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Predictors of Mucoid *Pseudomonas* Colonization in Cystic Fibrosis Patients

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Summary

Rationale: Chronic mucoid *Pseudomonas aeruginosa* within the airway in cystic fibrosis (CF) patients can determine prognosis. Understanding the risk factors of mucoid *P. aeruginosa* acquisition may change how we deliver care. This study aims to evaluate whether presence of risk factors reported to predict disease severity including gender, CFTR genotype, bacterial organisms in airway cultures, and serum levels of vitamins A and E, albumin, C-reactive protein, alpha 1-antitrypsin, and immunoglobulins increased the risk of mucoid *P. aeruginosa* acquisition. Methods: Primary endpoint was age at first transition from negative to positive culture for mucoid *P. aeruginosa*. Cox proportional hazards regression with time-dependent covariates examined development of mucoid *P. aeruginosa* infection and its association with longitudinally measured serum biomarkers, pulmonary function, and culture results for other organisms. Results: Median ages at CF diagnosis and at first culture were 0.55 and 5.7 years, respectively. Median number of cultures/patient was 17. Of the 323 subjects, 150 developed mucoid *P. aeruginosa* during a median 8.1 years' follow-up. In multivariate analysis, gender (relative hazard [RH] 0.55 for male vs. female, P=0.001), number of DF508 alleles (RH 1.66 for1 or 2 vs. 0, P=0.04), FEV1 % (RH 1.16 for 10% decrease, P=0.008), and most recent *Staphylococcus aureus* status (RH 0.24 for positive vs. negative, P< 0.0001) remained statistically significant. Conclusion: Female gender, number of DF508 alleles, decreased lung function, and lack of *S. aureus* on recent sputum culture are important risk factors for early detection of mucoid *P. aeruginosa*.

Keywords

cystic fibrosis; *Pseudomonas* colonization

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INTRODUCTION

Cystic fibrosis (CF) lung disease is characterized by chronic infection by *Pseudomonas aeruginosa*,[1,2] with reported prevalence increasing with age. Chronic *P. aeruginosa* within the CF airway is a well-known determinant of prognosis;[3] several studies demonstrate associations between *P. aeruginosa* colonization and subsequent lung function decline.[4–6] While not the first pathogen to colonize the CF lung, *P. aeruginosa* is the most common. Early infection with other pathogens, such as *S. aureus*, may prime the airway for later infection by *P. aeruginosa*.[7] Respiratory tract infections occur in 15–30% of infants, 30–40% of children, 60% of adolescents, and 80% of adults with CF.[6,8,9] Bronchoscopy data confirm *P. aeruginosa* colonization in ≤20% of CF children by age 2,[10] but, Burns et al. [1] showed that 39 of 40 CF young children <3 years of age had evidence of *P. aeruginosa* by serial oral pharyngeal cultures or antibody. Despite extensive inflammatory response to ward off colonization, most CF patients become infected with a dominant organism, usually *P. aeruginosa*, which can no longer be eradicated. Once this organism switches to the mucoid phenotype, lung function decline increases. Thus, the course of *P. aeruginosa* infection in most CF patients has three distinct stages: no *P. aeruginosa*; initial nonmucoid *P. aeruginosa*; and mucoid *P. aeruginosa* colonization.[1,6,11] Progressive pulmonary disease associated with chronic mucoid *P. aeruginosa* and airway inflammation appears to play a major role on the morbidity and mortality in CF patients,[12] and successful strategies have been developed to reduce chronic colonization by active early eradication.[4,13–15] However, elucidation of the risk factors for mucoid *P. aeruginosa* acquisition may lead to changes in management of patients with CF.

