Longitudinal Development of Mucoid Pseudomonas aeruginosa Infection and Lung Disease Progression in Children With Cystic Fibrosis

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Cystic fibrosis (CF) causes chronic lung disease in children with recurrent bacterial infections. Pseudomonas aeruginosa is the most common virulent respiratory pathogen in cystic fibrosis (CF), and appears to be a major limiting factor in overall survival in CF. Patients with CF who acquire P aeruginosa have 2.6 times higher risk of death, and P aeruginosa cross-infection has caused early CF deaths. Neonates with CF have structurally normal lungs and no P aeruginosa, but nonmucoid P aeruginosa is acquired after variable time periods. Recent observations suggest that nonmucoid P aeruginosa can possibly be eradicated by aggressive anti-P aeruginosa antibiotics, despite earlier views to the contrary. Mucoid P aeruginosa, a mutant phenotype of P aeruginosa, develops at a subsequent stage and produces exopolysaccharide/alginate, causing mucoidy and conferring resistance to phagocytosis and antibiotics. Mucoid P aeruginosa apparently cannot be eradicated by current antibiotics, becomes predominant with age, and predicts shortened CF survival. Thus, the course of P aeruginosa infection in patients with CF appears to occur in 3 distinct stages: no P aeruginosa, initial nonmucoid P aeruginosa, and mucoid P aeruginosa, whereby after colonization, P aeruginosa undergoes phenotypic conver-

Context Although Pseudomonas aeruginosa is the most common virulent respiratory pathogen in cystic fibrosis (CF), the longitudinal development of P aeruginosa infection and its effect on antibody responses and lung disease progression in children with CF remain unclear.

Objective To prospectively examine the epidemiology of P aeruginosa infection and its impact on CF pulmonary morbidity.

Design, Setting, and Patients We prospectively evaluated 56 CF patients at 2 CF centers in Madison and Milwaukee, Wis, from birth up to age 16 years between April 15, 1985, and April 15, 2004, with diagnoses made through the Wisconsin CF Neonatal Screening Project.

Main Outcome Measures Timing of nonmucoid P aeruginosa and mucoid P aeruginosa acquisition was assessed by first positive result. Longitudinal development from no P aeruginosa to nonmucoid P aeruginosa and from nonmucoid P aeruginosa to mucoid P aeruginosa was examined. Outcome measurements included antibody titers, respiratory symptoms, quantitative chest radiography, and pulmonary function tests.

Results Sixteen patients (29%) acquired nonmucoid P aeruginosa in the first 6 months of life. The age-specific prevalence of mucoid P aeruginosa increased markedly from age 4 to 16 years. Nonmucoid and mucoid P aeruginosa were acquired at median ages of 1.0 and 13.0 years, respectively. In contrast with the short transition time from no P aeruginosa to nonmucoid P aeruginosa, the transition time from nonmucoid to mucoid P aeruginosa was relatively long (median, 10.9 years) and could be slightly extended by brief/low anti-P aeruginosa antibiotic treatment. Antibody titers increased with both transitions, but the deterioration in cough scores, chest radiograph scores, and pulmonary function correlated best with transition from nonmucoid to mucoid P aeruginosa.

Conclusions Early prevention and detection of nonmucoid and mucoid P aeruginosa are critical because of early acquisition and prevalence. There is a window of opportunity for suppression and possible eradication (by aggressive anti-P aeruginosa treatment) of initial nonmucoid P aeruginosa. Mucoid P aeruginosa plays a much greater role in CF lung disease progression than nonmucoid P aeruginosa. Antibody titers, cough scores, and chest radiographs are early signs of nonmucoid P aeruginosa and especially mucoid P aeruginosa stages.

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sion from nonmucoid \textit{P. aeruginosa} to mucoid \textit{P. aeruginosa}.\cite{12} The timing of transformation from nonmucoid \textit{P. aeruginosa} to mucoid \textit{P. aeruginosa} in CF patients, however, remains unclear, as do the clinical consequences of such a change and the antibody responses that may protect against colonization.\cite{22}

To understand fully and better treat this virulent pathogen, the long-term epidemiology of \textit{P. aeruginosa} infection in CF and its effect on CF morbidity need to be delineated precisely. Although there are some short-term and cross-sectional studies on the acquisition of nonmucoid \textit{P. aeruginosa} and mucoid \textit{P. aeruginosa} using analyses of older and heterogenous CF patient groups with a wide age range,\cite{23,24} one of the greatest difficulties in gaining more knowledge about \textit{P. aeruginosa} in CF stems from the lack of longitudinal studies that systematically acquire information from early childhood. The Wisconsin CF Neonatal Screening Project,\cite{25-29} however, provides an opportunity to examine prospectively the epidemiology of \textit{P. aeruginosa} and its impact on CF pulmonary morbidity. Herein we report unique information on the longitudinal development of \textit{P. aeruginosa} infection and its effect on antibody responses and lung disease progression in children with CF from birth to age 16 years.

