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)

IQR 6.8-24.2, p = 0.051, Fig. 1A) and had more respiratory cultures obtained (median 11, IQR 10-22 vs. median 10, IQR 7-13, p < 0.03, Fig. 1B) than those who were *Trichosporon* negative. In the subset of patients that could perform spirometry (n = 10 *Trichosporon* positive, 340 *Trichosporon* negative), univariate analyses revealed that *Trichosporon* positive patients exhibited a trend towards lower initial percent predicted FEV_1 (median 55%, IQR 31.5-82.75% vs. median 76%, IQR 52-93%, p = 0.064, Fig. 1C) and had a significantly greater decline in percent predicted FEV_1 over time (median − 3.9%/year, IQR −11.2 to −0.8%/year vs. median − 1.3%/year, IQR −5.0 to −2.1%/year, p < 0.05, Fig. 1D).

Chryseobacterium species were identified in 34 cultures (0.69%) from 29 individual patients (6.1%). No patient had chronic infection (3 or more cultures) with *Chryseobacterium*, significantly different from the relative high rate of chronic infection observed with *Trichosporon* (p < 0.01). Patients with *Chryseobacterium* infection were younger than those without infection (median 12.6, IQR 2.3-20.5 years vs. median 16.0, IQR 7.4-24.5 years, p < 0.05, Fig. 2A), and they also had slightly more respiratory cultures obtained (median 11, IQR 8-16 vs. median 10, IQR 7-13, p < 0.05, Fig. 2B). In the subset with spirometry data available (n = 17 *Chryseobacterium* positive, 333 *Chryseobacterium* negative), initial percent predicted FEV_1 was similar in *Chryseobacterium* positive vs. negative patients (median 84%, IQR 72-92.5% vs. median 73%, IQR 50-93%, p = 0.19, Fig. 2C) as was decline in percent predicted FEV_1 over time (median − 1.9%/year, IQR −5.9 to −0.8%/year vs. median − 1.5%/year, IQR −4.9 to 1.8%/year, p = 0.49, Fig. 2D). Of note, no patients had positive cultures for both *Trichosporon* and *Chryseobacterium* over the time frame of the study.

To better assess clinical outcomes associated with these organisms, multivariate analyses were performed including *Trichosporon* or *Chryseobacterium* respiratory culture status as well as the culture status of common CF pathogens (*S. aureus*, ORSA, *P. aeruginosa*, and non pseudomonal glucose non-fermenters) plus age, gender, and nutritional status as potential confounders. For both organisms, multivariate models were constructed for initial percent predicted FEV_1 as well as change in FEV_1 over time (Tables 2 and 3). For initial FEV_1, age, nutritional failure, and infection with either *P. aeruginosa* or non-pseudomonal glucose non-fermenters were all negatively associated. *Trichosporon* infection was not associated with initial percent predicted FEV_1 but was strongly correlated with change in FEV_1 over time ($\beta = -0.16$, p = 0.003) in multivariate analyses. Initial FEV_1 was the only other variable predictive of changes in

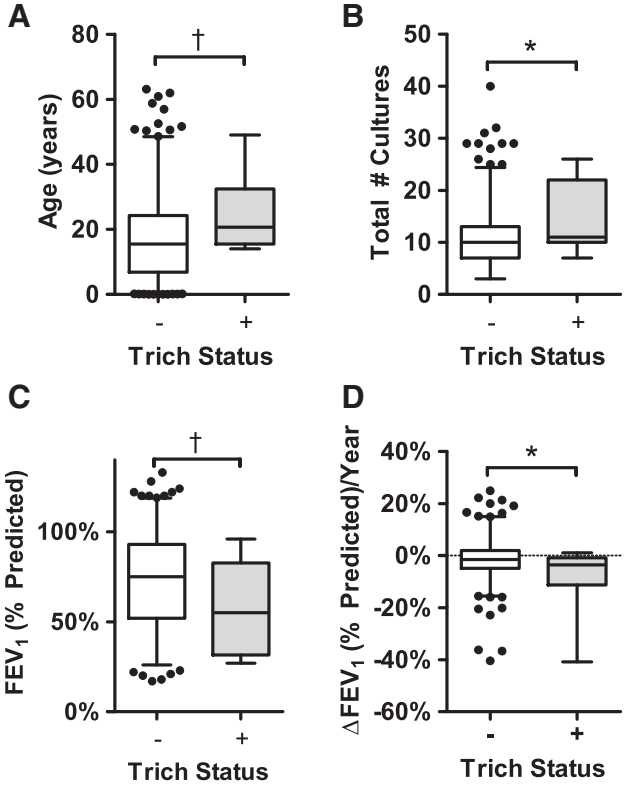

Fig. 1. Clinical characteristics of CF patients with *Trichosporon* infection. In univariate analyses, CF patients with at least one *Trichosporon* positive culture had **A)** a trend towards older age (p = 0.051), **B)** more cultures obtained (p < 0.03), **C)** a trend towards lower initial percent predicted FEV_1 (p = 0.06), and **D)** an increased rate of decline in percent predicted FEV_1 over time (p < 0.04) compared to patients with no *Trichosporon* positive cultures.

