

Changing Prevalence of Lower Airway Infections in Young Children with Cystic Fibrosis

Oded Breuer^{1,2}, Andre Schultz^{1,2,3}, Lidija Turkovic¹, Nicholas de Klerk¹, Anthony D. Keil^{2,4}, Siobhain Brennan¹, Joanne Harrison^{5,6,7}, Colin Robertson^{5,6,7}, Philip J. Robinson^{5,6,7}, Peter D. Sly^{8*}, Sarath Ranganathan^{5,6,7}, Stephen M. Stick^{1,2,3}, and Daan Caudri^{1,2,9}; on behalf of AREST CF[‡]

¹Telethon Kids Respiratory Research Centre, Telethon Kids Institute, and ³Division of Paediatric and Child Health, Faculty of Medicine, University of Western Australia, Perth, Western Australia, Australia; ²Perth Children's Hospital, Perth, Australia; ⁴Department of Microbiology, PathWest Laboratory Medicine WA, Perth, Western Australia, Australia; ⁵Department of Paediatrics, University of Melbourne, Melbourne, Australia; ⁶Murdoch Children's Research Institute, Parkville, Australia; ⁷Department of Respiratory and Sleep Medicine, Royal Children's Hospital, Parkville, Australia; ⁸Children's Health and Environment Program, Child Health Research Centre, The University of Queensland, Brisbane, Australia; and ⁹Department of Pediatrics/Respiratory Medicine, Erasmus MC, Rotterdam, the Netherlands

ORCID ID: 0000-0002-4775-9095 (O.B.).

Abstract

Rationale: Historical studies suggest that airway infection in cystic fibrosis initiates with *Staphylococcus aureus* and *Haemophilus influenzae*, with later emergence of *Pseudomonas aeruginosa*. *Aspergillus* species are regarded as relatively infrequent, late-occurring infections.

Objectives: To assess the prevalence and change in prevalence of early lower airway infections in a modern cohort of children with cystic fibrosis.

Methods: All infants diagnosed with cystic fibrosis after newborn screening participating in the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) cohort study between 2000 and 2018 were included. Participants prospectively underwent BAL at 3–6 months, 1 year, and annually up to 6 years of age. Lower airway infection prevalence was described. Changes in prevalence patterns were assessed longitudinally using generalized estimating equations controlling for age and repeated visits.

Measurements and Main Results: A total of 380 infants underwent 1,759 BALs. The overall prevalence and median age of first acquisition of the most common infections were as follows: *S. aureus*, 11%, 2.5 years; *P. aeruginosa*, 8%, 2.4 years; *Aspergillus* species, 11%, 3.2 years; and *H. influenzae*, 9%, 3.1 years. During the study, a significant decrease in prevalence of *P. aeruginosa* ($P < 0.001$) and *S. aureus* ($P < 0.001$) was observed with a significant change toward more aggressive treatment. Prevalence of *Aspergillus* infections did not significantly change ($P = 0.669$).

Conclusions: *Aspergillus* species and *P. aeruginosa* are commonly present in the lower airways from infancy. The decrease in prevalence of *P. aeruginosa* and *S. aureus* since 2000, coinciding with a more aggressive therapeutic approach, has resulted in *Aspergillus* becoming the most commonly isolated pathogen in young children. Further research is warranted to understand the implication of these findings.

Keywords: *Aspergillus*; *Pseudomonas aeruginosa*; BAL

(Received in original form October 12, 2018; accepted in final form February 27, 2019)

*P.D.S. is Associate Editor of *AJRCCM*. His participation complies with American Thoracic Society requirements for recusal from review and decisions for authored works.

‡The full membership of AREST CF is available at www.arestcf.org.

Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) is supported by Cystic Fibrosis Foundation Therapeutics, Inc., USA and Cystic Fibrosis Australia, as well as by National Health and Medical Research Council (NHMRC) grants APP1000896 and 1020555. O.B. has been supported by a Lowy Foundation Pediatric Fellowship arranged by AUSIMED (Australia/Israel Medical Research). D.C. received support for a research fellowship from the Rothwell Foundation, the Ter Meulen Grant of the Royal Netherlands Academy of Arts and Sciences, and the Sophia Children's Hospital Fund. None of the funding bodies were in any way involved in the data collection, interpretation of the data, or writing of the manuscript. S.M.S. is an NHMRC Practitioner Fellow and received support from Northern Star Resources.

Author Contributions: Study conception and design: O.B., A.S., S.M.S., and D.C. Data acquisition: O.B., D.C., and Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF). Data analysis: O.B., L.T., N.d.K., and D.C. Data interpretation: O.B., A.S., S.R., S.M.S., and D.C. Manuscript drafting: O.B. Manuscript revision: all authors, including AREST CF. Approval of final version of the manuscript: all authors, including AREST CF. Accountability for all aspects of the research: all authors, including AREST CF.

Correspondence and requests for reprints should be addressed to Oded Breuer, M.D., Telethon Kids Respiratory Research Centre, Telethon Kids Institute, University of Western Australia, P.O. Box 855, West Perth, WA 6872, Australia. E-mail: oded.breuer@telethonkids.org.au.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Crit Care Med Vol 200, Iss 5, pp 590–599, Sep 1, 2019

Copyright © 2019 by the American Thoracic Society

Originally Published in Press as DOI: 10.1164/rccm.201810-1919OC on February 27, 2019

Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the

Subject: Historically, *Staphylococcus aureus* pneumonia was the commonest cause of death in children with cystic fibrosis. After the introduction of antimicrobial treatments, other pathogens emerged as dominant organisms in what appeared to be an age-dependent sequence, with *S. aureus* and *Haemophilus influenzae* appearing early in disease followed by *Pseudomonas aeruginosa*. The association of infections with worse disease outcomes has focused attention on treatment of these organisms to improve survival. However, most studies of infections in young children with cystic fibrosis rely on data obtained from upper airway samples, which may not have accurately reflected lower airway infections. Furthermore, improved care, early diagnosis, and aggressive antibiotic treatment may have influenced the pattern of acquisition of lower airway pathogens and their contribution to lung disease.

