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IMPACT OF PSEUDOMONAS AND STAPHYLOCOCCAL INFECTION ON INFLAMMATION AND CLINICAL STATUS IN YOUNG CHILDREN WITH CYSTIC FIBROSIS

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Abstract

Objectives—To assess the effects of *Pseudomonas aeruginosa* (*Pa*) and *Staphylococcus aureus* (*Sa*) infection on lower airway inflammation and clinical status in young children with cystic fibrosis (CF).

Study design—We studied 111 children < 6 years of age who had two *Pa* positive oropharyngeal cultures within 12 months. We examined bronchoalveolar lavage fluid (BALF) inflammatory markers (cell count, differential, IL-8, IL-6, neutrophil elastase), CF-related bacterial pathogens, exotoxin A serology, and clinical indicators of disease severity.

Results—Young children with CF with both upper and lower airway *Pa* infection had higher neutrophil counts, IL-8 and free neutrophil elastase levels, increased likelihood of positive exotoxin A titers, and lower Shwachman scores compared with those with positive upper airway cultures only. *Sa* was associated with increased lower airway inflammation and the presence of both *Pa* and *Sa* had an additive effect on concentrations of lower airway inflammatory markers. BALF markers of inflammation increased with numbers of different bacterial pathogens detected.

Conclusions—Young children with CF with upper and lower airway *Pa* infection have heightened endobronchial inflammation and poorer clinical status compared with children with only upper airway *Pa* infection. The independent and additive effects of *Sa* on inflammation support the importance of polymicrobial infection in early CF lung disease.

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Keywords

cystic fibrosis; inflammation; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; bronchoalveolar lavage

I

Airway infection and inflammation are hallmarks of cystic fibrosis (CF). Chronic endobronchial infection with CF-related pathogens and associated neutrophil-dominated airway inflammation contribute to progressive obstructive pulmonary disease(1). However, the early natural history and relationship between airway infection, particularly with *Pseudomonas aeruginosa*, and extent of inflammation is still not completely understood. Numerous studies have examined infection and inflammation in infants and young children with CF through the use of bronchoscopy with bronchoalveolar lavage (BAL)(2–10). These studies demonstrate that lower airway infections with *P. aeruginosa*, *Staphylococcus aureus*, and *Haemophilus influenzae* occur in infants with CF(3–6,8–10). There is also considerable evidence that airway inflammation, characterized by neutrophil infiltration, elevated interleukin (IL)-8 concentrations, and free neutrophil elastase activity, begins early in life(2–10).

The presence of *P. aeruginosa* and other pathogens in the lower airway of infants with CF is hypothesized to increase the host inflammatory response and thereby accelerate the process of airway damage(9). Investigators report that the degree of endobronchial inflammation is related to the density of CF pathogens isolated from the lower airways(6,10). However, it is unclear whether the inflammatory response in early CF is pathogen specific. Two studies found that young children with *P. aeruginosa* did not have increased inflammation compared with those with other CF pathogens(7,8). Rosenfeld and colleagues reported higher BAL fluid (BALF) IL-8 levels but similar leukocyte and neutrophil counts in those infected with *P. aeruginosa* versus other pathogens(9) whereas Armstrong noted that the BALF samples with the highest concentrations of neutrophils and inflammatory markers grew *P. aeruginosa*(6). These studies are limited by relatively small sample sizes, especially *P. aeruginosa*-infected patients, and variable clinical status.

Although the negative impact of established *P. aeruginosa* infection in older individuals with CF is well documented(11–13), the clinical consequences of *P. aeruginosa* infections in younger children have not been fully characterized(14–16). There has been accumulating evidence in young children with CF that early *P. aeruginosa* infection does increase the risk of subsequent morbidity and mortality(17–19). Addressing the clinical significance of lower airway *P. aeruginosa* infection is hampered in part by the techniques for ascertaining *P. aeruginosa* infection in the CF airway. Oropharyngeal (OP) cultures are widely used as a surrogate for lower airway cultures in young children who are not expectorating, despite the fact that they lack sensitivity and diagnostic accuracy for lower airway *P. aeruginosa* infection (5,20,21). Also, children with CF frequently have a history of intermittent positive OP cultures for *P. aeruginosa* before becoming chronically infected in their lower airways(22).

