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CLEARANCE OF INITIAL MUCOID *PSEUDOMONAS AERUGINOSA* IN PATIENTS WITH CYSTIC FIBROSIS

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Abstract

Background—Accelerated lung function decline in cystic fibrosis (CF) is associated with mucoid *Pseudomonas aeruginosa* infection. Recent data suggests that mucoid *P.aeruginosa* may amenable to elimination from the airway. We aim to determine whether the initiation of an aggressive antibiotic eradication regimen upon initial discovery of mucoid *P.aeruginosa* in the CF airway could be successful in clearing the organism from the CF lung.

Methods—We performed a retrospective analysis of patients with CF who demonstrated new growth of mucoid *P.aeruginosa* in an airway culture between January 2003 and December 2008. The primary endpoint was clearance of mucoid *P.aeruginosa*, based upon the Leeds criteria, with no further growth of mucoid *P.aeruginosa* cultures within 12 months of the initial discovery and treatment. Factors associated with successful clearance were also evaluated.

Results—Forty-eight of 355 patients with CF had a new diagnosis of mucoid *P.aeruginosa* during the study period; fifteen patients underwent an eradication attempt, while 33 patients received no increase in therapy. We observed clearance of mucoid *P.aeruginosa* in 73.3% of patients undergoing an eradication attempt, whereas 36.6% of those that did not undergo attempted eradication cleared the organism at one year ($p<0.05$). Prolonged mucoid *P.aeruginosa* airway clearance (>24 months) for mucoid *P.aeruginosa* was seen in 60.0% in subjects undergoing

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eradication compared to 21.2% ($p=0.02$) in control patients. At the study conclusion, lung function was greater in subjects who underwent an eradication attempt than in patients who did not undergo an eradication attempt ($FEV_1\%$: 91.7% vs. 75.0%, $p=0.04$).

Conclusions—Clearance of initial mucoid *P.aeruginosa* from the airways of select patients with CF is possible with current antibiotic regimens, and the attempt may be associated with improved lung function.

Keywords

Cystic fibrosis; eradication; mucoid; *Pseudomonas aeruginosa*; therapy

1. Introduction

Cystic fibrosis (CF) is the most common lethal genetic disorder among Caucasians with an incidence of 1 in 3,000 live births and a carrier frequency of approximately 1 in 25 (1). Respiratory complications are the main causes of morbidity and mortality in patients with this disease. A vicious cycle of pulmonary infection by opportunistic bacteria and concomitant inflammation ultimately leads to bronchiectasis and eventual respiratory failure (2). Chronic infection with *Pseudomonas aeruginosa* is an important clinical event due to its well-described association with increased morbidity and mortality (3,4).

Although 95% of children with CF have evidence of intermittent *P.aeruginosa* infection by 3 years of age, it is unclear why *P.aeruginosa* is so efficient at colonization of the CF lung (5). When *P.aeruginosa* colonizes the CF lung, bacteria undergo phenotypic changes that perpetuate the infection. These changes include the induction of virulence factors and conversion to a mucoid phenotype in the presence of low oxygen tension, thick mucus production, and biofilm formation (6). Mucoid *P.aeruginosa* develops following infection with non-mucoid *P.aeruginosa* (7). The growth of mucoid *P.aeruginosa* differs from that of non-mucoid *P.aeruginosa*, which typically grows as a flat, uniform culture compared to the thick, organized alginate structure produced by mucoid *P.aeruginosa* (8). This exopolysaccharide/alginate confers resistance to both antibiotics and phagocytosis rendering mucoid *P.aeruginosa* infection more resistant to inherent immunologic defense and traditional antimicrobial therapy (7,9). Additionally, chronic mucoid *P.aeruginosa* infection is associated with accelerated decline in FEV_1 among both adults and children and has been shown to be a strong predictor of pulmonary decline relative to non-mucoid *P.aeruginosa* (10–12).

