

A longitudinal study of lung bacterial pathogens in patients with primary ciliary dyskinesia

M. C. Alanin¹, K. G. Nielsen², C. von Buchwald¹, M. Skov², K. Aanaes¹, N. Højby^{3,4} and H. K. Johansen^{3,5}

1) Department of Otolaryngology – Head and Neck Surgery and Audiology, 2) Danish PCD Centre, Paediatric Pulmonary Service, Department of Paediatrics and Adolescent Medicine, 3) Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet, 4) Institute of Immunology and Microbiology, University of Copenhagen and 5) Novo Nordisk Foundation Centre for Biosustainability, The Technical University of Denmark, Hørsholm, Denmark

Abstract

In patients with primary ciliary dyskinesia (PCD), impaired mucociliary clearance leads to an accumulation of secretions in the airways and susceptibility to repeated bacterial infections. The primary aim of this study was to investigate the bacterial flora in non-chronic and chronic infections in the lower airways of patients with PCD. We retrospectively reviewed the presence of bacteria from patients with PCD during an 11-year period and genotyped 35 *Pseudomonas aeruginosa* isolates from 12 patients with chronic infection using pulsed-field gel electrophoresis. We identified 5450 evaluable cultures from 107 patients with PCD (median age 17 years, range 0–74 years) (median age at diagnosis 7.8 years, range 0–63 years). *Haemophilus influenzae* was the most frequent microorganism. Other common pathogens were *P. aeruginosa*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Staphylococcus aureus*. The number of patients colonized with *P. aeruginosa* at least once varied from 11 to 44 patients (15–47%) annually, and 42 patients (39%) met the criteria for chronic infection at least once. *Pseudomonas aeruginosa* was more frequently isolated in teenagers and adults than children (p 0.02) and the prevalence was significantly lower in patients with preschool (<6 years) PCD diagnosis (p 0.04). Ten out of 12 patients (83%) were chronically infected with a unique clone-type of *P. aeruginosa*. No sharing of clone-types or patient-to-patient transmission was observed. In conclusion, PCD patients were infected by a unique set of bacteria acquired in an age-dependent sequence. *Pseudomonas aeruginosa* frequently colonizes the lower respiratory tract and the incidence of chronic infection was higher than previously reported.

Clinical Microbiology and Infection © 2015 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

Keywords: Ciliary motility disorders, Kartagener's syndrome, mucociliary clearance, *Pseudomonas aeruginosa*, pulse-field gel electrophoresis

Original Submission: 2 June 2015; **Revised Submission:** 21 August 2015; **Accepted:** 21 August 2015

Editor: F. Allerberger

Article published online: 2 September 2015

Corresponding author: M.C. Alanin, Department of Otolaryngology– Head and Neck Surgery and Audiology, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark

E-mail: mikkel.christian.alanin.01@regionh.dk

Introduction

Primary ciliary dyskinesia (PCD) is an autosomal recessive genetic disease caused by mutations in genes involved in ciliary structure and function. To date, mutations in 31 genes

covering approximately 60% of the PCD population have been described [1].

Normal ciliary movements play an integral part in the innate immune system and a critical role in airway defence. Due to impaired mucociliary clearance patients with PCD are predisposed to repeated or chronic infections of the upper and lower airways, which may lead to decreased lung function and bronchiectasis. Over time, 20% of PCD patients may develop end-stage lung disease [2].

Pseudomonas aeruginosa occasionally colonizes the lungs of patients with PCD and may cause destruction of the same character as demonstrated in patients with cystic fibrosis (CF). However, the pattern of bacterial flora in patients with PCD

has only been described in a few studies [2–4]. Several pathogens may contribute to pulmonary disease in PCD, including *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* [4,5]. As the available microbiological data in patients with PCD is incomplete, the primary aim of this study was to describe the general bacterial flora longitudinally, including the flora of chronic infections in the lower respiratory tract in patients with PCD, and to examine how the flora may change with patients' age.

Materials and methods

Study design and patients

We performed a retrospective study where we investigated the bacterial flora including the flora of chronic infections in the lower airways of patients with PCD during an 11-year period (January 2002 to December 2012). All Danish PCD patients are diagnosed and treated at the Danish PCD Centre at Rigshospitalet in Copenhagen. Inclusion criteria were (1) definitive PCD diagnosis based on presentation of the characteristic clinical phenotype and ciliary ultrastructural defects, abnormal ciliary function or a genetic mutation recognized to cause PCD [6] and (2) evaluable bacteriological data obtained from the laboratory database at the Department of Clinical Microbiology at Rigshospitalet.

