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Pulmonary exacerbations in cystic fibrosis with negative bacterial cultures

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Summary

Background—Pulmonary exacerbations are a major cause of morbidity in cystic fibrosis (CF) and likely contribute to lung function decline. Exacerbations are often associated with characteristic airway bacteria [CF related bacteria (CFRB)]. However, some patients do not have CFRB detected by culture during exacerbations.

Objectives—We sought to determine the proportion of airway cultures negative for CFRB during pulmonary exacerbations, and to characterize patients who were CFRB-negative versus CFRB-positive.

Methods—We performed a retrospective study of patients with CF admitted for a pulmonary exacerbation. Patients were classified as CFRB-positive or CFRB-negative based on admission airway cultures. Demographics, clinical presentation, lung function, history of chronic *P. aeruginosa* infection and improvement in lung function with treatment were compared between groups.

Main Results—There were 672 admissions for exacerbation involving 211 patients over five years. Seventeen percent were classified as CFRB-negative. Forty-one percent of bronchoalveolar lavage (BAL), 32% of throat and 10% of sputum samples were CFRB-negative. Among patients capable of expectorating sputum, the CFRB-negative group was younger, less likely to have chronic *P. aeruginosa*, had higher lung function and body mass index (BMI), and had a lower systemic inflammatory response on admission compared to those with CFRB-positive cultures. The two groups had similar numbers of patients with three or more signs and symptoms of a pulmonary exacerbation (88% versus 92%). Both groups returned to baseline lung function following treatment.

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Conflict of Interest Statement

ET Zemanick has no conflicts to disclose relevant to this manuscript.

BD Wagner has no conflicts to disclose relevant to this manuscript.

JK Harris has no conflicts to disclose relevant to this manuscript.

JS Wagener was previously employed by Genentech and has previously or is currently an advisor for Genentech, Gilead, GSK, Mpex, Novartis and Vertex.

FJ Accurso has no conflicts to disclose relevant to this manuscript.

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Conclusions—A significant number of patients with CF and pulmonary exacerbation did not have typical CF related bacteria detected by culture. Patients without CF related bacteria still had characteristic signs and symptoms of pulmonary exacerbation and responded to treatment. Understanding the causes of illness in these patients may improve the diagnosis and treatment of pulmonary exacerbations in CF.

Keywords

airway infection; sputum culture; bronchoalveolar lavage; lung function

Introduction

Pulmonary exacerbations, characterized by increased respiratory symptoms and worsening lung function, are a major cause of morbidity and decreased quality of life for patients with cystic fibrosis (CF).^{1,2} Recurrent pulmonary exacerbations are associated with long term decline in lung function and shortened survival.^{3–5} Patients with pulmonary exacerbations are frequently hospitalized and treated with intravenous antibiotics and augmented airway clearance therapy. Airway cultures are often collected during pulmonary exacerbations to direct antibiotic treatment, and these cultures frequently detect bacterial pathogens, most commonly *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Burkholderia cepacia* complex.^{6,7}

Despite the importance of pulmonary exacerbations in CF, their etiology is poorly understood.⁸ Postulated causes include acquisition of new bacterial pathogens, clonal expansion of colonizing bacteria, viral infections, increased host inflammatory response, chronic infection with *Aspergillus fumigatus*, and environmental insults.^{2,9} Nontuberculous mycobacterium species infect patients with CF, however their role in acute pulmonary exacerbations is unclear.¹⁰ Studies of pulmonary exacerbations frequently focus on patients with CF related bacteria, and intravenous antibiotics have been shown to improve clinical symptoms and lung function, suggesting bacterial infection as the etiology.^{11–13} Some patients with CF do not have typical bacteria detected even when pulmonary symptoms are present.^{14,15} Pulmonary exacerbation in these patients may be due to non-bacterial causes or to bacterial pathogens undetected by culture.

