Serology as a diagnostic tool for predicting initial 
Pseudomonas aeruginosa acquisition in children
with cystic fibrosis☆☆

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Received 24 April 2014; received in revised form 14 June 2014; accepted 16 June 2014
Available online 11 July 2014

Abstract

Rationale: Pseudomonas aeruginosa (Pa) serology could potentially be a useful adjunct to respiratory culture methods for the detection of initial or early Pa infection in patients with cystic fibrosis (CF).
Objective: To evaluate the utility of Pa serology to predict Pa isolation from respiratory (generally oropharyngeal) cultures in the subsequent 6 or 12 months among young children with CF from whom Pa had never been previously cultured. Pa serology was also evaluated in a group of healthy controls.
Methods: Children ≤ 12 years of age without prior isolation of Pa from respiratory cultures participating in the Early Pseudomonal Infection Control EPIC Observational Study (EPIC OBS) had annual serum samples for measurement of antibodies against alkaline protease, elastase and exotoxin A using a commercial kit; controls had a single serum sample. Logistic regression with generalized estimating equations was used to characterize associations between log10 serum antibody titers and first isolation of Pa from a respiratory culture within the subsequent 6 or 12 months, with adjustment for sex and age. Receiver operating characteristic curves were used to optimize antibody titer cutpoints by age group. The diagnostic properties of each antibody were estimated using these optimized cutpoints.
Results: Pa serology was evaluated in 582 children with CF (2084 serum samples) and 94 healthy controls. There was substantial overlap between serum antibody titers among controls, CF patients who did not acquire Pa (N = 261) and CF patients who did acquire Pa (N = 321). The maximum positive predictive value for first Pa positive culture within the ensuing 6 months was 76.2% and maximum negative predictive value was 72.1% for any antigen or combination of antigens; values were similar for 12 months.

Abbreviations: CF, cystic fibrosis; Pa, Pseudomonas aeruginosa; OP, oropharyngeal; BAL, bronchoalveolar lavage; EPIC OBS, Early Pseudomonas Infection Control Observational Study; CFFNPR, CFF National Patient Registry; GEE, generalized estimating equations; ROC, receiver operating characteristic; AUC, area under the curve; CFTR, cystic fibrosis transmembrane regulator; PPV, positive predictive value; NPV, negative predictive value.

☆ Financial support: Cystic Fibrosis Foundation grants OBSERV04K0 and EPIC0K0 to M. Rosenfeld, Seattle Children’s Hospital, Seattle, WA; Nemours Children’s Clinic Research Program, Jacksonville, FL.

☆☆ Prior presentation: Portions of this manuscript were presented as an oral and poster presentation at the 2012 North American Cystic Fibrosis Conference.

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1 Posthumous.
Conclusions: Pa serology does not appear useful for predicting first Pa positive oropharyngeal culture among young CF patients. © 2014 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

Keywords: Cystic fibrosis; Pseudomonas; Serology; ROC curves

1. Introduction

Progressive obstructive lung disease due to chronic airway infection and inflammation is the leading cause of morbidity and mortality in cystic fibrosis (CF), with the bacterial pathogen Pseudomonas aeruginosa (Pa) playing a prominent role [1]. Initial Pa acquisition generally occurs in early childhood [2,3], but is often transient [2] and may be limited to the upper airways [4]. In contrast, approximately 80% of CF adults has chronic airway Pa infection [5], which is associated with more rapid lung function decline [3,6], increased morbidity [3,7] and decreased survival [8,9]. Today, children with CF are routinely treated with antipseudomonal antibiotics upon first Pa isolation in an attempt to eradicate the organism [10,11]. Eradication of early Pa infection has a roughly 80% success rate [11–13] and has been shown to reduce the prevalence of chronic Pa infection in CF cohorts compared to historical controls [14–16].