Several studies have indicated that the eradication of non-mucoid strains of *P.aeruginosa* is possible with early aggressive treatment utilizing inhaled and oral antibiotics (13–18). The outcome for mucoid *P.aeruginosa* is less optimistic. Initial studies attempting to treat mucoid *P.aeruginosa* suggested that this phenotype could not be eradicated with current antibiotic therapies (19,20). Although novel therapeutics intended to eradicate mucoid *P.aeruginosa* are being tested in vitro and in animal models, human studies have rarely been reported (13,21). Indeed, the successful eradication of mucoid *P.aeruginosa* in patients with CF has been seldom reported in the literature (14,13,22). Recently, a report suggested that mucoid *P.aeruginosa* could be cleared in patients with CF using current antibiotic regimens (23), and complements studies indicating that factors other than mucoidy may be principally responsible for the chronic colonization (24).

Based on these studies, we hypothesized that aggressive combinations of inhaled, oral and/or intravenous antibiotics could lead to prolonged periods of negative airway cultures in patients with mucoid *P.aeruginosa*. In this report, we document our institution's experience

with CF patients found to have mucoid *P.aeruginosa* in airway cultures who underwent attempted eradication of this pathogen.

2. Methods

2.1 Subject selection and data collection

We conducted a retrospective chart review following approval of the institutional review board at the University of Alabama at Birmingham (UAB). Patients were identified using the PortCF database and confirmed via institutional electronic medical record. Chart review was conducted on all children with CF who were followed at the Pediatric CF Care Center at UAB over the study period. Patients were included in the analysis if they had a diagnosis of CF based on the 2008 CF Foundation guidelines,(25) had greater than two airway cultures, and had received care at our center during the study period of January 2003 through December 2008. The start date coincided with the development and implementation of a non-mucoid *P.aeruginosa* eradication protocol in 2003. Charts were excluded if the patient did not meet diagnostic criteria of CF, received care at another center, had fewer than three airway cultures, or received less than 12 months of care at our institution. Demographic data, microbiology culture results, CFTR genotype, and anti-*P.aeruginosa* treatment were extracted. Additionally, clinical features including the Body-Mass Index (BMI) percentile and percentage-predicted forced-expiratory volume in one second (FEV₁), if age-appropriate, were obtained at time of CF diagnosis, initial mucoid *P.aeruginosa* detection, 6 and 12 months following attempted eradication, and at the end of the study period. Number of admissions and number of hospitalized days were determined from the medical record. All pulmonary function and BMI data were extracted during outpatient visits.

2.2 Microbiological data

The institutional microbiologic surveillance program consists of quarterly sputum cultures in all CF patients during outpatient clinic visits and upon admission to the hospital. In patients unable to produce sputum, routine cultures were obtained by oropharyngeal swab or through induced sputum specimens. All respiratory cultures, both inpatient and outpatient, were evaluated for microbiologic growth in a standard CF specific protocol. All samples were cultured in the microbiology lab at Children's Hospital of Alabama. The cultures were plated on selective growth media including chocolate agar, trypticase soy agar with 5% sheep blood, Columbia CNA agar, MacConkey agar, and PC agar. Identification of the organisms was performed using standard microbiologic techniques and was consistent throughout the study period.

2.3 Eradication Protocol

The initial *P.aeruginosa* eradication protocol was implemented in 2003 with a subsequent revision in 2007. The initial protocol stipulated that all patients found to have new, non-mucoid *P.aeruginosa* should be placed on the treatment protocol that utilized oral antibiotics followed by three rounds of alternate month inhaled tobramycin. An airway culture was obtained at the conclusion of the treatment to document treatment outcome. The protocol was modifiable by either physician or patient preference to include intravenous antibiotics. Upon the discovery of mucoid *P.aeruginosa*, patients were treated with inhaled tobramycin and an additional antibiotic regimen. The decision to treat mucoid *P.aeruginosa* was determined by the treating physician. After the 2007 revision, discovery of a new mucoid *P.aeruginosa* stipulated that intravenous antibiotics should be used.