There is a paucity of data regarding the frequency of negative airway bacterial cultures in the setting of pulmonary exacerbation and the characteristics of these exacerbations. Our first aim was to determine the proportion of airway cultures negative for typical CF related bacteria at the time of admission for pulmonary exacerbation. Our second aim was to determine whether patients without CF related airway bacteria differed either at presentation or in response to treatment from those with detectable CF related bacteria. To determine the proportion of patients with CF who had airway cultures negative for CF related bacteria (CFRB) we retrospectively examined airway culture results (throat culture, sputum and bronchoalveolar lavage) from CF patients admitted to our hospital for pulmonary exacerbation over five years. Next, we compared clinical and laboratory characteristics at admission, and lung function response to treatment between patients with CFRB-negative and CFRB-positive airway cultures. Our hypothesis was that some patients requiring hospitalization for pulmonary exacerbation would not have typical CF related bacteria detected by airway culture, and that the characteristics and response to treatment of these patients would differ from those with CFRB-positive cultures, suggesting that the etiology of pulmonary exacerbation may differ between these two groups. The preliminary results of this study were previously presented.¹⁶

Materials and Methods

We reviewed the charts of all patients with CF admitted to the University of Colorado Denver affiliated Children's Hospital for a pulmonary exacerbation between May 2002 and May 2007. The Colorado Multiple Institutional Review Board approved this study. We queried our CF Clinical Care Center database for all patients admitted for a pulmonary exacerbation who had airway cultures (throat swab, sputum or bronchoalveolar lavage (BAL) fluid) obtained within seven days of admission. Patients admitted to the hospital with no record of airway culture within seven days of admission were excluded from further analysis.

The following demographic and diagnostic data were collected from the CF clinical database and the hospital's electronic medical record: gender, CF genotype, sweat test results, history of meconium ileus, age at admission, results of admission airway culture, airway culture results within 3 months prior to admission, history of prior or chronic *P. aeruginosa*, antibiotic treatment prior to admission, number of hospitalizations during the year prior to admission, body mass index (BMI), and systemic inflammatory markers [white blood cell count with differential (WBC), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR)]. Antibiotic treatment prior to admission was categorized as (1) recent antibiotic treatment, defined as new inhaled, oral or IV antibiotic prescribed in the two weeks prior to admission, or (2) chronic antibiotic treatment, defined as (a) inhaled tobramycin, (b) azithromycin or (c) other inhaled, oral or intravenous antibiotic prescribed for at least 2 months prior to admission. All airway cultures were performed according to standard Cystic Fibrosis Foundation guidelines.^{17,18} Chronic *P. aeruginosa* infection was defined as ≥ 3 positive cultures or mucoid *P. aeruginosa* detected in the 12 months prior to admission. Patients were classified by genotype as mild or severe based on published data.¹⁹ Due to the wide variety of treatment options, specific treatment regimens were not recorded; however, all patients received standard treatment for pulmonary exacerbations including intravenous antibiotics and augmented airway clearance treatments during their hospitalization.

Pulmonary function test results [forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁)] were recorded at baseline (defined as highest FEV₁ value recorded in the 12 months prior to admission), admission and discharge. Absolute values for FVC and FEV₁ were recorded and percent predicted values were calculated using Wang (for males ages 6 to 17 years; females ages 6 to 15 years) and Hankinson (for males ≥ 18 years; females ≥ 16 years) equations.²⁰⁻²²

Pulmonary exacerbation signs and symptoms were recorded as documented by the admitting physician, and consisted of: increased cough, increased sputum production, fever, weight loss, school or work absenteeism, increased respiratory rate, new findings on chest auscultation, decreased exercise tolerance or fatigue, decrease in FEV₁ of ≥ 10% predicted, decrease in pulse oximetry (defined as new or increased supplemental oxygen requirement or greater than 4% decline in room air oxygen saturation from previous well visit), and new infiltrate on chest radiograph.²³