The accurate detection of early Pa infection is problematic, as young children and those with mild lung disease typically do not expectorate sputum. Surveillance respiratory cultures in these patients are typically performed on oropharyngeal (OP) swabs. While OP cultures are known to have imperfect diagnostic accuracy compared to lower airway cultures [4,17], they are nonetheless standard of care in the U.S. and many other countries, and are widely used to guide treatment decisions [18], define stages of Pa infection [19] and predict clinical outcomes [20,21].

Serum titers of antibodies against Pa antigens have been shown to be elevated in chronic Pa infection [22,23] and to distinguish intermittent from chronic Pa colonization [24,25]. However, as most chronically infected patients expectorate sputum, the clinical utility of serology in this context is limited. In contrast, it has been suggested that Pa serology could prove a useful adjunct to respiratory culture methods for the detection of initial or early Pa infection [24,26,27], as serology has the potential advantages of being more accurate than upper airway cultures and less resource-intensive and invasive than BAL. The diagnostic accuracy of Pa serology relative to concurrent respiratory cultures remains controversial [17,28–30]. Importantly, several studies have demonstrated that positive Pa serology may precede initial isolation of Pa from both upper [2,3,25,29,31] and lower [2] airway cultures. If positive Pa serology could predict subsequent isolation of Pa from respiratory cultures, eradication therapy could potentially be initiated at an earlier stage to improve outcomes; this has been advocated [24] but not yet investigated.

The Early Pseudomonas Infection Control Observational Study (EPIC OBS) is a U.S. national prospective study to evaluate the risk factors for and clinical outcomes associated with isolation of Pa from respiratory cultures in a large cohort of children with CF who were Pa-culture negative at enrollment [32]. The objective of the current analysis was to evaluate the utility of Pa serology to predict subsequent Pa isolation from respiratory (generally OP) culture among young children with CF from whom Pa had never been previously cultured. We hypothesized that Pa serology would have acceptable diagnostic accuracy in predicting first isolation of Pa from respiratory cultures within the ensuing 6 or 12 months. As part of the current analysis, we also examined Pa serology and OP cultures in a cohort of children without CF undergoing elective surgical procedures at a single institution to assess the levels of anti-Pa antibodies in the unaffected population. Portions of this work have previously been published in abstract form.

2. Methods

2.1. Study participants and samples

The design of the EPIC OBS has been reported elsewhere [33,34]. Children with an established diagnosis of CF ≤ 12 years of age were enrolled at 59 accredited U.S. CF care centers between 2004 and 2006. Annual serum samples were collected for serology and banking, and the results of clinical respiratory cultures were recorded in the CFF National Patient Registry (CFFNPR). Eligibility criteria for participation in the current analysis were 1) no prior isolation of Pa from respiratory cultures since CF diagnosis, confirmed with CFFNPR data, 2) no loss to follow up or isolation of Pa from a respiratory culture in the first 120 days after enrollment [36] (as these individuals may have had Pa infection prior to enrollment), and 3) at least one serum sample collected. Written informed consent was obtained from the family of each participant and the study was approved by the Institutional Review Board at each participating site. Serum samples collected through 2009 and data collected through 2010 were included in the current analysis.

2.2. Non-CF controls

Otherwise healthy children ≤ 18 years of age undergoing a clinically indicated procedure that required sedation or anesthesia at Seattle Children’s Hospital, Seattle, WA, USA between September 2008 and February 2010 were recruited. Exclusion criteria included: (1) presence of indwelling catheters or devices (including myringotomy tubes) at enrollment or within the past year; (2) oral or IV antibiotic treatment within the past month; (3) presence of congenital or acquired immunosuppression; (4) history of cancer; (5) currently undergoing an otolaryngology or dental procedure; (6) immediate family member with CF; (7) blood transfusion within the past year. A serum sample for serology and an OP swab for culture were collected from each participant. The study was approved by the Seattle Children’s Hospital’s...
Hospital IRB and informed consent was obtained from all parents/guardians, as well as assent from participants as applicable. The respiratory culture results from this cohort have been previously published [34].