2.4 Definitions

P.aeruginosa status, either mucoid or non-mucoid, was considered positive if the organism was identified on any respiratory culture obtained from the patient. An eradication attempt was defined as an increase in antibiotic use, within one month, in response to a mucoid *P.aeruginosa* positive culture. The clearance definition was based on the Leeds criteria,(26) with patients considered to have cleared the mucoid *P.aeruginosa* infection if no mucoid *P.aeruginosa* had been isolated within the 12 months after the eradication attempt and a minimum of three negative cultures while off of antibiotics. Patients who did not undergo an eradication attempt were classified as free of mucoid *P.aeruginosa* if no further mucoid *P.aeruginosa* was demonstrated within 12 months of the initial positive airway culture.

2.3 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.0c for Mac (GraphPad Software, Inc., San Diego, CA). Continuous variables were described using means with standard deviations, or medians with ranges, and were analyzed using the Student's t-test, Mann-Whitney U test, or ANOVA, as appropriate. All tests were two-sided and p values <0.05 were considered statistically significant. Nominal data were analyzed utilizing Fisher's exact test. Clearance of mucoid *P.aeruginosa* at 6, 12, and 24-months was categorized as a nominal variable and analyzed using Fisher's exact test. Kaplan-Meier survival analysis was performed with mucoid *P.aeruginosa* clearance for the cohort that underwent attempted eradication and the group that received no increase in therapy in response to mucoid *P.aeruginosa* identification.

A portion of this data was previously presented as an abstract at the North American CF Conference in Minneapolis, MN (27).

3. Results

3.1 Study Enrollment and Baseline Data

Between January 2003 and December 2008, there were 337 patients identified with CF who had respiratory cultures obtained at our institution. One hundred-fourteen patients had never demonstrated growth of any *P.aeruginosa* phenotype, while 223 patients were culture positive for *P.aeruginosa*. Of these patients, 115 grew a nonmucoid phenotype and 108 grew a mucoid phenotype of *P.aeruginosa*. Sixty patients with a positive culture for mucoid *P.aeruginosa* were deemed to be colonized, as they had previous cultures collected prior to this study that were positive with mucoid *P.aeruginosa*. Thus, there were 48 patients who were discovered to have an initial growth of mucoid *P.aeruginosa* during the study period and fifteen of these underwent an eradication attempt (Figure 1).

The baseline characteristics of the subjects who had an airway culture positive for mucoid *P.aeruginosa* for the first time are summarized in Table 1. Patients who underwent an eradication attempt were younger at the time of initial mucoid *P.aeruginosa* detection when compared to patients who did not undergo an eradication attempt (81.0 months vs. 152.0 months, $P<0.001$) and had a greater sweat chloride level (117.4 mmol/L vs. 105.6 mmol/L, $P<0.05$). Previous non-mucoid *P.aeruginosa* was seen in 26.7% of the eradication cohort and in 51.5% of the non-eradication cohort, but this was not statistically significant ($p=0.12$). The gender, CFTR genotype, and type of airway culture for each subject who underwent eradication are summarized in Table 2. A detailed summary of each subject who underwent an eradication attempt is described in the Online Supplement.

All subjects were followed for a mean of 871 days with the eradication group having a mean observation duration of 918 days and the non-eradication group being followed for a mean

of 851 days. There was no difference in duration of observation between the two groups ($p=0.91$).

3.2 Eradication results

Eradication attempts utilized a combination of antibiotic approaches with thirteen (86.7%) subjects receiving oral antibiotics, eleven (73.3%) were given inhaled medications, and seven (46.7%) received intravenous antibiotics. The majority of patients received oral ciprofloxacin (thirteen subjects) and inhaled tobramycin (eleven subjects) during their eradication attempt. Seven subjects received intravenous tobramycin, five received ceftazidime, one received piperacillin/tazobactam, and one received timentim. No patients received colistin during their eradication attempt.

Of the 15 patients who underwent an eradication attempt, eleven subjects were cleared of their mucoid *P.aeruginosa* for greater than 12 months. In addition, nine of the eleven remained clear of mucoid *P.aeruginosa* for greater than 24 months and had a median of 14 negative cultures (range 4–24). There was a median follow-up duration of 46 months (range 28 to 61 months). All eradication attempts are summarized in Table 3. The group that remained clear of mucoid *P.aeruginosa* was younger at the time of CF diagnosis (median age 6 months, range 0–37 months) than those with recurrence of mucoid *P.aeruginosa* (median age 55 months, range 51–59 months) ($p=0.04$). Eradication success was more likely among those not previously documented to have grown non-mucoid *P.aeruginosa* in airway cultures ($p=0.04$).

