Environmental risk factors for the initial acquisition of *Pseudomonas aeruginosa* in young children with cystic fibrosis

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Environmental risk factors for the initial acquisition of *Pseudomonas aeruginosa* in young children with cystic fibrosis

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*Pseudomonas aeruginosa* (*Pa*) is the sentinel respiratory pathogen in cystic fibrosis (CF) patients. *Pa* respiratory tract infection typically occurs early in life; however, risk factors for initial *Pa* acquisition remain poorly understood. Although *Pa* in CF patients is generally acquired from the environment, to date, few studies have investigated specific environmental factors and their associations with initial *Pa* acquisition.

To address several gaps in the current literature regarding initial *Pa* acquisition, we conducted three retrospective studies to evaluate the potential role of environmental risk factors for initial *Pa* acquisition in young CF patients using data from the Cystic Fibrosis Foundation National Patient Registry, 2003-2009. First, we examined whether there was seasonal variation in rates of initial *Pa* acquisition and further evaluated potential seasonal variation within different climate zones. Second, we investigated whether there was residual spatial dependence of time to initial
Pa acquisition. Third, we evaluated the association of early life exposure to ambient air pollution (particulate matter <2.5 microns, PM_{2.5}) and time to Pa acquisition.

Results of the Poisson regression models evaluating seasonal acquisition of initial Pa demonstrated an increased rate of acquisition in summer (Incidence rate ratio [IRR]: 1.22; 95% confidence interval [CI]: 1.08, 1.39) and autumn (IRR: 1.34; 95% CI: 1.18, 1.52) seasons, compared to winter. Seasonal patterns were also found to differ by climate zones. In the spatial analysis of initial Pa acquisition, which employed a hierarchical Bayesian Weibull regression accommodating interval censored outcomes and using state of residence as the level of analysis, a moderately elevated spatial residual relative risk was found. An estimated 95% interval for the residual hazard ratio under one of the fitted models was 0.64 to 1.57 and the strongest positive association was observed in Southern states. Finally, an increase in PM_{2.5} exposure of 10 µg/cubic meter was associated with a 24% increase risk of Pa acquisition (95% confidence interval: 1-51%) in multivariate Weibull regression analysis.

The results of these studies suggest that environmental factors play a role in initial Pa acquisition in CF patients. Future studies to more fully elucidate the roles of these factors from both the individual and the pathogen perspectives could provide insight into the etiology of Pa acquisition in CF patients and inform prevention strategies.
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CHAPTER 1: Introduction

Cystic fibrosis

Cystic fibrosis (CF), an autosomal recessive disorder lacking a curative therapy, affects approximately 30,000 individuals in the United States and 70,000 persons worldwide (1). CF is the most common life shortening autosomal recessive disease in Caucasians and affects all races and ethnicities (1). CF is the result of a mutation in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a chloride channel expressed on the apical membrane of epithelial cells in the respiratory and gastrointestinal tracts. To date over 1,900 different mutations of the CFTR have been identified (2). Median survival for CF patients in the US is 37 years (1), with the overwhelming majority of deaths attributed to chronic progressive pulmonary disease (3).

CF lung disease begins with the depletion of the airway surface liquid, resulting in the inability of normal mucociliary clearance [3, 4]. This decreases the patient’s ability to normally clear aspirated pathogens leading to chronic endobronchial infection and a vigorous host inflammatory response. The lungs of CF patients become host to many pathogens, including Staphylococcus aureus, Burkholderia cepacia, Pseudomonas aeruginosa, Haemophilus influenzae, Stenotrophomonas maltophilia, and, increasingly, multiresistant Staphylococcus aureus, which are routinely identified from respiratory cultures of CF patients (1).

The sentinel respiratory pathogen in CF patients is Pseudomonas aeruginosa (Pa) (1), a hydrophilic, ubiquitous, opportunistic bacterium. Pa infection in CF patients can begin very early in life; the prevalence of Pa in respiratory cultures increases with age, from 10-30% at ages 0-5 years to 80% at ages ≥ 18 years (1). The incidence of Pa infection is also known to vary by age (4).
Initial \textit{Pa} isolates from CF patients generally exhibit the phenotypic characteristics resembling those of isolates from the environment. For example, they are usually non-mucoid (5), highly susceptible to antibiotics (5), and present at low density (6, 7). Over time, the distinct microenvironment in the CF airways allows selection of \textit{Pa} uniquely adapted for chronic, persistent infection. These organisms are more often mucoid, become increasingly antibiotic-resistant, are present at high density, and are virtually impossible to eradicate. Chronic \textit{Pa} infection is clearly associated with poorer clinical outcomes among CF patients (8-11).

The risk factors for and clinical impact of early \textit{Pa} infection are far less well understood, yet are of great importance to clinicians caring for young CF patients. The median age of initial acquisition of \textit{Pa} in children with CF is approximately two years of age (12), and delay in initial acquisition is associated with improved clinical outcomes (8). Early investigations of risk factors for early \textit{Pa} acquisition have focused largely on non-modifiable factors (gender, CF genotype, maternal education) or factors that may be due in part to undetected \textit{Pa} infection (respiratory admissions, height, weight). These studies have yielded conflicting results and have been limited by small sample sizes (N = 68 to 180) (13-15).

Due to the clinical importance and long term consequences of \textit{Pa} infection, recent investigations have focused on the setting of \textit{Pa} acquisition. Acquisition from the home environment has been shown to account for at most 20% of all cases (16), and other modes of initial acquisition including person-to-person transmission, nosocomial, and nebulizer equipment-related acquisition are rare. The fact that the diversity of initially acquired \textit{Pa} genotypes are similar to those collected from the environment (5, 17, 18), coupled with the environmental ubiquitousness of \textit{Pa} suggests that the majority of initial infection are due to environmental acquisition. Therefore, environmental characteristics should be investigated as
potential risk factors for initial acquisition of Pa in children with CF, including climatic factors, which have been shown to be associated with other Pa related infections (19, 20), and ambient air quality, given the documented adverse effects of particulate exposure on pulmonary exacerbation rates and lung function decline among CF patients (21).

**Overview of dissertation research**

The overall objective of this investigation was to evaluate geographic and environmental factors and their association with initial acquisition of Pa in the respiratory tract of children with CF enrolled in a large U.S. national patient registry. Three separate studies were conducted to evaluate different aspects of initial Pa acquisition. Specifically, we investigated whether: 1) incident Pa acquisition displayed seasonal variation; 2) state of residence affected risk of Pa acquisition, after adjusting for known risk factors for Pa acquisition and 3) early life exposure to particulate matter was associated with time to initial Pa acquisition in young children with CF.

In Chapter 2 (“Season is associated with Pseudomonas aeruginosa acquisition in young children with cystic fibrosis”), a retrospective study was conducted using the Cystic Fibrosis Foundation National Patient registry for the years 2003-2009. A total of 4,123 children were included in the study which compared seasonal incidence of initial Pa acquisition over a 7 year period; as well as, seasonal incidence within climate zones defined within the continental U.S. In this study, we also evaluated the seasonality of S. aureus acquisition, as a negative control.

In Chapter 3 (“Differential geographical risk of initial Pseudomonas aeruginosa acquisition in young U.S. children with cystic fibrosis”), we conducted a retrospective study of 3,601 children to examine whether state of residence affected risk of Pa acquisition among U.S. children with CF <6 years of age, using data from the Cystic Fibrosis Foundation National Patient Registry, 2003-2009. We employed a Bayesian hierarchical Weibull regression model
with interval censored outcomes. Spatial random effects, included at the state level and modeled using an intrinsic conditional autoregressive prior, allowed estimation of the residual spatial correlation. The regression portion of the model adjusted for demographic and disease characteristics potentially affecting Pa acquisition.

Finally, in Chapter 4 (“Fine particulate matter exposure and initial Pseudomonas aeruginosa acquisition in cystic fibrosis“), we conducted a retrospective analysis of the association of early life exposure to fine particulate matter <2.5 µm in aerodynamic diameter (PM$_{2.5}$) and initial Pa acquisition in children <6 years using Cystic Fibrosis Foundation National Patient Registry data from 2003-2009. Multivariable Weibull regression with interval censored outcomes was used to evaluate the association of time to initial Pa acquisition and PM$_{2.5}$ concentrations, which were estimated using inverse distance weighting methods or a nearest monitor approach based on a network of national air pollution monitors, for the year prior to patient’s birth.
References


CHAPTER 2: Season is associated with *Pseudomonas aeruginosa* acquisition in young children with cystic fibrosis

Abstract

*Pseudomonas aeruginosa*, the principal respiratory pathogen in cystic fibrosis (CF) patients, is ubiquitous in the environment. Initial *P. aeruginosa* isolates in CF patients are generally environmental in nature. However, little information regarding seasonality of *P. aeruginosa* acquisition is available. We conducted a retrospective study to evaluate the seasonality of initial *P. aeruginosa* acquisition in young children with CF in the USA using the Cystic Fibrosis Foundation National Patient Registry from 2003 to 2009. Additionally, we assessed whether seasonal acquisition varied by climate zone. A total of 4123 children met inclusion criteria and 45% (n = 1866) acquired *P. aeruginosa* during a mean 2.0 years (SD 0.2 years) of follow up. Compared with winter, increased *P. aeruginosa* acquisition was observed in summer (incidence rate ratio (IRR): 1.22; 95% CI: 1.07–1.40) and autumn (IRR: 1.34; 95% CI: 1.18–1.52), with lower acquisition observed in spring (IRR: 0.81; 95% CI: 0.70–0.94). Seasonal variations in *P. aeruginosa* acquisition rates in the temperate and continental climate zones were similar to those in the overall cohort. In contrast, no significant seasonal effect was observed in the dry climate zone. In a corresponding analysis, no seasonal difference was observed in the rate of acquisition of *Staphylococcus aureus*, another common CF respiratory pathogen. These results provide preliminary support that climatic factors may be associated with initial *P. aeruginosa* acquisition in CF patients. Investigation and identification of specific risk factors, as well as awareness of seasonal variation, could potentially inform clinical recommendations including increased awareness of infection control and prevention strategies.
**Introduction**