2.3. *Pseudomonas* serology

Serum samples were analyzed for titers of antibodies to the *Pa* antigens alkaline protease, exotoxin A, and elastase by Mediagnost® (Reutlingen, Germany) using their commercially available IgG enzyme immunoassay system [24,28,37]. Assays were batched and performed in duplicate. The lower limits of quantification were 0.41, 0.15 and 0.35 titer/ml for alkaline protease, exotoxin A and elastase, respectively (equal to 10 times the standard deviation of the blank). Intraassay variances were 4.39, 5.3 and 11.5% of the coefficient of variation (CV) and interassay variances were 5.7, 7.7 and 8.0% of the CV for alkaline protease, elastase and exotoxin A, respectively. The linearity of sample dilution has been proven for dilution range of 1:500 to 1:24,000 by the manufacturer. An independent evaluation of the test system has been conducted [24,28].

2.4. Statistical analyses

Logistic regression models were used to characterize associations between log_{10} serum antibody titers and first isolation of *Pa* from a respiratory culture within the subsequent 6 or 12 months, with generalized estimating equations (GEE) methods used to account for repeated observations per patient, and adjustment for sex, age (1–≤3 years, >3–≤6 years, >6 years) and time (days) between serum sample and respiratory culture. Receiver operating characteristic (ROC) curves were used to optimize antibody titer cutoffs to maximize the sensitivity and specificity of each antibody and by age group (0–<6 years, ≥6 years), using the area under the curve (AUC). The diagnostic properties of each serologic assay, including sensitivity, specificity, positive predictive value, and negative predictive value were estimated based on these optimized cutoffs. Similar analyses were performed for concurrent respiratory cultures (defined as collection within 3 weeks of the serum collection date) for comparison purposes. Analyses were performed using R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria) and SAS, version 9.2 (SAS Institute Inc., Cary, NC).

3. Results

A total of 1797 children with CF were enrolled in EPIC OBS between 2004 and 2006, of which 687 had no isolation of *Pa* from respiratory cultures prior to or during the first 120 days after enrollment. Of these 687 children, 582 had at least one serum sample collected during the observation period and therefore comprise the cohort for the current analyses. These 582 children contributed 2084 serum samples; 261 children (44.8%) had a first *Pa*-positive respiratory culture during the observation period and 321 remained *Pa*-negative. At baseline (Table 1), participants who subsequently acquired *Pa* were similar to those who remained *Pa* negative with respect to age and sex, but the proportion of children with a high risk *CFTR* genotype was higher in participants who acquired *Pa* (85.0% versus 65.4%, P < .0001), as was the prevalence of pancreatic insufficiency (91.2% versus 77.3%, P < .0001). A serum sample for serology and a simultaneous OP swab for culture were collected from 94 non-CF controls, who had a mean (SD) age of 8.5 (5.4) years; 54 were male. *Pa* was isolated from only 1 of the 94 OP cultures.

### Table 1

Baseline characteristics of children with CF.

<table>
<thead>
<tr>
<th>Age, years mean (SD)</th>
<th>Did not acquire <em>Pa</em> (N = 321)</th>
<th>Acquired <em>Pa</em> (N = 261)</th>
<th>Total (N = 582)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 years, n (%)</td>
<td>114 (35.5%)</td>
<td>106 (40.6%)</td>
<td>220 (37.8%)</td>
</tr>
<tr>
<td>&gt;3–6 years, n (%)</td>
<td>84 (26.2%)</td>
<td>66 (25.3%)</td>
<td>150 (25.8%)</td>
</tr>
<tr>
<td>&gt;6–12 years, n (%)</td>
<td>123 (38.3%)</td>
<td>89 (34.1%)</td>
<td>212 (36.4%)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>157 (48.9%)</td>
<td>127 (48.7%)</td>
<td>284 (48.8%)</td>
</tr>
<tr>
<td>CFTR functional class a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>210 (65.4%)</td>
<td>210 (85%)</td>
<td>420 (72.2%)</td>
</tr>
<tr>
<td>Low risk</td>
<td>53 (16.5%)</td>
<td>14 (5.4%)</td>
<td>67 (11.5%)</td>
</tr>
<tr>
<td>Not classified</td>
<td>51 (15.9%)</td>
<td>26 (10%)</td>
<td>77 (13.2%)</td>
</tr>
<tr>
<td>Missing</td>
<td>7 (2.2%)</td>
<td>11 (4.2%)</td>
<td>18 (3.1%)</td>
</tr>
<tr>
<td>Pancreatic sufficient b</td>
<td>73 (22.7%)</td>
<td>23 (8.8%)</td>
<td>96 (16.5%)</td>
</tr>
</tbody>
</table>