Comparison groups

We defined two patient groups based on admission culture detection of bacteria commonly associated with CF. Because patients could be admitted more than once during the study period, we examined each admission separately. Admissions were classified as CFRB-positive if any of the following bacteria were detected: *P. aeruginosa*, *S. aureus*, *H. influenzae*, *S. maltophilia*, *A. xylosoxidans*, or *B. cepacia complex*. Admissions were classified as CFRB-negative if none of these bacteria were detected. If other bacteria were

detected by culture, the results were recorded, but these admissions were still classified as CFRB-negative. Fungal culture results were recorded but were not used for classification. If patients had viral studies (nasopharyngeal aspirate or BAL samples) or nontuberculous mycobacterial cultures (sputum or BAL samples) obtained on admission, the results were recorded. Viral studies included culture or direct fluorescent antibody for the following viruses: adenovirus, cytomegalovirus, enterovirus/rhinovirus, influenza A and B, parainfluenza, and respiratory syncytial virus.

Statistical Analyses

Descriptive statistics were calculated using means and standard deviations for continuous variables and percentages for categorical variables. Generalized linear models were used to univariately compare each variable across culture result groups. The logit link was used for dichotomous variables and the log link was used for WBC, CRP and ESR, otherwise a linear regression was fit. Subgroup analysis was done for sputum, as opposed to throat or BAL samples at the time of admission. The repeated admissions were accounted for in the models using generalized estimating equations (GEE). In addition, a model was fit in which the culture result was the dependent variable and important variables were included multivariately. This final model aids in identifying important factors associated with the binary culture results while accounting for the repeated admissions for patients. P-values less than 0.05 were considered statistically significant. Adjustments for multiple comparisons were not made given the exploratory focus of this paper.²⁴ Less importance was placed on reducing the type I error in order to avoid potentially missing important findings which can be verified in future studies. All analyses were performed using SAS Version 9.2 software; the GEE models were fit using PROC GENMOD (SAS Institute Inc.: Cary, NC, 2008).

Results

There were 731 hospital admissions for CF related pulmonary exacerbations, but 59 (8%) were excluded because airway culture data was not available. The remaining 672 admissions involving 211 patients were included in our analysis. One hundred twenty-five patients (59%) were admitted more than once during the study period, and 40 (19%) had admissions in both the CFRB-negative and CFRB-positive group. Of those with admissions in both groups, 25 had a CFRB-positive admission followed by a CFRB-negative admission. There was no difference in the percent of admissions classified as CFRB-negative compared to CFRB-positive when examined by gender, ethnicity, genotype severity, history of meconium ileus or mode of diagnosis (newborn screen or conventional). Eighty-seven percent of patients carried two severe CF mutations.

Seventeen percent of admissions (n=116) involving 72 patients were classified as CFRB-negative. Eighty-three percent of admissions (n=556) involving 179 patients were classified as CFRB-positive. We examined our results by type of culture and found that 41% (95% CI, 27–55%) of BAL, 31% (23–39%) of throat, and 10% (6–15%) of sputum cultures were CFRB-negative. Most BAL (82%) and sputum (72%) samples were collected on day zero or one of admission. Throat swabs were more likely than BAL or sputum to be collected prior to admission. Subjects with BAL and throat cultures were not more likely to be on antibiotics prior to admission than those with sputum cultures after adjusting for age.

Patient characteristics at the time of admission are shown in Table 1. CFRB-negative patients were younger at the time of admission, less likely to have had an admission in the past year and less likely to be on chronic azithromycin therapy or to have chronic *P. aeruginosa*. BMI and FEV₁ percent predicted were higher at the time of admission in the CFRB-negative group and they were more likely to have been prescribed antibiotics in the 2

weeks prior to admission. Viral culture or direct fluorescent antibody (DFA) studies were sent on 17% of patients. Subjects with viral studies were slightly younger compared to those without viral studies (9.7 versus 10.6 years, $p<0.01$).

