Association of Lower Airway Inflammation With Physiologic Findings in Young Children With Cystic Fibrosis

Stacey L. Peterson-Carmichael, MD,1 William T. Harris, MD,1 Ruchika Goel, MD,2 Terry L. Noah, MD,1 Robin Johnson, RRT,1 Margaret W. Leigh, MD,1 and Stephanie D. Davis, MD1*

Summary. Background: The relationship between lower airway markers of inflammation and infection with physiologic findings is poorly understood in young children with cystic fibrosis (CF). The goal of this study was to evaluate the association of bronchoalveolar lavage fluid (BALF) markers of infection and inflammation, including mediators linked to airway remodeling, to infant lung function values in young children with CF undergoing clinically indicated bronchoscopy. Methods: Plethysmography and the raised volume rapid thoracoabdominal compression (RVRTC) technique were performed in 16 sedated infants and young children with CF prior to bronchoscopy. BALF was collected and analyzed for pathogen density, cell count, % neutrophils, transforming growth factor beta 1 (TGF-β1), matrix metalloproteinases (MMP), and interleukin-8 (IL-8). Results: There was a significant direct correlation between functional residual capacity (FRC), the ratio of residual volume to total lung capacity (RV/TLC) and FRC/TLC with % neutrophils (P < 0.05). Forced expiratory flows were inversely correlated to % neutrophils (P < 0.01). Lung function parameters did not differentiate those with and without lower airway infection; however, pathogen density directly correlated with FRC and inversely correlated with flows (P < 0.05). In a subset of the population, MMP-2 directly correlated with RV/TLC and inversely correlated with flows (P < 0.05) and TGF-β1 directly correlated with FRC (P < 0.05). Conclusions: Results from this study suggest that lower airway inflammation as well as mediators linked to airway remodeling play an active role in pulmonary deterioration in CF infants and young children undergoing clinically indicated bronchoscopy. Pediatr Pulmonol. 2009; 44:503–511.

Key words: infant pulmonary function tests; raised volume technique; plethysmography; bronchoalveolar lavage; bronchoscopy; forced expiratory flows; cystic fibrosis.

INTRODUCTION

Detecting early lung disease in infants and young children with cystic fibrosis (CF) may lead to earlier intervention and improved prognosis. Infant lung function testing may be helpful in characterizing the progression of early CF lung disease, but the relationship between physiologic markers of disease and lung infection or airway inflammation is not well defined. Dakin et al.1 reported that the concentration of bacterial pathogens and inflammatory markers (% neutrophils and interleukin [IL]-8 levels) directly correlated with air trapping in clinically stable CF children. An inverse correlation was also noted between specific compliance and pathogen concentration as well as % neutrophils. Correlation between markers of inflammation (% neutrophils, neutrophil number, total cell count, IL-8 levels, and leukotriene B4) and indices assessed with the low frequency forced oscillation technique have also been demonstrated.2 However, in both these studies, children

1Division of Pulmonology, Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.
2Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

This research was presented as an abstract at the 2005 North American Cystic Fibrosis Conference in Baltimore, Maryland.

Grant sponsor: NIH; Grant number: 5G32HL007106.

*Correspondence to: Stephanie D. Davis, MD, 130 Mason Farm Road, Campus Box 7217, Chapel Hill, NC 27599-7217.
E-mail: sddavis@med.unc.edu

Received 28 May 2008; Revised 8 January 2009; Accepted 8 January 2009.
DOI 10.1002/ppul.21044
Published online 13 April 2009 in Wiley InterScience (www.interscience.wiley.com).

© 2009 Wiley-Liss, Inc.
received general anesthesia to obtain measures of passive respiratory mechanics (single breath occlusion passive deflation), functional residual capacity (FRC) (nitrogen washout) or low-frequency forced oscillation. Obtaining lung function measures under general anesthesia is not feasible at most centers; though other studies have assessed lung function in sedated, non-intubated CF children.

In a cohort of symptomatic and asymptomatic CF infants and young children, identified by newborn screening (NBS), Nixon et al. reported that forced expiratory volumes (FEVs) assessed using the raised volume rapid thoracoabdominal compression (RVRTC) technique were not directly related to lower airway inflammation (% neutrophils, IL-8 levels, free neutrophil elastase). In this same study, a 10% reduction in forced expiratory volume measured at 0.5 sec (FEV$_{0.5}$) was noted in those subjects with lower airway infection identified by bronchoalveolar lavage. Forced expiratory flows (FEFs) were not reported in this study. Rosenfeld et al. followed CF infants longitudinally using bronchoscopy, clinical parameters, and partial FEFs. The results demonstrated no association between neutrophil density or IL-8 concentration and maximal flows at FRC. These four studies yield conflicting results; therefore, further evaluation of the relationship between lower airway inflammation and infection versus lung function in the CF infant and young child is warranted.