a High risk: both alleles with mutations in functional class 1, 2 or 3; low risk: at least one allele with a class 4 or 5 mutation; not classified: functional class not able to be determined based on mutations detected [46].

b Defined by pancreatic enzyme replacement therapy use in the CFF Registry.

Among the 261 children with CF who acquired *Pa* during the observation period, 169 (64.8%) had serum samples collected within 6 months prior to *Pa* isolation and all had a serum sample within 12 months prior to *Pa* isolation. A total of 68 (26.1%) had serum samples collected concurrently with *Pa* isolation, with a median elapsed time between the recorded date of serum collection and respiratory sample collection of 0.0 day (mean = 0.94 day, SD = 3.86 days) for this group.

Alkaline protease titers tended to be higher in CF patients than in controls, though these titers did not distinguish between CF patients who remained *Pa* negative and those who acquired *Pa* (Fig. 1). Exotoxin A titers tended to be highest in CF patients who remained *Pa* negative and all had a serum sample collected within 6 months prior to *Pa* isolation. A total of 29% and 16% had antibody titers ≥100 against alkaline protease, exotoxin A and elastase, respectively. Among 94 serum samples randomly selected from 94 age-matched CF children, 33%, 31% and 53%, respectively, had antibody titers below the limit of quantitation and 27%, 28%, and 21% had antibody titers ≥100 against alkaline protease, exotoxin A and elastase, respectively.

In logistic regression models, serum exotoxin A and elastase antibody titers, but *not* alkaline protease antibody titers, were
shown. Sample sizes are shown above the bars. Medians, 25th and 75th percentiles and ranges are depicted.

Fig. 1. Serum antibody titers in non-CF control children, children with CF that remained *Pa* negative for the subsequent year and children with CF that had initial isolation of *Pa* from a respiratory culture within the subsequent year. Box plots of serum titers of antibodies against *Pa* alkaline protease (left panel), exotoxin A (center panel), and elastase (right panel). Non-CF control children (hashed bars), children with CF that did not have *Pa* cultured in the subsequent year after serum collection (clear bars), and children with CF that had *Pa* cultured within the subsequent 12 months, 6 months, and concurrently with serum collection (gray bars) are shown. Sample sizes are shown above the bars. Medians, 25th and 75th percentiles and ranges are depicted.

statistically significant predictors of concurrent *Pa* isolation (P < .001), or *Pa* isolation within 6 months (P < .001) or 12 months (P ≤ .001) of serum collection among participants with CF, with odds ratios generally <2 (Fig. 2). Of the three *Pa* antigens, exotoxin A consistently had the largest effect size. Odds ratios decreased as the interval of time between serum collection and subsequent *Pa* isolation increased (Fig. 2).

ROC curve analyses and area under the curve (AUC) calculations for children with CF confirmed the relatively low diagnostic accuracy of antibody titers against all three antigens for predicting *Pa* isolation from respiratory cultures, with the highest AUC being 0.690 (a value of 0.5 indicates no association between the diagnostic test and the outcome). The rank order of association between antibody titers and probability of subsequent *Pa* isolation was exotoxin A > elastase > alkaline protease, and the AUCs were highest for concurrent isolation of *Pa* (Fig. 3).

Based on the ROC curves in Fig. 3, antibody titer cutpoints of ≥100 were used to test the accuracy of seropositivity compared to concurrent and subsequent *Pa* isolation (Table 2).