Infectious diseases often exhibit temporal and seasonal variation in incidence. These patterns depend upon the specific pathogen and host, the mode of transmission and the environmental characteristics (1, 2). *Pseudomonas aeruginosa (Pa)* is a ubiquitous environmental organism and the most significant pathogen in cystic fibrosis (CF) lung disease. The prevalence of *Pa* infection among CF patients increases with age, with positive respiratory tract cultures reported for approximately 20 to 30% of infants, 30 to 40% of children 2 to 10 years of age, 60% of adolescents and 80% of adults in the U.S. (3). Earlier initial *Pa* acquisition has been associated with increased morbidity and mortality (4-6). Patients with CF are initially infected with environmental isolates of *Pa* (7, 8). Regardless of treatment, patients commonly have a period of intermittent isolation of *Pa* from the respiratory tract before becoming chronically infected (9-11). Eventually there appears to be clonal selection of a specific *Pa* genotype that then undergoes genetic adaptation within the CF airway (8, 12).

While the seasonal acquisition of *Pa* in CF patients has been investigated (13, 14), these studies have been limited to small sample sizes in geographically homogenous areas. Insight into seasonal variations in the rate of *Pa* acquisition throughout the U.S. could elucidate climatic factors driving host-pathogen interactions and inform recommendations for monitoring respiratory cultures in young children with CF. The objectives of this study were therefore to: 1) evaluate the seasonal acquisition of *Pa* in young children with CF in the U.S. and 2) determine whether seasonal acquisition varied by climate zone.

**Methods**

We performed a retrospective study using Cystic Fibrosis Foundation (CFF) National Patient Registry data from January 1, 2003 to December 31, 2009. The Registry contains detailed
demographic and clinical information on CF patients treated at all U.S. CFF-accredited centers, who comprise >80% of diagnosed U.S. CF patients. Data are entered at each clinical encounter, including results of respiratory cultures. Since 2003, CFF clinical care guidelines have included obtaining quarterly cultures (i.e., four times per year) (15).

The study population included all patients in the Registry born in 2003 or later who had at least one respiratory culture recorded before two years of age and whose first recorded culture was negative for Pa. Therefore, only children up to six years of age were included in the study. The primary outcome was initial Pa acquisition (i.e., based on the first culture from which Pa was isolated). The primary exposure was season of Pa acquisition, defined as spring (March-May), summer (June-August), autumn (September-November), and winter (December-February).

Seasonality within the U.S. is geographically variable; therefore, we evaluated potential seasonal differences within climate zones using the revised Köppen-Geiger Climate classification (16). Briefly, in this classification scheme a total of five broad regions (Tropical, Dry/arid, Temperate, Continental, and Polar) are defined based on meteorological variables. We linked locations from individual-level zip code data from the CFF Registry, taken as the zip code in the year in which Pa was acquired or the year of the last clinical visit recorded in the Registry (for those remaining Pa free), to the Köppen-Geiger climate classification using ArcGIS version 10.1 (ESRI, Redlands, CA, USA). In the U.S. CF population, four climate zones are represented (tropical, dry/arid, temperate and continental) (Figure 1). Due to the minimal number of patients residing in the tropical zone (n=37), seasonal variability in this zone was not evaluated; these patients were retained in the overall seasonal analysis.
Descriptive statistics were produced. Student’s t-tests with unequal variances were used to compare continuous variables and chi-square tests compared categorical variables between acquisition status.

*Pa* incidence was calculated for each season over the study period. The denominator for these rates was the number of persons under observation and at risk for initial *Pa* acquisition during each season, while the numerator consisted of the cases occurring during that season. Due to the importance that culture frequency may play in observed incidence, the average number of cultures per person for each of the seasons was also evaluated.

To evaluate the association of season with *Pa* incidence, we used Poisson log-linear regression with quasi-likelihood and included the number of individuals at risk as the offset term. Winter season was the baseline for all comparisons, as we hypothesized that *Pa* incidence would be lowest during this season. Results are presented as incidence rate ratios (IRRs) and corresponding 95% confidence intervals (CIs). These analyses were then repeated for each climate zone separately.

We performed three sensitivity analyses. We evaluated whether the seasonal associations observed in the entire cohort were seen in individuals with different CFTR mutation classes (delta F508 homozygous, heterozygous and other), and age at date of culture (<2 or ≥2 years). We also limited the Poisson analyses by defining the persons at risk in each season as only those who had a culture recorded in the Registry during that season. Finally, hypothesizing that *Staphylococcus aureus* would not exhibit a seasonal pattern of acquisition, we replicated the above analyses with season of initial acquisition of *S. aureus* as the primary outcome of interest, among patients in the Registry born in 2003 or later who had at least one respiratory culture recorded before two years of age and whose first recorded culture was negative for *S. aureus*. 
The study was approved by the Cystic Fibrosis Foundation Registry Committee and the University of Washington IRB. All analyses were conducted using STATA 12.0 (StataCorp, College Station, TX).

Results

The study cohort consisted of 4,123 patients. Of these, 45% (n=1,866) acquired *Pa* while 55% (n=2,257) remained *Pa*-negative during a mean of 2.0 years (SD= 0.2 years) of observation (Figure 1). A total of 32,698 cultures were performed during follow-up in these individuals. The majority of cultures (90%) were oropharyngeal; however, other culture types included sputum (expectorated or induced) (8%) and bronchoscopic (1%). The demographic and clinical characteristics of the cohort are presented in Table 1. Children acquiring *Pa* tended to be diagnosed later, were less likely to be diagnosed by newborn screening and were more likely to be delta F508 homozygous compared to those that did not acquire *Pa*.

The overall *Pa* incidence rate during the study period was 16.5/1000 person-months (95% CI: 15.7-17.2) and the median age of *Pa* acquisition was 19 months. Figure 2 displays *Pa* incidence for each season during the study period. The highest rates of *Pa* acquisition were observed in the summer and autumn for each year, with peak *Pa* incidence occurring in autumn for all study years except 2008. The overall incidence rates in winter, spring, summer and autumn, respectively, were 15 (95% CI: 13-16), 12 (95% CI: 11-13), 18 (95% CI: 17-20) and 20/1000 person-months (95% CI: 18-22). Figure 2 also displays the average number of cultures in each season. It can be seen that the number of cultures per person per season steadily increased over the study period. At the beginning of the study period in 2003, the average number of cultures per patient per season was 0.4, rising to approximately one in 2009, likely
reflecting improved adherence to CFF guidelines regarding quarterly cultures. The average culture rate from 2003-2009 was similar for all seasons (0.9 cultures/person-season).

The results of Poisson regression models evaluating the association between Pa incidence and season of acquisition are presented in Table 2. Compared to winter, significantly higher Pa incidence was observed in summer (IRR: 1.22; 95% CI: 1.08-1.39) and autumn (IRR: 1.34; 95% CI: 1.18-1.52), while significantly lower Pa incidence was observed in spring (IRR: 0.81; 95% CI: 0.70-0.93).

The distribution of patients by climate zone was as follows: 289 (7%) in dry, 1,679 (43%) in temperate and 1,913 (49%) in continental climate zones. The overall Pa incidence in these regions was 14 (95% CI: 12-17), 17 (95% CI: 16-18) and 17/1000 person-months (95% CI: 16-18) for dry, temperate and continental climate zones, respectively. A minimal number of patients that changed zip code also changed climate zone (n=54) while under observation. The seasonal variation in Pa acquisition in the temperate and continental climate zones were similar to the overall cohort (Table 2). In contrast, a significant seasonal effect was not observed in the dry climate zone.

In sensitivity analyses, the seasonal pattern of Pa acquisition seen in the entire cohort was also seen in each CFTR mutation subgroup (delta F508 homozygous, heterozygous or other), among those with age at date of culture <2 or ≥2 years, and when the persons at risk in each season were defined as only those who had a culture recorded in the Registry during that season (data not presented).

Finally, we repeated the season of acquisition analyses for S. aureus. This study population differed slightly from that for the Pa analysis due to the larger number of individuals excluded due to first recorded culture positive for S. aureus (n=1,326). Thus, the study cohort
consisted of 3,196 patients. The demographic and clinical characteristics of this cohort were similar to those of the Pa acquisition cohort (Table 1). A total of 1,987 (62%) individuals acquired S. aureus during the study while 1209 (38%) remained S. aureus-negative. The overall S. aureus incidence was 26.7/1000 person-months (95% CI: 25.6-27.9) and the median age of S. aureus acquisition was 18 months. In contrast to the observed findings with Pa, there was little variation in S. aureus incidence by season (26/1000 person-months in spring [95% CI: 24-28]; 26/1000 person-months in summer [95% CI: 24-28]; 29/1000 person-months in autumn [95% CI: 26-31]; and 26/1000 person-months in winter [95% CI: 24-29]) (Figure 2). Poisson regression models showed no difference in S. aureus incidence by season for the cohort as a whole or in each climate zone (Table 2).