Recently, specific mediators have been linked to airway degradation and remodeling changes in children with CF. Transforming growth factor beta 1 (TGF-$\beta_1$) is an important modulator of inflammation and a potent stimulator of extracellular matrix production. Matrix metalloproteinases (MMP) are a family of proteases involved in extracellular degradation and airway remodeling. In induced sputum from children with CF, elevated MMP-9 levels have been associated with increased inflammation and diminished lung function. MMP-9 levels in bronchoalveolar lavage fluid (BALF) from CF children directly correlated with % neutrophils and significantly diminished after dornase alpha therapy when compared to an untreated group. In addition, increased levels of the ratio of MMP-9 to tissue inhibitor of metalloproteinases 1 (TIMP-1) in BALF have been associated with diminished forced expiratory volume at 1 sec (FEV$_1$) in patients with CF. However, the relationship of these factors to lung function in infants with CF is unknown.

We hypothesized that BALF inflammation (including mediators linked to airway remodeling) is associated with lung function changes in infants and young children with CF. The primary goal of this study was to correlate BALF markers of infection and inflammation with FEFs and lung volumes obtained using the RVRTC technique and plethysmography in infants and young children with CF undergoing clinically indicated bronchoscopy.

**MATERIALS AND METHODS**

**Subjects**

Over a 2-year period, infants and young children with CF who were scheduled for flexible bronchoscopy for clinical indications received infant lung function testing prior to the previously scheduled procedure. Confirmed diagnosis of CF was based on at least two of the following: (1) sweat chloride concentration of $>60$ mEq/mL, (2) two clinical features consistent with CF, or (3) genetic testing demonstrating two mutations associated with CF. Most children in the study were undergoing bronchoscopy to obtain lower respiratory cultures after first isolation of Pseudomonas on deep pharyngeal culture or to guide treatment of a pulmonary exacerbation, defined as an increase in baseline respiratory symptoms as determined by the subject’s primary pediatric pulmonologist. Standard practice within our institution is to perform bronchoscopy and bronchoalveolar lavage (BAL) on infants with new acquisition of Pseudomonas or with pulmonary symptoms not responding to outpatient therapy. Exclusion criteria included oxygen saturation of $<90\%$ on room air, severe upper airway obstruction, significant neurologic impairment or known seizure disorder, past adverse reaction to sedation, severe gastroesophageal reflux symptoms uncontrolled with anti-reflux medications, congenital heart disease, or arrhythmia. Informed consent was obtained from the subjects’ parents prior to bronchoscopy and infant lung function testing. With two subjects, infant lung function testing was performed for clinical purposes prior to bronchoscopy and a retrospective data review of these results was performed. This study was approved by the Committee for the Protection of the Rights of Human Subjects at the University of North Carolina School of Medicine.

**Infant Pulmonary Function Testing (IPFT) Measurements**

On either the day prior to or the day of scheduled bronchoscopy, subjects underwent IPFTs after sedation with chloral hydrate. Subjects received 75–125 mg/kg of chloral hydrate. Once asleep, each infant was placed in a whole body plethysmograph (Collins Infant Pulmonary Laboratory, serial # CF009) in a supine position. Lung volume measurements were tested prior to FEF maneuvers. FRC measures at end tidal inspiration (FRC$_{pleth}$) were obtained as described by Castile et al. An appropriately sized facemask was sealed around the infant’s nose and mouth with medical putty to prevent air leaks. The infant was occluded at end inspiration for at least two to three respiratory cycles. FRC was calculated by subtracting the lung volume above end-expiration from FRC$_{pleth}$. The final FRC represented the mean of a minimum of three separate occlusions.