The percentage of patients with recurrence of mucoid *P.aeruginosa* increased with duration of observation, but clearance remained persistent in the majority of subjects. There was no recurrence of mucoid *P.aeruginosa* in 73.2% of subjects within 6 months of the eradication attempt. At 24 months, 60% of subjects remained free from growth of mucoid PSA. Surveillance culture data collected from the subjects who did not undergo eradication documented the intermittent nature of initial mucoid infection with 62.5% of patients demonstrating a negative culture of *P.aeruginosa* at 6 months. By 24 months, however, only 26.9% remained mucoid *P.aeruginosa* negative ($p\text{-value}=0.018$, 24 months vs. 24 months). The outcome of eradication and the surveillance data are presented in Figure 2.

Kaplan-Meier analysis demonstrated prolonged mucoid *P.aeruginosa* clearance after an eradication attempt with 63.5% of subjects remaining free of mucoid *P.aeruginosa* for greater than 36 months. Prolonged time free from mucoid *P.aeruginosa* in the airway was seen in the cohort that underwent eradication when compared to the standard therapy cohort ($p=0.035$), with 50% of patients *P.aeruginosa* negative for 40 months in individuals treated with eradication attempt compared to 10.5 months in those without eradication. The Kaplan-Meier chart is depicted in Figure 3. The recurrence rate of mucoid *P.aeruginosa* was 23.5% per year observed in the eradication group and 58.9% in the control group ($p<0.001$).

There were fewer hospitalized days noted among patients who underwent eradication (mean 7.4 ± 4.8 days) compared to those who did not undergo an eradication attempt (mean 11.39 ± 47 days, $p=0.03$).

Among patients in the eradication cohort, FEV₁% data was available for 8 subjects for the entire study period, while seven subjects had incomplete data due to age related inability to perform spirometry at the study outset. Complete spirometry data were available from 31 of 33 patients in the non-eradication attempt group. Mean final percent-predicted FEV₁ among patients who underwent eradication attempt (FEV₁%: $91.65 \pm 21.8\%$) was significantly greater than those who did not undergo an eradication attempt (FEV₁%: $74.95 \pm 24.36\%$, $p<0.05$) at the study conclusion, whereas, no difference was discovered at the time of first

muroid *P.aeruginosa* detection (Figure 4A). FEV₁ increased by $11.31 \pm 10.8\%$ over a mean of 29 months in patients who underwent eradication, whereas the FEV₁ decreased by $2.39 \pm 12.9\%$ in those who did not undergo eradication ($p=0.01$). Multivariate regression analysis did not find a significant contribution to the change in FEV₁ due to age at muroid PSA detection, sweat test, age at diagnosis of CF, initial BMI percentile, or initial percent predicted FEV₁.

The median BMI percentile at the time of identification of muroid *P.aeruginosa* was the 39th percentile (range <1–93 percentile) for all subjects. There was no difference in BMI percentile at the study conclusion between the eradication attempt cohort ($48.9\% \pm 26.3$) and the non-eradication cohort ($41.7\% \pm 29.5$, $p=0.53$). The mean change in BMI percentile was not different between the eradication and non-eradication groups with a mean BMI percentile change of 8.62 ± 25.8 and 1.29 ± 16.9 , respectively (Figure 4B).