\section*{METHODS}

\subsection*{Study Design}

In brief, the Wisconsin CF Neonatal Screening Project\cite{25-29} is a randomized clinical trial conducted to assess neonatal screening for CF using a standardized evaluation and treatment protocol\cite{20} that prevented malnutrition. Written consent was obtained from parents, and the study was approved by the institutional review boards at University of Wisconsin, Madison, and Medical College of Wisconsin, Milwaukee. Half of Wisconsin's 650341 neonates born from April 15, 1985, through June 30, 1994, were randomly assigned to a group to be screened for CF and the other half to a nonscreened group. In this article, we report on the 56 CF cases from the screened group whose diagnoses were made through neonatal screening but who did not have meconium ileus. Any CF cases found subsequently in the nonscreened group were not included. We prospectively followed up these 56 cases every 6 weeks for their first year of life and every 3 months thereafter to age 16 years. At the discretion of physicians, patients could be treated with anti-\textit{P. aeruginosa} antibiotics if there were clinically significant infections, but patients did not routinely receive anti-\textit{P. aeruginosa} antibiotics after the first \textit{P. aeruginosa} detection, in accordance with the prevailing standard of care.

\subsection*{Outcomes}

Respiratory secretions were obtained from patients every 6 months by protocol and at all nonprotocol visits requiring cultures. Before patients could expectorate sputum, respiratory secretion samples were obtained by vigorous oropharyngeal swabbing.\cite{30,31} The secretion samples were cultured and antibiotic susceptibility testing was performed by disk diffusion. To evaluate antibody responses to \textit{P. aeruginosa}, serum samples were collected every 6 months coinciding with cultures. Antibody titers (expressed as log$_2$) were determined by an antibody capture immunopassay with antigen excess using cell lysate, exotoxin A, and elastase as antigens.\cite{31} Combined culture and serologic (cell lysate titer $\geq 8$) positive results were used to define the timing of the first appearance of nonmucoid \textit{P. aeruginosa} and culture for mucoid \textit{P. aeruginosa}. Lung disease outcomes included respiratory symptoms (cough and wheezing), quantitative chest radiography, and pulmonary function tests by spirometry. At each visit, cough and wheezing were reported by parents and examined by physicians, then both were scored.\cite{32} Chest radiographs were obtained every 6 months from diagnosis to age 4 years and annually thereafter and were scored using the Wisconsin (WCXR) and Brasfield (BCXR) methods.\cite{20,27} Spirometry generally was started when children reached age 4 years and was obtained every 6 months; forced expiratory volume in 1 second (FEV$_1$), forced vital capacity (FVC), FEV$_1$/FVC ratio, and forced expiratory flow between 25% and 75% of FVC (FEF$_{25-75}$%) were ensured as “acceptable” and calculated as percentage of predicted values.\cite{20,27}

\subsection*{Statistical Methods}

The age-specific prevalence\cite{1} of \textit{P. aeruginosa} was calculated. Kaplan-Meier survival curves and medians with 95% confidence intervals (CIs) were estimated for the timing of nonmucoid \textit{P. aeruginosa} and mucoid \textit{P. aeruginosa} acquisition and the transition time from first nonmucoid \textit{P. aeruginosa} acquisition to first mucoid \textit{P. aeruginosa} appearance. The Cox proportional hazards model was used to test the effect of anti-\textit{P. aeruginosa} antibiotics on the time to development of mucoid \textit{P. aeruginosa} from nonmucoid \textit{P. aeruginosa}, adjusting for center, sex, genotype, and pancreatic status. This was accomplished with a time-dependent indicator variable that switched from 0 to 1 at the time of the first anti-\textit{P. aeruginosa} treatment. The assumption of proportionality was assessed by analyzing the smoothed plot of the Martingale residuals vs time. The log-rank test was used to compare times for the transition from nonmucoid \textit{P. aeruginosa} to mucoid \textit{P. aeruginosa} between the anti-\textit{P. aeruginosa} and non–anti-\textit{P. aeruginosa} cohorts (children were initially classified as non–anti-\textit{P. aeruginosa} and became classified as anti-\textit{P. aeruginosa} when the previously mentioned time-dependent indicator switched to 1).