*Pediatric Pulmonology*
FEFs were then obtained from a raised lung volume using the RVRTC technique as previously described by Jones et al.\textsuperscript{12} and the American Thoracic Society/European Respiratory Society guidelines.\textsuperscript{13} Forced vital capacity (FVC), FEV\textsubscript{0.5}, FEF at 75\% of FVC (FEF\textsubscript{75}), and flows between 25\% and 75\% of FVC (FEF\textsubscript{25–75}) were measured. The flow–volume curve with the highest sum of FEF\textsubscript{25–75} and FVC that was also technically acceptable was chosen for analysis. Fractional lung volumes were determined from the following measures: FVC, FRC, and expiratory reserve volume (ERV). ERV was subtracted from the FRC to obtain residual volume (RV) and FVC was added to RV to obtain total lung capacity (TLC).

**Bronchoscopy and BAL**

A bronchoscopy with BAL was performed by the pediatric pulmonologist according to standard clinical practice either immediately after completion of IPFTs, or the next day. The location for BAL was based on radiographic findings, clinical examination, or bronchoscopic findings and was determined by the bronchoscopist. BAL was performed according to the established standard at our institution with instillation of 10 ml of non-bacteriostatic normal saline into each lavage site when patients are <10 kg. Patients between 10 and 20 kg receive 1 ml/kg of normal saline for each lavage site. Patients >20 kg receive 20 ml per lavage site. Two sites were lavaged for almost all subjects (12/16). In four instances, only one site was lavaged as determined by the pulmonologist performing the procedure. The average return of fluid during BAL for these 16 subjects was 52\% (range of 36.4–75\%). The BALF samples were all processed by the same two technicians using the standard methods as previously described by Muhlebach et al.\textsuperscript{14} BALF cell count was determined by using a hemocytometer. After centrifugation, a modified Wright–Giemsa stain was used and differential cell counts were assessed with 200 consecutive cells examined under light microscopy by an observer blinded to the IPFT results. Quantitative bacterial cultures, mycobacterial, qualitative viral and fungal cultures were performed by the UNC Hospitals clinical microbiology laboratories in accordance with standard protocol as detailed by Gilligan et al.\textsuperscript{15} A positive culture was defined as bacterial detection and organism identification as determined by our microbiology lab: ≥10\textsuperscript{5} colony forming units (CFU)/ml. Those cultures that only grew oropharyngeal flora were denoted as “negative cultures” for the purpose of this study. Measurement of MMP-2, MMP-9, and TIMP-1 was performed through a commercial vendor (Pierce Biotechnology, Woburn, MA) utilizing SearchLight\textsuperscript{TM} multiplex chemiluminescent sandwich ELISA assay. Measurement of TGF-β\textsubscript{1} and IL-8 utilized commercial ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

**Statistical Methodology**

The main IPFT outcomes were FVC, FEV\textsubscript{0.5}, FEF\textsubscript{75}, FEF\textsubscript{25–75}, FRC, ratio of residual volume (RV) to total lung capacity (TLC) defined as RV/TLC, and ratio of FRC to TLC defined as FRC/TLC.

Raw values of the flow variables and lung volumes were converted to Z-scores. To calculate the Z-scores, we used normative data from previous studies by Castile et al.\textsuperscript{11} and Jones et al.\textsuperscript{12} as reference values. Z-scores were calculated as the difference between observed and predicted values divided by the root mean square error (RMSE). For both flow variables and lung volumes, the Z-score models were adjusted for the length of each subject. To calculate the Z-scores for FVC, FEV\textsubscript{0.5}, FEF\textsubscript{75}, FEF\textsubscript{25–75}, we used the models and the RMSE published by Jones et al.\textsuperscript{12} For FRC, FRC/TLC, and RV/TLC, the raw data from Castile et al.\textsuperscript{11} were used to re-generate the quadratic regression equations and get the required RMSE, which was then used to generate the respective Z-scores.

Each of the six IPFT parameters was tested for correlation with lower airway inflammation represented by % neutrophils and IL-8 levels in BALF. The IPFT parameters were also tested for correlation with pathogen count in the culture-positive subjects represented by CFU/ml of BALF. IPFT measurements were also tested for association with the mediators linked to airway remodeling: TGF-β\textsubscript{1}, MMP-2, MMP-9, TIMP-1, and vascular endothelial growth factor (VEGF). Non-parametric Wilcoxon-rank-sum tests were carried out to compare the distributions of various pulmonary function measures between culture-positive and culture-negative subjects. For all analyses, the a priori alpha was set to 0.05. Statistical analysis software (SAS version 9.1.3) was used for all the analyses.\textsuperscript{16}