What This Study Adds to the

Field: Data from this unique longitudinal observational cohort indicate that infections with *P. aeruginosa* and *Aspergillus* species are prevalent from infancy. The presence of any individual bacterial pathogen does not predict the emergence of future dominant organisms. Reductions in the prevalence of *S. aureus* and *P. aeruginosa*, coinciding with a more aggressive treatment approach, has resulted in *Aspergillus* species becoming the commonest isolated pathogen in early life. Given the changing patterns of infection over time and the continued improvements in morbidity and mortality, the clinical significance and priority for treatment of organisms such as *Aspergillus* require further investigation to facilitate the development of rational future antimicrobial interventions to prevent progressive lung disease in early life.

Pathophysiological studies of cystic fibrosis (CF) lung infections have historically described a set of bacterial pathogens that are frequently acquired in an age-dependent sequence. Early infections were reported to be caused mainly by *Staphylococcus aureus* and *Haemophilus influenzae* (1, 2), whereas *Pseudomonas aeruginosa* was considered to appear later in the course of disease, after airways are “primed” by the earlier infections, to gradually become the predominant pathogen isolated in the respiratory secretions (2–4). More recent studies have shown that infection with *P. aeruginosa* occurs much earlier than believed previously, with a median age of first culture isolation ranging between 21 and 30 months (5–7). This early occurrence of *P. aeruginosa* is also reported in current patient registries. Nonetheless, data from these registries still show *S. aureus* and *H. influenzae* to be the predominant organisms cultured in preschool patients with CF (8, 9). Other infections routinely cultured in adult patients with CF, such as *Aspergillus* species and *Stenotrophomonas maltophilia*, are not commonly reported to occur in preschool children with CF. Importantly, many of these studies reporting the epidemiology of infections in patients with CF, including patient registries, use data from cultures obtained from upper respiratory tract secretions and not the lower airways. Upper respiratory cultures have been shown to lack sensitivity to accurately detect lower respiratory infections and are not routinely used to assess for the presence of fungal infections (5, 10–13). Furthermore, *S. aureus* and *H. influenzae* are organisms that often benignly colonize the upper respiratory tract, and thus upper respiratory cultures may overrepresent these pathogens (2).

Infection propagating inflammation is considered to be a major cause of the progressive lung disease seen in patients with CF (1, 14–16). Major changes in clinical practice over the past two decades (e.g., newborn screening, increasingly aggressive pseudomonal eradication regimens, antibiotic stewardship, improved airway clearance techniques, and novel CFTR [cystic fibrosis transmembrane conductance regulator] modulator therapies) (17–19) are likely to have had an impact on the frequency and nature of

pulmonary infections. Correctly identifying changes in the epidemiology of lower respiratory infection early in life in CF is essential for understanding the potential impact of available and emerging therapeutic interventions and of infection control measures to prevent progressive lung damage. The aims of this study were to describe the epidemiology of lower respiratory infection in a modern cohort of prospectively followed and aggressively treated children with CF diagnosed after newborn screening, to assess the associations between different infections, and to evaluate the change in infection prevalence during the 18 years of the study.

Methods

Study Population

All patients participating in the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) program at Princess Margaret Hospital (PMH; Perth, Western Australia, Australia) and Royal Children’s Hospital (RCH; Melbourne, Victoria, Australia) between January 2000 and February 2018 were included in the study. A more detailed version of the methods is provided in the online supplement. The AREST CF program consists of assessments at 3 months of age, 12 months of age, and annually until 6 years of age (20). The assessments include a bronchoscopy with BAL when clinically stable and a detailed clinical assessment. Since 2005, computed tomographic (CT) imaging of the chest has also been performed at the time of bronchoscopy. The program was approved by the ethics committee at each institution, and written informed consent was obtained from participants’ parents before each annual review.

Bronchoscopy, BAL Collection, and Processing

After induction of general anesthesia, a flexible bronchoscopy through a laryngeal mask airway was performed. BAL was collected in the right middle lobe and lingula (or worst affected lobe on chest CT scans) and sent for microbiological processing to test for the presence of bacteria, fungi, and viruses and for analysis of markers of inflammation (20–22).

Table 1. Study Population Characteristics at the Annual Reviews

Characteristic	Data
Study participants	380
Sex, M, n (%)	185 (49)
Pancreatic sufficiency, n (%)	57 (15)
Homozygous Δ F508, n (%)	184 (48)
Meconium ileus, n (%)	64 (17)
Patients per center, PMH/RCH	214/166
Total annual visits with BAL	1,759
At assessments age 3 mo	268
At assessments age 12 mo	293
At assessments age 2 yr	289
At assessments age 3 yr	268
At assessments age 4 yr	237
At assessments age 5 yr	217
At assessments age 6 yr	187
Visits per patient, median (range)	5 (1–7)
Age at visit, yr, median (range)	2.9 (0.2–6.7)

Definition of abbreviations: PMH=Princess Margaret Hospital; RCH=Royal Children's Hospital.

Clinical Routines during the Study Period

Chronic patient management in both centers followed international and national guidelines (17, 23–25). Clinical practice at PMH and RCH was to attempt *P. aeruginosa* eradication when detected on BAL. At PMH, the eradication protocol included 2 weeks of intravenous antibiotics followed by 4 weeks of inhaled tobramycin and oral ciprofloxacin (26). At RCH, at first, the protocol included a 3-month treatment with inhaled tobramycin and oral ciprofloxacin for the first and third months, and since 2016, a 2-month treatment with inhaled tobramycin.

At PMH, routine therapy for respiratory exacerbations included intravenous, inhaled, or oral antibiotics targeting *P. aeruginosa*. Routine therapy for respiratory exacerbations at RCH was driven by previous cultures (including oropharyngeal swabs); when these were unavailable or negative, gentamicin or later ceftriaxone and/or flucloxacillin were usually used as first-line empirical therapy. Importantly, children with CF followed at both centers were routinely prescribed antistaphylococcal prophylaxis (amoxicillin-clavulanic acid) from diagnosis until 2 years of age (27).

Statistical Analysis

Data were summarized by standard descriptive statistics analyzing the point prevalence of the most common pathogens cultured from the BAL samples at each age. Differences between groups were analyzed using Kruskal-Wallis and Mann-Whitney *U* tests as applicable.

The change in infection prevalence over the study period was investigated using generalized estimating equation models clustering for repeated visits in the same patients and adjusting for the patients' age at BAL. Statistical significance for a linear change in prevalence over time was calculated from these models, and the model output was used to plot the predicted prevalence for each pathogen over time using a polynomial fit. Mixed effects models were used to evaluate the change in treatment routines and outcomes during the study period and to evaluate if the probability of specific airway pathogens after 1 year of follow-up was associated with the presence of any pathogen at baseline. All analyses were performed using Stata version 15 software (StataCorp).