Discussion

We observed significant seasonal variation in the rate of initial Pa acquisition in a large U.S. cohort of young children with CF over a seven-year period. Compared to winter, incidence of initial Pa acquisition was significantly higher in summer and autumn and lower in spring. These seasonal differences in Pa acquisition rates were seen in the continental and temperate climate zones but not in the dry zone, though our power to detect seasonal variation in the dry zone was limited by the small number of individuals residing in this zone. We also evaluated seasonal patterns in S. aureus acquisition as a comparison with an outcome that we hypothesized would not have a seasonal difference and found no seasonal variation in S. aureus incidence rates in the overall cohort or in any climate zone. These results strongly suggest that climatic factors that vary with season influence initial Pa acquisition in CF patients.

To date, only two single centre studies have reported seasonal patterns of P. aeruginosa acquisition in children with CF, and to our knowledge no study has evaluated seasonal patterns
of *S. aureus* acquisition in children with CF. In a retrospective medical record review of 300 Danish CF patients from 1965 to 1990, Johansen and Høiby [14] also reported seasonal variation in acquisition, with higher acquisition of *P. aeruginosa* from October to March compared with April–September. Although no quantitative results were presented Farrell et al. [13] reported no differences in *P. aeruginosa* acquisition by season in 120 patients diagnosed through newborn screening in Wisconsin from 1985 to 1991, though their results may have been limited by small sample size.

Limited information is available regarding the seasonality of *Pa* infection in other settings. Perencevich and colleagues (17) investigated the seasonality of bacterial infections (including hospital acquired) among patients hospitalized at a single tertiary-care center from 1998-2005 and found increased cases of *Pa* and other gram negative bacteria in summer months compared to winter. Further, they found that for a 10% increase in temperature there was a 17% increase (95% CI: 4-31%) in *Pa* infections in warmer months (May to September) but not cooler months (October-April). Other studies investigated the temporality of *Pa* infections in otitis externa (18-20) and keratitis(21) and found increased rates of infection in summer and autumn seasons.

Our finding of seasonal variation in *Pa* acquisition rates suggest that meteorological conditions such as temperature, humidity or ambient air quality may be underlying risk factors for age at initial *Pa* infection (1). Such conditions could affect the density of *Pa* in the environment of children with CF or could play an indirect role by influencing the activities of children during each season. Several investigators have found an association between *Pa* prevalence and climatic factors, including warmer temperatures and higher humidity. Recently, Collaco and colleagues (22) investigated the role of environmental factors on CF lung disease in
three distinct cohorts. Though they did not focus specifically on initial acquisition of *Pa*, they showed that higher ambient temperature was associated with greater *Pa* prevalence in both the U.S and Australia. High humidity environments have been linked to *Pa* infections in non-CF infants (23), and rates of *Pa*-related keratitis have been shown to vary by Köppen climate zone in Australia (24). In addition, ambient air quality could be a risk factor for initial *Pa* acquisition, as higher concentrations of particular matter (PM$_{2.5}$) have been shown to adversely affect pulmonary exacerbation rates and lung function among CF patients (25).

Respiratory viruses have been demonstrated to be important causative agents of pulmonary exacerbations in CF patients (26), and virally-mediated damage to the respiratory epithelium could potentially predispose CF patients to bacterial airway infection (27). Interestingly, we did not observe higher rates of *Pa* acquisition in the winter, as might be expected if viral infections were the major risk factor for *Pa* infection.

Risk factors in the environments of CF patients for initial *Pa* infection remain poorly understood. With the recognition that initial *Pa* acquisition is environmental in nature, recent investigations have focused on home (28) and macro environmental factors (22) associated with *Pa* infection in CF patients. In one small study, acquisition from the home environment was shown to account for at most 20% of all cases (29). Other modes of acquisition including person-to-person transmission, nosocomial, and nebulizer equipment-related acquisition are rare. In a large prospective observational study, Rosenfeld, et al were unable to identify risk factors in the child’s environment (e.g., hot tub exposure, day care, breastfeeding) associated with age at initial *Pa* acquisition (28).

There are several limitations to the present investigation. First, the exact date of *Pa* acquisition was unknown for individuals; rather, the date of acquisition was considered to be the
date of positive culture. Second, in this observational study in young, generally pre-
expectorating children, approximately 90% of cultures were of upper respiratory tract samples
(oropharyngeal swabs) rather than lower respiratory tract samples (sputum or bronchoalveolar
lavage fluid). Oropharyngeal cultures are known to have moderate specificity and low
sensitivity for lower airway *Pa* (30). Thus, it is possible that seasonal patterns of lower airway
*Pa* incidence differ from those observed in this study, though the portal of entry for lower airway
infection is likely to be the upper airway. Third, we limited our analysis to initial *Pa* acquisition;
we did not evaluate the seasonal rates of chronic infection as we expected that the risk factors
would differ. Finally, we limited our cohort to children diagnosed with CF before the age of two
and with a maximum age of six (since the Registry only began collecting quarterly culture data
in 2003 and data were only available through 2009); this could affect the generalizability of our
results.

In summary, increased rates of initial *Pa* acquisition were observed in young CF patients
in summer and autumn compared to winter in this national study. Results of such analyses could
inform recommendations regarding prevention strategies and clinical care, including reinforcing
the importance of regular follow up visits with cultures, particularly during higher risk months
and identifying high risk populations that might benefit from more frequent monitoring. Similar
approaches could identify environmental risk factors for other CF pathogens such as
*Burkholderia cepacia*.
References


Table 1: Characteristics of the study cohorts, by *Pseudomonas aeruginosa* and *Staphylococcus aureus* acquisition status

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<td><em>Pa</em> acquired (n=1,866)</td>
<td><em>Pa</em> negative (n=2,257)</td>
<td><em>S. aureus</em> acquired (n=1,987)</td>
<td><em>S. aureus</em> negative (n=1,209)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47</td>
<td>52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48</td>
</tr>
<tr>
<td>Race/ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84</td>
<td>84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82</td>
</tr>
<tr>
<td>Black</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>11</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mean age (SD) at diagnosis, months</td>
<td>2.2 (4.0)</td>
<td>2.3 (4.2)</td>
<td>2.5 (4.3)</td>
<td>2.3 (4.4)</td>
</tr>
<tr>
<td>Identified by newborn screening (%)</td>
<td>35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50</td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53</td>
</tr>
<tr>
<td>CFTR mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔF508 homozygous</td>
<td>53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46</td>
</tr>
<tr>
<td>ΔF508 heterozygous</td>
<td>36</td>
<td>44</td>
<td>37</td>
<td>42</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>15</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

SD= standard deviation.

<sup>a</sup>P<0.05
Table 2: Seasonal incidence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* acquisition among children <6 years of age with cystic fibrosis in the United States from 2003-2009

<table>
<thead>
<tr>
<th>Climate Zone</th>
<th>Overall IRR (95% CI)</th>
<th>Dry IRR (95% CI)</th>
<th>Temperate IRR (95% CI)</th>
<th>Continental IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>REF</td>
<td>REF</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>Spring</td>
<td>0.81</td>
<td>0.69</td>
<td>0.83</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>(0.70-0.93)</td>
<td>(0.40-1.19)</td>
<td>(0.67-1.04)</td>
<td>(0.64-1.00)</td>
</tr>
<tr>
<td>Summer</td>
<td>1.22</td>
<td>0.91</td>
<td>1.14</td>
<td><strong>1.36</strong></td>
</tr>
<tr>
<td></td>
<td>(1.08-1.39)</td>
<td>(0.55-1.50)</td>
<td>(0.94-1.39)</td>
<td><strong>(1.12-1.64)</strong></td>
</tr>
<tr>
<td>Autumn</td>
<td>1.34</td>
<td>1.11</td>
<td><strong>1.21</strong></td>
<td><strong>1.55</strong></td>
</tr>
<tr>
<td></td>
<td>(1.18-1.52)</td>
<td>(0.69-1.78)</td>
<td>(1.01-1.47)</td>
<td>(1.28-1.88)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>REF</td>
<td>REF</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>Spring</td>
<td>0.99</td>
<td>0.96</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>(0.87-1.12)</td>
<td>(0.61-1.51)</td>
<td>(0.81-1.20)</td>
<td>(0.82-1.19)</td>
</tr>
<tr>
<td>Summer</td>
<td>0.98</td>
<td>0.82</td>
<td>0.96</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>(0.87-1.11)</td>
<td>(0.51-1.31)</td>
<td>(0.79-1.17)</td>
<td>(0.89-1.27)</td>
</tr>
<tr>
<td>Autumn</td>
<td>1.11</td>
<td>1.13</td>
<td>1.04</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>(0.99-1.25)</td>
<td>(0.74-1.74)</td>
<td>(0.86-1.25)</td>
<td>(0.97-1.38)</td>
</tr>
</tbody>
</table>

From Poisson regression models with the number of individuals at risk as the offset term. Winter season is the reference season. IRR= incidence rate ratio, CI= confidence intervals. Results in bold are statistically significant (p<0.05)
Figure 1: Climate zones of the continental United States based on the revised Koppen-Geiger climate classification (adapted from Peel, et al.(16))
Figure 2: Flow chart of study cohort for evaluation of acquisition of *Pseudomonas aeruginosa*.
Figure 3: Seasonal incidence rates for *Pseudomonas aeruginosa* acquisition among young children with cystic fibrosis in the United States from 2003-2009. Whiskers represent 95% confidence intervals.
Figure 4: Seasonal incidence rates for *Staphylococcus aureus* acquisition among young children with cystic fibrosis in the United States from 2003-2009. Whiskers represent 95% confidence intervals.
CHAPTER 3: Differential geographical risk of initial *Pseudomonas aeruginosa* acquisition in young U.S. children with cystic fibrosis

Abstract

*Pseudomonas aeruginosa* (*Pa*) is the sentinel respiratory pathogen in cystic fibrosis (CF) patients. We conducted a retrospective study to examine whether state of residence affected risk of *Pa* acquisition among U.S. children with CF <6 years of age, using data from the Cystic Fibrosis Foundation National Patient Registry, 2003-2009. The outcome was time to first isolation of *Pa* from a respiratory culture. We employed a Bayesian hierarchical Weibull regression model with interval censored outcomes. Spatial random effects, included at the state level and modeled using an intrinsic conditional autoregressive prior, allowed estimation of the residual spatial correlation. The regression portion of the model adjusted for demographic and disease characteristics potentially affecting *Pa* acquisition. A total of 3,608 children met inclusion criteria and were followed for an average of 2.1 (SD=1.6) years. *Pa* was cultured in 1,714 (48%) subjects. There was a moderately elevated spatial residual relative risk. An estimated 95% interval for the residual hazard ratio under one of the fitted models was 0.64 to 1.57; the strongest positive association was observed in Southern states. The fact that risk for *Pa* acquisition displayed spatial dependence suggests that regional factors, such as climate, may play an important role in *Pa* acquisition.

