patients with cystic fibrosis and the G551D mutation. Ann Am Thorac Soc 2020;17 (2):212–20.
[30] Jarosz-Griffiths HH, Scambler T, Wong CH, Lara-Reyna S, Holbrook J, Martinon F, et al. Different CFTR modulator combinations downregulate inflammation differently in cystic fibrosis. Elife 2020;9:e54556.
[31] Rowe SM, Heltshe SL, Gonska T, Donaldson SH, Borowitz D, Gelfond D, et al. Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. Am J Respir Crit Care Med 2014;190(2):175–84.
[32] Ribeiro CMP, Gentzsch M. Impact of airway inflammation on the efficacy of CFTR modulators. Cells 2021;10(11):3260.
[33] McNally P, Butler D, Karpievitch YV, Linnane B, Ranganathan S, Stick SM, et al. Ivacaftor and airway inflammation in preschool children with cystic fibrosis. Am J Respir Crit Care Med 2021;204(5):605–8.
[34] Casey M, Gabillard-Lefort C, McElvaney OF, McElvaney OJ, Carroll T, Heeney RC, et al. Effect of elexacaftor/tezacaftor/ivacaftor on airway and systemic inflammation in cystic fibrosis. Thorax 2023;78(8):835–9.
[35] Schaupp L, Addante A, Völler M, Fentker K, Kuppe A, Bardua M, et al. Longitudinal effects of elexacaftor/tezacaftor/ivacaftor on sputum viscoelastic properties, airway infection and inflammation in patients with cystic fibrosis. Eur Respir J 2023;62:2202153. https://doi.org/10.1183/13993003.02153-2022.
[36] Oudraad MC, Kuo W, Rosenow T, Andrinopoulou ER, Stick SM, Tiddens HA. Assessment of early lung disease in young children with CF: A comparison between pressure-controlled and free-breathing chest computed tomography. Pediatr Pulmonol 2020;55(5):1161–8.
[37] Rosenow T, Mok LC, Turkovic L, Berry LJ, Sly PD, Ranganathan S, et al. The cumulative effect of inflammation and infection on structural lung disease in early CF. Eur Respir J 2019;54(1):1801771.
[38] Bouma NR, Janssens HM, Andrinopoulou ER, Tiddens H. Airway disease on chest computed tomography of preschool children with cystic fibrosis is associated with school-age bronchiectasis. Pediatr Pulmonol 2020;55(1):141–8.