General care

Patients with a diagnosis of PCD are permanently affiliated with the outpatient clinic of the Danish PCD Centre and intentionally appointed every third month with clinical examinations, lung function measurement, and sputum samples. Blood samples are obtained annually for antibody measurements. Patients visit the same outpatient facility as children with CF and follow the same isolation strategy [7]. In brief, the isolated groups consist of (1) patients chronically infected *P. aeruginosa*, (2) patients without chronic *P. aeruginosa* infection including patients with intermittent *P. aeruginosa* colonization and (3) patients harbouring other Gram-negative bacteria.

Antibiotic treatment

Patients with positive lower respiratory tract samples are treated promptly even without the presence of clinical symptoms, and when *P. aeruginosa* is the disease-causing pathogen, patients are treated with colistin inhalation and oral ciprofloxacin for 21 days in patients without chronic *P. aeruginosa* infection. Patients with chronic *P. aeruginosa* infection are additionally treated every third month with a 2-week intravenous antibiotic course. Prophylactic antibiotic treatment is not used.

Bacteriological examination

Bacterial data were based on sputum samples or secretions obtained by endolaryngeal suction cultured at 37°C on standard agar media for 2 to 5 days. The media included a Sabouraud plate, a 7% NaCl plate, a 'Blue plate' (modified Conradi Drigalski's medium; State Serum Institute, Copenhagen, Denmark) selective for Gram-negative rods and non-selective media including 5% Danish blood agar and chocolate agar [7,8]. Gram-stained smears were examined using microscopy.

Lung infection status

We have extrapolated criteria and definitions developed for CF to the lung infection status of patients with PCD [9,10]. With modified 'CF Leeds criteria' [11] we defined the lung infection status as:

- *Chronic infection*, when >50% of the preceding 12 months' cultures were positive for the specific pathogen;
- *Intermittent colonization*, when ≤50% or less of the preceding 12 months' cultures were positive for the specific pathogen;
- *Free of colonization and infection*, when no growth has occurred in the lungs in the previous 12 months.

In order to be categorized according to these criteria, the patient should provide at least four samples of secretions from the lower airways during 1 year.

Spirometry

We calculated the median lung function for bacteriologically evaluable patients in 2012. For the remaining patients, we included the spirometry from the year of their latest evaluable bacteriological examination. Forced expiratory volume (FEV₁%) predicted and forced volume capacity (FVC%) predicted as per American Thoracic Society standards [12].

Precipitins

Specific precipitating antibodies against *P. aeruginosa* were measured annually using crossed immunoelectrophoresis at the Department of Clinical Microbiology, Rigshospitalet. Normal values are 0–1, and ≥2 precipitins is considered abnormal [13–15]. We did have an exception relative to the modified 'CF Leeds criteria': If a patient had more than one, but less than four positive bacteriological samples in combination with abnormal precipitins, we classified the patient as chronically infected.

Pulsed-field gel electrophoresis

Stored *P. aeruginosa* isolates were genotyped using pulsed-field gel electrophoresis (PFGE) as described previously [7]. The PFGE bands were evaluated visually according to the guidelines of Tenover *et al.* [16]. Isolates with differences in up to two

bands were considered identical. Both inter-patient and intra-patient relatedness were assessed.

Epidemiological terms

We used the period prevalence rate (PePR) to define the percentage of patients who harboured the bacteria in question in one or more examinations during a calendar year. The period prevalence rate for chronic infection (PePRchr) refers to the percentage of patients who could be classified as chronically infected during a calendar year according to our criteria.

Statistics

Statistical Analysis Software (SAS) version 9.3 was used for statistical analysis. Logistic regression using generalized estimating equations to take the clustered nature of the data into account was used to compare age group and age at diagnosis with the frequency of selected bacteria in the lower airways.

Ethics

The study was approved by the local ethics committee (H-1-2013-032).

Results

A total of 107 patients with PCD were included in the study (50 male and 57 female, median age 17 years, range 0–74 years). The median age at diagnosis was 7.8 years (range 0–63 years). A total of 5450 samples represented the lower airways (verified by microscopy). The median number of samples per patient per year was 5 (range 4–6). The patients had variable follow-up periods; the median follow up was 9 years (range 1–11 years). A median of 55 patients (range 38–70) per year were eligible for categorization according to lung infection status using the 'CF Leeds criteria' (Table 1). In all, 857 precipitin measurements were available in 103 out of the 107 patients (96%). The median precipitin was 1, range 0–35.