Airway cultures were obtained from 19% and 55% of subjects in the 30 and 90 days prior to admission respectively. Of those classified as CFRB-negative on admission, 11 of 116 (9%) had a positive culture in the month prior to admission. For those in the CFRB-positive group, 88% of cultures obtained within one month were positive compared to 38% positive in the CFRB-negative group. For cultures obtained within 3 months, 50% were positive for *P. aeruginosa*, 46% for *S. aureus*, 11% for *H. influenzae*, 9% for *S. maltophilia*, 4% for *A. xylosoxidans*, and 1% for *Burkholderia cepacia* complex. Cultures from CFRB-positive patients were more likely to grow *P. aeruginosa* (59% versus 15%, $p<0.01$) and *S. aureus* (51% versus 26%, $p<0.01$) compared to those from CFRB-negative patients. There was no difference in the detection rate for other CF pathogens.

Sputum culture subgroup analysis

Because of the significant difference in CFRB-negative culture frequency between airway sample types, and the tendency for younger children to have throat or BAL cultures more often than sputum, we performed a subgroup analysis of the 479 admissions involving 155 expectorating patients with sputum cultures obtained on admission. Fifty admissions (10%) were sputum CFRB-negative and 429 (90%) were sputum CFRB-positive. There was no difference in the percentage of admissions classified as sputum CFRB-negative when examined by gender, genotype severity, history of meconium ileus, or newborn screen versus conventional diagnosis. We compared subject characteristics at the time of admission (Table 1). As seen in the overall group, sputum CFRB-negative patients were younger, had less chronic *P. aeruginosa*, and higher BMI percent predicted than sputum CFRB-positive patients. Unlike the overall group, there was no difference in chronic or recent antibiotic use, including inhaled tobramycin and chronic azithromycin therapy, or in admissions in the past year between the sputum CFRB-negative and sputum CFRB-positive group.

We compared signs and symptoms of pulmonary exacerbation between groups. Sputum CFRB-negative patients were less likely to have cough (84% versus 95%, $p = 0.01$), exercise intolerance or fatigue (42% versus 62%, $p = 0.03$) and decreased oxygen saturations (14% versus 35%, $p<0.01$). There was no significant difference between groups in any of the remaining pulmonary exacerbation signs or symptoms including a 10% decline in percent predicted FEV₁ at admission (43% of sputum CFRB-negative compared to 63% of sputum CFRB-positive, $p=0.08$). There was also no difference in the number of patients with 3 or more signs and symptoms of pulmonary exacerbations between sputum CFRB-negative and sputum CFRB-positive patients. (88% versus 92%, $p= 0.33$).

Among sputum CFRB-positive patients, the most common bacteria detected at admission were *P. aeruginosa* ($n=315$, 73%) and *S. aureus* ($n = 208$, 48%). Twenty-eight percent ($n=118$) of patients were positive for both *P. aeruginosa* and *S. aureus*. Sputum CFRB-negative patients had other bacteria detected more frequently than sputum CFRB-positive patients (10% versus 2.6%, $p=0.02$). Other bacteria detected consisted of *Acinetobacter* spp, *Actinomyces* spp, *Enterobacter cloacae*, Group A *Streptococcus*, *Nocardia*, *Pseudomonas fluorescens*, *Pseudomonas mendocina*, *Ralstonia* spp, and *Serratia marcescens*. Ninety percent of sputum CFRB-negative samples were negative or contained only mixed upper respiratory flora. There was no difference in detection of *Aspergillus fumigatus* (40% versus 28%, $p=0.14$) or any fungal species (48% versus 34%, $p=0.09$) in the sputum CFRB-negative compared to the sputum CFRB-positive group. Viral studies (culture and/or DFA from nasal aspirate samples, or bronchoalveolar lavage samples) were more likely to be sent at the time of CFRB-negative admissions (18% versus 8%, $p=0.03$), but were not more

likely to be positive (11.1% versus 15.6%, $p=0.7$). There was no difference between groups in the number of nontuberculous mycobacterial cultures obtained on admission (64% in CFRB-negative versus 59% in CFRB-positive, $p=0.5$) or the number positive (15.6% versus 11.9%, $p=0.54$) (Figure 1).