Epidemiologic studies of *P. aeruginosa* in CF provide some insight into timing and risk factors for nonmucoid and mucoid *P. aeruginosa* colonization.[6,16] A recent prospective study by Li et al.[6] showed that mucoid *P. aeruginosa* played a much greater role in CF lung disease than nonmucoid *P. aeruginosa* in 56 children diagnosed by newborn screening. An older retrospective study by Demko et al.[17] reports the timing between initial nonmucoid *P. aeruginosa* cultures and the appearance of mucoid *P. aeruginosa. P. aeruginosa* colonization has been associated with meconium ileus, number of hospital admissions and early diagnosis of CF.[18] Another study by Wang et al.[19] suggests no difference in risk of *P. aeruginosa* acquisition between children diagnosed as newborns or in early childhood. Subsequent studies by Maselli et al.[16] of *P. aeruginosa* acquisition in children identified by newborn screen show a positive association among female gender, homozygous DF508 mutation, and S. *aureus* isolation and early detection of *P. aeruginosa* (median age of acquisition 8.1 years). Long term use of oral antibiotics that have no in vitro activity against *Pseudomonas* and integration of CF infants with older CF patients were also associated with increased risk of *P. aeruginosa* infection.[20–22] Finally, clinic exposures, aerosol use, lower level of mother's formal education, and female gender were significantly associated with earlier acquisition of mucoid *P.aeruginosa*.[17,23,24] However, none of these studies evaluated the development of mucoid *P. aeruginosa* infection and its association with serum biomarkers, *S. aureus* colonization, and lung disease progression.

With extensive clinical registry data collected at our center, we can evaluate whether factors that have been reported to predict lung disease severity increase the risk of *P. aeruginosa* acquisition. Of these, we analyzed gender, CFTR genotype, organisms in airway cultures, and serum levels of vitamins A and E, albumin, C-reactive protein (CRP), alpha 1-antitrypsin (AAT), and immunoglobulins.[25,26] Hypothesizing that some of these biomarkers and/or colonization with a specific organism predict mucoid *P. aeruginosa* colonization, we explored risk factors for acquisition of *P. aeruginosa* in infants, children, and adults with CF followed at Children's Hospital Boston. We report unique results on development of mucoid

P. aeruginosa infection and its association with *S. aureus* and lung disease progression in patients with CF.

METHODS

Study Population and Data Collection

In a study approved by the Hospital's Institutional Review Board, we examined all CF patients followed from 1993 to 2005 who were registered within a clinical and laboratory database at Children's Hospital Boston. The diagnosis of CF was documented in the medical record by pilocarpine iontophoresis sweat test (sweat chloride >60 mmol/L). The primary endpoint was the age at first transition from culture-negative to culture-positive for mucoid *P. aeruginosa*. Accordingly, patients were selected for analysis if they had two or more culture results available in the database, the first of which was negative for mucoid *P. aeruginosa*. Those patients who were negative for mucoid *P. aeruginosa* at first culture include both subjects with cultures negative for any *P. aeruginosa* as well as subjects with cultures positive for nonmucoid *P. aeruginosa*. Biomarker measures analyzed included those described in the literature as being correlated with lung function including serum CRP, total serum IgE, total serum IgG, serum AAT, albumin, vitamin A and E levels, total white blood cell and neutrophil counts, and neutrophil percent of total white blood cell count.[25,26] For each patient, we extracted all laboratory values and lung function test results from the medical record database at Children's Hospital Boston. Specifically, for pulmonary function testing, microbiologic data, genotype, and biomarker analysis data were extracted from the laboratory databases based on a CF diagnosis and downloaded into an ORACLE database. A structured query language reporting tool was run to join the hospital-wide laboratory values requested with the CF patient population followed at Children's Hospital Boston. Given the method for extracting data from various electronic sources and merging them, it was not possible to obtain an individual patient symptom history or medication information. However, as Children's Hospital Boston is an accredited CF Care Center, patients received standard CF care as outlined by the CF Consortium guidelines. Most patients did not receive antistaphylococcal prophylaxis. Anti-*pseudomonas* antibiotics were routinely given to patients with two positive CF cultures (either documented by deep throat cultures or bronchoalveolar lavage) in an attempt to eradicate *P. aeruginosa*.