Results

Three hundred eighty patients were included in the study. These patients underwent 1,759 annual assessments in which BAL samples were obtained and available for microbiological and cytokine analysis. Patients' characteristics are presented in Table 1.

Table 2. Specific Prevalence Characteristics of the Most Commonly Cultured Pathogens

	<i>Pseudomonas aeruginosa</i>	<i>Aspergillus</i> sp.	<i>Haemophilus influenzae</i>	<i>Staphylococcus aureus</i>
Total number of positive BALs	143/1,759 (8%)	192/1,759 (11%)	163/1,759 (9%)	199/1,759 (11%)
Coinfection with <i>P. aeruginosa</i>	—	23/192 (12%)	23/163 (14%)	27/199 (14%)
Coinfection with <i>Aspergillus</i> sp.	23/143 (16%)	—	21/163 (13%)	24/199 (12%)
Coinfection with <i>H. influenzae</i>	23/143 (16%)	21/192 (11%)	—	44/199 (22%)
Coinfection with <i>S. aureus</i>	27/143 (19%)	24/192 (13%)	44/163 (27%)	—
Coinfection with any pathogen	60/143 (42%)	65/192 (34%)	86/163 (53%)	93/199 (47%)
Median (IQR) age at first acquisition, yr*	2.4 (1.1–3.9)	3.2 (2.0–4.9)	3.1 (2.1–4.3)	2.5 (1.1–4.0)
Prevalence at age 6 yr	20/187 (11%)	33/187 (18%)	26/187 (14%)	27/187 (14%)
Cumulative incidence by age 6 yr	86/266 (32%)	106/266 (40%)	100/266 (38%)	107/266 (40%)

Definition of abbreviation: IQR=interquartile range.

Coinfection is defined as positive if both pathogens were cultured in samples taken from the same BAL procedure. See Figure E1 in the online supplement for analysis of coinfections specified by age. Cumulative incidence applies to 266 children who had reached the age of 6 years or more at the time of analysis. In the calculation of cumulative incidence, missing BALs were considered negative.

*Significance by Kruskal-Wallis test for difference in distribution in age of acquisition of first infection between any of the pathogens: $P < 0.001$; significance by Mann-Whitney *U* test for *P. aeruginosa* versus *Aspergillus*: $P = 0.007$, *P. aeruginosa* versus *H. influenzae*: $P = 0.012$, *P. aeruginosa* versus *S. aureus*: $P = 0.675$, *Aspergillus* versus *H. influenzae*: $P = 0.524$, *Aspergillus* versus *S. aureus*: $P = 0.001$, *H. influenzae* versus *S. aureus*: $P = 0.006$.

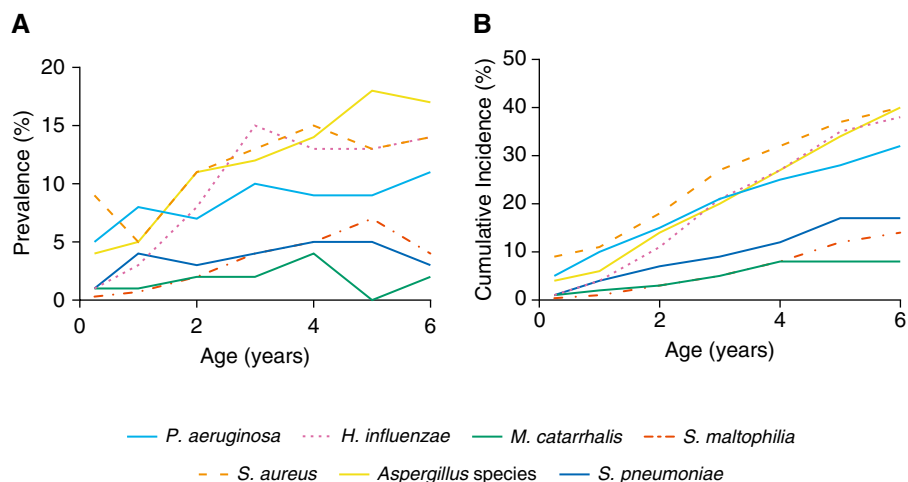


Figure 1. (A) Point prevalence and (B) cumulative incidence of the seven most commonly cultured lower respiratory tract infections, by patient age at the time of bronchoscopy.

Infection Prevalence

The most commonly cultured pathogens from the BAL samples of patients included in this study were *Aspergillus* species (11%), *S. aureus* (11%), nontypeable *H. influenzae* (9%), and *P. aeruginosa* (8%). Other less commonly encountered infections in BAL samples were *Streptococcus pneumoniae* (3%), *S. maltophilia* (3%), *Moraxella catarrhalis* (1.6%), methicillin-resistant *S. aureus* (0.7%), *Burkholderia* species (0.3%), *Achromobacter xylosoxidans* (0.2%), and nontuberculous mycobacteria (0.1%). Importantly, 55% of BAL sample results were negative for an infection.

The median ages of first acquisition of the most commonly encountered infections

were as follows: for *Aspergillus* species, 3.2 years; for *P. aeruginosa*, 2.4 years; for *S. aureus*, 2.5 years; and for *H. influenzae*, 3.1 years. The median age of first infection was not significantly different between *P. aeruginosa* and *S. aureus*, and both were significantly lower than the median age of first infection with *Aspergillus* species and *H. influenzae* ($P < 0.05$) (see Table 2).

There were no significant differences in the overall prevalence of infections with *P. aeruginosa* or *Aspergillus* species between medical centers (RCH and PMH), but infections with *S. aureus* and *H. influenzae* were significantly more prevalent in patients from RCH than in those from

PMH (15% vs. 8%; $P = 0.004$; 15% vs. 6%; $P < 0.001$, respectively) as previously reported (28). The point prevalence and cumulative incidence of infections according to age at BAL are presented in Figure 1. By 6 years of age, 89% of patients experienced at least one lower respiratory tract infection as detected in BAL; 32% of patients experienced at least one lower airway infection with *P. aeruginosa*; and 40% of patients experienced at least one infection with *Aspergillus* species (see Table 2 for additional information on infection prevalence and incidence).

