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Inflammatory Markers of Lung Disease in Adult Patients With Cystic Fibrosis

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Summary

Background—Progressive pulmonary disease associated with chronic bacterial infection and inflammation is the major cause of morbidity and mortality in cystic fibrosis (CF) patients. Identifying markers of inflammation that correlate with lung injury may be useful in monitoring disease progression and response to therapy. We hypothesized that levels of serum biomarkers would correlate with clinical course of CF as defined by pulmonary function testing (FEV₁).

Objective—To determine whether biomarkers of systemic inflammation correlate with lung function in adults with CF.

Methods—Retrospective cross-sectional analysis of 63 individuals >30 years of age diagnosed with CF in childhood and followed at Children's Hospital, Boston. We collected data on demographics, CFTR genotype, percent predicted forced expiratory volume in 1 sec (FEV₁), C-reactive protein (CRP), serum IgE and IgG, alpha₁-antitrypsin, total white blood cell and neutrophil counts, and percent neutrophils. We used univariate analyses and multivariate linear regression modeling to examine whether markers of systemic inflammation varied with FEV₁ (% predicted).

Results—In two-covariate models including CRP and one other marker, CRP ($P < 0.001$) and IgG ($P = 0.02$) were significantly associated with FEV₁ (% predicted). In the CRP and IgG model, percent predicted FEV₁ decreased by 4.91% ($P < 0.0001$) for each twofold increase in CRP and by 1.56% ($P = 0.02$) for each 100 mg/dl increase in IgG. Results were unchanged by adjustment for number of DF 508 CFTR alleles. There was no association between any other marker and FEV₁ (% predicted) after adjusting for CRP.

Conclusion—Severity of lung disease in long surviving adult CF patients is correlated with CRP and IgG levels. Our findings relating CRP and IgG levels and lung function provide a foundation for subsequent longitudinal studies and consideration of novel disease mechanisms and outcome measurements.

Keywords

cystic fibrosis; biological markers; airway inflammation; c-reactive protein in cystic fibrosis; lung function; IgG in cystic fibrosis

INTRODUCTION

Cystic fibrosis (CF) is an inherited multisystem disease characterized by progressive deterioration in lung function and pancreatic insufficiency attributed to dysfunction of a single gene encoding the CF transmembrane conductance regulator (CFTR).¹ The CFTR defect in the airways of CF patients affects a multitude of cellular functions, including transmembrane transport of Cl^- and Na^+ , Na^+/H^+ exchange, mucin secretion, and inflammatory signaling.^{2,3} This predisposes CF patients to chronic respiratory infections with *Pseudomonas aeruginosa*, resulting in progressive tissue damage due to airway inflammation.⁴⁻⁷ However, the course of the lung disease differs among patients, even those with the same CFTR mutation.⁸

Despite evidence of inflammation within the airway,^{9,10} it remains unclear which circulating biomarkers best reflect the airway inflammation and lung function of CF patients.¹¹ Demonstration of a consistent association of any of these markers with disease initiation or progression is lacking. Because airway inflammation plays a critical role in lung injury,^{5-8,12,13} markers of inflammation that correlate with or predict lung injury may be useful in monitoring disease progression and response to therapy.¹⁴ Ordonez and coworkers were the first to show that treatment of a pulmonary exacerbation in CF resulted in FEV₁ improvements that correlated with changes in several markers in induced sputum. Specifically, they observed reductions in *P. aeruginosa* and *Staphylococcus aureus* density, neutrophil count, IL-8 concentration, and neutrophil elastase activity.¹⁵ Compared to normal individuals, CF patients' sputum and bronchoalveolar lavage fluid (BAL) show an increased number of neutrophils; increased levels of the pro-inflammatory cytokines TNF α , IL-1 β , IL-6, IL-8, and leukotriene B₄¹⁴⁻¹⁹; and reduced levels of the anti-inflammatory cytokine IL-10.^{16,19-22} Although some studies showed an association of some markers of inflammation found in sputum or BAL with blood levels, others reported no correlation.²³⁻²⁷ Levels of circulating inflammatory markers have been shown to increase during a CF pulmonary exacerbation and to decline in response to antibiotic therapy, suggesting that systemic markers of inflammation reflect lung disease activity.²⁶ CRP concentrations increase in patients with pulmonary exacerbations of CF and decrease after antibiotic therapy.²⁸⁻³⁰ Two recent studies evaluated CRP levels and antibiotic use. After 12 months of treatment with azithromycin, median CRP values declined.^{31,32} In two studies comparing once-daily tobramycin therapy with three-times-daily treatment, CRP levels decreased in both treatment groups.^{28,29} Serum concentrations of IL-6 and neutrophil elastase- α_1 antiproteinase complex also diminish after antibiotic treatment.³³ These studies indicate that

inflammation plays a role in CF lung disease and that monitoring inflammatory markers may be clinically useful.