Baseline, admission and discharge spirometry data was available for 96% of expectorating patients. Lung function (FEV₁ percent predicted) was higher for sputum CFRB-negative compared to sputum CFRB-positive patients at baseline (84% versus 73%, $p=0.01$), admission (77% versus 59%, $p<0.01$) and discharge (83% versus 71%, $p=0.01$) (Figure 2). Percent change from baseline to admission in absolute FEV₁ was less in sputum CFRB-negative patients (−9.5% versus −16.4%, $p=0.03$), as was change with treatment (11.9% versus 28.8%, $p<0.01$). However, there was no difference between groups in FEV₁ change between discharge and baseline (0.9% change in CFRB-negative versus 2.4% change in CFRB-positive patients, $p=0.52$), suggesting that both groups returned to baseline after treatment. Lung function at discharge was not significantly different from baseline in either group (CFRB-negative, $p=0.6$; CFRB-positive, $p=0.16$). This study did not investigate differences in hospital treatment or use of specific intravenous antibiotics; however, length of hospitalization did not differ between groups.

White blood cell, CRP and ESR values were available for 94%, 85% and 72% of admissions respectively. Sputum CFRB-negative patients had lower white blood cell counts and lower absolute neutrophils. There was no difference between groups in CRP, ESR, absolute lymphocytes, monocytes or eosinophils (Table 2). Fewer sputum CFRB-negative patients had WBC counts and neutrophil percentages above the upper limit of normal than CFRB-positive patients (WBC > 12,000/ μ L, 16.7% versus 40.3%, $p=0.01$; neutrophils > 62%, 42.6% versus 60.1%, $p=0.03$).

Other findings

Gram stain data was available for 99% ($n=474$) of sputum samples. Seven samples were negative by gram stain for polymorphonuclear cells and bacteria, six in the CFRB-negative group and one in the CFRB-positive group. Polymorphonuclear cells were present in 75% of CFRB-negative samples and 94% of CFRB-positive samples ($p<0.01$). Polymorphonuclear cells and/or bacteria were present in 88% of CFRB-negative samples and 99% of CFRB-positive samples ($p<0.01$).

In order to investigate the association between several of the factors reported previously and culture results, a multivariate model was constructed (Figure 3). Patients with increasing age and chronic *P. aeruginosa* were less likely to have sputum CFRB-negative cultures [odds ratio 0.87 (0.80–0.95) for age and 0.11 (0.04–0.26) for chronic *P. aeruginosa*]. Female gender and the detection of airway fungus on admission culture were associated with a higher likelihood of sputum CFRB-negative cultures [odds ratio 2.48 (1.21–5.09) for female gender and 2.06 (1.04–4.07) for fungal infection]. Genotype severity, the presence of three or more signs and symptoms of pulmonary exacerbation and chronic tobramycin and/or azithromycin were not significantly associated with culture results.

Discussion

A significant number of patients with CF admitted for acute pulmonary exacerbations do not have typical CF related bacteria detected from airway cultures at the time of admission. We found that patients with CFRB-negative cultures were younger, less likely to have chronic *P. aeruginosa* infection, had better nutritional status and higher lung function than patients with CFRB-positive cultures. These findings persisted in a subgroup analysis of expectorating patients with CFRB-negative sputum cultures. Despite better lung function and nutritional

status, most sputum CFRB-negative patients had at least three signs and symptoms of pulmonary exacerbation and a decrease in lung function. Importantly, although sputum CFRB-negative patients had less FEV₁ percent decline on admission and less improvement after treatment, both groups experienced a similar return to baseline at discharge. Thus empiric treatment of patients with clinical signs and symptoms of pulmonary exacerbation may result in similar lung function improvements for patients with negative airway cultures compared to patients with detectable CF pathogens. Pulmonary exacerbations have been associated with lung function decline over one year.^{3,25} Our finding that both groups returned to baseline lung function suggests that this decline may not occur during the acute exacerbation period.