Sputum Culture Ascertainment

Sputum and deep throat swab specimens were cultured for bacterial pathogens. Colonization was defined as one positive microbiologic growth on culture. Cultures were performed in the Infectious Diseases Diagnostic Division at Children's Hospital Boston. Samples were plated on selective media for isolation of important CF pathogens: Trypticase soy agar with 5% sheep blood, MacConkey agar, oxidative-fermentative polymyxin B-bacitracin lactose agar, Haemophilus isolation agar and mannitol salt agar. Identification of organisms was performed by standard techniques, including the use of biochemical panels. In addition to *P. aeruginosa*, other organisms which were selectively cultured and identified included *S. aureus, Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Enterobac-teriaceae*, and *Burkholderia* species in the *B. cepacia* complex.

CFTR Genotype Analysis

Genomic DNA isolated from each subject was evaluated for the presence of any of 1000 CFTR gene mutations (Genzyme, Cambridge, MA or Ambry Genetics, Aliso Viejo, CA) as part of their clinical evaluation.

Pulmonary Function Measurements

Forced expiratory volume in 1 sec (FEV_1), forced vital capacity (FVC), and mid-peak flows ($FEF_{25–75}$) were determined by standard spirometry meeting American Thoracic Society criteria; absolute values were converted to a percentage of the predicted volume expected for a healthy individual of the same age, sex, and height on the basis of the regression equations developed by Knudson et al.[27,28]

STATISTICAL METHODS—We defined the "baseline" level of longitudinally measured laboratory and pulmonary function test measurements for an individual as the most recent measurement made on or before the date of the first culture for that subject or, if there were no such measurements, as the earliest available measurement taken after the first culture. In analyses of age at first positive culture, follow-up time was measured from birth, but each subject entered the risk set at the time of the first culture. Median age at first culture was estimated by a product-limit type estimate of the baseline hazard function from a Cox proportional hazards model with no covariates and late entry into the risk set.

The analysis of risk factors for acquisition of mucoid *Pseudomonas* also used the Cox model but with covariates. In this analysis, highly skewed laboratory measurements were transformed to a logarithmic scale for analysis (absolute WBC, lymphocyte, neutrophil and eosinophil counts, and serum levels of AAT, CRP, IgA, IgG, and IgM). Zero values were replaced by small positive values when necessary before taking logarithms. Modeling of risk factors proceeded as follows: except for gender and genotype, all covariates were measured longitudinally; laboratory and PFT measurements were analyzed as time-dependent covariates, with the most recently measured value as the covariate. In a preliminary analysis, the six PFT measurements (absolute liters and percent predicted for each of FEV_1, FVC, and $FEF_{25–75}$) were considered as a group in order to reduce the number of these highly correlated variables to a single predictor. When considering culture results as risk factors, we constructed two time-dependent indicator variables: one for any prior lifetime history of a positive culture and another for the most recent result. By considering these covariates simultaneously in a model, we were able to test whether a lifetime history of a positive culture was associated with any risk in addition to "current" status. We checked whether the relative hazard for each covariate in our final model varied according to age (≤ 10 vs. > 10 to ≤ 20 vs. >20 years) by testing for interaction between the covariate and age using a likelihood ratio test.

In additional analyses exploring the relationship between S. *aureus* and *P. aeruginosa*, age-specific prevalences of positive cultures were calculated by generalized estimating equations to account for clustering by subject. (Each subject could contribute several observations within an age interval.) In addition, we calculated the within-subject odds ratio between positivity for these two organisms using conditional logistic regression. This analysis uses only within-subject information to estimate the odds ratio and controls for all nontime-dependent patient characteristics by using each subject as its own control.

SAS version 9.1 was used for all analyses. Two-sided *P* values are reported, with *P* values < 0.05 considered statistically significant.