Evaluating Associations between Infections

The multivariate mixed effects model evaluating risk for a future infection showed that the risk for a lower respiratory tract infection with *P. aeruginosa*, *Aspergillus* species, or *S. aureus* was significantly increased with more than double the odds if the same pathogen was detected in the BAL in the previous year. None of the other pathogens seemed to increase the risk of later acquisition of *P. aeruginosa*. The risk for a future infection with *S. aureus* was also increased in children infected with *H. influenzae* (odds ratio, 2.2; 95% confidence interval, 1.2–4.0) at baseline, but it decreased for those infected with *P. aeruginosa* (odds ratio, 0.3; 95% confidence interval, 0.1–0.9) (Table 3).

Table 3. Association of Pathogens at Baseline with Pathogens at 1-Year Follow-up

Pathogen at Baseline	<i>Pseudomonas aeruginosa</i> after 1-yr Follow-up	<i>Aspergillus</i> sp. after 1-yr Follow-up	<i>Haemophilus influenzae</i> after 1-yr Follow-up	<i>Staphylococcus aureus</i> after 1-yr Follow-up
<i>P. aeruginosa</i>				
OR (95% CI)	2.4 (1.1–5.1)	1.3 (0.7–2.5)	0.5 (0.2–1.2)	0.3 (0.1–0.9)
P value	0.024	0.357	0.146	0.023
<i>Aspergillus</i> sp.				
OR (95% CI)	1.7 (0.9–3.0)	2.4 (1.2–4.5)	0.9 (0.4–1.7)	0.7 (0.3–1.5)
P value	0.084	0.008	0.721	0.353
<i>H. influenzae</i>				
OR (95% CI)	1.3 (0.7–2.6)	1.1 (0.6–2.1)	1.4 (0.7–2.8)	2.2 (1.2–4.0)
P value	0.381	0.713	0.373	0.010
<i>S. aureus</i>				
OR (95% CI)	1.3 (0.7–2.3)	0.9 (0.5–1.7)	1.3 (0.2–1.2)	2.4 (1.3–4.6)
P value	0.451	0.874	0.146	0.008

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

Results are from four separate multivariable mixed effects models in which multiple 1-year follow-up periods per patient were included. The correlation within the same patient was accounted for by the within-person random effect covariance matrix. All respiratory pathogens at baseline were included as possible predictors to assess their independent predictive ability. Models were corrected for age and pancreatic sufficiency.

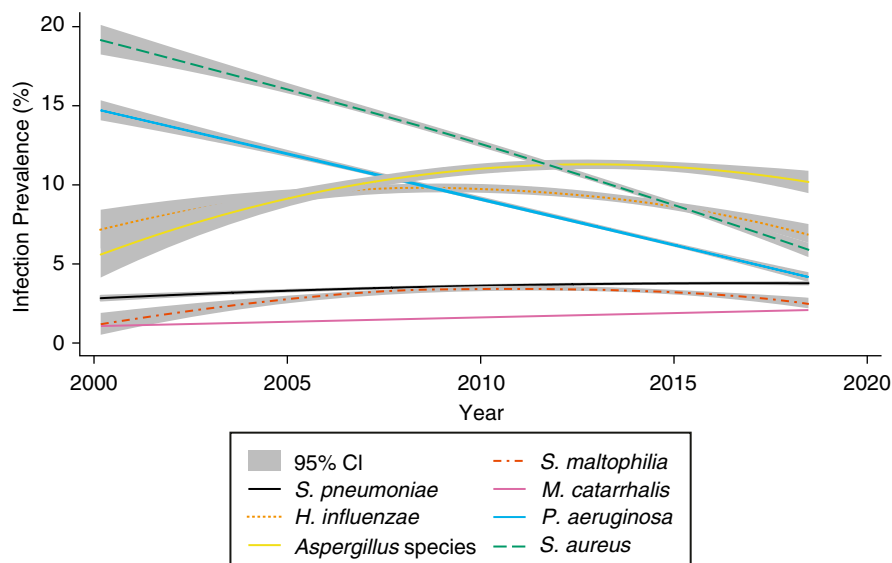


Figure 2. Change in lower respiratory infection prevalence during the study period (2000–2018) for the seven most commonly cultured lower respiratory tract infections, adjusted for age and repeated visits using the output of a generalized estimating equation model plotted with date of study visit as a fractional polynomial (twoway fplfitci procedure in Stata software). Coefficients of the generalized estimating equation models for a linear trend over time: *P. aeruginosa*, -0.09 (95% confidence interval [CI], -0.14 to 0.04 ; $P < 0.001$); *S. aureus*, -0.09 (95% CI, -0.13 to 0.05 ; $P < 0.001$); *Aspergillus* species, -0.01 (95% CI, -0.05 to 0.03 ; $P = 0.669$); *H. influenzae*, -0.04 (95% CI, -0.08 to 0.01 ; $P = 0.023$); *S. maltophilia*, -0.03 (95% CI, -0.14 to 0.02 ; $P = 0.529$); *S. pneumoniae*, 0.01 (95% CI, -0.06 to 0.07 ; $P = 0.874$); *M. catarrhalis*, 0.03 (95% CI, -0.08 to 0.14 ; $P = 0.571$).

Coinfection

Of 790 BAL cultures in which a pathogen was identified, in 24% (192), more than one pathogen was cultured (“coinfection”). A coinfection was most common with BAL results positive for both *S. aureus* and *H. influenzae*, and least common for BAL results positive for infections with *Aspergillus* species (Table 2). Figure E1 in the online supplement presents the change of coinfections with age.

Changes in Infection Prevalence, 2000–2018

During the study period, there were significant decreases in the prevalence of lower airway infections with *P. aeruginosa* ($P < 0.001$), *S. aureus* ($P < 0.001$), and *H. influenzae* ($P = 0.023$), as detected by BAL. *P. aeruginosa* infection prevalence gradually decreased from 12.3% to 9.8% to 5.9%, and *S. aureus* infection prevalence gradually decreased from 15.7% to 13.0% to 9.1% during the years 2000–2006, 2006–2012, and 2012–2018, respectively (Table 4). The relative magnitude of the decrease in prevalence was similar in each medical center, and the rate of decline was gradual over the study period (Figure 2). The

prevalence of the other encountered lower airway pathogens (*Aspergillus* species, *S. maltophilia*, *S. pneumoniae*, and *M. catarrhalis*) did not significantly change during the study period (Figure 2 and Table 4). The decrease in prevalence of *P. aeruginosa*, *S. aureus*, and *H. influenzae* has resulted in *Aspergillus* species emerging as the most commonly isolated lower airway pathogen cultured in recent years.