All airway culture results were analyzed to determine if a correlation exists between other microbiota and the ability to clear muroid *P.aeruginosa* from the airway. There were no associations found between eradication success and presence of *Aspergillus fumigatus* (N=0 eradication group, 1 no eradication; $p>0.5$), *Candida parapsilosis* (N=3, 7; $p>0.5$), *Candida albicans* (N=3, 7; $p>0.5$), non-muroid *P.aeruginosa* (N=14, 32; $p>0.5$), *Staphylococcus aureus* (N=7, 19; $p=0.25$), or Methicillin-resistant *S. aureus* (N=3, 14; $p=0.07$) in the initial muroid *P.aeruginosa* containing airway culture. Previous culture data was also assessed and found no difference in eradication success and prior airway cultures containing *A.fumigatus* (N= 0 for eradication group, 7 for non-eradication; $p=0.09$), *Candida albicans* (N= 2, 11; $p=0.30$), *S.aureus* (N= 6, 25; $p=0.08$), MRSA (N=4, 13; $p>0.5$), or *Candida parapsilosis* (N=1, 3; $p>0.5$). We did note an increase in clearance of muroid *P.aeruginosa* in subjects who had not previously grown non-muroid *P.aeruginosa* in the airway (N=n6, 26; $p=0.04$). Sample size limitations prevented analysis of the effect of non-muroid *P.aeruginosa* on duration of negative airway cultures.

There was no significant difference in clearance success of muroid *P.aeruginosa* based on the antibiotic therapy used, the age at initial muroid *P.aeruginosa* detection, gender, genotype, sweat chloride, initial BMI, or FEV₁ at the time of muroid *P.aeruginosa* detection. There was no difference between the subjects who remained clear of muroid *P.aeruginosa* and those who had a recurrence of muroid *P.aeruginosa* in regards to age at muroid *P.aeruginosa* detection, gender, sweat test, BMI, or genotype. Additionally, there was no statistical difference in *P.aeruginosa* susceptibility to tobramycin or ciprofloxacin between the subjects who successfully eradicated *P.aeruginosa* and those who did not clear the infection. Evaluation of antibiotic susceptibility was not predictive of the subjects who were successfully eradicated.

The success of the eradication protocol is also demonstrated in the frequency that muroid *P.aeruginosa* was found in CF airway cultures. The numbers of new muroid *P.aeruginosa* have significantly declined since the refinement of the eradication protocol. From 2003 until the refinement of the eradication protocol in 2007, there were a total of 41 subjects with initial airway cultures with growth of muroid *P.aeruginosa*. On average there were 10.25 new subjects with muroid *P.aeruginosa* discovered during this timeframe. After the implementation of the new eradication protocol; there were 4 subjects with initial muroid *P.aeruginosa* growth in 2007 and 3 in 2008.

Discussion

We describe the successful clearance of muroid *P.aeruginosa* with an increase in antibiotic therapy after the initial isolation of muroid *P.aeruginosa* from airway cultures in 73.3% of

patients who underwent attempted eradication compared to only 36.4% of patients treated without an eradication attempt. Importantly, attempted eradication was associated with a significant improvement in percent-predicted FEV₁ and a decrease in the number of hospitalized days among individuals who underwent an eradication attempt. These results suggest that eradication of initial mucoid *P.aeruginosa* can be successful in selected patients, and is associated with improved outcomes.

Previously, McPherson et al. described a retrospective analysis of mucoid *P.aeruginosa* clearance in the UK (23). In this report, they document the clearance of mucoid *P.aeruginosa* in 58% of patients for greater than one year with routine antibiotic use. They noted a similar long-term clearance of mucoid *P.aeruginosa* with routine antibiotic use (27% clearance at 2 years in McPherson et al. and 26.9% in the current study). Similar to the present study, McPherson et al. document an increase in FEV₁ among patients who cleared mucoid *P.aeruginosa*. They observed an increase in clearance of mucoid *P.aeruginosa* in patients with *Aspergillus fumigatus*, which was not recapitulated by the results of the present study. We document no change in prolonged clearance of *P.aeruginosa* in subjects with *Aspergillus* in airway cultures.

The central significant finding of the present study is that mucoid *P.aeruginosa* can be cleared in patients with an initial airway culture that documents *P.aeruginosa*. While McPherson documents similar findings of clearance of mucoid *P.aeruginosa*, they do not explicitly state the subjects who underwent eradication were treated with their initial mucoid *P.aeruginosa*. The current study focuses on the eradication results among patients treated upon the initial airway culture with mucoid *P.aeruginosa*. In addition, the present study contains the culture and treatment data of all subjects included in the study. There are no other CF care centers in the area that provide care to the subjects included in this study and therefore, the culture database allows a more comprehensive analysis than was performed in the McPherson study.