Spirometry was evaluable in 99 patients (93%). The median FEV₁% predicted was 77% (range 22–127%) and the median FVC% predicted was 93% (range 51–141%). Eight patients had no spirometry available because they were too young to comply.

Non-capsulate *H. influenzae*

Haemophilus influenzae was the most frequently isolated bacterium in our cohort, with a median PePR of 62% (range 46–80%). Annually, 7 to 23 patients (8–31%) were chronically infected. A median of one patient (range 0–5) had simultaneous chronic *H. influenzae* and *P. aeruginosa* infection. There was a negative association between the occurrence of *H. influenzae*

TABLE 1. Patient characteristics and the number of respiratory samples each year

Year	PCD patients included (males) ^a	Total number of representative samples for bacteriological culture each year (per patient median, range)	n ≥4 samples ^b
2002	56 (27)	345 (6, 1–17)	38
2003	71 (35)	439 (6, 1–25)	50
2004	74 (37)	482 (6, 1–32)	51
2005	75 (36)	455 (5, 1–25)	47
2006	78 (36)	421 (4, 1–22)	53
2007	92 (44)	496 (4, 1–23)	55
2008	91 (42)	535 (5, 1–21)	55
2009	94 (43)	556 (5, 1–19)	65
2010	94 (45)	561 (5, 1–15)	70
2011	95 (46)	573 (5, 1–16)	67
2012	99 (47)	587 (5, 1–16)	69

^aTo be included a patient should have at least one respiratory sample from the lower airways that year.

^bThese patients can be classified according to the 'Cystic Fibrosis Leeds criteria'.

and age, and the incidence differed significantly across age groups, p 0.002 (Fig. 1).

Pseudomonas aeruginosa

The median PePR was 32% (range 15–47), similar to 11–44 patients being intermittently colonized annually. Positive *P. aeruginosa* isolates originated from sputum samples in 92% and from secretions obtained by endolaryngeal suction in 8%. Forty-two patients (39%) met the criteria for chronic *P. aeruginosa* infection at least once during the study period and four of these were infected with the mucoid phenotype. Fig. 2 displays the course of infection in patients with chronic *P. aeruginosa* infection. The definition of chronic infection was based on culture = the modified 'CF Leeds criteria' in 30 patients (71%) and on a combination of precipitins and cultures in the remaining 12 patients (29%). A median of 4 (range 1–8) new patients became chronically infected each year, and an average of 11 patients (13%) met the definition of chronic infection annually. The median age at first chronic infection was 20 years (range 5–67 years). Chronic infection was cleared for a minimum of 1 year in 29 out of the 42 patients (69%); 20 patients were free of infection and nine continued as intermittently colonized. All patients were evaluable with at least four representative samples or a combination of sputum samples and precipitins in the year following chronic infection. During the study, 74 of 107 patients (69%) had one or more sputum samples positive for *P. aeruginosa*. The incidence of *P. aeruginosa* differed significantly across age groups p 0.02 (Fig. 1). We hypothesized that early PCD diagnosis was associated with a lower incidence of *P. aeruginosa*. The overall prevalence of *P. aeruginosa* in patients with a confirmed diagnosis before school age (<6 years) was 9% relative to 20% in patients with later diagnosis. The difference was statistically significant (p 0.04).

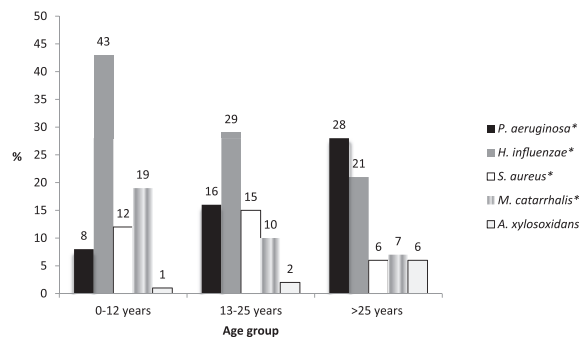


FIG. 1. Percentage of positive samples with *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis* and *Achromobacter xylosoxidans* in patients with primary ciliary dyskinesia according to age group. Patients were followed from 2002 to 2012. Children: 0–12 years, $n = 62$; teenagers and young adults: 13–25 years, $n = 54$; adults >25 years, $n = 38$. A patient can appear in more than one age group. * $p < 0.05$.

