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Original Article

Clinical outcomes in cystic fibrosis patients with *Trichosporon* respiratory infection☆

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Abstract

Background: Relationships between clinical outcomes and novel respiratory pathogens such as *Trichosporon* are not well understood.
Methods: Respiratory cultures from CF patients were screened for novel pathogens *Trichosporon* and *Chryseobacterium* as well as other pathogens over 28 months. Relationships between microbiologic and clinical data were assessed using univariate and multivariate methods.
Results: Of 4934 respiratory cultures from 474 CF patients, 37 cultures from 10 patients were *Trichosporon* positive. Patients with positive *Trichosproron* cultures had a greater decline in FEV_1 over time (−3.9%/year vs. -1.3%/year, $p < 0.05$), whereas *Chryseobacterium* did not influence lung function. These findings were confirmed in multivariate analyses that included age, gender, and other common pathogens as confounders. Treatment of *Trichosporon* infected patients was associated with improved lung function.
Conclusions: Trichosporon can be recovered from a small but clinically meaningful fraction of CF patients. The presence of *Trichosporon*, but not *Chryseobacterium*, is associated with greater declines in lung function.

Keywords: Chryseobacterium; lung function

1. Introduction

Cystic fibrosis is characterized by persistent infection with a variety of respiratory pathogens, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* the most common. The contributions of these and many other pathogens to CF lung disease have been relatively well defined through epidemiological studies that examine relationships between respiratory infection and clinical outcomes, most often lung function. For example, multiple studies have demonstrated lung function declines more rapidly in CF patients infected with *Pseudomonas aeruginosa*, *Achromobacter* species, and *Burkholderia cepacia* complex. While not definitive, such associations are generally used to identify the pathogens that should be more aggressively targeted.

Over the past several years, several potential novel pathogens have been recovered with greater frequency from CF respiratory secretions. One such potential pathogen is *Trichosporon*, a soil fungus that can be an opportunistic pathogen [1]. *Trichosporon* species are generally refractory to treatment with amphotericin and the echinocandins, and strains have been reported with reduced susceptibility to azoles as well [1,2]. These resistances raise the possibility that aggressive anti-fungal treatment of allergic bronchopulmonary disease due to *Aspergillus* and *Scedosporium* may be responsible for the emergence of this organism [3]. Over

☆ Many of these findings were previous presented in abstract form at the American Thoracic Society meeting held in Denver, Colorado in 2105: Esther CR Jr., Lin F-C, Kerr A, Gilligan P. Clinical outcomes of Trichosporon or Chryseobacterium respiratory infection in cystic fibrosis. Am J Respir Crit Care Med, 2015

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the past decade, several case studies suggest that *Trichosporon* can be recovered from culture of CF respiratory secretions [4] and may be associated with clinical deterioration in CF patients [5–7].

To better understand the potential pathogenicity *Trichosporon*, we performed a prospective evaluation of respiratory infection with *Trichosporon* species within all CF patients in our center over 28 month period. Associations to clinical outcome measures including percent predicted FEV_1, FEV_1 decline over time, and nutritional status were then examined using univariate and multivariate analyses to establish links between infection and disease. As a control, we performed a similar analysis with another potential novel pathogen *Chryseobacterium*, a glucose non-fermenting gram negative bacillus that was first identified in CF respiratory cultures in 2002 [8], with subsequent case studies suggesting that it may be a respiratory pathogen in CF [9,10]. Our goal was to provide the comparative studies needed to define associations between these pathogens and clinical outcomes.