In two multicenter clinical trials conducted by the Cystic Fibrosis Foundation Therapeutics Development Network (CFF-TDN)(23,24), bronchoscopy and BAL were performed to assess lower airway microbiology and inflammation in young children with CF who had at least two prior OP cultures positive for *P. aeruginosa*. We hypothesized that young children with CF who have both upper and lower airway *P. aeruginosa* infection would have a greater degree of endobronchial inflammation compared with subjects with only upper airway *P. aeruginosa* infection. We also sought to compare the presence of *P. aeruginosa* exotoxin A

serologic response, nutritional status, and other clinical characteristics in these young patients, as well as the effects of *S. aureus* on lower airway inflammation. In this multicenter study, we were able to conduct the largest analysis to date assessing the impact of infection with *P. aeruginosa* and *S. aureus* on lower airway inflammation and clinical status in a diverse group of young children with CF.

METHODS

Study Design

This cross-sectional study examined data from all subjects who had baseline BAL performed as part of two sequential multicenter clinical trials conducted by the CFF-TDN(23,24). These studies were conducted with Institutional Review Board approval at 12 centers between 2000 and 2005. Written informed consent was obtained for all enrolled subjects.

Inclusion criteria for both studies were the same, including: age ≥ 6 months and < 6 years, diagnosis of CF, and at least one historical OP culture positive for *P. aeruginosa* within 2 weeks to 12 months before screening. Subjects with a *P. aeruginosa*-positive OP culture at screening were eligible for a baseline evaluation including bronchoscopy and BAL performed within 3 weeks of the screening visit(23). Baseline evaluation included physical examination, modified Shwachman score (maximum score of 75, no radiograph score)(26), OP culture, and blood draw for *P. aeruginosa* exotoxin A serology.

Laboratory Studies

Bronchoscopy and BAL were conducted at each site according to a standard operating procedure developed by the CFF-TDN (described in complete detail in reference 28). In brief, BAL was performed in a segmental bronchus of the lingula (primary site) or right middle lobe (secondary site). Sterile non-bacteriostatic physiologic saline was instilled through the bronchoscope in three aliquots of 1 mL/kg each (maximum 30 mL, minimum 10 mL). The BALF was pooled into a single sample, placed immediately on ice, and processed for quantitative cultures, total cell counts and differential cell counts, urea, cytokines (IL-8, IL-6), and free neutrophil elastase activity. BAL specimens were cultured for the following bacterial pathogens: *P. aeruginosa*, *S. aureus*, *H. influenzae*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*. Cultures with growth of any of the above CF bacterial pathogens (lower limit of detection of 20 cfu/mL) were considered positive. The concentrations of BALF inflammatory mediators reported in this manuscript were not corrected for urea dilution.

OP cultures, quantitative BALF cultures and *P. aeruginosa* exotoxin A serology titers were performed at the CFF-TDN Microbiology Core (Children's Hospital and Regional Medical Center, Seattle, WA). A positive result for exotoxin A serology was defined as a titer of 1:200 or higher(22). BALF cytology was performed at the CFF-TDN Cytology Core (Case Western Reserve, Cleveland, OH) and inflammatory markers were measured at the CFF-TDN Inflammatory Mediator Core (The Children's Hospital, Denver, CO). All laboratory methodologies have been previously described(23).

Statistical Analysis

Descriptive statistics and graphical displays were used for summarizing data by study group, with groups defined based on CF pathogens isolated from BALF culture. Variables were log-transformed as needed. Univariate comparisons between groups were performed using t-tests or linear regression for continuous variables and chi-squared statistics for categorical variables. Linear regression models were also used to assess whether presence of *P. aeruginosa* and presence of *S. aureus* from baseline BALF culture were predictive of lower airway

inflammatory marker concentrations obtained at baseline BAL. In these models, coefficient estimates reflected the mean difference in inflammatory marker concentration between subjects having a positive versus negative culture for each pathogen, after adjusting for whether the other pathogens were also isolated; these models also accounted for presence of CF pathogens other than *P. aeruginosa* and *S. aureus*. P-values were not adjusted for multiple comparisons. Analyses were performed using Stata (version 9.2) and S-plus (version 6.2).