**RESULTS**

A total of 16 CF infants and young children (12 males) completed the study, achieved research quality data, and were included in the final data analyses. Eleven of the 16 subjects were inpatients with a history of persistent or worsening cough who underwent testing at the start of a course of antibiotics for the treatment of a pulmonary exacerbation. One subject was an outpatient referred from an outside institution for bronchoscopy but was at clinical baseline, and four additional subjects were outpatients who underwent flexible bronchoscopy for first isolation of *Pseudomonas aeruginosa* via deep pharyngeal culture. The ages of the subjects ranged from 18 to 167 weeks with a median age of 86 weeks. Subject demographics are shown in Table 1.
Bronchoscopic and BALF Findings

Quantitative bacterial and fungal cultures were performed on the BALF. Pathogens were recovered (Table 2) in 9 of the 16 (56.3%) subjects. Three of these subjects (33.3%) had more than one pathogen isolated from their BAL cultures. The bacterial pathogens isolated were: *P. aeruginosa* in 5/9 subjects (55.6%), *Oxacillin-sensitive Staphylococcus aureus* (OSSA) in 3/9 (33.3%), *Haemophilus influenzae* in 2/9 (22.2%), and *Oxacillin-resistant Staphylococcus aureus* and *Stenotrophomonas maltophilia* in one subject each. In one subject, *Scopulariopsis* species, a fungal pathogen, was isolated. Although the range of results for BALF in inflammation was broad in our study population, the overall median % neutrophils was 35.7% and the median % neutrophils in the culture-positive subjects was 48.3%. In 10 of the 16 subjects, there was sufficient BALF to measure a series of secondary endpoints related to airway inflammation, degradation, or remodeling. Results of these assays are also shown in Table 2.

IPFT Findings

For illustrative purposes, the raw IPFT data for FEF_{25–75}, FRC, and RV/TLC have been plotted overlaying the regression curves for the normative data from Castile et al.\(^1\) and Jones et al.\(^1\) in Figures 1–3. The mean (SD) of Z-scores for all six IPFT parameters is also demonstrated in Table 3.

Eight subjects (50%) demonstrated evidence of airway obstruction (defined as FEV_{0.5}, FEF_{75}, and/or FEF_{25–75} with Z-score values of < −2). In our data set, FEF_{75} was the most sensitive marker for detection of airflow obstruction in this population. Twelve of the 16 subjects (75%) demonstrated evidence of hyperinflation and/or air trapping defined as FRC_{pleth}, RV/TLC, and/or FRC/TLC with Z-score values > 2.

Relationships Between IPFT and BALF Constituents

When assessing the relationship between lower airway inflammation (represented by % neutrophils) and lung volumes; a positive and statistically significant correlation was seen with Z-scores of FRC (correlation coefficient rho = 0.66, \(P < 0.01\); Fig. 4), RV/TLC (rho = 0.574, \(P < 0.05\); Fig. 5), and FRC/TLC (rho = 0.574, \(P < 0.05\)) versus % neutrophils, suggesting that air trapping increases as inflammation increases. Similar assessment between % neutrophils and various flow measurements revealed a negative and statistically significant correlation between Z-scores of FEF_{75} (rho = −0.67, \(P < 0.01\); Fig. 6) and FEF_{25–75} (rho = −0.63, \(P < 0.01\)) versus % neutrophils, suggesting that small airways flow decreases as inflammation increases. The relationship between IL-8, a potent

---

**TABLE 1—Demographic Profile (n = 16)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
<td>86 (18–167)</td>
</tr>
<tr>
<td>Weight (kg), Z-score(^1)</td>
<td>9.85 (6–13.2) (-1.43 (±1.31))</td>
</tr>
<tr>
<td>Length (cm), Z-score(^1)</td>
<td>76.7 (61.5–92) (-1.18 (±1.11))</td>
</tr>
<tr>
<td>Weight/length ratio (%), Z-score(^1)</td>
<td>12 (10–15) (-0.64 (±1.09))</td>
</tr>
<tr>
<td>Sex ratio, M/F</td>
<td>12/4</td>
</tr>
</tbody>
</table>

\(^1\)Expressed as mean (±SD).\(^30,31\)