PFGE

We genotyped 35 *P. aeruginosa* isolates from 12 patients using PFGE. The median timespan between first and last isolates in each individual was 2.0 years (range 0.4–3.6 years). Intra-patient genetic relatedness of *P. aeruginosa* isolates was found in 10 of 12

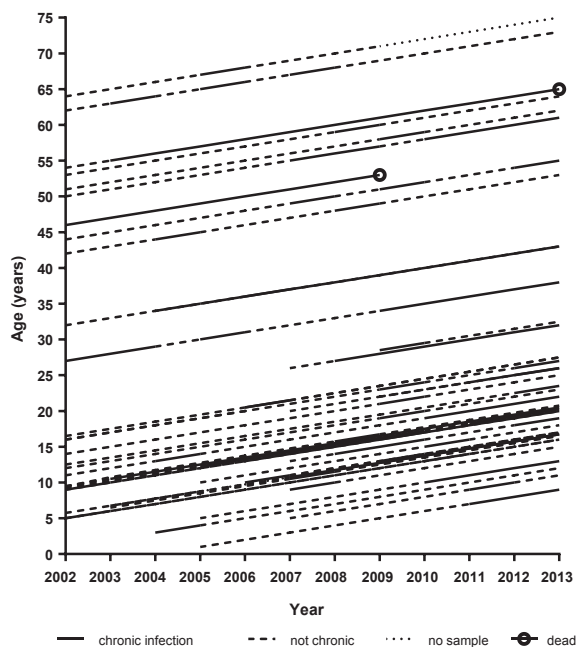


FIG. 2. Lexis diagram showing the course of *Pseudomonas aeruginosa* infection in 42 patients with primary ciliary dyskinesia who qualified as chronically infected at least once between 2002 and 2012. Courses are displayed from the first evaluable sample until the last observation in 2012. All but one patient (small dots) was evaluable. Chronic infection was based on modified 'Cystic Fibrosis Leeds criteria'.

chronically infected patients (83%). In two patients, we observed a shift in clone-type. We observed no sharing of clone-types between the 12 PCD patients and no patient-to-patient transmission. We provide an example of genetic relatedness and an example of a change in clone-type in [Supporting Information online](#).

Other bacteria

The number of patients with PCD colonized and chronically infected with *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Achromobacter xylosoxidans*, *Aspergillus fumigatus* and *M. catarrhalis* is given in [Supporting Information online](#). Fig. 1 illustrates how different Gram-negative bacteria vary by age and reports if the incidence differs significantly ($p < 0.05$) across age groups.

Non-tuberculous mycobacteria (*Mycobacterium abscessus*) was isolated once from a single patient in 2010.

Discussion

In this comprehensive, retrospective study we present an overview of the bacterial flora from the lower respiratory tract in patients with PCD followed during an 11-year period in the Danish national PCD Centre. We also present novel data concerning the genetic relatedness of *P. aeruginosa* strains in individual patients with PCD who have chronic infections.

In patients with PCD, the progression of lung disease may result from continuous pulmonary infections, inflammation and a change in the lower airway pathogens over time. *Haemophilus influenzae* and *M. catarrhalis* dominate in children, whereas *P. aeruginosa* is more common in teenagers and adults. This infection pattern resembles patients with CF, who are infected by a unique set of bacteria acquired in an age-dependent sequence; early infections are most frequently caused by *Staphylococcus aureus* and *H. influenzae*, and later *P. aeruginosa* and other Gram-negative bacteria take over [17]. However, in CF the diversity of bacteria decrease with age [18], which contrasts with our results (Fig. 1), and the pathogens described in the present study seem consistent with the pathogens demonstrated in patients with non-CF bronchiectasis [19,20].

Pseudomonas aeruginosa frequently colonizes the lower respiratory tract in patients with PCD and for the first time we found a significantly increased incidence with increasing age, which is only partially shown in previous studies [2,4]. The observation that early PCD diagnosis is correlated with a significant lower prevalence of *P. aeruginosa* is new. Chronic infection was observed in 39% of all patients at least once. This is markedly higher than previously reported; Maglione *et al.* [3] reported chronic infection in 8 out of 158 (5%) children with PCD. The difference may be because of differences in age, frequency of sputum sample collection and the criteria used to define chronic infection.