Introduction

Cystic fibrosis (CF) is the most common life shortening autosomal recessive disease in Caucasians and affects all races and ethnicities. CF is characterized by chronic lower airway bacterial infection and progressive lung function decline which is associated with high morbidity and premature death. *Pseudomonas aeruginosa* (*Pa*), a ubiquitous gram-negative bacterium, is
the most important respiratory pathogen in CF patients. Prevalence of *Pa* in respiratory cultures increases with age from ~30% at ages 0-5 years to 80% at ages ≥ 18 years (1). Initial clinical *Pa* isolates are present at low density (2, 3) and generally exhibit phenotypic characteristics of environmental isolates in that they are non-mucoid (4) and highly susceptible to antibiotics (4). Initially, *Pa* infection is typically intermittent and asymptomatic; however, over time, *Pa* adapts to the unique milieu of the CF lower airway. Infection eventually becomes chronic, characterized by biofilm formation, with mucoid (5, 6) and antibiotic resistant (7-10) strains. Earlier *Pa* acquisition is associated with worse clinical outcomes (7); consequently, early identification and aggressive eradication strategies are recommended to delay or prevent chronic infection (11-13).

The fact that the genotypes of initial *Pa* isolates are similar to those of environmental isolates (4, 14, 15), coupled with the ubiquitous nature of *Pa* in the environment, suggests that the majority of initial infections in CF patients are environmentally acquired. While patient-level risk factors for initial *Pa* infection have been evaluated (8, 16-21), few studies have investigated potential environmental risk factors (21, 22). Recently, warmer annual ambient temperature was shown to be associated with increased *Pa* prevalence and earlier age at *Pa* acquisition (22), suggesting that risk of *Pa* acquisition could potentially be geographically dependent. Further, initial *Pa* acquisition has been shown to vary between seasons; with differential seasonal patterns observed for climate zones (23).

If macroenvironmental factors are contributing to the risk of *Pa* acquisition in the CF population, then differential acquisition should be expected within a large geographic area in which variation in these factors is present. In this case, for patients residing in close proximity to each other, we would expect some degree of residual spatial correlation in risk of *Pa* acquisition.
after adjustment for patient-level characteristics known to be associated with Pa acquisition. To date, no study has investigated spatial patterns of initial Pa acquisition in any CF population; such an investigation could potentially inform our understanding of Pa acquisition. The purpose of this investigation was to evaluate the residual spatial correlation of initial Pa acquisition in young children with CF, after adjusting for known risk factors for Pa acquisition.

**Methods**

**Study population and design**

We conducted a retrospective study to describe the geographic distribution of and evaluate potential residual spatial dependence for initial Pa acquisition in young children with CF using the U.S. Cystic Fibrosis Foundation National Patient Registry data from 2003-2009. The Registry is a national database containing a wide range of demographic and encounter-based clinical data on approximately 80% of all U.S. CF patients. The study population consisted of all CF patients born after December 31, 2002 with a recorded household zip code in the lower contiguous 48 states. Thus, participants were ≤6 years of age at study completion. Patients were excluded if they did not have a respiratory culture recorded prior to two years of age. In order to evaluate incident Pa cases, patients in whom Pa was isolated from the first recorded culture were also excluded. Using zip code data from the Registry, state of residence was identified using ArcGIS 10.1 software (ESRI; Redlands, CA, USA). Individuals were assigned to the state in which they resided in either the year in which the first Pa positive culture was obtained or the last clinical visit recorded for those children that remained Pa free during observation.

The primary outcome of interest was time to initial Pa acquisition, defined as first Pa-positive culture recorded in the Registry. Respiratory cultures are generally obtained quarterly (i.e. four times per year) (11) in accordance with CFF clinical care guidelines. In these young
children, the source of respiratory samples is generally an oropharyngeal swab. This study was approved by the Institutional Review Board of the University of Washington and the Cystic Fibrosis Foundation Registry Committee.

**Statistical analysis**

Descriptive statistics were produced and compared between children who acquired *Pa* during follow-up and those that remained *Pa* free. A chi-squared test was used to test the differences in proportions by *Pa* acquisition status for categorical variables and t-tests with unequal variances were used for continuous variables. To describe the geographic distribution of the study population, the total number of children in each state and the national distribution of participants, by *Pa* acquisition status, were mapped. To further describe *Pa* acquisition, the proportion of patients acquiring *Pa* and median time to first positive *Pa* culture were also plotted at the state level.

The exact date of *Pa* acquisition was unknown for each individual; rather, acquisition was only known to occur within an interval time period between the date of first *Pa*-positive culture and the date of the last prior *Pa*-negative culture. Thus, Weibull regression with interval censored outcomes was used to evaluate time to *Pa* acquisition. Study participants entered the risk set upon their first recorded encounter in the Registry and were right censored if they remained *Pa* free at last encounter recorded prior to January 1, 2010. The Weibull (2 parameter) density function can be expressed as $T \sim \text{Weibull}(\alpha, \lambda)$, where $T$, time to event, is the random variable of interest and $\alpha$ and $\lambda$ are the shape and scale parameters, respectively. Considering interval censored outcomes, an individual’s outcome, $T_i$, can be expressed as a triple $(T_{i0}, T_{iL}, \delta_i)$, whereby $T_{i0}$ represents the time for the interval’s left hand endpoint, which for all children in the present study represents the first recorded negative *Pa* culture; for those
remaining $Pa$ free, $T_{le}$ represents the time of right censoring. Similarly, $T_{hi}$ represents the time corresponding to the right hand endpoint for the acquisition interval; for individuals acquiring $Pa$ represents the date of first positive culture and for individuals remaining $Pa$ free this represents an unobserved time. $\delta_i$ is a censoring indicator and defined by $Pa$ acquisition status.

All statistical models were adjusted for a priori factors potentially associated with $Pa$ acquisition, including sex, race (white vs non-white), ethnicity (Hispanic vs non-Hispanic), insurance status (any private insurance vs no private insurance), cystic fibrosis transmembrane conductance receptor (CFTR) functional class (minimal, residual, or unclassified) (18, 24, 25), age at diagnosis (months), diagnosis by newborn screening (yes/no), and culture frequency (defined as the number of cultures per number of days to $Pa$ acquisition or censoring). CFTR functional class was defined as follows: minimal function (“severe”): both mutations in Class I, II, or III (includes the most common CFTR mutation, deltaF508, a class II mutation); residual function (“mild”): one or both mutations in Class IV or V.

Spatial modeling

Previous applications of spatial regression models to time to event analyses with interval censored outcomes are limited (26, 27). If an outcome is spatially dependent, then ignoring this can (usually) lead to an underestimation of standard errors, resulting in overly narrow interval estimates and subsequent, incorrect inference. This (potential) spatial dependence therefore violates the independence assumption for observations required for generalized linear models (GLM). The more flexible class of generalized linear mixed models (GLMM) can accommodate such dependence. While frequentist methods are common in many GLMM applications, Bayesian methodology, utilizing Markov chain Monte Carlo (MCMC) has become the predominant choice for spatial applications. Recently, integrated nested Laplace approximation
INLA (28) has emerged as a computationally efficient alternative to MCMC for performing Bayesian analysis when distributions, including the Weibull (26), can be expressed as a latent Gaussian model.

To evaluate the potential spatial dependence of time to initial Pa acquisition, we used INLA for the previously described Weibull regression model with interval censored outcomes. Our approach was to evaluate Pa acquisition using the state as the areal unit of analysis. A finer level of analysis was not possible due to the sparseness of data at, for example, the county level. The so-called convolution random effects model (29) includes two random effects terms, an unstructured, independent random effect \( V_j \) assigned for each state \( j \) and a spatially structured random effect \( U_j \). We model the latter as an intrinsic conditional autoregression (ICAR) for each state. The ICAR model borrows information from “neighboring” areal units (defined in our study as states sharing a common boundary). Thus, \( V_j \) are independent whereas \( U_j \) depend on the random effects \( U \) of neighboring areas. The spatial Weibull hazard model can be expressed as

\[
\lambda(y; z, \alpha) = \alpha \gamma^{\alpha-1} \exp(\eta_i) = \mathbf{Z}_i^T \beta + V_{j(i)} + U_{j(i)},
\]

and \( j(i) \) denotes the state \( j \) within which individual \( i \) resides. Additionally, \( \mathbf{Z}_i^T \) is the design matrix; \( V_j \) is a Gaussian Markov random field where \( U_j \sim N(0, \sigma_u^2) \). The variances \( \sigma_u^2 \) and \( \sigma_v^2 \) represent unknown hyperparameters, with priors taken from inverse Gamma \((a, b)\) distributions \((IG)\). Spatial and independent variances are on different scales and are not directly comparable, \( \sigma_u^2 \) is a conditional variance, based on \( U_k, k \in \partial_j \), while \( \sigma_v^2 \) is a marginal variance. The proportion of variance explained by the structured spatial component was estimated by the empirical posterior marginal variance.
For the independent and spatially structured variances we took IG(1, 0.026) priors. These priors are such that a 95% interval for the residual hazard ratio (HR) is 0.5 to 2, and are based on the residual HRs following a log Student t distribution with 2 degrees of freedom (30). The model was completed by assigning improper flat priors for $\beta$.