In addition to studying each antigen independently, the predictive ability of combinations of seropositivity for the three antigens was analyzed. The ability of positive serology to predict subsequent *Pa* isolation (positive predictive value, PPV) was generally poor, ranging from 34.1% (95% CI 26.0, 43.3) to 61.1% (95% CI 43.5, 76.4). The greatest PPV was achieved when all three antibody titers were positive, and the PPV increased with the length of time allowed after serum collection for detection of a positive culture. The latter finding is at least in part due to the fact that predictive values are influenced by prevalence (whereas sensitivity and specificity are not). The prevalence of isolation of *Pa* within 6 months and 12 months of serum sample collection were 34% and 45%, respectively. Among children with CF who had serum antibody titers ≥100 for all three *Pa* antigens, 56.2% went on to have *Pa* cultured within 6 months and 61.1% within a year.

The ability of negative serology to predict no subsequent isolation of *Pa* (negative predictive value, NPV) was better than the PPV, ranging from 54.4% (95% CI 49.6, 59.1) to 70.7% (95% CI 65.6, 75.4). The NPV of exotoxin A antibody titers <100 was greater than that of other antibodies, either alone or in combination with exotoxin A. The NPV for serology decreased with increasing time from serum collection to culture, again, in part due to fact the predictive values are influenced by prevalence. For instance, only 70.7% of children with exotoxin A antibody titers <100 remained *Pa* negative for up to 6 months, and only 59.7% was *Pa* negative for up to a year after serum collection (Table 2).

Fig. 2. Odds ratios for the effect of log10 serum antibody titers on risk of concurrent *Pa* isolation and *Pa* isolation within 6 months and 12 months after serum collection in children with CF. A logistic regression model was adjusted for patient sex and age (1–≤3 years, >3–≤6 years, >6 years). Odds ratios (log scale) are shown for risk of concurrent *Pa* isolation (clear circles), *Pa* isolation within 6 months after serum collection (gray circles), and within 12 months (black circles) after serum collection. Bars represent 95% confidence intervals for odds ratios.
3.2. Effect of age-specific cutpoints

Serum antibody titers were generally lower in children with CF less than 6 years of age than in older children (Fig. 4—online supplement). Thus, we constructed ROC curves CF by age group (1 to <6 and ≥6 years old) (Fig. 4—online supplement). Using these data, optimal antibody titer cutpoints for each age group were determined using serum sample level modeling (Table 3—online supplement). Age-specific titer cutpoints improved the diagnostic properties of serologic assays for prediction of Pa isolation (Table 4—online supplement). For example, the best PPV for Pa isolation in the subsequent 6 months when titers for all three antibodies were ≥100 improved from 56.2% (CI 37.9, 73.2) (Table 2) to 76.2% (CI 52.5, 90.9) using age-specific cutpoints (Table 4—online supplement). Cutpoints optimized by age produced a much smaller improvement in the NPV for Pa isolation in the subsequent 6 months, going from 67.8% (CI 61.8, 73.3) to 72.1% (CI 66.5, 77.1) for presence of ≥1 antibody above the cutpoint (Table 2 and online supplement Table 4). The highest positive and negative predictive values for concurrent Pa isolation and isolation in the subsequent 6 or 12 months using both sets of antibody titer cutpoints are shown in Fig. 5 (online supplement).

4. Discussion

Based on reports by our group [2] and others [31] that positive serology may precede first isolation of Pa from respiratory cultures, we aimed to assess whether Pa serology could accurately predict subsequent isolation of Pa from oropharyngeal cultures in

Table 2

Seropositivity (titers ≥100) as a predictor of concurrent or subsequent Pa isolation among children with CF.