We hypothesized that one factor accounting for the heterogeneity in pulmonary disease severity is variation in host immune responses. To test this hypothesis, a cohort of CF patients with stable and well-documented disease status was evaluated in a cross-sectional analysis to determine the relationship between serum biomarkers of inflammation and severity of CF lung disease. We hypothesized that levels of serum biomarkers would correlate with clinical course of CF as defined by pulmonary function testing (FEV₁). Biomarker measures analyzed included those described in the literature as being correlated with lung function such as serum CRP, total serum IgE, total serum IgG, serum alpha₁-antitrypsin (AAT), total white blood cell and neutrophil counts, and neutrophil percent of total white blood cell count.

We sought to assess the cross-sectional relationship of biomarkers of inflammation to lung disease severity in a cohort of CF patients. An association between serum biomarkers and lung function determined in a cross-sectional analysis would be necessary prior to proceeding to a larger, more complex, and costly longitudinal analysis. Markers of airway inflammation may be a sensitive measure to determine disease initiation or progression and could help determine the efficacy of new therapies and help determine novel drug therapies. These findings may also have implications for defining the pathophysiology of CF lung disease, stratifying patients, monitoring therapy, and identifying new therapeutic targets.

METHODS

Study Population and Data Collection

We examined a unique group of CF patients aged ≥ 30 years, with the longest history of the disease followed at Children's Hospital in Boston. They represent a large cohort of CF subjects who have stable, well-documented disease status. The study was approved by the Hospital's Institutional Review Board. The diagnosis of CF was documented in the medical record by pilocarpine iontophoresis sweat test (sweat chloride >60 mmol/L). Sixty-five patients met these criteria, but two had no marker data and were excluded, leaving 63 for inclusion in the analysis. For each patient, we extracted the most recent laboratory values and lung function test results from the medical record that were obtained when the patient was at his or her stable baseline and not during a pulmonary exacerbation. We identified eligible CF patients and collected laboratory data by mining the clinical laboratory database at Children's Hospital, Boston. Specifically, for pulmonary function testing and biomarker analysis, data were extracted from the laboratory databases and down-loaded into an ORACLE database. An SQL reporting tool was run to join the hospital-wide laboratory values requested to the CF patient population followed at Children's Hospital. Given the method for extracting data from various electronic sources and merging them together, it was not possible to obtain a symptom history or medication information.

We chose a cross-sectional analysis for this initial study design to examine the relationship between the biomarkers and lung function. Eighty percent of the inflammatory markers were measured on or before the date of the lung function test (median 20 days prior). The time

interval between lung function tests and measurements of inflammatory markers varied between and sometimes within patients. Half of the laboratory measurements were done within ± 3 months of the lung function measurement, and 79% of the laboratory measurements were made within ± 12 months. For two patients, the most recent lung function test results available were at age < 30 years. Laboratory measures included those described in the literature as being correlated with lung function: serum CRP, total serum IgE, total serum IgG, AAT, total white blood cell and neutrophil counts, and neutrophil percent of total white blood cell count. We also evaluated the number of DF 508 alleles, because it is the most common CFTR mutation in the white population.

Genotype Analysis

Genomic DNA isolated from each subject was evaluated for the presence of any of 1,000 CFTR gene mutations (Ambry Genetics, Aliso Viejo, CA) as part of clinical evaluation.

Pulmonary Function Measurements

Forced expiratory volume in 1 sec (FEV_1) was determined by standard spirometry meeting American Thoracic Society criteria; absolute values were converted to a percentage of the predicted volume expected for a healthy individual of the same age, sex, and height on the basis of the regression equations developed by Knudson.^{34,35} Each patient was assigned a disease severity group according to classification of the Epidemiological Study of Cystic Fibrosis (ESCF) for patients ≥ 30 years of age: severe, $FEV_1 < 45.5\%$ predicted; moderate, $45.5\text{--}50.9\%$; mild $50.9\text{--}59.8\%$; and very mild/normal, $\geq 59.8\%$.³⁶ Because only eight patients were in the moderate disease severity category, this group and the mild severity group were pooled for the analysis. Review of hospitalization dates within the year of ascertainment of biomarkers and pulmonary function tests showed that only one FEV_1 value was obtained during a bronchitic exacerbation. However, this measurement was similar to multiple measurements in the same patient during the previous 3 years. A bronchitic exacerbation was defined as worsening of symptoms, as indicated by declining lung function and FEV_1 change of greater than 15% predicted, because such a decrease is a strong predictor of clinician-diagnosed pulmonary exacerbation.³⁷