Changes in Clinical Routines and Outcomes, 2000–2018

Changes in clinical routines and outcomes are presented in Tables 5 and E1. Overall, during the study period, there was a significant increase in the use of chronic therapies for routine treatment of CF lung disease (azithromycin, hypertonic saline, and dornase alfa), as well as increased use of antipseudomonal oral and inhaled antibiotics (ciprofloxacin and inhaled tobramycin) (Tables 5 and E1). Intravenous antibiotic treatment (both outpatient and inpatient), which included treatment for eradication of newly acquired *P. aeruginosa* infections, did not significantly change during the study period, which is suggestive of a more aggressive approach targeting

P. aeruginosa despite increasing proportions of negative culture results.

When assessing changes in outcome measures during the study period, we found that body mass index, FEV₁ at 6 years of age, and number of admissions for respiratory exacerbations did not significantly change. However, lower respiratory inflammation, including neutrophil elastase concentrations and neutrophil percentage in BAL samples, significantly decreased during the study period (Table 5).

Discussion

In this study, to better understand the epidemiology of lower respiratory infections in young children with CF, we demonstrated that lower airway infections with *P. aeruginosa*, *Aspergillus* species, *S. aureus*, and *H. influenzae* are all prevalent from early infancy. We observed that the prevalence of *P. aeruginosa*, *S. aureus*, and *H. influenzae* significantly decreased in the last two decades and that these changes coincided with the general increased use of oral and inhaled antipseudomonal antibiotics and inhaled mucolytic therapies. An important observation resulting from the decrease in prevalence was that *Aspergillus* species are now the most prevalent lower respiratory pathogens identified in our cohort of children. The emergence of *Aspergillus* species as the most dominant pathogens suggests that further studies should be undertaken to determine the clinical significance of this observation and the contribution of *Aspergillus* infections to early progressive lung disease.

Infections with *P. aeruginosa* begin in early childhood, and *P. aeruginosa* gradually becomes the major respiratory pathogen in patients with CF (5–7). A commonly proposed concept is that infections with *P. aeruginosa* follow earlier infections with other pathogens that “prime” the airway for colonization (2, 3). In our cohort of young children, although *P. aeruginosa* is one of the earliest encountered lower respiratory infections, we show that the risk for lower airway infections with *P. aeruginosa* is not significantly increased by prior infection with *S. aureus* or *H. influenzae*.

Another major finding in our study is the significant decrease in the prevalence of

Table 4. Infection Prevalence, according to Year of Study and Patient Age at Time of Bronchoscopy (Repeat Visits Included)

	2000–2006				2006–2012				2012–2018			
	0–2 yr (n = 92)	3–4 yr (n = 57)	5–6 yr (n = 22)	All (n = 171)	0–2 yr (n = 272)	3–4 yr (n = 239)	5–6 yr (n = 211)	All (n = 722)	0–2 yr (n = 304)	3–4 yr (n = 236)	5–6 yr (n = 326)	All (n = 866)
<i>Pseudomonas aeruginosa</i> , % (n)	9.8% (9)	14.0% (8)	18.2% (4)	12.3% (21)	7.8% (21)	10.9% (26)	11.4% (24)	9.8% (71)	4.3% (13)	5.9% (14)	7.4% (24)	5.9% (51)
<i>Aspergillus</i> sp., % (n)	4.3% (4)	12.3% (7)	13.6% (3)	8.2% (14)	6.6% (18)	14.2% (34)	13.7% (29)	11.2% (81)	3.6% (11)	11.0% (26)	18.4% (60)	11.2% (97)
<i>Staphylococcus aureus</i> , % (n)	13.0% (12)	19.3% (11)	18.2% (4)	15.7% (27)	8.1% (22)	15.0% (36)	17.1% (36)	13.0% (94)	4.3% (13)	10.6% (25)	12.3% (40)	9.1% (78)
<i>Haemophilus influenzae</i> , % (n)	6.5% (6)	7.0% (4)	0% (0)	5.8% (10)	5.1% (14)	16.3% (39)	13.2% (28)	11.2% (81)	1.3% (4)	9.7% (23)	13.8% (45)	8.3% (72)

Definition of abbreviation: n = number of annual assessments with BAL.

Table 5. Evaluation of Effect of Calendar Year on Changes in Treatment Routines and Outcomes during Study Period (2000–2018)

Management (Routine Medication Use in the Year before the Annual Assessment)	Odds Ratio per 1-yr Increase in Calendar Year (95% Confidence Interval), P Value		
	0–2 yr (n = 366 Assessments in 208 Patients)	3–4 yr (n = 397 Assessments in 238 Patients)	5–6 yr (n = 501 Assessments in 239 Patients)
Azithromycin use	1.7 (1.1 to 2.9), 0.028	1.5 (1.1 to 2.0), 0.015	1.3 (1.1 to 1.6), 0.013
Hypertonic saline use	NA	1.3 (1.0 to 1.7), 0.098	1.2 (1.0 to 1.4), 0.089
Dornase alfa use	2.1 (1.1 to 4.1), 0.028	1.8 (1.3 to 2.4), 0.001	2.1 (1.6 to 2.8), <0.001
Ivacaftor use	NA	NA	NA
Ivacaftor–lumacaftor use	NA	NA	NA
Oral amoxicillin–clavulanic acid use	1.1 (1.0 to 1.3), 0.081	1.0 (0.9 to 1.1), 0.851	0.9 (0.8 to 1.0), 0.051
Inhaled tobramycin use	1.3 (1.1 to 1.5), 0.008	1.2 (1.1 to 1.3), 0.008	1.1 (1.0 to 1.2), 0.021
Ciprofloxacin use	NA	1.1 (0.9–1.3), 0.311	1.1 (0.9 to 1.3), 0.069
Use of intravenous antibiotics	1.1 (1.0 to 1.2), 0.046	0.9 (0.8–1.0), 0.297	1.0 (0.9–1.2), 0.965
			1.3 (1.1 to 1.5), <0.001
			1.2 (1.1 to 1.4), 0.010
			1.7 (1.5 to 2.0), <0.001
			NA
			NA
			1.0 (0.9 to 1.1), 0.825
			1.1 (1.1 to 1.2), <0.001
			1.1 (1.0 to 1.2), 0.026
			1.0 (0.9–1.1), 0.648
			NA
			NA