The effect of \textit{P. aeruginosa} development on outcomes was assessed by generalized estimating equations (GEEs) with an independence working correlation using the identity link\cite{33} and adjusting for center, sex, genotype, pancreatic status, and age. Status of nonmucoid \textit{P. aeruginosa} and mucoid \textit{P. aeruginosa} was 0 until the first positive result and 1 thereafter. Three vari-
ables were used to incorporate the timing of *P. aeruginosa* into GEE models: (1) duration—no *P. aeruginosa* was the time from birth to first nonmucoid *P. aeruginosa* acquisition; (2) duration—nonmucoid *P. aeruginosa* was the time from first nonmucoid *P. aeruginosa* acquisition to first mucoid *P. aeruginosa* appearance; and (3) duration—mucoid *P. aeruginosa* was the time since first mucoid *P. aeruginosa* appearance. Duration—no *P. aeruginosa*, duration—nonmucoid *P. aeruginosa*, and duration—mucoid *P. aeruginosa* add up exactly to and are confounded by age; consequently, age was excluded from models with these 3 variables. Analysis of these variables allowed us to determine whether the data were consistent with abrupt or gradual changes in outcomes after the first positive result. If there were abrupt changes, regression coefficients for *P. aeruginosa* status would be significant and otherwise would be nonsignificant; if *P. aeruginosa* status had no significant effect, it was dropped from the model. Whether duration with *P. aeruginosa* had a significant effect was assessed by testing whether slope differences for duration—nonmucoid *P. aeruginosa* vs duration—no *P. aeruginosa* and duration—mucoid *P. aeruginosa* vs duration—nonmucoid *P. aeruginosa* were significant.

Cox models adjusting for center, sex, genotype, and pancreatic status were used to test the effect of *P. aeruginosa* development on the time to change in disease status using reported cough score greater than 1 (ie, cough in morning or with postural drainage or frequent productive cough), WCXR score greater than 5 (ie, mild irreversible lung disease), 26 BCXR score less than 21 (ie, mild irreversible lung disease), 26 and the percentage of predicted values of FEV₁, FVC, FEV₁/FVC, and FEF₂₅₋₇₅% less than 82.4%, 82.6%, 89.25%, and 67.9%, respectively. SAS, version 8.00 (SAS Institute Inc, Cary, NC) and S-Plus, version 3.4 (Mathsoft Inc, Seattle, Wash) statistical software were used for statistical analyses. All tests were regarded as significant at P < .05.

**RESULTS**

The demographic and clinical characteristics of the 56 children are shown in the Table. As expected, they had a younger-than-average mean (SE) age of diagnosis (13.9 [5.1] [median, 6.9] weeks) compared with 106.4 (1.8) (median, 33.9) weeks for 6692 patients born during 1985-1994 and reported to the CF Foundation Registry. A total of 1921 cultures were collected with an interval of 4.04 (0.16) months between cultures. As shown in Figure 1, during the