Statistical Analysis

Unadjusted associations were assessed with Spearman correlations, t -tests, and analysis of variance, as appropriate. Nonparametric Wilcoxon and Kruskal-Wallis tests yielded similar results (data not shown). We used linear regression to assess the adjusted associations between markers and lung function, using percent predicted FEV_1 values as the outcome variable in the models. CRP and IgE were logarithmically transformed for this analysis to reduce the skewness of their distributions. After adjustment for CRP, only one other marker contributed significantly to the model, leading to a two-covariate “final” model. Residual plots confirmed that model assumptions were not violated. We then checked whether additional adjustment for genotype changed the results by adding an indicator variable for homozygous DF 508 genotype. To assess whether the associations differed by genotype, we tested for interaction between CFTR genotype and each marker. The measurements of inflammatory markers and lung function were not necessarily made concurrently for each

patient. We performed several analyses to assess whether the time interval between measurements affected the study conclusions. To assess confounding, we compared estimated model coefficients with and without inclusion of the time interval in the final model. We also tested for interaction between the interval length and marker value to evaluate whether the associations between markers and FEV₁ differed, depending on the interval between measurements. Finally, we repeated these analyses using the absolute value of the time interval. Two-sided P-values are reported, with $P < 0.05$ considered statistically significant.

RESULTS

Table 1 illustrates the characteristics of the 63 study participants at the time of the lung function testing. Their mean age was 39.8 years; the oldest subject was 59 years old. There was no association between age and either the percent predicted FEV₁ (Spearman correlation 0.11, $P = 0.39$) or the number of DF 508 alleles (analysis of variance $P = 0.66$). The percent predicted FEV₁ levels were similar in patients homozygous for DF 508 (mean $\pm$ SD: 53.8 ± 22.5) and patients with zero or one DF 508 allele (mean $\pm$ SD: 51.8 ± 22.2 ; t -test $P = 0.74$).

Table 2 shows the distribution of each biomarker and its association with percent predicted FEV₁ in an unadjusted analysis. The markers most strongly correlated with percent FEV₁ were CRP ($\rho = -0.63$), IgG ($\rho = -0.34$), AAT ($\rho = -0.47$), WBC ($\rho = -0.26$), and absolute neutrophil count ($\rho = -0.22$). Each correlation is unadjusted for the other markers or other covariates. AAT and CRP were highly correlated with each other ($\rho = 0.73$). Figure 1 shows the inverse association between lower FEV₁ percent predicted and higher CRP levels.

In a series of two-covariate linear regression models including CRP and one other marker, CRP was highly significant in all models ($P < 0.001$) and the associated slope coefficient did not change appreciably. The only other significant marker in these models was IgG ($P = 0.02$); P -values for other markers were all >0.36 . The model with CRP and IgG indicates that the percent predicted FEV₁ decreases by 4.91% ($P < 0.0001$) for each twofold increase in CRP and by 1.56% ($P = 0.02$) for each 100 mg/dl increase in IgG (Table 3).

Adjusting for DF 508 genotype by adding an indicator variable for homozygous genotype to the model did not appreciably affect these results. Similar analyses suggested that age was also not a confounder or effect modifier. We evaluated the association between age and number of DF 508 alleles to show that having an allele associated with severe disease did not influence our results. Interactions with genotype were not significant, indicating that the associations shown in Table 3 were similar for patients homozygous for DF 508 and patients with other genotypes. Model results were unaffected by adjusting for the time interval between measurement of FEV₁ and inflammatory markers, using either the positive or negative difference in days or the absolute value of the difference. Interactions between marker values and time intervals were also not significant.

DISCUSSION

We have demonstrated correlations between level of FEV₁ in CF and variations in serum CRP and IgG levels. These associations were not confounded or modified by the number of DF 508 alleles.