The results of our study are consistent with a smaller cohort of patients recently described in Australia (14). In this report, six patients who were found to have mucoid *P.aeruginosa* in BALF and underwent treatment with IV ticarcillin-clavulanate and tobramycin for 14 days. Mucoid *P.aeruginosa* was eradicated in four of the six children and they remained free of *P.aeruginosa* for a median of 42.5 months (range 3–69 months). The present study is more favorable than previous results reported in a recent North American study in which two of six patients (median age 3.0 years) successfully cleared mucoid *P.aeruginosa* utilizing inhaled tobramycin for 28 or 56 days (13). It is possible that the use of inhaled tobramycin as monotherapy for mucoid *P.aeruginosa* and the prolonged period between detection of *P.aeruginosa* and initiation of treatment contributed to the lower success rate reported by Gibson et al. Other studies have utilized a combination of intravenous, oral, and nebulized antibiotics in eradication protocols for non-mucoid *P.aeruginosa*. Nixon et al. documented successful clearance of mucoid *P.aeruginosa* in only 1 of 13 patients using intravenous antibiotics followed by oral and inhaled medications (28). The frequency of surveillance in that report may contribute to the high recurrence of mucoid *P.aeruginosa*, as they obtained BALF culture data annually versus our practice, which utilized quarterly microbiologic surveillance. The corresponding delay in initiating therapy may have contributed to decreased eradication success in this study.

The median age at detection of mucoid *P.aeruginosa* in the eradication attempt cohort was 81 months (range 31–201), while the median age of mucoid *P.aeruginosa* detection was 152 months (range 69–237) in the patients who did not undergo eradication. The attempted eradication cohort acquired mucoid *P.aeruginosa* at a significantly younger age than was previously reported by Li et al. and Levy et al. who observed median ages of acquisition of

156 months and 135 months, respectively, potentially reflecting advances in surveillance mechanisms over time (7,29). The younger age of patients who underwent an eradication attempt in this study may contribute to the high percentage of success that we report, as they may have less extensive airway damage when compared to older patients. The younger age of the eradication cohort may also reflect the physician bias of being more aggressive in patients who may be perceived to have a longer lifespan if therapy is successful. Finally, the decision to treat younger patients may reflect the more aggressive posture that parents have adopted toward the treatment of CF. Further study is warranted to evaluate the issue of eradication in younger patient cohorts.

Attempted eradication was associated with an improved percentage-predicted FEV₁ in this study, suggesting a more aggressive treatment stance toward mucoid *P.aeruginosa* potentially reduces rates of lung function decline. Additionally, as 33.3% of our subjects grew mucoid *P.aeruginosa* in the initial airway culture obtained at the time of diagnosis (median age 55 months), they did not benefit from early therapeutic intervention. Both of these observations underscore the utility of neonatal screening for CF now instituted in the U.S., which should facilitate early microbiologic surveillance and treatment in CF, and allow *P.aeruginosa* detection prior to the inception of pulmonary symptoms.

There are limitations that are inherent to this study, including its retrospective design and relatively small sample size. Selection bias is also a concern with the study design as the eradication attempts of mucoid *P.aeruginosa* were not systematic and were initiated based on clinical judgment. The eradication protocol that debuted in 2003 was aimed at eradicating non-mucoid *P.aeruginosa*, and was not specifically focused on the mucoid phenotype of the organism. The implementation of the eradication protocol did not change the number of eradication attempts, but did codify the treatment approach. Patients who were deemed candidates for eradication at our center were younger and were treated with a more aggressive initial therapeutic posture, however, other factors not captured in our study may also have contributed to successful eradication. Prior to the refinement of the formal eradication protocol in at our center in 2007, numerous therapeutic regimens were utilized to treat mucoid *P.aeruginosa*. The variability of eradication therapies used throughout the study period limits our ability to define the most effective eradication strategy for mucoid *P.aeruginosa*. The small sample size likely also limits identification of demographic variables or other factors that could be associated with successful clearance of *P.aeruginosa*.