RESULTS

Subject Characteristics

One hundred eleven infants and young children with CF underwent a baseline bronchoscopy. Subjects were categorized into three groups based on BALF culture results: BALF positive for *P. aeruginosa* (and possibly other CF bacterial pathogens), BALF negative for *P. aeruginosa* but positive for other CF bacterial pathogens, and BALF negative for all CF bacterial pathogens. The clinical characteristics of subjects in each group at the time of BAL are summarized in Tables I (available at www.jpeds.com) and Table II. Demographic and descriptive characteristics were similar between the study groups (Table I). A similar percentage of subjects were BALF culture positive for *S. aureus* in the two groups with positive BALF cultures. *H. influenzae* was the bacterial pathogen most commonly isolated in the subjects who were BALF negative for *P. aeruginosa* but positive for other CF pathogens. Height and weight percentiles were not significantly different among the groups although they tended to be lower in subjects with positive BALF cultures (Table II). For those subjects 2 years of age and older, body mass index percentiles did not differ significantly between groups (data not shown). The main differences between the groups were the exotoxin A serology status and modified Shwachman illness severity scores. Subjects who were BALF positive for *P. aeruginosa* had a higher prevalence of positive exotoxin A titers compared with the other study cohorts ($p=0.02$, Table I). Modified Shwachman scores were lower on average among the cohort who was BALF positive for *P. aeruginosa*, indicating more severe clinical disease (Table II). The lower Shwachman scores in this group were due primarily to lower physical examination and nutrition subscores.

Bronchoalveolar Lavage Fluid Markers of Inflammation

The BALF measurements of inflammation for each group are summarized in Table III. All subjects had active airway inflammation as determined by elevated inflammatory markers compared with published normal values(3,5,8). Comparing the three groups, subjects who were BALF positive for *P. aeruginosa* had significantly greater BALF total cell counts, neutrophil percentages and counts, IL-8 concentrations, and free neutrophil elastase activity. The group whose BALF was negative for *P. aeruginosa* but positive for other CF pathogens had an intermediate degree of lower airway inflammation. Among the cohort who was positive for *P. aeruginosa*, BALF markers of inflammation tended to be higher in the subjects with mucoid strains of *P. aeruginosa* versus those with non-mucoid strains (Table IV; available at www.jpeds.com). Measures of lower airway inflammation were also compared between subjects grouped according to whether or not they had *S. aureus* isolated from the lower airways. Subjects with BALF cultures positive for *S. aureus* ($n=27$) had increased concentrations of BALF inflammatory markers compared with subjects with negative cultures for *S. aureus* ($n=84$), as follows (mean $\pm$ SD): neutrophil count ($\log_{10}$ cells per mL), 5.9 ± 0.5 vs. 5.2 ± 0.7 ($P<0.0001$); percent neutrophils, $54.0\% \pm 21.2$ vs. $33.7\% \pm 23.6$ ($P=0.0003$); IL-8 ($\log_{10}$ pg/mL), 3.4 ± 0.5 vs. 2.6 ± 0.6 ($P<0.0001$); and detectable free neutrophil elastase activity, 46% vs. 17% ($p=0.003$). BALF markers of inflammation tended to be higher in the subjects with *S. aureus* versus those with *H. influenzae* (data not shown).

Both BALF neutrophil counts ($r=0.60$, $p<0.001$) and IL-8 levels ($r=0.59$, $p<0.001$) (data not shown) were significantly related to combined lower airway *P. aeruginosa* density and *S. aureus* density. The subjects co-infected with *P. aeruginosa* and *S. aureus* tended to have the highest associated measurements of inflammation. Further analyses revealed that several BALF markers of inflammation increased with number of different bacterial pathogens detected (Table V). For instance, those subjects with three organisms detected in their BAL cultures had the highest associated amount of airway inflammation.