**TABLE 2—BALF Data**

<table>
<thead>
<tr>
<th>Primary BALF variables (n = 16)(^1)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive bacterial or fungal culture</td>
<td>9/16 (56.3%)</td>
</tr>
<tr>
<td>Pathogen density (CFU/ml)(^2)</td>
<td>500,000 CFU/ml (10,000–40,010,000)</td>
</tr>
<tr>
<td>(culture-positive subjects only, n = 9)</td>
<td></td>
</tr>
<tr>
<td>BALF cell count (/ml)</td>
<td>755,000 (45,000–43,500,000)</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>35.7% (3–87%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Secondary BALF variables (n = 10)(^1)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (pg/ml)</td>
<td>2,441 (341–20,157)</td>
</tr>
<tr>
<td>TGF-(\beta_1) (pg/ml) (n = 11)</td>
<td>107 (45–354)</td>
</tr>
<tr>
<td>MMP-2 (pg/ml)</td>
<td>1,396 (1,106–5,436)</td>
</tr>
<tr>
<td>MMP-9 (pg/ml)</td>
<td>306,347 (6,503–4,007,100)</td>
</tr>
<tr>
<td>TIMP-1 (pg/ml)</td>
<td>57,002 (30,730–234,500)</td>
</tr>
<tr>
<td>MMP-9/TIMP-1</td>
<td>3.45 (0.1–17.1)</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>537 (229–1,892)</td>
</tr>
</tbody>
</table>

\(^1\)Data are represented as median (range) with respective sample size of n except where indicated otherwise.

\(^2\)Pathogen density does not include oropharyngeal flora.

*Pediatric Pulmonology*
neutrophil chemoattractant, and the various IPFT parameters approached statistical significance ($P = 0.06$ for both $\text{FEF}_{25-75}$ and $\text{FEF}_{75}$), although IL-8 and % neutrophils were strongly correlated with each other ($\rho = 0.86$, $P < 0.001$).

When assessing the relationship between IPFT measures and CFU/ml in the culture-positive subjects, a positive and statistically significant correlation between pathogen density and FRC ($\rho = 0.80$, $P < 0.0001$) was seen. Similarly, significant negative correlations between pathogen density and $\text{FEF}_{75}$ ($\rho = -0.70$, $P < 0.05$) and $\text{FEF}_{25-75}$ ($\rho = -0.70$, $P < 0.05$) were seen.

Additionally, the Z-scores for $\text{FEV}_{0.5}$, $\text{FEF}_{25-75}$, and $\text{FEF}_{75}$ did not differ significantly between subjects with lower airway infection based on culture positivity, as compared to subjects without lower airway infection. Similarly, no significant difference was seen between the Z-scores for the different lung volumes for the above two groups.

For the subset of subjects in whom sufficient BALF was available to measure secondary endpoints previously linked to airway remodeling in CF, MMP-2 and TGF-β1 demonstrated significant correlation with certain IPFT parameters. TGF-β1 correlated directly with FRC ($\rho = 0.65$, $P < 0.05$). MMP-2 inversely correlated with $\text{FEF}_{25-75}$ ($\rho = -0.78$, $P < 0.01$), $\text{FEF}_{75}$ ($\rho = -0.74$, $P < 0.05$), and directly correlated with RV/TLC ($\rho = 0.66$, $P < 0.05$). MMP-9, TIMP-1, MMP-9/TIMP-1 ratio, and VEGF did not correlate significantly with any IPFT variables.

**DISCUSSION**

This study evaluated the relationship of markers of lower airway infection and inflammation to physiologic measures of lung function assessed using the raised volume technique and plethysmography in a young CF population at the time of clinically indicated bronchoscopy. Our findings demonstrate that in this population, subjects with evidence of increased lower airway inflammation, as measured by % neutrophils in BALF, had significantly diminished flows as well as significant hyperinflation and air trapping. In the infants that had evidence of lower airway infection, pathogen density was significantly higher in those with more diminished flows. Our data from the subset of subjects with sufficient BALF for additional study also suggest that certain mediators linked to airway remodeling (MMP-2 and TGF-β1) are also associated with these physiologic markers of airway disease.