An additional limitation of this study is the lack of BALF analysis, which was precluded by the retrospective design of the present study. While BALF culture is considered the gold standard for the diagnosis of lung pathogens in CF, it is relatively invasive and can have significant heterogeneity of culture results (30). Our institution uses oropharyngeal and sputum culture data for microbiologic surveillance. Although, oropharyngeal culture is not as sensitive or specific as BALF for the diagnosis of *P.aeruginosa*, it does have a >90% negative predictive value (31). Thus, the patients with repeated negative oropharyngeal cultures likely have a high correlation with BALF culture results. Neither Anti-Pseudomonas antibody responses nor genetic analysis of the *P.aeruginosa* organisms are routinely used at our center. Whether these represent factors that contribute to successful eradication remains unknown. Recently it has been suggested that underlying virulence factors, independent of mucoidy, are important predictors of sustained *P.aeruginosa* clearance (32). Our results are consistent with this observation, as we observed a relatively high clearance of mucoid *P.aeruginosa* organisms in the treated population.

The data obtained by this study demonstrate successful clearance in 73.3% of patients with new acquisitions of mucoid *P.aeruginosa* with 60.0% of patients achieving prolonged (>24 month) mucoid *P.aeruginosa* free respiratory cultures. This may have a positive long-term

clinical impact as suggested by the improved FEV₁ seen in patients who underwent attempted eradication. Although the significance remains to be determined, the prolonged elimination of mucoid *P.aeruginosa* due to eradication attempt is likely to be beneficial, and deserves further study. Indeed, following analysis of these data, our center has been more aggressive and systematic in attempting eradication of all *P.aeruginosa* on initial culture from the airway since these data were available. Perhaps as a result of this, we have experienced a decline in mucoid *P.aeruginosa* rates. From 2003 until the 2007 protocol revisions, we experienced 10.25 cases of initial mucoid *P.aeruginosa* growth per year. After the implementation of the new protocol we have seen 3.5 cases of mucoid *P.aeruginosa* per annum.

This study recapitulates the findings by McPherson et al. that mucoid *P.aeruginosa*, especially on first detection, may be amenable to antibiotic pressure resulting in long-term clearance of the airway from this organism(23).

We suggest that larger, prospective studies are indicated to further define the efficacy of mucoid *P.aeruginosa* eradication and to determine the variables that influence success. In the interim, our findings suggest that select patients with CF who are found to have mucoid *P.aeruginosa* can be successfully eradicated if early aggressive interventions are initiated at the first detection of mucoid *P.aeruginosa* and is associated with improved outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BALF	Broncho-alveolar lavage fluid
BMI	Body-mass index
CF	Cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
FEV₁	Forced-expiratory volume in one second
OP	oropharyngeal

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Figure 1.

Enrollment of subjects. *Pseudomonas aeruginosa* positive cultures were defined as detection of *P.aeruginosa* in any culture result. *P.aeruginosa*=PsA

Figure 2.

Number of individuals with a positive *P.aeruginosa* culture over time. The number of participants are plotted with regard to their culture status and the treatment they received. Positive culture results represent the participant had a culture with growth of mucoid *P.aeruginosa* at that time and remained positive subsequently. **A.** Culture results at 6 months. **B.** Culture results at 12 months **C.** Culture results at 24 months. Analysis was performed using Fisher's exact test with a p-value of <0.05 was considered to be significant.

Survival without mucoic PsA on an airway culture

Months	0	3	6	12	18	24	36	48	60
Eradication	14	14	12	12	12	10	8	5	1
No Eradication	33	28	24	14	11	7	6	3	1

Figure 3.

Survival curve of clearance of mucoic *Pseudomonas aeruginosa* culture negative survival. Mucoic *P.aeruginosa* detection was defined as the appearance of mucoic *P.aeruginosa* on any airway culture. The percentage of patients with clearance of mucoic *P.aeruginosa* from the airway was 73.3% and 36.3% for the eradication attempt cohort and for the routine care cohort at 12 months. The Mantel-Cox test demonstrated a p value of 0.035.

Figure 4.