We used linear regression models to assess the independent effects of *P. aeruginosa* and *S. aureus* lower airway infection on BALF measurements of inflammation (Table VI; available at www.jpeds.com). The presence of *P. aeruginosa* and *S. aureus* were significant independent predictors of BALF total cell counts, neutrophil percentages and counts, and IL-8 concentrations. For example, the presence of *P. aeruginosa* in BALF culture was associated with an approximate 20% increase in percent neutrophils ($P<0.001$), independent of the effect of *S. aureus*, and presence of *S. aureus* was associated with an approximate 15% increase in percent neutrophils ($P=0.004$), independent of the effect of *P. aeruginosa*. Potential interactions between *P. aeruginosa* and *S. aureus* on BALF inflammatory marker concentrations were also examined. This was accomplished by adding an interaction term to each of the models shown in Table VI. There were no significant interactions detected, suggesting the effects were additive and not synergistic. Regression analyses did not include free neutrophil elastase because many of the subjects had undetectable levels.

Analyses also revealed that modified Shwachman scores were lower in those subjects with detectable free neutrophil elastase activity in BALF compared with those without detectable neutrophil elastase (63.5 ± 8.8 vs. 69.7 ± 5.5 , $p<0.0001$). Neutrophil elastase detection was collinear with *P. aeruginosa* infection (i.e., most instances of detectable neutrophil elastase occurred in subjects with BALF positive for *P. aeruginosa*), making it difficult to determine whether *P. aeruginosa* infection or neutrophil elastase detection is a better predictor of Shwachman scores.

DISCUSSION

In this large multicenter study, we have demonstrated that young children with CF with lower airway *P. aeruginosa* infection (i.e. BALF positive for *P. aeruginosa*) had a greater degree of endobronchial inflammation (higher neutrophil counts, IL-8 levels, and free neutrophil elastase activity) and poorer clinical status (lower modified Shwachman scores) compared with those with only upper airway *P. aeruginosa* positive cultures. Lower airway *S. aureus* infection was also related to BALF markers of inflammation and subjects co-infected with *P. aeruginosa* and *S. aureus* tended to have the highest amount of airway inflammation. Further, BALF markers of inflammation increased with numbers of different bacterial pathogens detected. The presence of free neutrophil elastase, primarily seen in subjects with *P. aeruginosa* in the lower airways, was associated with lower modified Shwachman scores. Almost half of the children with *P. aeruginosa* in their lower airways had detectable free neutrophil elastase activity. This contrasts with the absence of free neutrophil elastase in all subjects negative for CF pathogens in their BALF and 86% of children who were negative for *P. aeruginosa* but positive for other pathogens in their BALF. Previous studies have documented the presence of free neutrophil elastase in the BALF of infants and young children with CF(2,3,6), but in this study we can link its detection with worse clinical status. Because free neutrophil elastase is believed to be a contributor to airway injury in CF(27), young children with *P. aeruginosa* and free neutrophil elastase in their lower airways are likely at risk for more rapid development of lung damage. These data provide compelling evidence that the presence of *P. aeruginosa* in the lower airways is clinically important in infants and young children with CF. Our study, which included more subjects infected with *P. aeruginosa* than previous single center or regional studies, expands

upon prior observations that *P. aeruginosa* infection is coupled with a worse clinical course beginning in early childhood(15–19,28). This suggests that the initial presence of *P. aeruginosa* in the lower airways is not a benign process of colonization, but rather one of active infection with associated lung disease and clinical consequences.