Both CRP and IgG are markers of active inflammation and may play a role in the inflammatory cascade within the CF lung. However, the role of these or other inflammatory markers is not clearly defined. CRP has been shown to correlate directly with severity of airflow obstruction and inversely correlate with the use of systemic and inhaled corticosteroids.³⁸ Serum concentrations of CRP are thought to differentiate an exacerbation of chronic obstructive pulmonary disease from daily symptom variation.³⁹ The hypothesis that inflammation contributes independently to progression of CF lung disease is also supported by the clinical findings of the anti-inflammatory agents ibuprofen and azithromycin retarding lung disease progression.^{32,40} In addition, proteomic analysis of sputum from patients with CF showed that IgG levels were a strong predictor of FEV₁.⁴¹ Our study confirms that host responses as measured by IgG and CRP levels, are markers of lung disease severity. These serum markers may thus be useful as outcome measures to assess baseline clinical status in CF patients and responses to therapies.

By evaluating the associations among several clinical inflammatory markers and pulmonary disease severity as defined by percent predicted FEV₁, we were able to demonstrate an association between higher host IgG and CRP levels and clinical disease severity. For patients with elevated IgG levels and lower FEV₁, the IgG levels may represent a hyperimmune response that is ineffective against infection and possibly destructive to the airway. In addition, patients with low IgG antibody levels have a better prognosis^{42,43} suggesting that once chronic infection sets in, a host inflammatory response is initiated that changes this status. It appears that the inability of the immune effectors generated to effectively clear *P. aeruginosa* results in continued B cell and T-cell stimulation, which likely contributes to the progression of lung disease. In addition to antigen-specific immune activation, elevated levels of serum IgG may be produced in response to *P. aeruginosa* surface polysaccharides,^{44,45} which have been shown to mitogenically activate human B cells and stimulate immunoglobulin synthesis. Overall, elevated serum IgG is clearly a marker of increased inflammatory responses to pathogens, and finding a correlation with lung function in CF patients is consistent with the conclusion that inflammation is an important factor in deterioration in respiratory function in CF.

C-reactive protein (CRP) is an acute-phase reactant that not only increases during an inflammatory response but augments the immune response through complement activation, tissue damage, and activation of endothelial cells.^{46,47} Serine proteinases in the lung are normally kept in check by antiproteinases and with time are eventually overwhelmed by high proteinase levels in CF lungs.⁴⁸ AAT is also an acute-phase reactant that increases with the other inflammatory markers. However, it is plausible that the elevated levels of AAT, which correlated highly with CRP levels, are necessary to ameliorate the damage caused by a poorly inhibited inflammatory response and offset the lung damage that takes years to manifest. CRP has a short serum half-life and thus could be a valuable surrogate marker of

inflammation for measuring the efficacy of treatment interventions in patients or during clinical trials. In addition, the association between CRP and FEV₁ was changed little by adjusting for other markers, further supporting the potential value of specific CRP determinations as a single surrogate among the candidate markers.

Our study has certain limitations. Although this was the largest cohort of adult CF patients 30 years of age followed at one center described to date, our sample size was limited to 63 patients. Although it represents an interesting and informative CF population, it does not include very young patients or those with rapidly progressing disease who die before the age of 30 years. Thus, our findings might not be applicable to these groups or other distinct CF populations. However, the relationship that we found between pulmonary disease severity, as measured by FEV₁, serum CRP, and IgG as markers of systemic inflammation were not confounded or modified by age or the number of DF 508 alleles. This suggests that it may be possible to generalize the results to other CF populations, including to younger patients. Although we might expect the older CF patients to have worse lung function or fewer DF 508 alleles, age and number of DF 508 alleles did not differ among subjects in the different lung disease severity categories. This is likely due to a survivor effect or selection bias but also suggests the possibility of a role for modifier genes in the clinical course of CF in these patients. Only one of the FEV₁ values was obtained during a bronchitic exacerbation; therefore, the use of IV antibiotics should not have affected the relationship, we found between clinical status and CRP and IgG levels. However, we do not have data about other treatments that might confound the CRP and PFT relationship. Because we used clinical laboratory and pulmonary function data, it was impossible to obtain concurrent data points and the time interval between ascertainment of these values was not consistent. While this may decrease the precision of our estimates, it should not affect the validity of the results, as evidenced by the fact that incorporating the time interval between measurements into the model in various ways had negligible effects on conclusions. Most laboratory values were obtained within a few months of the corresponding pulmonary function test. Although serum laboratory measures and pulmonary function tests were not perfectly contemporaneous, there is no evidence that the magnitude of the association depends on how closely the two measurements were made. In addition, the retrospective study design did not allow us to identify and evaluate other biomarkers that have been shown to correlate with inflammation in CF such as IL-8, IL-6, or tumor necrosis factor- α . Another limitation of the study is the cross-sectional design, which not only precludes the evaluation of associations between changes in marker values and changes in lung function, but also prevented us from assessing whether the observed associations are predictive. Although we believe our associations are robust, longitudinal data on a larger, unselected group of CF patients would address these concerns.