RESULTS

Of 542 CF patients identified, 10 had no culture results available and 18 had only one, leaving 514 subjects with two or more cultures. Of these, 323 were negative and 191 were positive for mucoid *P. aeruginosa* at first culture. Table 1 shows the clinical characteristics of these two cohorts. Although similar by gender and genotype, those who were positive for mucoid *P. aeruginosa* at the first culture tended to be much older and have a longer history

of CF before the first available culture result. The remainder of this analysis was restricted to the 323 patients who were negative for mucoid *P. aeruginosa* at first culture. This baseline was obtained at a median age of 5.7 years. The median number of cultures per patient was 17, collected over a median follow-up of 8.1 years. Table 2 shows characteristics of the CF cohort, who were negative for mucoid *P. aeruginosa* at first culture, at baseline. The median age at CF diagnosis was 0.55 years with 90th percentile 7.82 years. The cohort was 52% male. Forty-six percent of the patients were DF508 homozygous and 35% were heterozygotes. Thirty-four percent of the cohort had positive cultures for nonmucoid *P. aeruginosa*. Clinical serum marker levels and lung function measurements confirm that the values were performed at baseline and not during a pulmonary exacerbation. For laboratory values and lung function measurements, when a value measured at or before the first culture date was not available, the earliest measurement after the first culture was used as the baseline value. For the blood cell counts and other laboratory measurements, the baseline values were measured at or before the first culture in approximately 50–70% of subjects; in 75–90% of subjects the first measurement was no later than 12 months after the first culture. For lung function measurements, approximately 80% were measured at or before and 97% were measured no later than 12 months after the first culture. Table 3 shows the number and percentage of patients who were culture-positive for each organism at any time during follow-up. Also shown are the actuarial estimates of median age at first positive culture. For mucoid *P. aeruginosa* this median age is 11.3 years. Eleven patients died between ages 10 and 45 years (median 24 years).

Table 4 depicts the univariate analysis of the association between each potential risk factor and the risk of acquisition of mucoid *P. aeruginosa*. Gender, decreased lung function, and decreased vitamin A levels were significantly associated with an elevated risk of mucoid *P. aeruginosa*. In addition, both a lifetime history and the most recent status of infection with *S. aureus* were associated with a lower risk of *P. aeruginosa*. Other characteristics that were marginally significant ($P < 0.10$) include presence of at least one DF508 allele, lifetime history of *S. maltophilia* infection, and the most recent serum levels of neutrophils, AAT, total protein, and vitamins A and E. These variables were retained for further consideration in multivariable models.

As a preliminary step in multivariable modeling, we investigated the two *S. aureus* measures in adjusted analyses. When lifetime culture history and most recent culture status of *S. aureus* were considered together in a model, only the recent status was significant [recent positive, RH = 0.20, 95% CI (0.14, 0.30), $P < 0.0001$; lifetime history of any positive, RH = 1.35, 95% CI (0.83, 2.20), $P = 0.23$]. On the basis of these results, we considered only the most recent *S. aureus* status in further multivariable models.

Six different lung function measures were all highly significant in univariate analysis. We explored them as a group with a view toward reducing the number of correlated predictors before building multivariable models. For each of FEV_1, FVC, and $FEF_{25–75}$, when the pair of variables (e.g., percent predicted FEV_1 and FEV_1 liters) was considered together in a model, the liter variable was clearly the least significant (all P values > 0.16). We then considered the three percent predicted variables in pairs. After adjusting for percent predicted FEV_1, the other variables both had P values > 0.54. Therefore, we retained only percent predicted FEV_1 in further models.

Starting with the highly significant risk factors from the univariate analysis and preliminary modeling steps, we considered adding other candidate factors one at a time and arrived at the final model shown in Table 5. This model includes gender, genotype, percent predicted FEV1, and most recent *S. aureus* culture status. All of these predictors remained statistically

significant when any of the other candidate predictors was added to this model, and none of the other predictors was significant (all P values ≥ 0.10).

We also considered modeling DF508 genotype as a three-level variable (0, 1, 2 alleles). The relative hazards (RH) for 1 versus 0 alleles (RH = 1.63) and for 2 versus 0 alleles (RH = 1.69) were very similar, therefore the two-level variable used in our final model (RH = 1.66 for 1 or 2 alleles vs. 0 alleles) is preferred. In order to evaluate possible biases introduced by inclusion of subjects whose first available culture was at a late age, and subjects whose first available lung function test results were after their first culture results, we re-evaluated our final model restricted to two subgroups: subjects whose first available culture was at age <10 years, and subjects whose "baseline" lung function was measured at or before the date of their first available culture. The results were very similar to those shown in Table 5, suggesting that conclusions were not influenced by inclusion of subjects with missing early data. Finally, we checked for heterogeneity of the RH across age groups; none was found.