The formulation of the spatial model allows us to simultaneously: 1) estimate the residual spatial dependence, and 2) investigate the impact of spatial correlation on regression coefficients for the predictors of time to $Pa$ acquisition. Results for regression coefficients are presented as HRs and the associated 95% confidence intervals (CI) for models with no random effects and as credible intervals for Bayesian random effects models. The random effect terms can be interpreted as the effect of state of residence on time to $Pa$ acquisition for each subject. At the state level, quartiles for the non-spatial and spatial random residual relative risks were plotted on a map for each state.

We evaluated the sensitivity to the prior specifications by using different IG distributions for the random effect variances. Five priors were chosen for the variances of the random effects terms. These were chosen to represent the 95% posterior probability as outlined by Wakefield (31), and included IG(0.5, 0.0164), IG(0.5, 0.006), and IG(0.5, 0.0014), exponential distributions with 1 degree of freedom and 95% ranges for the residual HRs of (0.1, 10), (0.25, 4), (0.5, 2), respectively. Additionally, the IG priors IG(1.0, 0.2864), IG(1.0, 0.104), were used, which correspond to alternative Student $t$ distributions with 2 degrees of freedom and 95% ranges of (0.1, 10) and (0.25,4), respectively. All $P$ values are 2-sided and a $P$ value of less than 0.05 was considered statistically significant. All analyses were performed using the R (Version 2.15.2) statistical environment (32).
Results

A total of 3,608 children were included in the final analysis, of which 48% (n= 1,714) acquired *Pa* during a mean observation period of 2.1 (SD 1.6) years. The mean number of recorded respiratory cultures per participant was 8.3 (SD 6.1). The median time to *Pa* acquisition was 470 days (75th percentile – 25th percentile: 195-634 days). Demographic characteristics of the study population by *Pa* acquisition status are presented in Table 3. Patients remaining *Pa* free were more likely to be male and to have been diagnosed by newborn screening, while those acquiring *Pa* were more likely to receive public insurance, be ΔF508 homozygous and have CFTR mutations with minimal function.

The nationwide distribution of the study population, by state, is presented in Figure 5 and by *Pa* acquisition status in Figure 6. The proportion of patients acquiring *Pa* over the study period varied by state and ranged from 21-71% (Figure 7). The distribution by state of median age at first *Pa* positive culture for children acquiring *Pa* is presented in Figure 8, by quartile of age (in days). Generally, delayed acquisition was observed in the Western states compared to the rest of the country.

Results from unadjusted and adjusted Weibull survival models for time to *Pa* acquisition, including multivariate regression models with and without spatial random effects, are presented in Table 4. Comparing the adjusted Weibull regression models it can be seen that including independent random effects for state did not materially change the observed associations for other potential risk factors. For example, the HR for the effect of gender was comparable for all models, indicating minimal confounding by location for these variables. Although newborn screening for CF was a state-by-state policy during this time period; associations with time to *Pa* acquisition varied little between models. Interpretation of covariate coefficients in the models
without random effects takes the usual form (i.e., the change in the hazard ratio associated with a unit change in the covariate of interest, with all other covariates held constant) while for the random effects models we add to this “for individuals in the same state (or in states with the same random effects)”.

In the multivariate regression models including only an independent random effect term for each state, the posterior estimated standard deviation of the residual HR was 1.26 (95% credible interval: 1.17, 1.38), indicating there is excess variability. An estimate of the 95% interval estimate for the residual HRs is therefore $\exp(\pm 1.96 \times 0.23) = (0.64, 1.57)$. Here the “residual” refers to excess risk associated with the independent random effects after accounting for other covariates. Similarly, inclusion of independent and spatial random effects indicated that there was a moderate, statistically significant independent random component associated with time to $Pa$ acquisition (HR=1.20; 95% credible interval: 1.12, 1.20). For this model, inclusion of the structured spatial random effect had minimal effect on the residual risk associated with the independent random effect term. The estimated proportion of the total residual variability of the log hazard ratio explained by the spatial component was approximately 45%, based on the empirical variance of $U_j$.

Figures 9 and 10 depict the mapping of the residual relative risk of the spatial and independent random effect terms. As is evident, the spatial random effect terms display a high level of spatial structure and smoothness, with the highest spatial dependence observed in the Southern states and the lowest levels observed in the Western and Northeastern states. As expected, the independent random effects terms do not display any clear geographic pattern.

Interpretation of results did not materially change when different priors were applied (Table 5). The variance attributable to the independent random effect (states) was decreased
when the spatial random effects were included in the models, as expected. The variances of the spatial random effects terms were slightly higher when an inverse Gamma prior based on the posterior residual HRs following an exponential distribution was placed on the random effects compared to when an inverse Gamma based on a log Student $t$ distribution was used.

**Discussion**

The identification of factors associated with initial $Pa$ acquisition is of great importance to the clinical management of CF patients. We have demonstrated that time to initial $Pa$ acquisition in young children with CF in the U.S. exhibits moderate residual spatial correlation, with the strongest positive associations observed in the southern U.S. These results suggest that macroenvironmental factors such as temperature, humidity or air pollution may be contributing to the risk of $Pa$ acquisition, particularly in the southern states.

Strengths of this investigation included a large, national study population and individual-level covariate data to adjust for previously reported factors associated with incident $Pa$ acquisition. Further, in contrast to previous investigations of $Pa$ acquisition, we accounted for the interval censored nature of the outcome. We are unaware of any other studies that have employed spatial methodologies for investigating $Pa$ acquisition; therefore, comparison to other studies is limited.

Effect estimates of patient-level covariates in the non-spatial Weibull regression models were similar to those obtained in a recent investigation by Rosenfeld and colleagues (21) reporting on a corresponding Registry study population and subjects enrolled in a large U.S. observational study (33, 34). Importantly, in the present investigation, the estimated effect sizes for the covariates were not significantly altered after inclusion of the random spatial and non-spatial components, indicating that confounding by location was most likely not present,
presumably because the covariates did not have a strong spatial pattern. In the present investigation, the mean age at initial Pa acquisition was 1.2 years, an earlier age than previously reported (19-22), likely due to our eligibility criteria which included having a first clinical encounter and initial negative Pa culture prior to two years of age, as well as limiting our cohort to children <6 years of age.

The complex interplay between genetic and environmental factors in explaining the heterogeneity of CF lung disease is an area of active investigation. In the CF Twins and Siblings Study, Collaco and colleagues (35) demonstrated that approximately half of the lung function variation in this cohort was explained by environmental/stochastic factors as opposed to genetic factors. In this same cohort, genetic factors contributed little to initial Pa acquisition (36). Thus, identification of specific environmental factors contributing to this variation is paramount to understanding the natural history of disease.

Spatial epidemiology provides a powerful set of statistical tools for understanding the etiology of infectious diseases. The roles of environmental factors in infectious disease outbreaks, spread and acquisition have been well described (37). For example, climatic conditions are important factors in the distribution of vectors and may also increase host susceptibility to infection. In the context of Pa acquisition in CF patients, initial acquisition is generally considered to be from the environment rather than transmitted between patients, presenting a somewhat unusual infectious disease paradigm. Identification of geographic variability in Pa acquisition in this study suggests that there may be factors such as climate associated with environmental Pa proliferation and/or affecting individual susceptibility or exposure to Pa. It may be important in future work to consider applications to predict Pa acquisition in both space and time.
Our analysis employed a Bayesian GLMM. The GLMM is a rich family of models characterized by inclusion of normal random effect term(s), the distinguishing feature from the more traditional GLM. The Bayesian framework is flexible, and in our applications included spatially structured random effects. A limitation of implementing Bayesian GLMMs, often performed with WinBUGS (38), is the computing time required. INLA provides a relatively fast computation method to accommodate such models, as well as accommodating a wide variety of other models including generalized additive models. We found that interval censored data, a common occurrence in other epidemiological settings, can be easily implemented using the INLA package in R using a Weibull distribution. Additionally, INLA also allows for Cox type GLMM survival models to be implemented; however, this requires specifying the baseline hazard to be piecewise constant (i.e., modeling the baseline hazard with knots).