Change in percent-predicted FEV₁ and BMI during an eradication attempt. **4A.** Mean percent-predicted FEV₁ with the 95% confidence intervals are presented. Final FEV₁ refers to the FEV₁ percent-predicted data obtained at the study conclusion. **4B.** Mean BMI percentiles with the 95% confidence intervals are presented. BMI= Body Mass Index; FEV₁= forced expiratory volume in one second.

Table 1Baseline characteristics of patients with newly acquired mucoid *P.aeruginosa*, by attempted eradication status

	No Eradication Attempt (N=33)	Eradication Attempt (N=15)	P value
Age at CF diagnosis, months ^a	6.3 (0–223)	7.0 (0–134)	0.684
Age at mucoid PA detection, months ^a	152.0 (69–237)	81.0 (31–201)	<0.001
Initial BMI, percentile	39.9 ± 29.5	40.3 ± 26.8	0.958
Initial FEV ₁ , percent predicted	79.9 ± 22.2 ^b	86.9 ± 19.6 ^c	0.422
Gender, female	13 (39.3)	8 (53.3)	0.531
Genotype, F508del homozygous	20 (60.6)	7 (46.7)	0.531
Prior non-mucoid PA infection	17 (51.5)	4 (26.7)	0.124
Sweat chloride, meq/L	105.6 ± 2.7 ^d	117.4 ± 2.9 ^e	0.016

Definition of abbreviations: CF = Cystic Fibrosis; BMI = Body-Mass Index; FEV₁ = Forced expiratory volume in one second; PA = *Pseudomonas aeruginosa*

Data are expressed as mean ± SD or number (with percentage in parenthesis) except where noted. P-values determined by the Student t-test for continuous variables or Fisher's exact test for nominal data.

^aMedian age and range

^bFEV₁ data available for 31 subjects.

^cFEV₁ data available for 8 subjects

^dSweat chloride data available for 21 subjects.

^eSweat chloride data available for 9 subjects.

Table 2

Characteristics of patients with initial mucoid *Pseudomonas aeruginosa* growth in airway culture who underwent an eradication attempt.

Subject Number	Gender	Genotype	Type of Culture
1	Female	F508del/F508del	OP
2	Female	F508del/L467P	S
3	Female	F508del/F508del	OP
4	Female	F508del/R815X	OP
5	Male	F508del/F508del	OP
6	Male	F508del/F508del	S
7	Female	F508del/F508del	OP
8	Female	F508del/N1303K	S
9	Male	F508del/F508del	S
10	Male	F508del/F508del	OP
11	Female	F508del/G551D	OP
12	Male	F508del/G551D	S
13	Female	F508del/G551D	OP
14	Male	F508del/F508del	S
15	Male	F508del/N1303K	S

Definition of Abbreviations: OP = oropharyngeal culture; S = sputum culture

Table 3

Summary of mucoid *Pseudomonas aeruginosa* eradication attempts.

Subject Number	Duration of all PA clearance	Number of cultures clear of all PA	Duration of mucoid PA clearance	Number of cultures clear of mucoid PA	PA phenotype at next detection	Inhaled antibiotics	Oral antibiotics	IV antibiotics
1 ^a	22	10	24	14	NM	X	X	X
2 ^a	52	14	52	14	None	X	X	
3	19	17	19	17	M		X	X
4 ^a	52	14	52	14	None	X	X	
5	13	4	53	24	NM			X
6	46	17	46	17	M	X	X	
7 ^a	61	19	61	19	None	X	X	
8 ^a	5	2	30	9	NM	X	X	
9	10	2	28	4	NM	X	X	
10 ^a	3	1	40	9	NM	X	X	
11 ^a	0	0	43	16	NM		X	X
12	0	0	5	1	M			X
13	0	2	0	2	M	X	X	X
14	5	2	4	2	M	X	X	X
15	7	1	2	1	M	X	X	

Definition of abbreviations IV = intravenous; M = mucoid; NM = non-mucoid; PA = *Pseudomonas aeruginosa*; X = therapy used in eradication attempt

Data are expressed as number of months or number of cultures obtained.

^aSubjects who remained airway culture negative for mucoid *P.aeruginosa* at the conclusion of the study period.