The observation that *S. aureus* colonization is associated with a lower risk of *P. aeruginosa* colonization was investigated further. Table 6 shows age-specific prevalence of these two organisms. The prevalence estimates take into account the clustering due to each subject's possible contribution of several samples within a single age interval. From birth to adulthood, age-specific prevalences of mucoid *P. aeruginosa* increase from less than 5% to over 40%. *S. aureus* prevalence is increased slightly to just over 80% in the first 15 years, then decreases, especially after age 24. These trends are broadly consistent with the hypothesis that the presence of *S. aureus* is protective against mucoid *P. aeruginosa* but do not demonstrate a within-subject association. In a more direct comparison, we used conditional logistic regression to estimate the within-subject odds ratio (OR) between the two prevalences. The association was again consistent with the proportional hazards analysis: OR = 0.43, 95% CI (0.34, 0.53), $P < 0.001$. To check the possibility of residual confounding by age, we added age to the model. The OR changed only slightly, OR = 0.41, 95% CI (0.32, 0.51), $P < 0.001$.

DISCUSSION

We examined clinical registry data for risk factors that may be associated with the initial detection of mucoid *P. aeruginosa* from a cohort of 323 CF patients followed at Children's Hospital Boston. Our findings indicate that female gender, presence of at least one DF508 allele, lower percent predicted FEV_1, and a negative *S. aureus* sputum culture were predictive of mucoid *P. aeruginosa*. The relative hazards for mucoid *P. aeruginosa* colonization in males versus females was 0.55, and the earlier acquisition of mucoid *P. aeruginosa* in females has been previously postulated to contribute to their diminished survival.[17] These genotype and gender findings are consistent with results demonstrating that both of these characteristics are associated with initial detection of *P. aeruginosa* and poor outcomes in CF. Our findings confirm a gender difference for mucoid *P. aeruginosa* acquisition likely contributing to the more rapid lung function decline in females with CF.[16,17,29,30] CF patients with 1 or 2 versus 0 DF508 mutations had an increased risk of mucoid *P. aeruginosa*. As a deficiency in CFTR protein in CF patients is thought to impair the clearance of *P. aeruginosa*,[31,32] this finding is consistent with the decreased CFTR function evident in patients homozygous for DF508. Similarly, while there is genetic heterogeneity in patients homozygous for the DF508 mutation, patients with DF508 mutations generally have more severe lung disease than CF patients with either one DF508 allele or other non-DF508 genotypes.[33] At any given age, among people who never tested positive for mucoid *P. aeruginosa*, those with lower lung function are more likely to convert to positive mucoid *P. aeruginosa* than those with higher lung function. While these results suggest that decreased lung function is a predecessor to mucoid *P. aeruginosa*, representative

papers in the literature showed that mucoid *P. aeruginosa* may also be associated with a subsequent further decline in lung function.[3,6] We also found that laboratory serum biomarkers did not predict mucoid *P. aeruginosa* acquisition in the multivariate analysis. Finally, we found that a CF patient's airway does become colonized by progression from no *P. aeruginosa*, initial nonmucoid *P. aeruginosa*, and then mucoid *P. aeruginosa*,[1,6,11] confirming prior studies. This progression could be due to the host's process in mediating innate immunity to mucoid *P. aeruginosa* infection. An ongoing inflammatory response likely acts as a "double-edged sword" and suggests that: mediators of immunity and inflammation, when produced in a physiologically appropriate fashion in response to infection, mediate resistance; failure of this response leads to infection; and ongoing production of the same mediators contributes to immunopathology and lung function decline.