There are several limitations to our study. First, in our study approximately 90% of respiratory cultures were from oropharyngeal swabs. The sensitivity of oropharyngeal cultures for detecting Pa in the lower respiratory tract is low (44%); therefore, results reported herein may more accurately reflect upper airway and not lower airway colonization. Second, the spatial analysis was conducted at the state level as our sample size did not allow analysis at a finer geographical level. An assumption of the modeling approach was a common state level effect so that within-state differences could have been masked. Finally, spatial correlation of health outcomes is often related to underlying differences in processes of care (affecting the endpoint) or unmeasured confounding variables such as socioeconomic status. We did adjust for CF care center and culture frequency; however, residual confounding could still remain after adjustment for insurance status.
In conclusion, we demonstrated that there are significant geographic differences in time to \textit{P}a\ acquisition in young children with CF. Results of this spatial analysis provide evidence to justify the conduct of future studies to identify potential climatic and environmental risk factors for \textit{P}a\ acquisition.
References


Table 3. Distribution of Demographic and Disease Characteristics Among Young Children with Cystic Fibrosis Who Acquired or Remained *Pseudomonas aeruginosa* Free, United States, 2003-2009

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Acquired Pa during follow-up (n=1,714)</th>
<th>Remained Pa negative (n=1,909)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>48</td>
<td>52</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>White (%)</td>
<td>92</td>
<td>92</td>
<td>0.61</td>
</tr>
<tr>
<td>Hispanic (%)</td>
<td>11</td>
<td>10</td>
<td>0.61</td>
</tr>
<tr>
<td>Any private insurance (%)</td>
<td>53</td>
<td>54</td>
<td>0.26</td>
</tr>
<tr>
<td>ΔF508 mutation category (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homozygous</td>
<td>53</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Heterozygous</td>
<td>36</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>CFTR mutation class (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimal</td>
<td>73</td>
<td>59</td>
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<tr>
<td>Residual</td>
<td>6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>22</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis, months (mean)</td>
<td>2.3</td>
<td>2.2</td>
<td>0.36</td>
</tr>
<tr>
<td>Diagnosis by newborn screening (%)</td>
<td>35</td>
<td>49</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator
Table 4: Results of Spatial and Nonspatial Weibull Regression Models Evaluating Time to *Pseudomonas aeruginosa* Acquisition in Young Children with Cystic Fibrosis, United States, 2003-2009

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Multivariate non-spatial model</th>
<th>Multivariate with area level effect only</th>
<th>Multivariate Spatial Model</th>
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<td>(0.82, 1.08)</td>
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Standard deviation of random effect(s) (median)

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<th></th>
<th>Independent ($\sigma_v$)</th>
<th>Spatial ($\sigma_u$)</th>
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<tr>
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<td>0.23</td>
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<td>(0.11, 0.30)</td>
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<td>(0.09, 0.36)</td>
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Abbreviations: HR, hazard ratio; CI=confidence interval, unless otherwise specified; CFTR, cystic fibrosis transmembrane conductance receptor

*CI reflects credible interval (2.5 and 97.5%) based on Inverse Gamma (1.0, 0.026) prior(s)*
Table 5: Estimated Posterior Median and Corresponding 95% Credible Interval for the Standard Deviation of the Independent and Spatial Random Effects for Hierarchical Multivariable Models With and Without Spatial Random Effects

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<th>Prior Specification for the Inverse Variance of Random Effect Terms</th>
<th>InvGa (0.5, 0.016)</th>
<th>InvGa (0.5, 0.006)</th>
<th>InvGa (0.5, 0.0014)</th>
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<td>Independent effect ($\sigma_p$)</td>
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<td>0.23</td>
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<td>(0.16, 0.33)</td>
<td>(0.15, 0.33)</td>
<td>(0.21, 0.38)</td>
<td>(0.18, 0.34)</td>
</tr>
<tr>
<td>Adjusted spatial model with independent and spatial random effects</td>
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<td></td>
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<tr>
<td>Independent effect ($\sigma_p$)</td>
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<td>0.19</td>
<td>0.20</td>
<td>0.25</td>
<td>0.21</td>
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<tr>
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<td>(0.11, 0.31)</td>
<td>(0.11, 0.32)</td>
<td>(0.13, 0.35)</td>
<td>(0.19, 0.36)</td>
<td>(0.14, 0.31)</td>
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<tr>
<td>Spatial random effects ($\sigma_u$)</td>
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<td>0.15</td>
<td>0.13</td>
<td>0.31</td>
<td>0.24</td>
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<tr>
<td></td>
<td>(0.08, 0.38)</td>
<td>(0.05, 0.36)</td>
<td>(0.04, 0.29)</td>
<td>(0.20, 0.47)</td>
<td>(0.15, 0.24)</td>
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</tbody>
</table>

Abbreviations: InvGa, inverse Gamma distribution
Figure 5: Geographic distribution of young children with cystic fibrosis included in the study cohort.
Figure 6: Geographic distribution of the study cohort by *Pseudomonas aeruginosa* acquisition status during follow-up.

NOTE- Each symbol represents one subject.
Figure 7: Proportion of study population acquiring *Pseudomonas aeruginosa*, by state.
Figure 8: Median days to $Pa$ acquisition among children acquiring *Pseudomonas aeruginosa*, by state.
Figure 9: Residual relative risk for spatial (smoothed) random effects, from adjusted hierarchical spatial Weibull regression model for time to initial *Pseudomonas aeruginosa* acquisition in young children with cystic fibrosis, 2003-2009.
Figure 10: Residual relative risk for independent random effects, from adjusted hierarchical spatial Weibull regression model for time to initial *Pseudomonas aeruginosa* acquisition in young children with cystic fibrosis, 2003-2009.
CHAPTER 4: Fine particulate matter exposure and initial *Pseudomonas aeruginosa* acquisition in cystic fibrosis

Abstract

Rationale: Increasing evidence suggests that exposure to ambient air pollution contributes to the severity of cystic fibrosis (CF) respiratory disease in school age children and adults, but this association has not been evaluated in young children.

Objectives: To investigate the association of early life exposure to fine particulate matter <2.5 µm in aerodynamic diameter (PM$_{2.5}$) and initial *Pseudomonas aeruginosa* acquisition in young children with CF.

Methods: Retrospective analysis of initial *Pseudomonas* acquisition in children <6 years using Cystic Fibrosis Foundation National Patient Registry data from 2003-2009. PM$_{2.5}$ exposure was estimated using inverse distance weighting methods based on air pollution monitors within 30 miles of place of residence, for the year prior to patient's birth. Multivariable Weibull regression with interval censored outcomes evaluated the association of time to initial *Pseudomonas* acquisition and PM$_{2.5}$ concentrations.

Measurements and Main Results: A total of 3,575 children met inclusion criteria and 48% (n=1,711) acquired *Pseudomonas* at a median age of 15 months (25th-75th percentiles: 9-25 months). An increase in PM$_{2.5}$ exposure of 10 µg/cubic meter was associated with a 24% increase risk of *Pseudomonas* acquisition (95% confidence interval: 1-51%). Interpretation of the results was similar when PM2.5 was analyzed as quartiles of exposure. For example, compared to the lowest quartile, a non-significant increased risk of *Pa* acquisition was observed for the second (hazard ratio [HR]=1.15; 95% CI: 0.98, 1.35) and third (HR= 1.15; 95% CI: 0.99,
1.34) quartiles with a significant increased risk for the highest quartile (HR=1.21; 95% CI: 1.04, 1.42) of IDW PM2.5 within 30 miles.

Conclusions: Increased PM$_{2.5}$ exposure was associated with earlier *Pseudomonas* acquisition in young children with CF.

**Introduction**

Young children are particularly susceptible to the deleterious effects of outdoor ambient air pollution (1, 2). Children engage in more outdoor activities (3) and have higher ventilation rates (4), leading to a proportionately greater exposure to air pollution than adults. The correspondingly smaller lung surface area (5), immature immune system and inability to successfully detoxify pollutants (6) may contribute to early life vulnerability to respiratory insults. Prenatal and early life (7) are potentially critical windows during which air pollution (8) can result in impaired lung development (9, 10) and increased risk of respiratory diseases (11-16).

Cystic fibrosis (CF) is characterized by chronic endobronchial infection and inflammation with progressive declining pulmonary function, often resulting in premature mortality. Given the significant heterogeneity within the CF genotype-phenotype relationship, non-genetic influences are estimated to account for approximately half of the variation in CF lung disease (17). Increasing evidence suggests a link between air pollution exposure and morbidity in school age children and adults with CF, including lung function decline and pulmonary exacerbations (18-21). Even in infants and young children with CF, the pulmonary microenvironment is already characterized by inflammation (22, 23) and bronchiectasis (24). Because decline in lung function (25) occurs at this early age, which may be irreversible (26), identification of risk factors contributing to early life morbidity is clinically important.
Pseudomonas aeruginosa (Pa), a ubiquitous gram-negative bacterium, is the sentinel respiratory pathogen in CF and infection frequently occurs in the first 6 years of life. Earlier age of acquisition is associated with increased morbidity and mortality (27); yet, risk factors for initial Pa acquisition remain poorly understood (28). Air pollution can induce mitochondrial oxidative stress in the airway epithelium, resulting in increased inflammation and an inability to successfully clear pathogens (29). However, little information is available regarding CF respiratory microbiology and air pollution exposure (30). Therefore we conducted a large national retrospective study to evaluate the association of ambient outdoor fine particulate matter (< 2.5 µm in aerodynamic diameter) (PM$_{2.5}$) exposure and initial Pa acquisition. We hypothesized that increasing PM$_{2.5}$ levels would be associated with increased risk of Pa acquisition.

**Methods**

**Study Population and Design**

We conducted a retrospective analysis of outdoor PM$_{2.5}$ exposure and initial Pa acquisition in young children with CF using the Cystic Fibrosis Foundation Patient Registry data from 2003-2009. This national database includes demographic and encounter-based data for all CF patients treated at CFF accredited centers throughout the US. Current clinical recommendations include quarterly clinical visits (ie four times per year) with respiratory microbiology testing (31). This study was approved by the Institutional Review Board of the University of Washington and the Cystic Fibrosis Foundation Registry Committee.