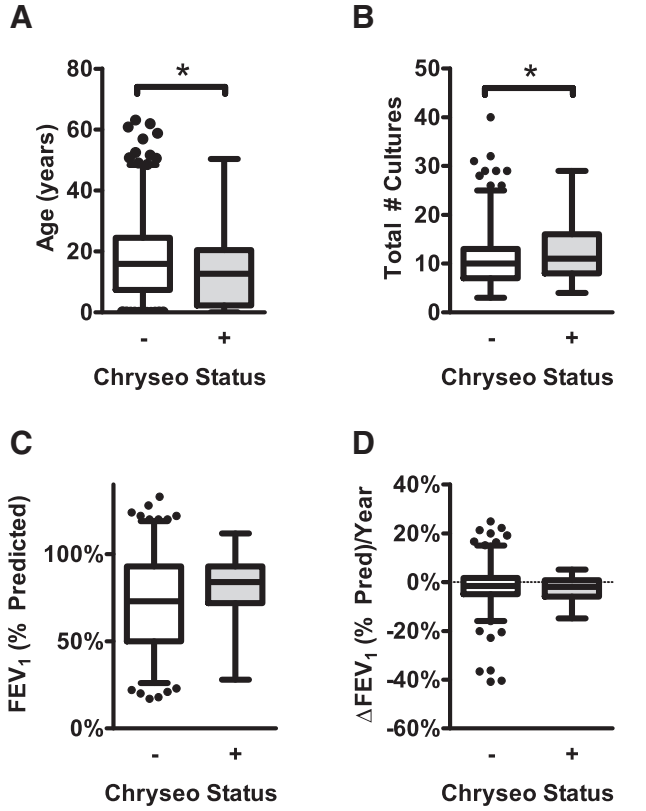

Fig. 2. Clinical characteristics of CF patients with *Chryseobacterium* infection. In univariate analyses, CF patients with at least one *Chryseobacterium* positive culture were **A)** younger (p < 0.05), **B)** had more cultures obtained (p < 0.05), **C)** had no significant difference in initial predicted FEV_1 (p = 0.14), and had **D)** no difference in percent predicted FEV_1 over time (p = 0.49) compared to patients with no *Chryseobacterium* positive cultures.

Table 2
Multivariate analysis of Trichosporon infection.

	Initial FEV_1		ΔFEV_1 over time	
Variable	β	p-value	β	p-value
FEV_1, % predicted	-	-	−0.161	0.015
Age	−0.486	0.000	−0.114	0.063
Nutritional Failure	−0.323	0.000	0.067	0.229
Staphylococcus sps	0.054	0.215	0.083	0.133
ORSA	−0.080	0.070	−0.097	0.087
Pseudomonas sps.	−0.117	0.005	−0.017	0.749
Non-*Pseud* GNR	−0.102	0.012	−0.066	0.202
Trichosporon	−0.039	0.336	−0.155	0.003

FEV_1 over time.In contrast, *Chryseobacterium* culture status was not associated with the initial percent predicted FEV_1 or with change in FEV_1 over time in multivariate analysis. Interestingly, there was a trend ($\beta = 0.078$, $p = 0.051$) towards improved percent predicted FEV_1 in patients with *Chryseobacterium* respiratory infection.

Among the *Trichosporon* infected patients, we identified six who underwent antifungal treatment directed against this pathogen and had lung function values obtained within a week of treatment initiation. Initial treatment regimens consisted of itraconazole (200–400 mg twice daily), voriconazole (200–400 mg twice daily), or fluconazole (400 mg once daily) and lasted an average of 97 ± 33 days. Lung function values obtained approximately three months after start of treatment (average 102 ± 23 days) were significantly improved relative to initial values (Fig. 3).