The independent and additive impact of *S. aureus* on lower airway disease in these infants and young children with CF is another important finding in this study. Those subjects with lower airway infection with *S. aureus* had elevated neutrophil counts and percentages, and increased IL-8 levels, in comparison to subjects with negative BALF cultures. Further, our linear regression models revealed that the presence of both *P. aeruginosa* and *S. aureus* had a significant additive effect on the different measures of lower airway inflammation. In fact, the coefficient estimates in Table VI suggest that there may be a more pronounced effect of *S. aureus* on all of the inflammatory markers, with the exception of percent neutrophils. These data add to the prior observational finding that CF infants less than 2 years of age with both *S. aureus* and *P. aeruginosa* in their initial OP cultures had increased mortality during the first 10 years after diagnosis, lower radiographic scores, and significantly reduced lung function compared with subjects with other bacteria detected or negative OP cultures(15). Therefore, *S. aureus* should be considered an important lower airway pathogen, independent of *P. aeruginosa* status. This is especially relevant as *S. aureus* is the most common pathogen detected in young children with CF and its prevalence appears to be increasing in the CF population(29).

In this study, only 59/111 patients had a positive BAL culture for *P. aeruginosa* after having at least two positive OP cultures for *P. aeruginosa* within the preceding year, including one at a screening visit within 3 weeks of the baseline visit. It can only be speculated whether this is because BAL dilutes the bacteria and may result in negative detection or due to absence of bacteria in the lower airway. Despite the poor diagnostic accuracy of OP cultures for lower airway *P. aeruginosa* infection in this and prior studies (5,20,30), the need for bronchoscopy to ascertain the presence of *P. aeruginosa* in the lower airways of young non-expectorating children with CF is debatable. Our data indicate that merely having a history of two or more positive OP cultures for *P. aeruginosa* was associated with lower airway inflammation as even the group with negative BALF bacterial cultures had elevated inflammatory markers compared with control children from previous BAL studies(3,5,8). This would be an argument supporting antibiotic treatment for positive OP cultures in an effort to eradicate *P. aeruginosa* from the upper and possibly lower airway and to potentially reduce lower airway inflammation(23). If the goal is to reduce endobronchial inflammation by treating infection, then bronchoscopy is not always necessary because multiple positive OP cultures of *P. aeruginosa* are associated with airway inflammation. However, our data raise a new potential value for bronchoscopy – the detection of lower airway polymicrobial infection. This would help target treatment of *P. aeruginosa* and *S. aureus* and other pathogens to reduce the additive and marked lower airway inflammatory response. This study further highlights the need for investigations of anti-inflammatory therapies in young children with CF. If effective anti-elastase therapy were available, then identifying free neutrophil elastase in the lower airways would be an additional value of bronchoscopy.

This multicenter study involved the largest number of young children with documented lower airway *P. aeruginosa*. These data provide a more diverse and larger CF population with which to explore these associations than has been examined in previous smaller, and mainly single center, studies. Despite these strengths, a limitation of this analysis is that it only assesses cross-sectional associations. It does not allow for assessment of the evolution over time of the natural history of infection, particularly with *P. aeruginosa*, and inflammation in young children with CF. Another limitation is that regional heterogeneity in lower airway infection and inflammation as assayed by BAL may confound observed associations(31,32). Further, there

are no data available to allow comparisons to children of a similar age who have had repeated negative OP cultures and either negative or positive BALF cultures. This would be helpful in establishing the relevance of positive OP cultures for *P. aeruginosa* to lower airway inflammation. It is important to remember that our study population is not representative of all young children with CF or those with a new acquisition of *P. aeruginosa* because our cohort had evidence of prior *P. aeruginosa* infection (at least two *P. aeruginosa*-positive OP cultures within 12 months).

In conclusion, young children with CF with both upper and lower airway *P. aeruginosa* have heightened endobronchial inflammation and worse clinical status compared with subjects with only upper airway *P. aeruginosa* infection. Lower airway infection with *S. aureus* was associated with increased lower airway inflammation and the presence of both *P. aeruginosa* and *S. aureus* had a significant additive effect on the inflammatory response. Lower airway bacterial density correlated with markers of airway inflammation and BALF markers of inflammation increased with numbers of different bacterial pathogens detected. These data highlight the importance of optimizing early intervention strategies to treat both infection and inflammation in young children with CF.