Inflammatory markers have previously been considered indicative of lung function in CF patients, but prior analyses did not evaluate the joint effects of these inflammatory markers on pulmonary function in CF nor measure the magnitude of their impact. Only by considering the joint effects were we able to demonstrate that, after controlling for CRP level, several markers that appear important in unadjusted analysis do not contribute independently to lung function. We also unexpectedly found that the number of DF 508 alleles did not affect the association between inflammatory markers and lung function, in

spite of the expectation that a significant portion of older patients with good lung function would likely represent CF patients with mutant CFTR alleles associated with less severe disease. Overall, these data suggest that measurement of IgG and CRP levels in CF patients could offer clinically important prognostic information.

Subsequent investigations will need to include a longitudinal analysis to further clarify the cause and effect of CRP levels and selected markers of inflammation and lung disease severity. Identification of factors that explain the observed variability in pulmonary outcome could be valuable both in furthering our understanding of CF pathophysiology and in evaluating the efficacy of therapies.

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ABBREVIATIONS

AAT	alpha ₁ -antitrypsin
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CRP	C-reactive protein
FEV₁	forced expiratory volume in 1 sec.

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Fig. 1. Plot of FEV₁ percent predicted versus C-reactive protein. Each black dot represents individual CF patient value of FEV₁ and CRP; solid black line is the univariate regression line

TABLE 1
Characteristics of Study Population

Study population	
N	63
Age in years: Mean ($\pm$ SD)	39.8 (6.3)
Females/males	29/34
CFTR genotype, N (%)	
Homozygous DF508	23 (42)
Heterozygous DF508	23 (42)
Other genotype	9 (16)
Unknown	8 (13)
Disease severity, N (%)	
Normal/very mild	19 (30)
Mild	11 (17)
Moderate	8 (18)
Severe	25 (40)
% Predicted FEV ₁ : Mean ($\pm$ SD)	54.4 (24.1)

Age is at the time, the FEV₁ % predicted was obtained.

Each patient was assigned a disease severity group based on FEV₁ values, using the Epidemiological Study of Cystic Fibrosis (ESCF) classification for patients ≥ 30 years of age: severe, FEV₁ $\leq 45.5\%$ predicted; moderate, $>45.5\text{--}50.9\%$; mild, $>50.9\text{--}59.8\%$; very mild/normal, $>59.8\%$.

TABLE 2
Marker Distributions and Associations With Lung Disease Severity

Marker	N	Median (10th, 90th) ²	<u>Median, by lung disease severity¹</u>			Corr ³	P-value ³
			None	Mild/mod	Severe		
C-reactive protein (mg/L)	57	6.4 (1.1, 128.0)	2.4	5.15	20.95	−0.63	<0.0001
IgE (mg/dl)	50	28 (7.5, 373)	20	28	37	0.08	0.59
IgG (mg/dl)	62	1235 (891, 1781)	1120	1361	1385	−0.34	0.007
AAT (mg/dl)	61	151 (120, 235)	144	151.5	167	−0.47	0.0001
WBC (1000×/mm ³)	63	9.62 (5.77, 15.59)	9.21	8.78	11.77	−0.26	0.04
Neutrophil (1000×/mm ³)	61	6.85 (3.54, 12.25)	6.7	6.4	9.0	−0.22	0.08
Neutrophil (%)	61	74.1 (59.4, 86.2)	71.0	75.0	75.0	−0.13	0.30

AAT, alpha₁-antitrypsin; WBC, total white blood cell count.

¹ Each patient was assigned a disease severity group based on FEV₁ values using the ESCF classification for patients ≥30 years of age: severe, FEV₁ <45.5% predicted; moderate, >45.5–50.9%; mild, >50.9–59.8%; very mild/normal, >59.8%.

² 10th and 90th percentiles.

³ Nonparametric Spearman correlation with % predicted FEV₁ and test of non-zero correlation.

TABLE 3
Multivariate Regression of % FEV₁ on Markers of Inflammation

Marker	Slope±SE	P-value
Log CRP	-4.91 ¹ ±0.96	<0.0001
IgG, mg/dl	-1.56 ² ±0.63	0.02

¹Change in %FEV₁ per twofold change in CRP.

²Change in %FEV₁ per 100 mg/dl change in IgG.