| Table. Demographic and Clinical Characteristics of the 56 Patients From Birth to Age 16 Years |
|-------------------------------------------------|----------------|
| **Characteristics**                             | **No. (%)** |
| **Center**                                      |              |
| Madison                                         | 28 (50)      |
| Milwaukee                                       | 28 (50)      |
| **Sex**                                         |              |
| Female                                          | 21 (38)      |
| Male                                            | 35 (62)      |
| **Genotype**                                    |              |
| ΔF508/ΔF508                                     | 33 (59)      |
| ΔF508/other                                     | 23 (41)      |
| **Pancreatic status**                           |              |
| Insufficiency/probable insufficiency            | 50 (89)      |
| Sufficiency/probable sufficiency                | 6 (11)       |
| **Age of diagnosis**, mean (SE) [median], wk    | 13.9 (5.1) [6.9] |
| **Total cultures, No.**                         | 1921         |
| **Interval between cultures, mean (SE), mo**    | 4.04 (0.16)  |
| **Ever use of anti-*Pseudomonas aeruginosa* antibotics** |          |
| No *P. aeruginosa* (n = 56)                     | 13 (23)      |
| Nonmucoid *P. aeruginosa* (n = 53)              | 30 (57)      |
| Mucoid *P. aeruginosa* (n = 27)                 | 19 (70)      |
| Hazard ratio (95% CI) for developing mucoid *P. aeruginosa* among 53 nonmucoid *P. aeruginosa* patients (adjusted for covariates) |     |
| Receiving anti-*P. aeruginosa* vs not receiving anti-*P. aeruginosa* | 0.09 (0.02-0.39) | .001 |
| Time to mucoid *P. aeruginosa* among 53 nonmucoid *P. aeruginosa* patients (based on log-rank), y |         |
| Receiving anti-*P. aeruginosa* vs not receiving anti-*P. aeruginosa* | 11.01 (8.61-15.12) | .31 |
| Not receiving anti-*P. aeruginosa*              | 9.48 (5.99-14.40) |
| Time to mucoid *P. aeruginosa* among 47 nonmucoid *P. aeruginosa* patients (based on log-rank using culture results only), median (95% CI), y |       |
| Receiving anti-*P. aeruginosa* vs not receiving anti-*P. aeruginosa* | 9.02 (6.89-15.12) | .03 |
| Not receiving anti-*P. aeruginosa*              | 6.10 (4.47-8.21) |

Abbreviation: CI, confidence interval.

*Data are expressed as No. (%) unless otherwise noted.
†No tests were performed for center, sex, genotype, or pancreatic status.
‡Based on aerosol, intravenous, and oral anti-*P. aeruginosa* antibiotics.
first 6 months of life, 71% of the patients had no P aeruginosa, but the percentage of patients with no P aeruginosa decreased quickly, and all patients reaching age 13 years acquired P aeruginosa. Sixteen patients (29%) acquired nonmucoid P aeruginosa in the first 6 months of life. The age-specific prevalence of nonmucoid P aeruginosa increased from birth to age 4 years (86%), then decreased when mucoid P aeruginosa predominated. One patient developed mucoid P aeruginosa at age 1.43 years (the earliest we observed), after showing nonmucoid P aeruginosa at 0.53 years. The age-specific prevalence of mucoid P aeruginosa increased from age 4 years (4%) to 16 years, when 92% of all 13 patients reaching that age developed mucoid P aeruginosa. All patients who developed mucoid P aeruginosa had acquired nonmucoid P aeruginosa first.

As shown in Figure 2A and 2B, nonmucoid P aeruginosa was acquired at a median age of 1.0 (95% CI, 0.6-1.5) years, and mucoid P aeruginosa developed at a median of 13.0 (95% CI, 10.0-14.9) years. In contrast with the short transition from no P aeruginosa to nonmucoid P aeruginosa, the transition from nonmucoid P aeruginosa to mucoid P aeruginosa was relatively long, with a median of 10.9 (95% CI, 8.6-14.0) years (Figure 2B). As shown in the Table, patients with nonmucoid P aeruginosa had a significantly higher rate of anti-P aeruginosa antibiotic use than patients with no P aeruginosa, while patients with mucoid P aeruginosa had a nonsignificantly higher rate than patients with nonmucoid P aeruginosa. Among 53 patients with nonmucoid P aeruginosa, those receiving anti-P aeruginosa antibiotics had a significantly lower hazard and a longer transition time than those not receiving anti-P aeruginosa antibiotics (Table). Even those not receiving antibiotic treatment, however, had a relatively long transition time from nonmucoid to mucoid P aeruginosa, 9.48 years (by culture) or 6.10 years (by culture). The approximate incidence rate of aminoglycoside resistance to mucoid P aeruginosa in the anti-P aeruginosa and non-anti-P aeruginosa groups among nonmucoid P aeruginosa patients was 27% vs 11% of cultures (P = .02) and 43% vs 22% of patients (P = .38).

Longitudinal patterns of antibody titers for 3 antigens (Figure 3) show that there are abrupt elevations in antibody titers with transition from no P aeruginosa to nonmucoid P aeruginosa and second elevations with transition from nonmucoid P aeruginosa to mucoid P aeruginosa. Longitudinal analyses revealed that compared with patients with no P aeruginosa, those with nonmucoid P aeruginosa had significant abrupt increases in antibody titers for cell lysate (4.15; P < .001), exotoxin A (4.57; P < .001), and elastase (2.63; P < .001); compared with patients with nonmucoid P aeruginosa, those with mucoid P aeruginosa had significant abrupt increases in antibody titers for cell lysate (1.59; P < .001), exotoxin A (1.26; P = .007), and elastase (2.46; P < .001).