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Appendix- list of study members; online only

The Inhaled Tobramycin in Young Children Study Group included the following site investigators who participated in protocol review and study conduct: Michael Konstan (Case Western Reserve University, paid consultant to Chiron and Novartis), Barbara Chatfield (University of Utah), George Retsch-Bogart (University of North Carolina), David A. Waltz (Children's Hospital Boston/Harvard University, employed by Novartis Institutes for BioMedical Research, Inc.), James Acton (University of Cincinnati), Pamela Zeitlin (Johns Hopkins University), Peter Hiatt (Baylor College of Medicine), Richard Moss (Stanford University, Advisory Board member for Novartis [speaker/consultant], Genetech [speaker/consultant], Vertex, Inspire, PTC, Mpex, Ardis, Johnson & Johnson, Sequoia Sciences, and AOP Lantibio), Jeffrey Wagener (University of Colorado, employed by Genetech), Greg Omlor (Children's Medical Center of Akron), Drucy Borowitz (Women and Children's Hospital of Buffalo), and Margaret Rosenfeld (University of Washington).

Table 1
Descriptive characteristics of study subjects at time of baseline bronchoscopy

		BAL negative for all CF bacterial pathogens (n=31)	BAL Pa negative but other pathogens positive (n=21)	BAL Pa positive (n=59)
Sex	Males	18 (58%)	8 (38%)	30 (51%)
	Females	13 (42%)	13 (62%)	29 (49%)
Age at enrollment (yrs)	Mean (SD)	2.9 (1.8)	3.0 (1.7)	3.3 (1.7)
Age at 1 st Pa+ culture (yrs)*	Mean (SD)	1.8 (1.6)	2.4 (1.7)	2.3 (1.7)
Time since 1 st Pa+ culture (yrs)*	Mean (SD)	0.7 (1.1)	1.3 (1.5)	1.0 (1.2)
CF genotype†	ΔF508/ΔF508	16 (52%)	11 (52%)	33 (56%)
	ΔF508/other	13 (42%)	9 (43%)	17 (29%)
	other/other		1 (5%)	6 (10%)
Other CF pathogens‡	<i>S. aureus</i>		7 (33.3%)	20 (33.9%)
	<i>H. influenzae</i>		17 (80.9%)	13 (22.0%)
	<i>S. maltophilia</i>		5 (23.8%)	3 (5.1%)
	<i>A. xylosoxidans</i>		1 (4.8%)	2 (3.4%)
Exotoxin A serology§	Positive	7 (22.6%)	8 (38.1%)	32 (54.2%)
	Negative	21 (67.7%)	11 (52.4%)	20 (33.9%)
	Not done	3 (9.7%)	2 (9.5%)	7 (11.9%)

* Date and age of first historical culture (almost all were OP) positive for *P. aeruginosa* were reported for 23/31 subjects with negative BALF cultures, 14/21 subjects with BALF negative for *P. aeruginosa* but positive for other CF pathogens, and all subjects with BALF cultures positive for *P. aeruginosa*.

† Genotype data was unavailable for 2 subjects with negative BALF cultures and 3 subjects with BALF cultures positive for *P. aeruginosa*.

‡ Prevalence of other CF bacterial pathogens isolated from their BALF culture.

§ $p = 0.02$, by non-parametric test for trend across ordered groups.

Table 2
Clinical indicators of nutritional status and disease severity by study group

	BAL negative for all CF bacterial pathogens (n=31)	BAL Pa negative but other pathogens positive (n=21)	BAL Pa positive (n=59)	P-value
Weight percentile *	43.1 (29.3)	34.6 (26.3)	33.3 (27.3)	0.31
Height percentile *	41.1 (30.1)	32.0 (19.5)	33.1 (29.1)	0.40
Shwachman history subscore *	24.5 (1.3)	24.0 (1.4)	23.8 (2.1)	0.17
Shwachman exam subscore *	23.5 (1.8)	22.4 (2.5)	20.8 (3.5) [‡]	<0.001
Shwachman nutrition subscore *	23.1 (3.1)	23.1 (2.2)	21.4 (3.8) [‡]	0.03
Modified Shwachman score *	71.1 (5.2)	69.5 (4.9)	66.1 (7.9) [‡]	0.002