Pulmonary exacerbations are important contributors to morbidity and worsening lung damage in CF.² Treatment of pulmonary exacerbations relies on airway culture, and empiric treatment is used if bacteria are not detected.⁸ Little is known about pulmonary exacerbations in patients without typical CF related bacteria. A few authors have previously examined non-bacterial pulmonary exacerbations in CF.^{15,26} Petersen and colleagues studied non-bacterial respiratory infections in 116 CF patients followed over an eight month period.¹⁵ Sputum or laryngeal cultures were obtained at regular intervals and during exacerbations (mean 2.9 exacerbations/patient). Serum antibody responses to viruses, *Chlamydia* spp., *Mycoplasma pneumonia* and *P. aeruginosa* were monitored. Eighteen percent of exacerbations had no definite microbial etiology determined, a finding very similar to ours. Pribble and colleagues studied 59 CF patients during 80 pulmonary exacerbations to determine if patients with non-bacterial infections (defined as viral infections or *Mycoplasma pneumoniae*) were clinically distinguishable from patients without non-bacterial infections.²⁶ They compared patients with influenza (10%), other non-bacterial infections (19%) and no non-bacterial infections (71%) and found that influenza was associated with more severe exacerbations. However, 98% of patients also had CF associated bacteria (*P. aeruginosa* or *B. cepacia*) detected on admission. Other researchers have also focused on the role of viral and atypical bacterial infections in pulmonary exacerbations, but these patients often have concurrent CF related airway bacterial infections.²⁷⁻²⁹

There are a number of reasons that airway cultures may not detect expected bacteria at the time of pulmonary exacerbation including (1) false negative cultures, (2) failure of culture to detect anaerobes or other fastidious bacteria, and (3) pulmonary exacerbations related to viral infections, atypical bacteria or other non-bacterial causes.^{2,30,31} False negative cultures may occur due to inadequate sampling or interference by previously administered antibiotics.^{32,33} Culture positive samples were more likely to contain polymorphonuclear cells or bacteria, suggesting that inadequacy of samples may have contributed to negative culture findings. However, gram stain evaluation has been shown to have poor correlation with culture results for CF sputum samples.³⁴ Culture interference by antibiotics is a valid concern in our study, given the high number of patients on antibiotics at the time of admission. Overall, more CFRB-negative patients were prescribed antibiotics in the two weeks prior to admission than CFRB-positive patients. However, this finding did not persist among expectorating patients. In addition, chronic antibiotic use was not more common among patients with CFRB-negative cultures compared to patients with CFRB-positive culture. Unexpectedly, we found that more patients in the CFRB-negative group were on inhaled tobramycin than had chronic *P. aeruginosa* as defined by our study. We examined the records of a portion of CFRB-negative patients without chronic *P. aeruginosa* infection, but on inhaled tobramycin, and found that most had either a distant history (> 1 year prior to admission) of *P. aeruginosa* infection or chronic infection with another gram-negative bacterium such as *S. maltophilia* or *B. cepacia* complex.

A second possible explanation for CFRB-negative cultures is the presence of difficult to cultivate bacteria such as anaerobes. Recent studies demonstrate that the CF airway microbiome is diverse with polymicrobial infections common.^{6,31,35} Using strict anaerobic conditions, Worlitzsch and colleagues found obligate anaerobes, including *Staphylococcus saccharolyticus*, *Peptostreptococcus prevotii* and *Actinomyces*, in 58% of CF sputum samples during exacerbation.³⁶ Molecular techniques based on bacterial 16S rRNA gene amplification are now available that can identify bacteria without the need for culture.^{37,38} Using molecular techniques Sibley and colleagues found that the *Streptococcus milleri* group was the numerically dominant organism in 39% of acute pulmonary exacerbations.³⁹ Further studies are needed to elucidate the role of these bacteria in pulmonary exacerbations. We found that a higher proportion on CFRB-negative cultures contained other bacteria, not typically related to CF, than CFRB-positive cultures. However, the bacteria detected were variable with no particular organism dominating our findings.