Cough and wheezing scores were associated with P aeruginosa development. Figure 4 shows that transition from no P aeruginosa to nonmucoid P aeruginosa increased reported cough scores, and transition from nonmucoid to mucoid P aeruginosa caused further increase. Longitudinal analyses revealed that compared with patients with no P aeruginosa, those with nonmucoid P aeruginosa had nonsignificant increases in cough scores by report/examination; compared with patients with nonmucoid P aeruginosa, those with mucoid P aeruginosa had significant abrupt increases in cough scores by report (0.38; P = .005) and on examination (0.35; P = .003). Additionally, the wheezing scores of patients with nonmucoid P aeruginosa and those with mucoid P aeruginosa were not significantly different by report/examination from wheezing scores of those with no P aeruginosa and nonmucoid P aeruginosa, respectively.

Chest radiograph scores varied with P aeruginosa stages. As shown in Figure 4, during the no P aeruginosa period, WCXR and BCXR scores gener-
ally remained normal over time, and transition from no \( P \) aeruginosa to nonmucoid \( P \) aeruginosa slightly worsened chest radiograph scores; however, transition from nonmucoid \( P \) aeruginosa to mucoid \( P \) aeruginosa led to significant deterioration in chest radiograph scores. Longitudinal analyses revealed that the change from no \( P \) aeruginosa to nonmucoid \( P \) aeruginosa was associated with nonsignificant worsening in WCXR scores (1.10; \( P = .20 \)) and BCXR scores (−0.48; \( P = .21 \)), while transition from nonmucoid \( P \) aeruginosa to mucoid \( P \) aeruginosa caused significant/pronounced abrupt deterioration in WCXR scores (6.52; \( P < .001 \)) and BCXR scores (−2.34; \( P < .001 \)).

Pulmonary function also changed with \( P \) aeruginosa development. Figure 5 reveals little influence of the change from no \( P \) aeruginosa to nonmucoid \( P \) aeruginosa, whereas transition from nonmucoid \( P \) aeruginosa to mucoid \( P \) aeruginosa significantly altered percentage of predicted FEV1, FVC, and \( \text{FEF}_{25-75}\%\). Specifically, compared with patients with no \( P \) aeruginosa, those with nonmucoid \( P \) aeruginosa showed only a gradual decline (slope difference) in percentage of predicted FEV1/FVC (−0.49; \( P = .03 \)). However, compared with patients with nonmucoid \( P \) aeruginosa, those with mucoid \( P \) aeruginosa had significant abrupt declines in percentage of predicted FEV1 (−12.13; \( P = .02 \)) and FVC (−9.15; \( P = .007 \)) and gradual declines in percentage of predicted FEV1/FVC (−1.42; \( P = .003 \)) and \( \text{FEF}_{25-75}\%\) (−4.65; \( P < .001 \)).

Patients developed nonmucoid \( P \) aeruginosa at median age 1.0 year and mucoid \( P \) aeruginosa at age 13.0 years; the median transition time from mucoid to nonmucoid \( P \) aeruginosa was 10.9 years.

The longitudinal outcomes during \( P \) aeruginosa development were plotted as a function of age using locally weighted regressions with smoothing. Patients contributed measurements for no \( P \) aeruginosa prior to infection with nonmucoid \( P \) aeruginosa, then contributed nonmucoid \( P \) aeruginosa data after the first acquisition of nonmucoid \( P \) aeruginosa, and contributed mucoid \( P \) aeruginosa data after the first appearance of mucoid \( P \) aeruginosa. Thus, at each age, the 3 lines represent measurements taken from 3 different groups, ie, no \( P \) aeruginosa, nonmucoid \( P \) aeruginosa, and mucoid \( P \) aeruginosa.
Patients with nonmucoid *P. aeruginosa* and those with mucoid *P. aeruginosa* had nonsignificantly higher hazards of development of changes in disease status than patients without *P. aeruginosa* and patients without mucoid *P. aeruginosa*, respectively. The brief/low anti-*P. aeruginosa* antibiotic exposures in our treatment regime did not result in significant delays of lung disease progression.