Our data differ from previous study populations in which BALF markers of inflammation and/or infection were compared to infant lung function. These prior
studies mainly focused on screening asymptomatic infants, whereas most of our subjects were receiving diagnostic bronchoscopy for pulmonary exacerbation or new acquisition of *Pseudomonas*. In addition, the lung function techniques and measures used in three of these prior studies are not comparable to the raised volume and plethysmographic techniques used in this study.\(^1,2,4\) Nixon et al.\(^3\) and Linnane et al.\(^17\) did evaluate infant lung function using the raised volume technique; however, unlike our study, lower airway inflammation was not associated with worsening lung function. In one of these studies, the group\(^3\) only compared FEVs to markers of airway inflammation and did not evaluate FEFs. Like Nixon et al.\(^3\) and Linnane et al.,\(^17\) we did not see an association between FEV\(_{0.5}\) and lower airway inflammation. Thus, FEVs may not be as sensitive a measure of lower airway disease as FEFs. We also demonstrated that FRC, RV/TLC, and FRC/TLC were significantly elevated in the presence of increased % neutrophils. To our knowledge, no other published studies have demonstrated an association between lower airway inflammation and fractional lung volumes in CF infants.

Unlike previous studies, our subject pool was composed primarily of hospitalized young children who had a higher infection rate with *Pseudomonas*. Our population would therefore be more likely to have significant airway obstruction as compared to previous publications.\(^1,3,17\) This may limit the generalizability of our results, as our population was in fact “sicker” than the infants and young children in some other relevant studies. Our results demonstrate that the taller (older) patients had more severe airways disease. Though Linnane et al.\(^17\) did not find a correlation between inflammation and diminished IPFT parameters; they did provide evidence of pulmonary deterioration with increasing age, specifically in the group of “healthy” CF infants older than 6 months of age. Together, these findings may support the concept of pulmonary deterioration as CF infants age, even in the absence of infection, suggesting other potential factors such as markers of airway remodeling.

Though viral surveillance using rapid antigen screening was not routinely used, viral cultures were performed on the subjects with recovery of only one virus (cytomegalovirus). As it may be more difficult to culture viral pathogens; non-bacterial pathogens could well have contributed to symptoms, decreased infant lung function measures, and an altered inflammatory milieu in any of our 16 subjects. A larger sample size would help to clarify the role of non-bacterial pathogens in our clinical outcomes.

<table>
<thead>
<tr>
<th>Lung function measurements</th>
<th>Z-score mean (± SD), n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV(_{0.5}) (ml)</td>
<td>-1.49 (1.94)</td>
</tr>
<tr>
<td>FEF(_{25-75}) (ml/sec)</td>
<td>-2.48 (2.66)</td>
</tr>
<tr>
<td>FEF(_{25-75}) (ml/sec)</td>
<td>-2.33 (2.84)</td>
</tr>
<tr>
<td>FVC (ml)</td>
<td>-0.76 (1.58)</td>
</tr>
<tr>
<td>FRC(_{pleth}) (ml)</td>
<td>3.55 (4.49)</td>
</tr>
<tr>
<td>RV/TLC</td>
<td>2.22 (2.28)</td>
</tr>
<tr>
<td>FRC/TLC</td>
<td>2.82 (2.41)</td>
</tr>
</tbody>
</table>

![Fig. 4](image1.png)  
**Fig. 4.** Relationship of the Z-score of FRC to % neutrophils. The plus sign (+) represents each individual subject.

![Fig. 5](image2.png)  
**Fig. 5.** Relationship of the Z-score of RV/TLC to % neutrophils. The plus sign (+) represents each individual subject.

 ---

*Pediatric Pulmonology*
Several hypotheses regarding the roles of inflammation and infection in destruction of the CF airways exist. The early inciting factors involved in increased airway inflammation in young CF children remain unclear, and may include factors both inherent in CF epithelia and secondary to infection or abnormal mucus clearance.\(^{18,19}\) Increased inflammation in the absence of infection has been demonstrated in infants with CF.\(^{20}\) In addition, free neutrophil elastase has been shown to be associated with a deterioration in FEV\(_1\) in children and adults with CF\(^{21}\) and IL-8, neutrophil elastase, total cell and neutrophil counts have been inversely correlated to FEV\(_1\) despite a lack of association with bacterial density in CF children.\(^{22}\) Due to limited BALF, we were unable to assess neutrophil elastase levels as an endpoint. Early structural damage has been associated with airway inflammation, as demonstrated by our group\(^ {23}\) where we reported that IL-8 levels and % neutrophils in BALF from CF infants with a pulmonary exacerbation were significantly higher in the most diseased lobe as compared to the lobe with less disease as identified by high resolution chest CT (HRCT).