Outcome	Difference per 1-yr Increase in Calendar Year (95% Confidence Interval), P Value		
	0–2 yr (n = 555 Measurements in 284 Patients)	3–4 yr (n = 483 Measurements in 271 Patients)	5–6 yr (n = 518 Measurements in 244 Patients)
Inflammation			
IL-8 concentration (log scale)	0.03 (–0.01 to 0.06), 0.064	–0.01 (–0.04 to 0.04), 0.945	0.01 (–0.03 to 0.05), 0.656
Neutrophil elastase (log scale)	–0.05 (–0.07 to 0.03), <0.001	–0.06 (–0.1 to 0.02), 0.004	–0.06 (–0.1 to 0.01), 0.020
% Neutrophils in BAL cell count	–1.7 (–2.1 to 1.3), <0.001	–1.6 (–2.2 to 1.0), <0.001	–0.9 (–1.6 to 0.23), 0.009
Admission for respiratory exacerbations	0.06 (–0.12 to 0.23), 0.521 (n = 281 assessments in 161 patients)	–0.13 (–0.71 to 0.44), 0.647 (n = 302 assessments in 198 patients)	0.10 (–0.08 to 0.28), 0.287 (n = 247 assessments in 185 patients)
FEV ₁ at 6 yr of age (z-score)	—	—	–0.05 (–0.13 to 0.04), 0.273 (n = 132 patients)
BMI at 6 yr of age	—	—	0.02 (–0.08 to 0.12), 0.657 (n = 152 patients)

Definition of abbreviations: BMI = body mass index; NA = number of patients too small to perform analyses (use of ciprofloxacin under 2 yr, n = 5; use of hypertonic saline under 2 yr, n = 2; use of ivacaftor under 6 yr, n = 2; use of ivacaftor–lumacaftor under 6 yr, n = 0).

Bold indicates $P < 0.05$. Results were derived from mixed effects logistic regression models for assessment of patient management and mixed effects linear regression models for assessment of patient outcomes. Analyses are clustered for repeated measures in the same patient and corrected for pancreatic insufficiency, age, sex, and homozygosity for $\Delta F508$.

lower airway *P. aeruginosa*, *S. aureus*, and *H. influenzae* infections during the study period. Because of the observational nature of the study, causality cannot be assessed for this observation, but a similar decrease in incidence and prevalence of *P. aeruginosa* infections in older patients with CF, and recently also in infants diagnosed after newborn screening, has previously been reported (29–31). Our study focuses on a young cohort of children, mostly naive to previous infections, and represents most of the children in the two centers with comprehensive follow-up from diagnosis after newborn screening and direct evaluation of lower airway pathogens (13). The decrease in prevalence of common bacterial pathogens was gradual and center independent. During the study period, patient management significantly changed toward a more aggressive treatment approach with increased use of antipseudomonal antibiotics and use of chronic therapies. This is consistent with international guidelines (17, 27, 32) and is also evident in data from the Cystic Fibrosis Foundation Patient Registry (8). Other factors, such as improved infection control, airway clearance techniques, and antibiotic stewardship, were not evaluated in this study but may have also contributed to the change in infection prevalence. These factors clearly represent a general improvement in care, in line with current international guidelines, that is likely to have contributed to the decrease in prevalence of bacterial pathogens in our and other cohorts (29–31, 33).

S. aureus is often considered to be the first pathogen cultured from respiratory secretions in young patients with CF (1–3), with an overall reported prevalence of 30–70% (2, 8, 9). In our cohort, the median age of first infection with *S. aureus* was not significantly different from that of *P. aeruginosa*, and the overall prevalence of *S. aureus* lower airway infection was only 11%. We speculate that this low prevalence in *S. aureus* infection and increased age of first acquisition in our cohort is due to the routine prescription of antibiotic prophylaxis against *S. aureus* used in both participating centers. Furthermore, lower airway sampling used in our study, which avoids upper airway contamination seen with oropharyngeal swabs and sputum cultures, may also have contributed to the lower prevalence of *S. aureus* and

H. influenzae than in studies that rely on oropharyngeal cultures (10). The antistaphylococcal prophylactic routine as well as the gradual increased use of azithromycin during the study period may also explain the gradual decline in the prevalence of *S. aureus* and *H. influenzae* infections contrary to recent reports (8). Importantly, previous studies assessing antistaphylococcal prophylaxis raised concerns of increased incidence of overall *P. aeruginosa* infections and risk for first acquisition of *P. aeruginosa* infections in the patients receiving therapy (34, 35). However, as mentioned above, we have observed a significant decrease in *P. aeruginosa* infections during our study period despite the routine use of *S. aureus* prophylactic therapy, possibly in view of an aggressive *P. aeruginosa* eradication and treatment approach.

Aspergillus species, mainly *Aspergillus fumigatus*, are prevalent fungal pathogens isolated from the CF airway (36, 37). In patients with CF, they are usually regarded as a late-occurring pathogen, possibly after frequent exposure to broad-spectrum antibiotic therapy (3, 38, 39). Surprisingly, therefore, our study demonstrates that *Aspergillus* species are the commonest organism cultured, usually as an isolated pathogen, from the lower respiratory airways of young patients with CF. Other recent studies that have evaluated lower airway samples in pediatric patients with CF also reported a higher prevalence of *Aspergillus* infections than expected (40, 41), strongly suggesting that our observations are generalizable in the context of modern approaches to CF therapy. These studies evaluated lower airways, whereas most centers routinely use oropharyngeal samples for pathogen surveillance in preschool children with CF. Oropharyngeal samples are not routinely processed for fungal pathogens and lack sensitivity for their detection (40, 41).

On the basis of our longitudinal observations, the dominance of *Aspergillus* species may be explained by the declining prevalence of other common bacterial pathogens and increased use of antibiotics. However, with the relatively common isolation of *Aspergillus* species at 3 months of age and overall stable prevalence during the 18 years of the study, despite the increased use of antibiotics, it is

possible that *Aspergillus* infection might be inherent to the pathophysiology of early CF lung disease as previously suggested (2, 42, 43). Because we have previously reported that *Aspergillus* species are associated with lower airway neutrophilic inflammation in young children (44), the role of these organisms in the progression of early lung disease requires further investigation. Currently, no guidelines recommend routine treatment of *Aspergillus* infections, and therapy of *Aspergillus* infections is aimed largely at patients meeting criteria for a diagnosis of allergic bronchopulmonary aspergillosis. However, several studies suggest that respiratory infections with *Aspergillus* are associated with worse clinical outcomes (36, 41, 44–47) and that treatment might be beneficial (48). Thus, there is a need to improve fungal surveillance and culturing detection techniques in young children to better understand the prevalence and clinical significance of early *Aspergillus* infections to develop appropriate interventions.