2. Methods

2.1. Microbiological and clinical data

All respiratory samples from CF patients at our institution were prospectively screened on routine CF cultures for the presence of *Trichosporon* and *Chryseobacterium* species as well as other CF respiratory pathogens over a 28 month period (January 2009 – April 2011). Routine CF culture was performed by inoculation of respiratory samples onto chocolate agar, Columbia CNA agar with 5% sheep blood, MacConkey agar, mannitol salt agar and *Burkholderia cepacia* selective agar. Cultures were examined daily for 4 days. Gram-negative glucose non-fermenters including *Chryseobacterium* were identified using the VITEK 2 GN panel (bioMérieux, Durham, NC). Yeast-like organisms that were present in pure or predominant amount or had at least a confluent growth at the primary zone of inoculation were identified. During the study period, *Trichosporon* was identified by the colony and Gram stain morphologies and was reported as *Trichosporon* species [11]. The waxy, heaped and often wrinkled colonies with or without a mycelial fringe, and presents of arthroconidia, true hyphae, pseudohyphae and blastoconidia were considered to be indicative of *Trichosporon* species. Archived isolates that were available from a subset of patients were identified by sequencing of the internal transcribed spacer (ITS) region and the D1/D2 region of the 28S ribosomal subunit, as described previously [12] and were compared with sequences in NCBI BLASTN database. Clinical patient data was abstracted from the Port CF database. Patients with solid organ transplant during or prior to the study period were excluded from analysis. Lung function was recorded as percent predicted FEV_1, and values obtained at less than 6 years of age were excluded as being potentially unreliable. Change in lung function over time was defined as the slope by linear regression of all available values. Change over time was not calculated for patients with fewer than three lung function values or less than three months of data available. This study was approved by the UNC IRB (14–3272).

2.2. Statistical analysis

Relationships between microbiologic and clinical data were assessed using univariate methods, with Student's T-test or Mann–Whitney based on the normality of the datasets. Multivariate methods were performed using generalized linear mixed effects models with random intercepts, which controlled for confounders and presence of repeated measures. The normality and equal variance assumption of the responses in the linear regression model was carefully checked using adjusted residuals, and no clear violation of the statistical assumptions was found. Analyses were performed in GraphPad Prism v5.0 (La Jolla, CA) or SAS version 9.2 (SAS, Cary, NC).

3. Results

A total of 4934 respiratory cultures from 474 individual CF patients were included in the analysis, with an average of 10 cultures per patient over the 28 month study period (range 3–40, Table 1). As expected, *S. aureus* and *P. aeruginosa* species were the most common pathogens, present on at least one culture over the study period in 86% and 85% of patients, respectively. Of the remaining pathogens identified in >1% of cultures, all were relatively well described in the CF literature (>30 references on PubMed) with the exception of *Trichosporon* and *Chryseobacterium* species (<10 references for each).

Trichosporon species were identified in 37 cultures (0.75% of all cultures) from 10 individual patients (2.1% of all patients). Six of the 10 patients had evidence of chronic infection, with three or more *Trichosporon* positive cultures. Eight of the 10 patients had received inhaled antibiotics within the past six months, and two had a history of itraconazole treatment for allergic bronchopulmonary aspergillosis. Archived isolates from 7 patients, including 4 of those with chronic infection, were viable for species identification by sequencing of the ITS and D1/D2 regions. All were identified as *T. mycotoxinivorans*.

Several common CF pathogens were recovered in similar rates in *Trichosporon* positive and negative patients, including *Pseudomonas* (90% in positive, 84% in negative), *Staphylococcus* (90% vs. 86%), and other molds (60% vs. 51%). Non-pseudomonal gram negative rods, including *Achromobacter*, *Burkholderia*, *Ralstonia*, and *Stenotrophomonas* species, were recovered at higher rates in *Trichosporon* positive patients (80% vs. 45%, $p < 0.05$), and there was a trend towards higher rates of oxacillin resistant *Staphylococcus aureus* (ORSA) (70% vs. 43.1%, $p = 0.11$).

Patients positive for *Trichosporon* had a trend towards older age (median 20.7 years, IQR 15.5-32.4 vs. median 15.5 years,

Table 1
CF Patient Demographics

n =	474
Age (years)	15.6 (IQR 7-24.2)
Gender (% male)	48.8%
Initial FEV1 (% predicted)	75 (IQR 51-93)
# Cultures	10 (IQR 7-13)