**COMMENT**

Our longitudinal study defines the long-term epidemiology of *P. aeruginosa* infections in children with CF. To our knowledge, this is the first longitudinal study of *P. aeruginosa* stages in CF children from birth and the first to evaluate antibody and clinical progression serially in such developmental stages. Our findings on the interval from nonmucoid *P. aeruginosa* to mucoid *P. aeruginosa* and the chronological implications of anti-*P. aeruginosa* antibiotic therapy also provide new, clinically important information. During the longitudinal development of *P. aeruginosa* infection in CF patients from birth to age 16 years, 16 patients (29%) acquired nonmucoid *P. aeruginosa* in the first 6 months of life and 1 patient developed mucoid *P. aeruginosa*, the marker of poor survival in CF, at age 1.43 years. In those who reached age 16 years, 92% developed mucoid *P. aeruginosa*. These findings reveal that children with CF can acquire nonmucoid *P. aeruginosa* and mucoid *P. aeruginosa* very early in life and that the prevalence of mucoid *P. aeruginosa* increases markedly as patients age. Therefore, early prevention and detection of nonmucoid *P. aeruginosa* and mucoid *P. aeruginosa* are critical.

**Figure 4.** Respiratory Symptoms and Chest Radiographs by Age for Patients With No, Nonmucoid, and Mucoid *Pseudomonas aeruginosa*

Wisconsin chest radiograph scores range from 0 to 100, with 0 being best; Brasfield chest radiograph scores range from 0 to 25, with 25 being best.

**Figure 5.** Predicted Pulmonary Function by Age for Patients With No, Nonmucoid, and Mucoid *Pseudomonas aeruginosa*

Lower limits of normal were 82.4%, 82.6%, and 67.9%, respectively, for forced expiratory volume in 1 second, forced vital capacity, and forced expiratory flow between 25% and 75% of forced vital capacity.
The median acquisition ages for nonmucoid *P. aeruginosa* (1.0 year) and mucoid *P. aeruginosa* (13.0 years) suggest that nonmucoid *P. aeruginosa* occurs during infancy and early childhood, while mucoid *P. aeruginosa* generally begins later. Our longitudinal observation on the timing of mucoid *P. aeruginosa* agrees with the mean age (12.5 years) found in cross-sectional analyses by Ballmann et al. The short transition time we observed from no *P. aeruginosa* to nonmucoid *P. aeruginosa* (median, 1 year) and the relatively long transition time from nonmucoid *P. aeruginosa* to mucoid *P. aeruginosa* (median, 10.9 years) further suggest that patients with CF typically acquire nonmucoid *P. aeruginosa* soon after birth but that nonmucoid *P. aeruginosa* gradually develops into mucoid *P. aeruginosa*. Initial nonmucoid *P. aeruginosa* can possibly be eradicated by aggressive anti-*P. aeruginosa* treatment, but once mucoid *P. aeruginosa* is established, eradication seems impossible, and a life-threatening situation develops. In 1 study, 4 children with CF died before age 7 years after acquiring mucoid *P. aeruginosa*. Therefore, the relatively long interval from nonmucoid *P. aeruginosa* to mucoid *P. aeruginosa* provides a window of opportunity for suppression and possible eradication (by aggressive anti-*P. aeruginosa* treatment) of initial nonmucoid *P. aeruginosa* to prevent/postpone transition to mucoid *P. aeruginosa*. Our assessment of anti-*P. aeruginosa* antibiotics suggests that such therapy slightly extends the window by prolonging the interval from nonmucoid to mucoid *P. aeruginosa*. However, one must consider the ratio of risk (antibiotic resistance) to benefit of use of an antibiotic that may not eradicate the organism. We found that there was a trend for anti-*P. aeruginosa* antibiotic use to be associated with development of antibiotic resistance. Studies to compare the risk and benefit of anti-*P. aeruginosa* treatment are warranted.

Our data show that patients with no *P. aeruginosa* had relatively normal chest radiograph scores and pulmonary function, nonmucoid *P. aeruginosa* acquisition slightly worsened these outcomes, and transition from nonmucoid *P. aeruginosa* to mucoid *P. aeruginosa* led to significant deterioration in these outcomes. Similarly, Ballmann et al and Parad et al in cross-sectional analyses of older children and adults, found that mucoid *P. aeruginosa* infection was associated with decline in percentage of predicted FEV1. Our findings suggest that nonmucoid *P. aeruginosa* does not cause dramatically altered lung structure/function and that preventing transformation to mucoid *P. aeruginosa* might help preserve these children's lungs, but patients with mucoid *P. aeruginosa* have dramatically impaired lung structure and function. Furthermore, our examinations of other pathogens, such as *Staphylococcus aureus* and *Haemophilus influenzae*, did not show such a unique pattern or impact on lung disease. Therefore, longitudinal *P. aeruginosa* development leads to lung disease progression in children with CF, and mucoid *P. aeruginosa* plays a much greater role than nonmucoid *P. aeruginosa*.