The design of the present study, which includes annual lower respiratory BAL cultures, may have underestimated the prevalence of transient infections. However, the large number of patients included and the longitudinal design are likely to have provided robust estimates of prevalence of persistent infection, particularly because the trends were similar in two geographically separated centers. Patients participating in the study were routinely prescribed antistaphylococcal prophylaxis in the first 2 years of life. Hence, our results may not reflect infection rates in populations with infrequent use of antistaphylococcal antibiotics.

The main strengths of the present study are the large number of patients included from two geographically distinct CF centers over nearly two decades, high participation rates, standardized sampling of lower respiratory secretions, and the longitudinal design. This enabled us to accurately describe the prevalence of lower respiratory pathogens in young patients with CF, which is essential to best understanding the inflammatory responses to lung infections in early life (1, 13, 15, 16).

Although our observations are the result of a unique surveillance approach, the conclusions are likely to be generalizable. The decline in prevalence of common pathogens that we have observed in young children is also reported for older patients from international data registries. Importantly, our data explain the emergence of *Aspergillus* species as common pathogens.

Furthermore, the changing patterns of infection have been associated with increased antibiotic use consistent with international recommendations and as reported in national patient registries. The clinical significance of the dominance of *Aspergillus* species requires further investigation to develop rational antimicrobial interventions aimed at

preventing progressive lung disease in young children with CF. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Catherine Gangell (Curtin University, Perth, Australia) for assistance with this study.

References

1. Armstrong DS, Grimwood K, Carlin JB, Carzino R, Gutiérrez JP, Hull J, et al. Lower airway inflammation in infants and young children with cystic fibrosis. *Am J Respir Crit Care Med* 1997;156:1197–1204.
2. Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* 2002;15:194–222.
3. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003;168:918–951.
4. Govan JR, Nelson JW. Microbiology of lung infection in cystic fibrosis. *Br Med Bull* 1992;48:912–930.
5. Burns JL, Gibson RL, McNamara S, Yim D, Emerson J, Rosenfeld M, et al. Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. *J Infect Dis* 2001;183:444–452.
6. West SE, Zeng L, Lee BL, Kosorok MR, Laxova A, Rock MJ, et al. Respiratory infections with *Pseudomonas aeruginosa* in children with cystic fibrosis: early detection by serology and assessment of risk factors. *JAMA* 2002;287:2958–2967.
7. Heltshe SL, Khan U, Beckett V, Baines A, Emerson J, Sanders DB, et al. Longitudinal development of initial, chronic and mucoid *Pseudomonas aeruginosa* infection in young children with cystic fibrosis. *J Cyst Fibros* 2018;17:341–347.
8. Cystic Fibrosis Foundation Patient Registry annual data report 2016. 2016.
9. Australian Cystic Fibrosis Data Registry annual report 2015. 2015.
10. Armstrong DS, Grimwood K, Carlin JB, Carzino R, Olinsky A, Phelan PD. Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis. *Pediatr Pulmonol* 1996;21:267–275.
11. Ramsey BW, Wentz KR, Smith AL, Richardson M, Williams-Warren J, Hedges DL, et al. Predictive value of oropharyngeal cultures for identifying lower airway bacteria in cystic fibrosis patients. *Am Rev Respir Dis* 1991;144:331–337.
12. Rosenfeld M, Emerson J, Accurso F, Armstrong D, Castile R, Grimwood K, et al. Diagnostic accuracy of oropharyngeal cultures in infants and young children with cystic fibrosis. *Pediatr Pulmonol* 1999;28:321–328.
13. Breuer O, Caudri D, Akesson L, Ranganathan S, Stick SM, Schultz A, AREST CF. The clinical significance of oropharyngeal cultures in young children with cystic fibrosis. *Eur Respir J* 2018;51:1800238.
14. Armstrong DS, Hook SM, Jansen KM, Nixon GM, Carzino R, Carlin JB, et al. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. *Pediatr Pulmonol* 2005;40:500–510.
15. Sagel SD, Gibson RL, Emerson J, McNamara S, Burns JL, Wagener JS, et al.; Inhaled Tobramycin in Young Children Study Group; Cystic Fibrosis Foundation Therapeutics Development Network. Impact of *Pseudomonas* and *Staphylococcus* infection on inflammation and clinical status in young children with cystic fibrosis. *J Pediatr* 2009;154:183–188.
16. Sagel SD, Kapsner R, Osberg I, Sontag MK, Accurso FJ. Airway inflammation in children with cystic fibrosis and healthy children assessed by sputum induction. *Am J Respir Crit Care Med* 2001;164:1425–1431.
17. Borowitz D, Robinson KA, Rosenfeld M, Davis SD, Sabadosa KA, Spear SL, et al.; Cystic Fibrosis Foundation. Cystic Fibrosis Foundation evidence-based guidelines for management of infants with cystic fibrosis. *J Pediatr* 2009;155(6 Suppl): S73–S93.
18. Heltshe SL, Mayer-Hamblett N, Burns JL, Khan U, Baines A, Ramsey BW, et al.; GOAL (the G551D Observation-AL) Investigators of the Cystic Fibrosis Foundation Therapeutics Development Network. *Pseudomonas aeruginosa* in cystic fibrosis patients with G551D-CFTR treated with ivacaftor. *Clin Infect Dis* 2015;60:703–712.
19. Gilligan PH, Downey DG, Elborn JS, Flume PA, Funk S, Gilpin D, et al. “Pathogen eradication” and “emerging pathogens”: difficult definitions in cystic fibrosis. *J Clin Microbiol* 2018;56:e00193–18.
20. Sly PD, Brennan S, Gangell C, de Klerk N, Murray C, Mott L, et al.; Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF). Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. *Am J Respir Crit Care Med* 2009;180:146–152.
21. Berry LJ, Sheil B, Garratt L, Sly PD; AREST CF. Stability of interleukin 8 and neutrophil elastase in bronchoalveolar lavage fluid following long-term storage. *J Cyst Fibros* 2010;9:346–350.
22. D’Sylva P, Caudri D, Shaw N, Turkovic L, Douglas T, Bew J, et al. Induced sputum to detect lung pathogens in young children with cystic fibrosis. *Pediatr Pulmonol* 2017;52:182–189.
23. Button BM, Wilson C, Dentice R, Cox NS, Middleton A, Tannenbaum E, et al. Physiotherapy for cystic fibrosis in Australia and New Zealand: a clinical practice guideline. *Respirology* 2016;21:656–667.
24. Lahiri T, Hempstead SE, Brady C, Cannon CL, Clark K, Condren ME, et al. Clinical practice guidelines from the Cystic Fibrosis Foundation for preschoolers with cystic fibrosis. *Pediatrics* 2016;137:e20151784.
25. Saiman L, Siegel JD, LiPuma JJ, Brown RF, Bryson EA, Chambers MJ, et al.; Cystic Fibrosis Foundation; Society for Healthcare Epidemiology of America. Infection prevention and control guideline for cystic fibrosis: 2013 update. *Infect Control Hosp Epidemiol* 2014;35(Suppl 1):S1–S67.
26. Douglas TA, Brennan S, Gard S, Berry L, Gangell C, Stick SM, et al. Acquisition and eradication of *P. aeruginosa* in young children with cystic fibrosis. *Eur Respir J* 2009;33:305–311.
27. Castellani C, Duff AJA, Bell SC, Heijerman HGM, Munck A, Ratjen F, et al. ECFS best practice guidelines: the 2018 revision. *J Cyst Fibros* 2018;17:153–178.
28. Ramsey KA, Hart E, Turkovic L, Padros-Goossens M, Stick SM, Ranganathan SC. Respiratory infection rates differ between geographically distant paediatric cystic fibrosis cohorts. *ERJ Open Res* 2016;2:00014–2016.
29. Salsgiver EL, Fink AK, Knapp EA, LiPuma JJ, Olivier KN, Marshall BC, et al. Changing epidemiology of the respiratory bacteriology of patients with cystic fibrosis. *Chest* 2016;149:390–400.
30. Razvi S, Quittell L, Sewall A, Quinton H, Marshall B, Saiman L. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995 to 2005. *Chest* 2009;136:1554–1560.
31. Hoch H, Sontag MK, Scarbro S, Juarez-Colunga E, McLean C, Kempe A, et al. Clinical outcomes in U.S. infants with cystic fibrosis from 2001 to 2012. *Pediatr Pulmonol* 2018;53:1492–1497.