In addition and perhaps more importantly, we found that a positive *S. aureus* culture is associated with a lower risk of mucoid *P. aeruginosa*. This finding contradicts previous investigations that demonstrate an association between more frequent positive *S. aureus* cultures and existing infection with mucoid *P. aeruginosa*.[16,34] However, the *Patient Registry 1999 Annual Data Report* from the Cystic Fibrosis Foundation[8] showing a rise in *P. aeruginosa*, as *S. aureus* colonization diminishes, supports our results. We found that both a lifetime history of *S. aureus* infection and a recent history of *S. aureus* infection were associated with a lower risk of first acquisition of *P. aeruginosa* colonization. However, when both were considered together, only the recent history was significant. Coupled with the observations that *S. aureus* tends to be acquired early in life and maintains a high age-specific prevalence, this finding suggests that, if *S. aureus* has a protective effect, it is short-lived. It may be that transition of *S. aureus* status from positive to negative creates an opportunity for *P. aeruginosa* colonization. The conditional logistic regression analysis also supports this idea by demonstrating that, within an individual, a change in infection status from positive to negative for one organism tends to be associated with a change in the opposite direction for the other organism. Lending further support to these results, studies by Ratjen et al.[20] and Stutman et al.[21] found that patients receiving continuous anti-staphylococcal antibiotic therapy had a significantly higher rate of *P. aeruginosa* acquisition than patients receiving only intermittent or no antibiotic therapy. They concluded that continuous therapy with antistaphylococcal antibiotics directed against *S. aureus* increases the risk of colonization with *P. aeruginosa*. Although the use of continuous therapy with antistaphylococcal antibiotics remains controversial,[35] our analysis also suggests that this strategy favors early colonization with *P. aeruginosa*.

While our study design and analyses are unique and some of our findings novel, our study has certain limitations. We, as well as others, used oropharyngeal (OP) and/or sputum cultures[16,19,22,24] in our analyses, which may have imposed a selection bias as the younger patients, unable to expectorate, may not have provided as many samples as the older patients. However, as depicted in Table 6, the number of cultures per subject was fairly constant across age strata. Although mucoid *P. aeruginosa* is considered the predominant respiratory bacterial pathogen in adult CF patients with one particular study showing a prevalence rate of 92% in 16 year olds, our study shows lower prevalence rates, for example 26% in 15–19 year olds (Table 6). In comparison to the Li et al. paper referenced, we have a much larger sample size contributing to prevalence estimates with 124 patients contributing to the 15–19 age range compared with 13 patients age 16 in the Li paper. The lack of inclusion of a chest X-ray assessment either by a modified Shwachman or other scoring system could also be perceived as a limitation of this study especially in light of recent studies showing that chest X-ray changes may be more sensitive in detecting early pulmonary involvement in CF than spirometry.[36] However, there is no agreed upon chest X-ray or CT scan scoring system among CF centers.

The limitations of our registry database include the taking of measurements at multiple time points for clinical reasons and not in the consistent method usual for study purposes. The historical database is also clustered toward more data on the sicker CF patients, as these patients would have more clinic visits and values in the database. These limitations should not affect the reproducibility of our results. The strengths of this database include having numerous measurements on a large cohort of CF patients, with multiple observations, followed at one center over a 13-year period. Collection of study subjects from a single center affords some control over variation in environmental factors, which are difficult to control in multicenter studies.

In conclusion, female gender, presence of at least one DF508 allele, decreased lung function, and a lack of *S. aureus* isolation on the most recent sputum culture are important risk factors for early detection of mucoid *P. aeruginosa*. Recognition of these risk factors should lead to earlier aggressive antibiotic and efficacious treatments to delay chronic infection with mucoid *P. aeruginosa*. Furthermore, our analysis suggests that prophylactic anti-staphylococcal antibiotics may favor earlier colonization with mucoid *P. aeruginosa*.