Transitions both from no *P. aeruginosa* to nonmucoid *P. aeruginosa* and from nonmucoid *P. aeruginosa* to mucoid *P. aeruginosa* led to abrupt increases in antibody titers, suggesting that antibody responses closely correlate with *P. aeruginosa* stages. Antibody-mediated immunity of *P. aeruginosa* infection may have been specialized to defend against this virulent pathogen and memory enhanced to recurrent/severe *P. aeruginosa* infection. During nonmucoid *P. aeruginosa* infection, B lymphocytes apparently recognize the presence of initial *P. aeruginosa* antigens and up-regulate antibody production. Thus, patients with CF mount immediate but moderate humoral responses to nonmucoid *P. aeruginosa*. At the mucoid *P. aeruginosa* stage, however, antibody-mediated immunity may be memory enhanced against large amount of antigens and promptly creates much greater antibody production. Therefore, rising antibody titers are early signs of nonmucoid *P. aeruginosa* and mucoid *P. aeruginosa* stages; ie, relatively low titers indicate nonmucoid *P. aeruginosa* and high titers indicate mucoid *P. aeruginosa*. We also demonstrated that dramatic cough score and chest radiograph changes were associated with progressive lung infections that led to impaired pulmonary function. Therefore, cough scores and chest radiographs may also signal nonmucoid *P. aeruginosa* and, especially, mucoid *P. aeruginosa* stages and potentially guide therapeutic decisions.

Early prevention and detection of nonmucoid *P. aeruginosa* and mucoid *P. aeruginosa* and prompt suppression and possible eradication of nonmucoid *P. aeruginosa* are critical in CF therapy. At the time of diagnosis following symptoms, one third of CF patients in the United States are already colonized with *P. aeruginosa*. Neonates with CF have histologically normal lungs and no *P. aeruginosa* and can be identified through screening, which provides an opportunity for environmental precautions, infection control such as cohort isolation to eliminate *P. aeruginosa* cross-infection, antibiotic prophylaxis, and/or immunotherapy to be implemented for prevention at early ages. Screening and routine monitoring also allow early detection of *P. aeruginosa* in CF and can facilitate suppression and possible eradication of initial nonmucoid *P. aeruginosa*. Thus, pulmonary benefits can potentially be achieved, such as prolonging the no *P. aeruginosa* period and postponing or preventing the transition from nonmucoid to mucoid *P. aeruginosa*, thus potentially reducing lung disease progression in children with CF. Because of the limitation of available patients in our study, we used a sample size of 56; thus, our power for detecting small effect sizes may be limited. Future studies with larger populations would be helpful for detecting lung disease progression in response to *P. aeruginosa* development.

In conclusion, early prevention and detection of nonmucoid *P. aeruginosa* and mucoid *P. aeruginosa* is critical because of early acquisition and prevalence. There is a window of opportunity for suppression and possible

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eradication of initial nonmucoid P aeruginosa. Mucoid P aeruginosa plays a much greater role in CF lung disease progression than nonmucoid P aeruginosa. Antibody titers, cough scores, and chest radiographs are early signs of the stages of nonmucoid P aeruginosa and, especially, mucoid P aeruginosa.

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Study concept and design: Li, Kosorok, Farrell. Acquisition of data: Farrell, Laxova, West, Green, Collins, Rock, Splainger. Analysis and interpretation of data: Li, Kosorok, Farrell, Laxova, Green. Drafting of the manuscript: Li, Farrell, Laxova. Critical revision of the manuscript for important intellectual content: Li, Kosorok, Farrell, West, Green, Collins, Rock, Splainger. Statistical analysis: Li, Kosorok, Farrell, Collins. Obtained funding: Farrell. Administrative, technical, or material support: Kosorok, Farrell, Laxova, West, Green, Rock, Splainger. Financial Disclosures: None reported. Funding/Support: This work was supported by the National Institutes of Health grants DK 34108 and MO1 RR03186 and Cystic Fibrosis Foundation grant A001-00.

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