32. Mogayzel PJ Jr, Naureckas ET, Robinson KA, Mueller G, Hadjiliadis D, Hoag JB, *et al.*; Pulmonary Clinical Practice Guidelines Committee. Cystic fibrosis pulmonary guidelines: chronic medications for maintenance of lung health. *Am J Respir Crit Care Med* 2013;187:680–689.
33. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010;23:299–323.
34. Ratjen F, Comes G, Paul K, Posselt HG, Wagner TO, Harms K; German Board of the European Registry for Cystic Fibrosis (ERCF). Effect of continuous antistaphylococcal therapy on the rate of *P. aeruginosa* acquisition in patients with cystic fibrosis. *Pediatr Pulmonol* 2001;31:13–16.
35. Hurley MN, Fogarty A, McKeever TM, Goss CH, Rosenfeld M, Smyth AR. Early respiratory bacterial detection and antistaphylococcal antibiotic prophylaxis in young children with cystic fibrosis. *Ann Am Thorac Soc* 2018;15:42–48.
36. Reece E, Segurado R, Jackson A, McClean S, Renwick J, Grealley P. Co-colonisation with *Aspergillus fumigatus* and *Pseudomonas aeruginosa* is associated with poorer health in cystic fibrosis patients: an Irish registry analysis. *BMC Pulm Med* 2017;17:70.
37. Valenza G, Tappe D, Turnwald D, Frosch M, König C, Hebestreit H, *et al.* Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. *J Cyst Fibros* 2008;7:123–127.
38. Milla CE, Wielinski CL, Regelman WE. Clinical significance of the recovery of *Aspergillus* species from the respiratory secretions of cystic fibrosis patients. *Pediatr Pulmonol* 1996;21:6–10.
39. Cheer SM, Waugh J, Noble S. Inhaled tobramycin (TOBI): a review of its use in the management of *Pseudomonas aeruginosa* infections in patients with cystic fibrosis. *Drugs* 2003;63:2501–2520.
40. Saunders RV, Modha DE, Claydon A, Gaillard EA. Chronic *Aspergillus fumigatus* colonization of the pediatric cystic fibrosis airway is common and may be associated with a more rapid decline in lung function. *Med Mycol* 2016;54:537–543.
41. Harun SN, Wainwright CE, Grimwood K, Hennig S; Australasian Cystic Fibrosis Bronchoalveolar Lavage (ACFBAL) Study Group. *Aspergillus* and progression of lung disease in children with cystic fibrosis. *Thorax* 2019;74:125–131.
42. Skov M, Pressler T, Jensen HE, Høiby N, Koch C. Specific IgG subclass antibody pattern to *Aspergillus fumigatus* in patients with cystic fibrosis with allergic bronchopulmonary aspergillosis (ABPA). *Thorax* 1999;54:44–50.
43. Baxter CG, Dunn G, Jones AM, Webb K, Gore R, Richardson MD, *et al.* Novel immunologic classification of aspergillosis in adult cystic fibrosis. *J Allergy Clin Immunol* 2013;132:560–566.e10.
44. Gangell C, Gard S, Douglas T, Park J, de Klerk N, Keil T, *et al.*; AREST CF. Inflammatory responses to individual microorganisms in the lungs of children with cystic fibrosis. *Clin Infect Dis* 2011;53:425–432.
45. Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. *Chest* 2010;137:171–176.
46. McMahon MA, Chotirmall SH, McCullagh B, Branagan P, McElvaney NG, Logan PM. Radiological abnormalities associated with *Aspergillus* colonization in a cystic fibrosis population. *Eur J Radiol* 2012;81:e197–e202.
47. Fillaux J, Brémont F, Murriss M, Cassaing S, Rittié JL, Tétu L, *et al.* Assessment of *Aspergillus* sensitization or persistent carriage as a factor in lung function impairment in cystic fibrosis patients. *Scand J Infect Dis* 2012;44:842–847.
48. Shoseyov D, Brownlee KG, Conway SP, Kerem E. *Aspergillus* bronchitis in cystic fibrosis. *Chest* 2006;130:222–226.