Finally, pulmonary exacerbations may be brought on by other causes including viruses, atypical bacteria, fungi, nontuberculous mycobacterial infections or environmental causes.² Our study was not designed to determine the etiology of exacerbations in patients with CFRB-negative cultures. However, we did examine viral, nontuberculous mycobacterium and fungal cultures when available. We found that only a small percentage of patients had viral studies done at the time of admission and that few of these studies were positive. Patients with viral studies were slightly younger compared to those without viral studies. As these patients were also more likely to have negative bacterial culture, this finding may explain the higher number of viral studies obtained in the CFRB-negative group. The low number of viral studies obtained overall limits our interpretation. There was also no difference in the number of nontuberculous mycobacterial cultures obtained or the number positive between groups. Thus it does not appear nontuberculous mycobacterium infections played a causative role in the majority of CFRB-negative patients. *Mycoplasma* and *Chlamydia* infections may have contributed to some pulmonary exacerbations, but studies for atypical bacteria are not routinely done on our CF patients. In addition, previous studies of CF pulmonary exacerbations have not demonstrated a strong link between pulmonary exacerbation and atypical bacterial infections.^{15,27,40} After controlling for other variables, a positive fungal culture on admission increased the odds of having a CFRB-negative culture. A recent publication found an association between chronic infection with *A. fumigatus* and pulmonary exacerbation.⁹ Although we did not differentiate between chronic and intermittent infection with *A. fumigatus*, it is possible that some exacerbations were associated with chronic fungal infection.

Overall, throat and BAL cultures were more likely to be negative than sputum culture. This may be because non-expectorating CF patients tend to be younger and have less bacterial colonization than those who spontaneously expectorate sputum or because of decreased sensitivity of throat swab and BAL compared to sputum.^{41,42} Although BAL is often considered the gold standard for detection of lung infection in CF, the sensitivity of BAL may be reduced if only one lobe is sampled, if sample contamination with lidocaine occurs, or due to sample dilution.⁴²⁻⁴⁴ In our multivariate analysis of expectorating patients, female gender was associated with a higher likelihood of negative cultures. The reasons for this finding are unclear, but may be due to differences in etiology of exacerbations or adequacy of sputum samples between males and females.

As a retrospective study of pulmonary exacerbations, our study has important limitations. Subject information was not collected in a standardized way, and it is possible that pulmonary exacerbation symptoms were underreported, however this bias should have impacted both groups equally. Viral and nontuberculous mycobacterial cultures were not collected on all patients and atypical bacterial studies were not obtained. Our medication

history relied on medical records around the time of admission; therefore, no assessment of patient compliance could be made. This study did not seek to compare treatment regimens; thus, we did not collect treatment data. Instead we sought to compare lung function response based on negative or positive culture results to standard clinical care practices at our institution. We limited our time frame to a period when no changes occurred in our microbiology laboratory with respect to processing airway cultures to limit discrepancies in bacterial detection. Due to the wide age range, not all patients were able to perform lung function testing at the time of admission. However, when we limited our analysis to expectorating patients, 96% had baseline, admission and discharge lung function results available. One advantage of our study is that we are a large CF center that has been prospectively collecting data for many years; thus, we were able to comprehensively examine a large number of admissions.

In conclusion, we found that a significant number of CF patients experiencing a pulmonary exacerbation did not have expected bacteria detected in admission airway cultures. Although these patients had better lung function and nutritional status than those with positive cultures, they still had significant symptoms of a pulmonary exacerbation and decreased lung function. It is possible that routine cultures missed bacteria due to technique, prior antibiotic therapy, viral or atypical bacterial infections, or the presence of difficult to cultivate microbes. Future studies into molecular microbial diagnostic techniques may offer improved sensitivity and a broader view of the CF airway microbiome, helping to elucidate the cause and optimal treatment of CF pulmonary exacerbations.