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TABLE 1

Characteristics of Patients by Mucoid *P. aeruginosa* Status at First Culture

	Mucoid *P. aeruginosa* positive (N = 191)	**Mucoid *P. aeruginosa* negative (N =323)**	***P*-value**
Male gender	96(52%)	167 (52%)	0.78
# DF508 alleles[1]			0.69
0	34(20%)	59 (19%)	
1	65(38%)	108 (35%)	
2	71(42%)	141 (46%)	
Age at first culture (years)	18.9 (9.2, 34.4)	5.7 (0.3, 21.0)	<0.001
Years from diagnosis to first culture[2]	14.6 (2.5, 29.3)	1.9 (0.0, 16.5)	<0.001

For categorical characteristics, data are n (%) and *P* values are from Fisher's exact test. For continuous characteristics, data are median (10th percentile, 90th percentile) and *P* values are from the Mann– Whitney–Wilcoxon test.

[1] N = 170 mucoid *P. aeruginosa* positive, N =308 mucoid *P. aeruginosa* negative with genotype data.

[2] N = 170 mucoid *P. aeruginosa* positive, N = 300 mucoid *P. aeruginosa* negativewith known date ofdiagnosis.

TABLE 2

Baseline Characteristics for 323 Patients Negative for Mucoid *P. aeruginosa* at First Culture

Characteristic	N	n (%) or median (10th, 90th)[1]
Male gender	323	167 (52%)
Number of DF508 alleles	308	
0		59 (19%)
1		108 (35%)
2		141 (46%)
Age at diagnosis (years)	300	0.55 (0.02, 7.82)
Culture status at baseline		
Nonmucoid *P. aeruginosa* positive	323	110 (34%)
Staphylococcus aureus positive	323	251 (78%)
Stenotrophomonas maltophilia positive	323	2 (0.6%)
Burkholderia cepacia complex positive	323	3 (0.9%)
Blood counts		
WBC count (× 1,000/mm^3)	323	10.15 (6.53, 14.84)
Lymphocyte count (× 1,000/mm^3)	321	3.46 (1.73, 6.74)
Lymphocyte percent	321	37.8 (18.0, 57.0)
Neutrophil count (× 1,000/mm^3)	321	4.6 (2.6, 9.1)
Neutrophil percent	321	49.1 (30.3, 71.4)
Eosinophil count (× 1,000/mm^3)	321	0.19 (0.07, 0.47)
Eosinophil percent	321	2.0 (0.8, 4.9)
Other markers		
CRP (mg/dl)	316	0.00 (0.00, 1.03)
AAT (mg/dl)	321	151 (109, 216)
Total protein (g/dl)	320	6.9 (5.7, 7.9)
Albumin (g/dl)	323	3.8 (3.2, 4.2)
IgA (mg/dl)	321	101 (26, 256)
IgG (mg/dl)	321	781 (328, 1480)
IgM (mg/dl)	321	93 (42, 190)
Vitamin A (mcg/dl)	319	34.8 (16.0, 53.0)
Vitamin E (mg/L)	319	7.6 (3.0, 14.3)
Lung function		
FEV_1 percent of predicted	293	98 (66, 128)
FVC percent of predicted	293	102 (76, 127)
$FEF_{25–75}$ percent of predicted	293	84 (28, 129)
FEV_1 liters	293	1.31 (0.79, 2.90)
FVC liters	293	1.54 (0.93, 3.73)
$FEF_{25–75}$ liters	293	1.48 (0.62, 2.92)

[1] 10th and 90th percentiles.

TABLE 3

Number and Percent of Patients Who Tested Positive for Each Organism at Some Time During Follow-Up

Organism	n (%)	Median age (years) at first positive
Nonmucoid *P. aeruginosa*	262 (81%)	2.9
Mucoid *P. aeruginosa*	150 (46%)	11.3
S. aureus	310 (96%)	0.46
S. maltophilia	50 (15%)	28.6
Burkholderia cepacia complex	32 (10%)	Not reached

Median age at first positive culture is also shown.