<table>
<thead>
<tr>
<th>Pa isolation concurrent with serum collection</th>
<th>Individual Pa antigens with antibody titers ≥100</th>
<th>Number of Pa antigens with antibody titers ≥100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaline protease</td>
<td>Exotoxin A</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>35.3 (24.4, 47.9)</td>
<td>54.4 (41.9, 66.4)</td>
</tr>
<tr>
<td>Specificity</td>
<td>75.0 (69.8, 79.6)</td>
<td>75.9 (70.8, 80.5)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>23.3 (15.8, 32.9)</td>
<td>32.7 (24.4, 42.3)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>84.3 (79.4, 88.3)</td>
<td>88.6 (84.0, 92.0)</td>
</tr>
<tr>
<td>Pa isolation within 6 months after serum collection</td>
<td>24.9 (18.7, 32.2)</td>
<td>39.1 (31.7, 46.9)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>74.8 (69.6, 79.4)</td>
<td>77.6 (72.5, 81.9)</td>
</tr>
<tr>
<td>Specificity</td>
<td>55.7 (50.0, 61.2)</td>
<td>83.9 (79.2, 87.6)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>23.4 (15.8, 32.9)</td>
<td>32.7 (24.4, 42.3)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>84.3 (79.4, 88.3)</td>
<td>88.6 (84.0, 92.0)</td>
</tr>
<tr>
<td>Pa isolation within 12 months after serum collection</td>
<td>23.0 (18.1, 28.7)</td>
<td>35.6 (29.9, 41.8)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>74.8 (69.6, 79.4)</td>
<td>77.6 (72.5, 81.9)</td>
</tr>
<tr>
<td>Specificity</td>
<td>55.7 (50.0, 61.2)</td>
<td>83.9 (79.2, 87.6)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>42.6 (34.4, 51.2)</td>
<td>56.4 (48.4, 64.0)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>54.4 (49.6, 59.1)</td>
<td>59.7 (54.8, 60.6)</td>
</tr>
</tbody>
</table>

a Proportion of patients with antibody titers ≥100 among those who had subsequent Pa isolation with confidence intervals.

b Proportion of patients with antibody titers <100 among those who remained culture negative with confidence intervals.

c Proportion of patients who had subsequent Pa isolation among those with antibody titers ≥100 with confidence intervals.

d Proportion of patients who remained culture negative among those with antibody titers <100 with confidence intervals.
a cohort of Pa negative patients. If so, routine surveillance of Pa serology might potentially allow for earlier detection of Pa infection and therefore earlier initiation of Pa eradication therapy, potentially improving outcomes. Unfortunately, we found that positive serology was not able to accurately predict isolation of Pa in the ensuing 6 or 12 months. In our cohort, even using antibody titer cutoffs optimized for age, the positive predictive value of Pa serology for predicting isolation of Pa from a respiratory culture in the ensuing 6 months was 76.2% and the negative predictive value was 72.1%. In other words, about 1 in 4 children predicted based on positive serology to become Pa positive in the next 6 months did not actually acquire Pa during that time period, while 1 in 4 children predicted by negative serology to remain Pa negative in the next 6 months in fact became Pa positive during the same period.

Isolation of new Pa from oropharyngeal cultures in children with CF is not a clinical endpoint, but rather an admittedly imperfect diagnostic test [4], and it seems clear that some of the inability of serology to predict subsequent Pa isolation in our study is a result of the limitations of our chosen “gold standard” rather than of Pa serology. Molecular methods for detection of Pa infection are gaining traction [24–27,31] and had we utilized such techniques, our outcomes may have been different. However, because treatment decisions in the U.S. are still generally made today based on Pa isolation from conventional upper airway cultures [18], we felt it was reasonable to test the ability of serology to act as a diagnostic surrogate for Pa isolation from these cultures. The presence of substantial titers of antibodies to Pa in children without CF (Fig. 1) and the relatively poor predictive power of optimized serology in children with CF (Fig. 5—online supplement) suggest that serology as we have studied it would not be a particularly useful diagnostic surrogate for upper airway culture. In addition, Douglas et al. [17] found very similar results to ours when comparing Pa serology to simultaneous lower airway cultures obtained by BAL. Whether or not intervening with anti-pseudomonal antibiotics when positive serology is first detected might improve outcomes is an entirely different question from the one we evaluated, one requiring a randomized, controlled trial in which clinical endpoints rather than surrogate outcomes such as oropharyngeal cultures are evaluated.

