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Original Article

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

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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 $\log_{10}$ 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.

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Conclusions: *Pa* serology does not appear useful for predicting first *Pa* positive oropharyngeal culture among young CF patients.

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 [35] ≤ 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 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