TABLE 4

Univariate Proportional Hazards Models Predicting First Culture Positive for Mucoid *P. aeruginosa*

Covariate	Comparison	RH	95% CI	*P*-value
Gender	Male vs. female	0.60	(0.43, 0.83)	0.002
DF508 alleles	1 or 2 vs. 0	1.54	(0.98, 2.41)	0.06
Culture history				
S. aureus	Ever vs. never +	0.38	(0.25, 0.58)	<0.0001
S. aureus	Recent + vs. –	0.23	(0.17, 0.32)	<0.0001
BCC	Ever vs. never +	1.32	(0.61, 2.86)	0.48
BCC	Recent + vs. –	0.64	(0.16, 2.63)	0.54
S. maltophilia	Ever vs. never +	1.58	(0.97, 2.57)	0.07
S altophilia	Recent + vs. —	1.24	(0.44, 3.51)	0.69
Lung function				
FEV_1 %	10% points ↓	1.20	(1.11, 1.30)	<0.0001
FEV_1 liters	1 L ↓	1.75	(1.32, 2.30)	<0.0001
FVC %	10% points	1.19	(1.09, 1.30)	0.0001
FVC liters	↓ 1 L ↓	1.56	(1.22, 2.01)	0.0004
$FEF_{25–75}$ %	10% points	1.12	(1.05, 1.18)	0.0002
$FEF_{25–75}$ liters	↓ 1 L ↓	1.40	(1.16, 1.70)	0.0005
Blood counts				
WBC count	Twofold ↑	1.20	(0.85, 1.68)	0.30
Lymphocyte count	Twofold ↑	1.13	(0.83, 1.55)	0.43
Lymphocyte %	10% points ↑	0.95	(0.80, 1.13)	0.55
Neutrophil count	Twofold ↑	1.26	(0.97, 1.63)	0.09
Neutrophil %	10% points ↑	1.05	(0.90, 1.22)	0.57
Eosinophil count	Twofold ↑	1.00	(0.86, 1.16)	0.99
Eosinophil %	1% point ↑	1.00	(0.92, 1.09)	0.95
Other markers				
AAT	Twofold ↑	1.65	(0.98, 2.77)	0.06
Albumin	1 g/dl ↑	0.77	(0.51, 1.17)	0.22
CRP	Twofold ↑	1.01	(0.95, 1.08)	0.70
Total protein	1 g/dl ↑	0.81	(0.63, 1.03)	0.09
IgA	Twofold ↑	1.05	(0.89, 1.25)	0.55
IgG	Twofold ↑	1.10	(0.76, 1.59)	0.62
IgM	Twofold ↑	1.04	(0.84, 1.31)	0.70
Vitamin A	1 mcg/dl ↑	0.98	(0.97, 0.99)	0.008
Vitamin E	1 mg/L ↑	0.96	(0.93, 1.00)	0.08

RH, relative hazard; CI, confidence interval; BCC, *Burkholderia cepacia* complex; FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity; $FEF_{25–75}$, mid peak flows.

TABLE 5

Multivariable Proportional Hazards Models Predicting First Culture Positive for Mucoid *P. aeruginosa*

Covariate	Comparison	RH	95% CI	*P*-value
Gender	Male vs. female	0.55	(0.38, 0.79)	0.001
DF508	1 or 2 vs. 0 alleles	1.66	(1.02, 2.72)	0.04
FEV_1 %	10% point ↓	1.16	(1.06, 1.26)	0.0008
S. aureus	Most recent + vs. –	0.24	(0.17, 0.34)	<0.0001

TABLE 6

Age-Specific Prevalences of Mucoid *P. aeruginosa* and *S. aureus*

Age (years)	Number of Samples	Number of subject	Samples per subject	Prevalence	
				Mucoid *P. aeruginosa* (%)	*S. aureus* (%)
				P. aeruginosa (%)	(%)
0–4	1,071	155	6.9	3.5	71.9
5–9	1,470	199	7.4	10.4	75.0
10–14	1,373	168	8.2	16.5	81.5
15–19	963	124	7.8	26.1	77.2
20–24	450	68	6.6	26.6	77.3
25–29	259	34	7.6	37.7	63.7
30–34	170	22	7.7	44.0	62.0
≥35	160	19	8.4	41.0	56.6