Though ours is the first study to evaluate the utility of Pa serology in predicting subsequent first isolation of Pa from respiratory cultures among Pa negative patients, several prospective studies have evaluated the diagnostic utility of serology compared to concurrent respiratory cultures in early Pa infection, with conflicting results. Douglas et al. [17], reported similarly low positive and negative predictive values of serology in young CF patients undergoing BAL. Others have reported higher diagnostic accuracy [29,30,38]. These differences can be attributed to the specific antigens evaluated, the choice of cutpoint for defining a positive titer, the prevalence of Pa in the population tested, and the source of respiratory cultures (sputum vs. oropharyngeal swabs vs. BAL) [25].

In the early development of ELISA assays for Pa antibodies, several investigators looked for serum Pa antibodies in non-CF children with no history of Pa infection [23,39–41]. To our knowledge, ours is the first study to evaluate Pa serology in non-CF control children in whom concurrent OP cultures were performed. Among the 94 control children in our study, in whom Pa was isolated from an OP culture in only one, there was nonetheless evidence of possible prior Pa infection based on Pa serologic titers >100. Interestingly, van Ewijk et al. [42] performed serial OP cultures in 20 children with CF and 19 unrelated healthy controls each time the children presented with signs and symptoms of a viral respiratory infection during one viral season, and isolated Pa from 37% of the controls and 30% of the children with CF. The Pa persisted in the children with CF whereas it was rapidly cleared from the control children. Thus, the respiratory tract may be a common site of Pa infection in healthy children. Other potential sources of mucosal invasion could include the skin, gastrointestinal or genitourinary tracts. Whether or not these healthy children truly had experienced Pa infection, the lack of specificity of Pa antibodies to children with CF is one reason why Pa serology may be less useful in detecting early Pa infection in children with CF.

Our study has several limitations in addition to sampling oropharyngeal swabs rather than BAL for assessment of respiratory cultures. First, serum for serology was only collected annually, so conclusions regarding more frequent sampling cannot be drawn. However, routine annual blood tests are standard of care in the U.S. Secondly, since positive and negative predictive values are affected by prevalence, the observation that PPV increases while NPV decreases with increased observation time after serum collection is in part due to the increased prevalence of Pa-positivity with increasing observation time. Similarly, our results should not be generalized to populations with widely different rates of Pa acquisition. Finally, we did not evaluate the effect of Pa eradication treatment on Pa serology, though Ratjen et al. [30] and Anstead et al. [37], have both demonstrated that Pa antibody titers may be useful in monitoring response to treatment. Finally, we chose to evaluate Pa serology using a commercial assay so that it could easily be adopted by clinical laboratories. We measured IgG antibodies to elastase, alkaline phosphatase and exotoxin A, as these antibodies have been extensively evaluated in CF [17,22,24,25,27–31,37]. Our results should not be generalized to serologic assays for antibodies to other antigens, other antibodies (i.e. IgM, IgA) to these antigens, or non-commercial kits for the antibodies we studied [43]. It is possible that serology to other antigens, such as flagellar antigens, might have yielded different results [44,45].

In conclusion, our results demonstrate that Pa serology is only modestly accurate in predicting subsequent first isolation of Pa from upper respiratory cultures among Pa negative CF patients. Thus, the role of Pa serology in the routine monitoring of early Pa infection remains unclear.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2014.06.005.

Acknowledgments

We wish to thank Bruce Marshall, MD, and the Cystic Fibrosis Foundation for providing the National Patient Registry data and Barbara Mathewson for providing the analytic programming
support. We also wish to thank all the site investigators and research coordinators, as well as all the participants in the EPIC Observational Study and their families. This study was funded by US Cystic Fibrosis Foundation grant EPIC 09K0.

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