The study population consisted of children with an established diagnosis of CF born after December 31, 2002 (i.e., ≤6 years of age at study completion), with a valid household residential zip code in the lower contiguous 48 states. Patients were excluded if they did not have a
respiratory culture recorded prior to two years of age; in order to evaluate incident \textit{Pa} cases, patients in whom \textit{Pa} was isolated from the first recorded culture were also excluded.

\textbf{Pa Acquisition}

The primary outcome was time to initial \textit{Pa} acquisition, defined as first \textit{Pa} positive respiratory culture while on study. The majority of respiratory cultures (90\%) in this young age range were collected from oropharyngeal samples. In this study, the exact date of \textit{Pa} acquisition was unknown, rather acquisition was only known to occur within a time interval, defined as the date of positive \textit{Pa} culture (right hand endpoint of acquisition interval) and date of previous negative respiratory culture (left hand endpoint of acquisition interval).

\textbf{Particulate Matter Exposure}

Annual summaries of PM$_{2.5}$ data were obtained from the Air Quality System, a national network of federally and locally-funded air monitoring stations throughout the U.S. Over the study period, monitoring stations came on-line and went off-line; therefore, monitors contributing data varied between years.

The primary exposure of interest was the mean yearly concentration (\(\mu g/m^3\)) of PM$_{2.5}$ in the year prior to birth for each child. Year prior to birth was chosen because it captured exposure prior to disease onset and accounted for the secular decline in U.S. air pollution levels. To assign individual-level PM$_{2.5}$ exposure from monitoring stations, latitude and longitude coordinates were geocoded using ArcGIS 10.1 (ESRI, Redlands, CA). A similar procedure was employed for each child using the residential zip code centroid taken from the zip code at first entry into the Registry. Due to the various methods available for PM$_{2.5}$ assignment, we evaluated several PM$_{2.5}$ exposure metrics. PM$_{2.5}$ exposure was classified using inverse distance weighting (IDW) procedures, assuming an exponential decay model (based on distance), for monitors located
within 10, 30, and 50 miles of the zip code centroid for each individual’s place of residence. Therefore, individuals not residing within these predefined radii were not assigned an exposure. The primary PM$_{2.5}$ exposure metric was based on the IDW within 30 miles, in accordance with the previously published CF literature (20, 30); however, complete results for each metric are provided. A nearest monitor approach for PM$_{2.5}$ classification was also performed, which was accomplished by identifying the nearest air monitoring station for each child. Sensitivity of exposure was further evaluated by quartile of PM$_{2.5}$ for each of the previously described metrics.

Statistical Analysis

Demographic and disease characteristics were compared between children who acquired *Pa* during follow-up and those that remained *Pa* free. Student’s t tests with unequal variances compared continuous variables and chi-square tests compared categorical variables between these groups.

Multivariable Weibull regression with interval censored outcomes was used to evaluate the association of PM$_{2.5}$ and time to initial *Pa* acquisition. For this analysis, children entered the risk sets upon date of first clinical encounter recorded in the Registry. Subjects were right censored if they remained *Pa* free at last clinical encounter recorded prior to January 1, 2010; censoring date for these individuals was taken as the date of last clinical visit. For children who acquired *Pa* during follow up, interval censored outcomes were constructed as previously described.

All multivariable models were adjusted a priori for the following potential confounding variables: sex, race (White vs non-White), ethnicity (Hispanic vs non-Hispanic), insurance status (any private vs. no private), cystic fibrosis transmembrane receptor (CFTR) functional class (class I and II vs. class III, IV and V) (32-34), diagnosis by newborn screening (yes/no), year of
first clinical encounter, urban/rural status using the Rural Urban Commuting Area coding Version 2.0 (35) (defined as urban, large rural, small rural, or isolated), and culture frequency (defined as number of cultures performed per number of days under observation prior to censoring or *Pa* acquisition). Results are presented as hazard ratios (HR) with corresponding 95% confidence intervals (CI) for a 10μg/m³ increase in PM$_{2.5}$ exposure. In sensitivity analyses evaluating the categorical quartiles of PM$_{2.5}$ exposure, the referent group for analyses was the lowest quartile of exposure.

**Results**

A total of 3,575 children were included in the cohort of which 1,711 (48%) acquired *Pa* during a mean follow-up of 1.8 years (SD=1.6 years). The majority of patients resided in urban areas (75%), followed by large rural (13%), small rural (6%), and isolated (6%) areas. The demographic and disease characteristics of the study population by *Pa* acquisition status are presented in Table 6. In general, children who acquired *Pa* during follow up were more likely to be female, non-white, and have a CFTR mutation class of I and II (minimal CFTR function; includes the most common mutation, ΔF508). The median age of first positive *Pa* culture was 15 months (25th- 75th percentiles: 9- 25 months).

The median distance to the nearest air monitoring station was 11.1 miles (25th- 75th percentiles 5.2- 24.7 miles). For the study population, 91% (n=3,252) resided within 50 miles of a monitoring station, while 80% (n=2,861) and 46% (n=1,628) resided within 30 and 10 miles, respectively. The overall mean PM$_{2.5}$ exposure was 12.1μg/m³ based on the nearest monitor approach and was 12.3μg/m³ when based on each of the three IDW metrics (Table 7). PM$_{2.5}$ exposure was slightly higher for those who acquired *Pa* during follow-up compared to those that remained *Pa* free for all four exposure metrics.
Table 8 summarizes the results of the multivariable regression models for the association of PM$_{2.5}$ and time to $Pa$ acquisition. After adjustment for potential confounding variables, significant associations were observed for all PM$_{2.5}$ measurements, except for the IDW values within 10 miles (HR= 0.96; 95% CI: 0.76, 1.22); which included only 46% of the entire study cohort. For example, a 10μg/m$^3$ increase in PM$_{2.5}$ for individuals residing within 30 miles of an air pollution monitor was associated with a 23% increased risk of $Pa$ acquisition (95% CI: 1-49%) and a similar 26% increased risk (95% CI: 6-50%) was observed in the nearest monitor analysis.

Interpretation of the results was similar when the PM$_{2.5}$ metrics were analyzed as quartiles. The highest quartile of exposure was generally associated with the largest effect size (Figure 11). For example, compared to the lowest quartile of IDW PM$_{2.5}$ within 30 miles, a non-significant increased risk of $Pa$ acquisition was observed for the second (HR: 1.15; 95% CI: 0.98, 1.35) and third (HR: 1.15; 95% CI: 0.99, 1.34) quartiles with a significant (HR: 1.21; 95% CI: 1.04, 1.42) increased risk for the fourth quartile.

**Discussion**

In this large, national retrospective investigation we found a significant association between early life exposure to PM$_{2.5}$ and time to $Pa$ acquisition in young children with CF: each 10μg/m$^3$ increase in PM$_{2.5}$ was associated with an increased risk of $Pa$ acquisition (HR: 1.24; 95% CI: 1.01, 1.51). In addition, these associations were consistent across exposure metrics and the strongest associations were observed for the highest PM$_{2.5}$ concentrations. There are several strengths of the current investigation. First, this was a large national cohort enrolled over a seven year period. Also, evaluating exposure data in the year prior to birth ensured that the exposure
was evaluated prior to *Pa* acquisition and allowed for the known declining secular trends in PM\textsubscript{2.5} levels occurring in the U.S.

Although children with CF are potentially highly susceptible to adverse effects from air pollution exposure, there has been minimal prior evaluation of its association with *Pa* acquisition. In the only comparable study, Collaco and colleagues (30), evaluated the effects of environmental factors, including PM\textsubscript{2.5} exposure, on lung function and *Pa* prevalence. Employing the average PM\textsubscript{2.5} exposure value for the year 2006 of the nearest monitor within 30 miles among 677 individuals, the authors reported an increased odds ratio of *Pa* prevalence (OR= 1.12; 95% CI: 1.01, 1.23) for a 10μg/m\textsuperscript{3} increase in PM\textsubscript{2.5}. There are several important differences in study design between the present study and the one performed by Collaco et al. First, the median age at *Pa* acquisition between these study populations differed significantly and is most likely due to differing inclusion criteria. Second, while both studies used similar air pollution monitor approaches, we evaluated an inverse distance weighted summary measure of PM\textsubscript{2.5} in the year prior to birth. Given the potential for exposure misclassification using the zip code centroid, IDW is an alternative approach to a nearest monitor analysis to account for the misclassification of residential location and spatial variability in PM\textsubscript{2.5} concentrations.

The identification of risk factors associated with initial *Pa* acquisition is of great importance to the clinical management of CF patients and currently, remain relatively uncharacterized. More severe CFTR mutations are known risk factors; however, no personal exposures have been previously described (28). Phenotypically, initial *Pa* isolates closely resemble environmental strains, in that they are antibiotic susceptible and non-mucoid (36-38) and initial *Pa* acquisition is considered to be environmental in nature. Thus, identification of potential environmental factors is paramount to understanding the natural history of disease.
The results of this study suggest a likely moderate contribution of ambient air pollution to the risk of initial \( Pa \) acquisition. This hypothesis is consistent within the CF setting; yet the mechanisms by which exposure to particulate matter may increase risk should be further explored. Such mechanisms may include potentiation of the underlying chronic airway inflammation characteristic of CF or increased exposure to oxygen free radicals. Future studies may consider the differential effects of prenatal and postnatal exposure and whether other bacterial and viral infections in this population are also associated with increased levels of PM\(_{2.5}\) and other air pollutants.