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Figure 1. Microbiology results for expectorating patients

Sputum CFRB-negative cultures were more likely to have other bacteria than sputum CFRB-positive cultures. There was no difference in detection of fungi, viruses or nontuberculous mycobacterium. Viral and nontuberculous mycobacterial cultures were obtained in 9% and 59% of patients respectively. *Statistically significant difference with $p=0.02$

Figure 2. Spirometry for expectorating patients

Mean FEV₁ at baseline, admission and discharge for sputum CFRB-negative and sputum CFRB-positive patients. (Mean ± 2 SE) N = 479 admissions *Statistically significant difference with p < 0.01

Figure 3. Multivariate Associations for expectorating patients

Odds ratio of sputum CFRB-negative airway culture on admission is increased for females compared to males and for patients with positive fungal culture. Older age and chronic *P. aeruginosa* infection were associated with decreased chance of sputum CFRB-negative culture. (Odds ratio with 95% confidence limits displayed)

CFRB-negative and CFRB-positive admission characteristics. Recent antibiotic treatment defined as oral or intravenous antibiotics prescribed in 2 week prior to admission; Chronic *P. aeruginosa* infection defined as 3 cultures positive for *P. aeruginosa* and/or mucoid *P. aeruginosa* detected in 12 months prior to admission. P-values calculated using logistic regressions with generalized estimating equations to account for repeated measures.

Table 1

	CFRB-Negative (n = 116)	CFRB-Positive (n = 556)	p-value	Sputum CFRB- Negative (n=50)	Sputum CFRB- Positive (n=429)	p-value
Age (years $\pm$ SD)	10.1 $\pm$ 5.8	15.5 $\pm$ 6.6	<.01	13.8 $\pm$ 3.7	17.5 $\pm$ 5.2	<.01
1 admit in past year, n (%)	62 (53)	393 (71)	0.01	36 (72)	328 (76)	0.57
Length of Stay (days $\pm$ SD)	10.2 $\pm$ 7.7	11.8 $\pm$ 8.9	0.08	13.3 $\pm$ 9.9	12.4 $\pm$ 9.7	0.54
Chronic <i>P. aeruginosa</i> , n (%)	19 (16)	316 (57)	<.01	8 (16)	287 (67)	<.01
BMI, % predicted ($\pm$ SD)	39 $\pm$ 25	28 $\pm$ 24	<.01	36 $\pm$ 23	26 $\pm$ 23	0.03
Admission FEV ₁ , % predicted ($\pm$ SD)	81 $\pm$ 22	62 $\pm$ 25	<.01	77 $\pm$ 21	59 $\pm$ 24	<.01
Antibiotic Therapy, n (%):						
Inhaled tobramycin	42 (36)	246 (44)	0.20	20 (40)	206 (48)	0.30
Chronic azithromycin	47 (41)	298 (54)	0.03	23 (46)	251 (59)	0.11
Inhaled tobramycin or azithromycin	65 (56)	369 (66)	0.06	32 (64)	308 (72)	0.24
Any chronic antibiotics	74 (64)	395 (71)	0.20	36 (72)	333 (78)	0.41
Recent antibiotic treatment	67 (58)	241 (44)	0.01	26 (52)	173 (41)	0.16

Table 2

Comparison of systemic inflammatory markers between sputum CFRB-negative patients and sputum CFRB-positive patients. Total white blood cell and absolute neutrophil counts were lower in sputum CFRB-negative patients.

	Sputum CFRB Negative (n=50) Mean (SE)	Sputum CFRB Positive (n=429) Mean (SE)	p-value
White blood cell count ($\times 10^3/\mu\text{l}$)	9.7 (0.05)	11.7 (0.04)	<.01
Neutrophils, absolute ($\times 10^3/\mu\text{l}$)	6.0 (0.4)	7.8 (0.4)	<.01
Lymphocytes, absolute ($\times 10^3/\mu\text{l}$)	2.5 (0.1)	2.5 (0.1)	0.92
Monocytes, absolute ($\times 10^3/\mu\text{l}$)	0.72 (0.05)	0.72 (0.03)	0.94
Eosinophils, absolute ($\times 10^3/\mu\text{l}$)	0.25 (0.05)	0.25 (0.02)	0.99
ESR (mm/hr)	14.1 (0.22)	21.2 (0.08)	0.06
CRP (mg/dl)	2.32 (0.31)	3.19 (0.11)	0.32