* Results shown are the mean (SD) for each study group. P-values reflect a global test performed after fitting a linear regression model that included two predictor variables representing study group and "BAL negative for all CF bacterial pathogens" as the reference group. A significant P-value can be interpreted as indicating that at least two of the groups were significantly different with respect to the mean value of the clinical parameter. All p-values are unadjusted for multiple comparisons.

[‡] p <0.001 relative to the reference group.

[#] p <0.05 relative to the reference group.

Table 3

BALF inflammatory markers by study group

	BAL negative for all CF bacterial pathogens (n=31)	BAL Pa negative but other pathogens positive (n=21)	BAL Pa positive (n=59)	P-value
Log ₁₀ total white cells (per mL) [*]	5.6 (0.3)	5.9 (0.3) [‡]	6.0 (0.4) [‡]	<0.0001
Log ₁₀ neutrophils (per mL) [*]	4.8 (0.6)	5.4 (0.5) [‡]	5.6 (0.7) [‡]	<0.0001
Percent neutrophils [*]	22.1 (14.4)	33.6 (17.6) [§]	49.3 (25.9) [‡] //	<0.0001
Log ₁₀ IL-8 (pg/mL) [*]	2.3 (0.6)	2.8 (0.7) [‡]	3.1 (0.6) [‡]	<0.0001
Log ₁₀ IL-6 (pg/mL) [*]	0.9 (0.5)	1.2 (0.5)	1.2 (0.5)	0.06
Neutrophil elastase detected ^{**}	0/30 (0.0%)	3/21 (14.3%)	23/56 (41.1%)	<0.001

^{*} Results shown are the mean (SD) for each study group. Subjects with missing data were as follows: total white cells (n=8), neutrophils (n=9), percent neutrophils (n=8), IL-8 (n=4), and IL-6 (n=3). Samples with values reported as below the lower limit of quantitation (LLQ) were assigned a value equal to one-half the LLQ for summarization. P-values reflect a global test performed after fitting a linear regression model that included two predictor variables representing study group and "BAL negative for all CF pathogens" as the reference group. A significant P-value can be interpreted as indicating that at least two of the study groups were significantly different with respect to the mean value of the inflammatory marker. All p-values are unadjusted for multiple comparisons.

[‡] p < 0.001 relative to the reference group.

[‡] p < 0.01 relative to the reference group.

[§] p < 0.05 relative to the reference group.

// p = 0.003 relative to "BAL Pa negative but other pathogens positive" group.

^{**} Results shown are the number and proportion of subjects with free neutrophil elastase detected in their BALF. 4 subjects had missing data. p-value is based on a chi-squared test.

BALF inflammatory markers among BALF *P. aeruginosa* positive subjects, by mucoidy status

Table 4

	BAL with non-mucoid Pa (n=44)	BAL with mucoid Pa isolated (n=15)	P-value
Log ₁₀ total white cells (per mL) *	5.9 (0.4)	6.1 (0.4)	0.08
Log ₁₀ neutrophils (per mL) *	5.5 (0.7)	5.9 (0.6)	0.02
Percent neutrophils *	44.5 (25.7)	62.7 (22.1)	0.02
Log ₁₀ IL-8 (pg/mL) *	3.0 (0.6)	3.2 (0.5)	0.16
Log ₁₀ IL-6 (pg/mL) *	1.2 (0.5)	1.1 (0.4)	0.60
Neutrophil elastase detected [†]	14/41 (34.1%)	9/15 (60.0%)	0.08

* Results shown are the mean (SD) for each study group. Among the 59 BAL Pa positive subjects, subjects with missing data were as follows: total white cells (n=6), neutrophils (n=7), percent neutrophils (n=6), IL-8 (n=2), and IL-6 (n=1). Samples with values reported as below the lower limit of quantitation (LLQ) were assigned a value equal to one-half the LLQ for summarization. P-values were obtained from t-tests with unequal variances.