We have shown in the current study that inflammation, whether in the presence or absence of infection, is associated with decreases in flows and increased air trapping. Our results were influenced by two outliers with high neutrophil counts (>80%); however, we can conclude that high levels of inflammation correlated with decreased flows and hyperinflation, but at lower levels of inflammation, this relationship was less clear. Interestingly, flow values and lung volumes did not significantly differ between the CF children with lower airway infection as compared to the children without infection. Three of seven culture negative subjects received outpatient antibiotics prior to bronchoscopy and infant lung function testing implying the possibility of pathogen clearance. However, our overall findings suggest that lower airway inflammation may be a critical component leading to flow limitation, distal mucous plugging, and air trapping in early CF.

Airway tissue degradation and remodeling may be outcomes of chronic inflammation in CF and other pulmonary disorders. In a somewhat older population of CF children than ours, Hilliard et al.\(^ {5}\) demonstrated that increased matrix breakdown products were associated with decreased lung function, and that reticular basement membrane thickening correlated with BALF TGF-\(\beta_1\) levels. Our data for TGF-\(\beta_1\) and FRC appear to be consistent with these previous data and add support to the concept of a relationship between mediators of airway remodeling and lung function in the youngest CF population. More extensive studies focused on MMP, TGF-\(\beta_1\), and other mediators of airway remodeling as well as reticular basement membrane thickness are needed to validate our findings. However, there may be a rationale for therapeutic strategies altering the airway matrix as a means of minimizing lung damage in the CF population.

With the recent implementation of NBS in over 40 states, identifying markers of early CF lung disease is critical for developing potential outcome measures for future therapeutics as well as helping the CF clinician in his or her care of the young child afflicted with this disease. Early detection of markers of airway inflammation and remodeling via flexible bronchoscopy and concurrent monitoring of infant lung function may provide sensitive means of detecting airway disease. Though there is still some controversy over absolute benefit of CF diagnosis soon after birth on long-term health and life expectancy, it is a rational presumption that better monitoring of the infant population will improve nutritional and respiratory outcomes as well as long-term survival.\(^ {24}\) CF lung abnormalities during infancy are classically silent with respect to historical or physical examination findings in suggesting the presence of disease. This “clinical silence” occurs despite the presence of small airways disease characterized by distal mucous plugging leading to peripheral airway dilatation.\(^ {25}\) Using the raised volume technique, investigators have demonstrated the presence of early airway abnormalities\(^ {26–28}\) despite no previous history of respiratory disease.\(^ {29}\) We confirmed these previously published findings in our CF population undergoing clinically indicated bronchoscopy. Findings in our study aid in clarifying the etiology of these previously reported abnormalities by demonstrating an association between lower airway inflammation and lung function abnormalities in young children with or without infection.

Our results suggest that inflammation is a critical process in pulmonary deterioration during CF exacerbation in infants and young children, much like in older children and adults. The significantly elevated levels of MMP-2 in association with decreased FEF\(_{75}\), FEF\(_{25-75}\), and increased air trapping (RV/TLC) as well as the

---

**Fig. 6. Relationship of the Z-score of FEF\(_{75}\) to % neutrophils. The plus sign (+) represents each individual subject.**
association of elevated TGF-β₁ levels with increased FRC were based on a relatively small subset of our study population and thus need to be further validated. However, it is not difficult to envision how these and other markers of airway remodeling might be used for scoring severity of early CF lung disease, particularly if less invasive sampling techniques can be developed. Meanwhile, the use of bronchoscopy, BALF markers, and physiologic measures may provide sensitive means of detecting airway disease even prior to onset of symptoms or chronic infection. Identifying and validating these early outcome measures will prove useful for helping the CF community target the appropriate therapy for future intervention trials in the youngest population.

ACKNOWLEDGMENTS

We would like to thank Dr. Robert Castile for providing the raw plethysmography and lung volume values from a set of normative controls. We would like to thank Jim Ronk, RRT for his assistance in performing the infant lung function testing and Kathy Abode, RN for assisting in scheduling the joint procedures (infant lung function tests and bronchoscopy). We would also like to thank Justin Hubbard, MS and Paula Murphy for processing the BALF samples and Dr. Marianne Muhlebach for reviewing our microbiology methodology. Dr. Peterson-Carmichael was supported by an NIH training grant (5G32HL007106). Dr. Harris was supported by a Cystic Fibrosis training grant (Harris07A0). Dr. Davis received a Harry Shwachman Cystic Fibrosis Clinical Investigator Award (DAVIS00Q0).

REFERENCES