4. Discussion

Both *Trichosporon* and *Chryseobacterium* species were recovered from respiratory cultures in a small but meaningful fraction of patients with CF. However, clinical features associated with infection were very different for the two organisms. *Trichosporon* respiratory infection was more often chronic and tended to occur in older, sicker individuals. In contrast, *Chryseobacterium* was not associated with chronic infection in this study and was more often isolated from younger patients a trend towards better lung function. More importantly, *Trichosporon*, but not *Chryseobacterium*, was associated with greater decline in lung function over time in both univariate and multivariate analyses controlling for other markers of disease severity. While this association does not necessarily indicate causation, the results suggest that *Trichosporon* infection may lead to progressive lung damage.

Table 3
Multivariate analysis of Chryseobacterium infection.

	Initial FEV_1		ΔFEV_1 over time	
Variable	β	p-value	β	p-value
FEV_1, % predicted	-	-	−0.149	0.027
Age	−0.488	0.000	−0.118	0.056
Nutritional Failure	−0.325	0.000	0.065	0.250
Staphylococcus sps	0.051	0.233	0.085	0.128
ORSA	−0.086	0.050	−0.107	0.062
Pseudomonas sps.	−0.113	0.007	−0.013	0.808
Non-*Pseud* GNR	−0.110	0.006	−0.079	0.131
Chryseobacterium	0.078	0.051	−0.020	0.706

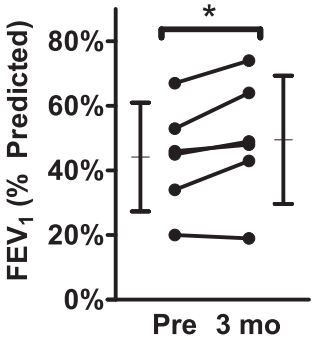

Fig. 3. Antifungal treatment in patients with *Trichosporon* infection. In patients with *Trichosporon* respiratory infection, improvements in percent predicted FEV_1 were observed three months after initiation of antifungal treatment. * = $p < 0.05$.

The relationship between *Trichosporon* respiratory infection and deterioration in lung function is consistent with previous case series. Kroner et al. reported "greater decline in FEV_1 than expected" in 4 of 8 CF patients infected with *Trichosporon*, with some patients improving after initiation of antifungal therapy [6], and similar anecdotal relationships have been described in other reports [4,6]. However, this is the first study to systematically examine clinical outcome measures in CF patients with *Trichosporon* respiratory infection relative to those that did not culture this pathogen.

These data suggest that treatment of *Trichosporon* respiratory infection may be indicated in CF in the setting of clinical decline. Most *Trichosporon* species are susceptible to triazole antifungals, though resistance to other antifungal classes has been reported [1,2]. Our evaluation of patients treated with these agents for *Trichosporon* demonstrated modest improvements in lung function after antifungal therapy, but these retrospective data should be interpreted very cautiously. Most patients were being simultaneously treated with other antibiotics targeted towards other respiratory pathogens, and it is impossible in this retrospective study to determine which therapies were most associated with clinical improvement. Nevertheless, these results suggest that controlled trials of antifungal therapy are indicated to determine the role and utility of antifungal therapy for affected patients. Although concern has been raised that previous treatment with azole antifungals might contribute to emergence of *Trichosporon* respiratory infection [3], we found a history of previous azole treatment in only two of the ten *Trichosporon* infected patients, which is insufficient to draw any conclusions regarding a potential association.

Although *T. ashaii*, *T. mucoides*, *T. asteroides*, and *T. inkin* account for the vast majority of invasive human *Trichosporon* infections [13,14], our available isolates were all identified as *T. mycotoxinivorans*. Previous case reports and case series have shown that the clinical spectrum of *T. mycotoxinivorans* ranges from chronic infections to fulminant diseases, and this species has been recognized as a potential pathogen in CF patients [4–7]. However, pathogenesis underlying the relationship between *T. mycotoxinvorans* infection and patients with CF has not been studied.

The lack of relationship between *Chryseobacterium* respiratory infection and clinical outcome measures is somewhat surprising given previous reports suggesting pathogenic status, though objective measures of decline have not been reported [9,10]. The fact that patients with *Chryseobacterium* positive cultures actually had a trend towards higher lung function values suggests that this organism is a less virulent, somewhat sporadic pathogen. However, we cannot rule out the possibility that *Chryseobacterium* respiratory infection could be problematic in a subset of individuals.

In summary, our cohort analysis suggest that *Trichosporon*, but not *Chyrseobacterium* respiratory infection is associated with decline in lung function over time. This data should help guide treatment decisions for CF patients infected with these pathogens.

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