Published studies on the role of air pollution and outcomes in CF patients have generally focused on lung function decline and pulmonary exacerbations using either longitudinal or cross-sectional designs. In an analysis of the CFF Registry data from 2000, Goss, et al. (20), using 2000 yearly summaries for ambient air pollution, found that an increase of 10\( \mu \)g/m\(^3\) PM\(_{2.5}\) was associated with increased odds of having two or pulmonary exacerbations in 2000 (OR= 1.21; 95% CI: 1.07, 1.33) and a mean FEV1 decline of 155ml (95% CI: 115, 194). Goeminne and colleagues (19), conducting a case-crossover study of 215 patients treated at a CF referral clinic in Belgium over a 12-year period found increased odds of pulmonary exacerbation on days with higher PM\(_{10}\) exposure (OR=1.11; 95% CI: 1.05, 1.17). Jassal et al (21) found no association between pulmonary exacerbation frequency and proximity to roadways among 145 children treated at the Children’s Hospital Los Angeles. In a smaller study of 103 patients treated at an outpatient clinic in Sao Paulo, Brazil, Farhat and colleagues (18) reported an association between ozone and pulmonary exacerbations; however, this association was only reported for a two day lag in exposure, with no other associations observed for other pollutants (PM\(_{10}\), NO\(_2\), SO\(_2\), and CO).
There are several limitations to our study. First, air pollution exposure was limited to outdoor ambient PM$_{2.5}$; other air pollutants and indoor air quality were not considered in this study. Similarly, due to limitations associated with the use of residential zip code data, proximity to major roadways, an exposure of increasing importance when studying potential effects from air pollution exposure, could not be evaluated in this study. Second, the use of the zip code centroid could result in ecological bias if the areal level exposure estimates do not accurately reflect individual exposure; however, PM$_{2.5}$ concentrations have been shown to correlate well between monitors (39).

Third, regional differences in practice patterns may account for some variation in initial $Pa$ acquisition. To account for the potential treatment effects by CF Center, we also performed analyses in which individuals were clustered by CF treatment Center; however, results did not materially change. Fourth, oropharyngeal cultures were the primary source of outcome ascertainment. Rosenfeld at al. (40) evaluated the sensitivity and specificity of oropharyngeal cultures compared to bronchoalveolar lavage, the gold standard for $Pa$ identification, and found a moderate sensitivity (44%) and high specificity (95%) for oropharyngeal cultures in detecting lower airway colonization. Thus, results obtained herein may more accurately reflect upper airway colonization.

Finally, the observed moderate effect sizes may indicate a true association of PM$_{2.5}$ exposure and $Pa$ acquisition or may be potentially confounded by other unknown factors, such as exposure to additional unmeasured pollutants or to environmental tobacco smoke (ETS). Unfortunately, data on ETS exposure in the Registry was limited. During follow-up, approximately 55% of participants did not have ETS data recorded in the Registry, precluding any meaningful analysis. Additionally, socioeconomic status (SES) may be a potential
confounding factor. In our analysis, we adjusted for insurance status (any Private Insurance vs no private insurance) as an SES indicator.

In conclusion, this study provides evidence supporting an association between increased early life PM$_{2.5}$ exposure and initial $Pa$ acquisition in young children with CF. These results are in accordance with previous studies that have found associations between ambient air pollution levels and other CF outcomes (pulmonary exacerbations and lung function decline). Future studies that correlate early childhood lung inflammation, $Pa$ acquisition and air pollution levels could potentially inform our understanding of the natural history of $Pa$ infection. Given the lack of strategies available to patients to prevent $Pa$ acquisition, additional studies that can elucidate risk factors for initial $Pa$ acquisition are needed.
References


31. Borowitz D, Robinson KA, Rosenfeld M, Davis SD, Sabadosa KA, Spear SL, Michel SH, Parad RB, White TB, Farrell PM, Marshall BC, Accurso FJ. Cystic fibrosis foundation


37. Foght JM, Westlake DW, Johnson WM, Ridgway HF. Environmental gasoline-utilizing isolates and clinical isolates of Pseudomonas aeruginosa are taxonomically indistinguishable by chemotaxonomic and molecular techniques. Microbiology 1996;142 ( Pt 9):2333-40.


Table 6: Distribution of demographic and disease characteristics among young children with cystic fibrosis from 2003-2009, by *Pseudomonas aeruginosa* acquisition status

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<th><strong>Pseudomonas aeruginosa acquisition status</strong></th>
<th>Remained <em>Pa</em> free (n=1,864)</th>
<th>Acquired <em>Pa</em> during follow up (n=1,711)</th>
<th>P value</th>
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<td>Male (%)</td>
<td>966 (52)</td>
<td>821 (48)</td>
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</tr>
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<td>925 (54)</td>
<td>0.270</td>
</tr>
<tr>
<td>RUCA classification (%)</td>
<td></td>
<td></td>
<td>0.322</td>
</tr>
<tr>
<td>Urban</td>
<td>1,410 (76)</td>
<td>1,250 (73)</td>
<td></td>
</tr>
<tr>
<td>Large rural</td>
<td>228 (12)</td>
<td>238 (14)</td>
<td></td>
</tr>
<tr>
<td>Small rural</td>
<td>114 (6)</td>
<td>117 (7)</td>
<td></td>
</tr>
<tr>
<td>Isolated</td>
<td>105 (6)</td>
<td>99 (6)</td>
<td></td>
</tr>
<tr>
<td>Diagnosed by newborn screening (%)</td>
<td>950 (51)</td>
<td>615 (36)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Mean age at diagnosis, months (SD)</td>
<td>2.2 (4.0)</td>
<td>2.2 (4.0)</td>
<td>0.769</td>
</tr>
<tr>
<td>ΔF508 Mutation category (%)</td>
<td></td>
<td></td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Homozygous</td>
<td>772 (41)</td>
<td>904 (53)</td>
<td></td>
</tr>
<tr>
<td>Heterozygous</td>
<td>825 (44)</td>
<td>625 (37)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>267 (14)</td>
<td>182 (11)</td>
<td></td>
</tr>
<tr>
<td>CFTR Mutation class (%)</td>
<td></td>
<td></td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Minimal</td>
<td>1,152 (62)</td>
<td>1,291 (75)</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>235 (13)</td>
<td>98 (6)</td>
<td></td>
</tr>
<tr>
<td>Unclassified</td>
<td>463 (25)</td>
<td>310 (18)</td>
<td></td>
</tr>
</tbody>
</table>

Definition of abbreviations: *Pa*= *Pseudomonas aeruginosa*; RUCA= rural urban commuting area; SD= standard deviation; CFTR= cystic fibrosis transmembrane receptor

†P<0.05
Table 7: Distribution of PM$_{2.5}$ ($\mu$g/m$^3$) concentrations in year prior to birth for young children with cystic fibrosis from 2003-2009, overall and by Pseudomonas aeruginosa acquisition status

<table>
<thead>
<tr>
<th>Exposure Metric</th>
<th>Overall</th>
<th>Remained Pa free</th>
<th>Acquired Pa during follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (25$^{th}$-75$^{th}$ percentiles)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Nearest monitor</td>
<td>12.1 (2.8)</td>
<td>12.0 (10.2-13.9)</td>
<td>11.9 (2.8)</td>
</tr>
<tr>
<td>IDW 50 miles</td>
<td>12.3 (2.6)</td>
<td>12.4 (10.5-14.0)</td>
<td>12.1 (2.6)</td>
</tr>
<tr>
<td>IDW 30 miles</td>
<td>12.3 (2.7)</td>
<td>12.4 (10.4-14.0)</td>
<td>12.1 (2.7)</td>
</tr>
<tr>
<td>IDW 10 miles</td>
<td>12.3 (2.8)</td>
<td>12.3 (10.3-14.0)</td>
<td>12.2 (2.8)</td>
</tr>
</tbody>
</table>

Definition of abbreviations: Pa= Pseudomonas aeruginosa; SD=standard deviation; IDW=inverse distance weighted
Table 8: Multivariable Weibull regression with interval censored outcomes for the association of PM$_{2.5}$ and time to *Pseudomonas aeruginosa* acquisition for young children with cystic fibrosis, 2003-2009

<table>
<thead>
<tr>
<th>Exposure metric</th>
<th>Study Population (n)</th>
<th>Multivariable Analysis</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nearest Monitor</td>
<td>3,575</td>
<td>1.26</td>
<td>(1.06, 1.50)</td>
</tr>
<tr>
<td>IDW: 10 miles</td>
<td>1,628</td>
<td>0.99</td>
<td>(0.77, 1.27)</td>
</tr>
<tr>
<td>IDW: 30 miles</td>
<td>2,861</td>
<td>1.24</td>
<td>(1.01, 1.51)</td>
</tr>
<tr>
<td>IDW: 50 miles</td>
<td>3,252</td>
<td>1.22</td>
<td>(1.01, 1.49)</td>
</tr>
</tbody>
</table>

Definition of abbreviations: HR=hazard ratio; CI=confidence interval; IDW=inverse distance weighted

All regression models are adjusted for: sex, race, ethnicity, insurance status, rural urban commuting area, diagnosis by newborn screening, age at diagnosis, and CFTR mutation class.

Results of regression models reflect the hazard ratio associated with a 10 μg/m$^3$ increase in PM$_{2.5}$ exposure.
Figure 11: Adjusted hazard ratios (95% confidence intervals) for time to *Pseudomonas aeruginosa* acquisition in relation to categoric quartiles of PM$_{2.5}$ exposure in young children with cystic fibrosis from 2003-2009. The referent group for each analysis was the lowest quartile of PM$_{2.5}$ exposure. All models were adjusted for sex, race, ethnicity, insurance status, rural urban commuting area, diagnosis by newborn screening, age at diagnosis, and CFTR mutation class.

![Graph showing adjusted hazard ratios](image)

**Definition of abbreviations**: IDW=inverse distance weighted
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