[†] Results shown are the number and proportion of subjects with free neutrophil elastase detected in their BALF. There were 3 subjects with missing data. The P-value is based on a chi-squared test.

Table 5
BALF inflammatory markers by number of CF pathogens detected*

	0 CF pathogens (n=31)	1 CF pathogen (n=40)	2 CF pathogens (n=33)	3 CF pathogens (n=7)	P-value
Log ₁₀ total white cells (per mL) [†]	5.6 (0.3)	5.8 (0.3)	6.1 (0.4)	6.2 (0.5)	<0.0001
Log ₁₀ neutrophils (per mL) [†]	4.8 (0.6)	5.3 (0.6)	5.7 (0.6)	5.8 (0.7)	<0.0001
Percent neutrophils [‡]	22.1 (14.4)	39.2 (26.2)	51.2 (22.9)	49.2 (18.3)	<0.0001
Log ₁₀ IL-8 (pg/mL) [‡]	2.3 (0.6)	2.7 (0.5)	3.2 (0.5)	3.7 (0.7)	<0.0001
Log ₁₀ IL-6 (pg/mL) [‡]	0.9 (0.5)	1.0 (0.5)	1.4 (0.4)	1.6 (0.6)	<0.0001
Neutrophil elastase detected [‡]	0/30 (0.0%)	9/38 (23.7%)	13/32 (40.6%)	4/7 (57.1%)	<0.001

* CF pathogens included *P. aeruginosa*, *S. aureus*, *H. influenzae*, *S. maltophilia*, and *A. xylosoxidans*. No subject had *B. cepacia* isolated. The number of subjects with inflammatory marker measurements included: total white cells (n=103), neutrophil count (n=102), percent neutrophils (n=103), IL-8 (n=107), IL-6 (n=108), and free neutrophil elastase (n=107).

[†] Results shown are the mean (SD) for each group. The P-value for each marker was obtained from a linear regression evaluating the linear association between number of pathogens and marker concentration.

[‡] Results shown are the number and proportion of subjects with free neutrophil elastase detected in their BALF. The P-value is based on a chi-squared test.

Table 6
Presence and independent effects of *P. aeruginosa* and *S. aureus* on BALF inflammatory markers

Response variable*	Predictor variable	Coefficient estimate [†]	95% confidence interval	P-value
Log ₁₀ total white cells (per mL) [n=103]	<i>P. aeruginosa</i> present	0.23	0.08, 0.38	0.003
	<i>S. aureus</i> present	0.37	0.20, 0.55	<0.001
Log ₁₀ neutrophils (per mL) [n=102]	<i>P. aeruginosa</i> present	0.47	0.24, 0.71	<0.001
	<i>S. aureus</i> present	0.58	0.29, 0.86	<0.001
Percent neutrophils [n=103]	<i>P. aeruginosa</i> present	20.09	11.54, 28.65	<0.001
	<i>S. aureus</i> present	14.96	4.88, 25.03	0.004
Log ₁₀ IL-8 (pg/mL) [n=107]	<i>P. aeruginosa</i> present	0.44	0.23, 0.66	<0.001
	<i>S. aureus</i> present	0.67	0.42, 0.91	<0.001
Log ₁₀ IL-6 (pg/mL) [n=108]	<i>P. aeruginosa</i> present	0.10	-0.09, 0.28	0.30
	<i>S. aureus</i> present	0.33	0.12, 0.55	0.003

* The sample size for each model (shown in the left-most column) was less than the total number of subjects due to missing data for inflammatory marker concentrations. Samples with values reported as below the lower limit of quantitation (LLQ) were assigned a value equal to one-half the LLQ for model purposes.

[†] The coefficient estimate for each pathogen reflects the mean difference in inflammatory marker concentration between subjects with cultures positive vs. negative for that pathogen, after adjusting for whether the other pathogen was also isolated.