However, the criteria that we adopted from patients with CF [10] seem valid, as 83% of chronically infected patients harboured the same clone-type for years. Another explanation for the higher rate of chronic infection is the possibility of cross-infections from other PCD patients; however, genetically unrelated isolates were found in the 12 patients, indicating a lack of transmission between patients and a successful isolation strategy.

Importantly, part of the sputum data from 54 children from the present study was also included in the above-mentioned study by Maglione et al. [3], which is a limitation. However, there are several major differences between the two studies. Maglione et al. focused mainly on nutrition and lung function. Our study focuses on the bacterial flora from the lower airways verified by microscopy in a national centre with well-established treatment regimens in the entire study period. Maglione et al. did not exclude samples with only oral flora and included children from different European centres, which may have different treatment regimens. Further, Maglione et al. did not correlate bacterial flora with age or age at diagnosis, or discuss definitions of infection status, which are novel analyses.

The observed chronic infection pattern with intermediate periods without infection may be unique for PCD and different from that observed in CF. The reason for this difference may be that patients with PCD have a milder lung disease compared with CF patients, which is exemplified by a later age at diagnosis 7.8 years versus 6–12 months in CF. In CF, eradication of *P. aeruginosa* from chronically infected lungs is rarely achieved [21]. The observed infection pattern in PCD may support the possibility of a non-pulmonary bacterial reservoir for repeated pulmonary infections, which may be the upper airways [22] as in patients with CF [23–25]. Future studies are needed to examine a potential benefit of sinus surgery in patients with PCD.

Our results indicate that *P. aeruginosa* is a common and important pathogen in PCD, colonizing 15–47% of patients annually. This is supported by Davis et al. [5], who found *P. aeruginosa* in the lungs of 9% of children with PCD in a cross-sectional study. However, chronic lung infection with *P. aeruginosa* may have a smaller impact on lung function in PCD patients than in CF patients, which is in agreement with previous studies [3,5,26] that found no correlation between lower respiratory tract pathogens and lung function.

In agreement with previous studies [2–5,27], non-capsulate *H. influenzae* was the most frequently isolated bacterium. Chronic infection was common, but most patients were not chronically infected in successive years, suggesting an effective treatment regimen. However, to date no controlled trials examining the effect of antibiotic eradication therapy in PCD patients have been published [6].

Non-tuberculous mycobacteria were isolated from only one patient during the study period, which contradicts previous

reports by Davis et al. [5], who found non-tuberculous mycobacteria in 3% of children with PCD, and Noone et al. [2], who isolated non-tuberculous mycobacteria in 10% of adult PCD patients. A possible explanation for the low prevalence of non-tuberculous mycobacteria may be a limited diagnostic focus on these bacteria until recent years in our centre.

We find it relevant that a uniform definition of infection status was established in patients with PCD, as this could be useful concerning strategies for antibiotic therapy, cohort-isolation, and for publication purposes and comparative clinical studies. We adopted definitions established in CF to define lung infection status in PCD, which may not be optimal. Chronic infection implies persistence of the bacteria in the focus (lungs/sputum). It may seem contradictory that some patients categorized with chronic infection are later considered not chronically infected. Hence, it may be beneficial to introduce a heritable component to the criteria as suggested in CF by Green et al. [28], where persistent infection is defined as three consecutive years of culture data with at least one positive culture in at least two of the three years. Another limitation is the exclusive use of standard culture techniques, which may underestimate the diversity of potential pathogens from the lower airways [18]. However, using microscopy on all samples ensures representation from the lower airways.

The levels of precipitins against *P. aeruginosa* are used in our centre because serology may be more sensitive than cultures in detecting early chronic respiratory tract infections [17]. However, as in the case of vaccination, antibodies are still measurable long after the antigen/bacteria have been eliminated (immunological memory), so detection of antibodies in culture-negative patients where the bacteria have been successfully eliminated by antibiotics is not indicative of actual chronic infection. The use of precipitins in patients with PCD therefore requires further research.

Here, we present a comprehensive study of a national cohort of 107 Danish patients with PCD including extensive longitudinal microbiological follow up for 11 years, allowing new insights into our understanding and definition of bacterial colonization and infection in the lower airways of patients with PCD.

Transparency declaration

The authors declare that they have no conflicts of interest.

Authors' contributions

All authors contributed to the conception of this work, revised it critically for important intellectual content, interpreted the

data, and approved the final version to be published. MA acquired and analysed data and drafted the work.

Funding

The Candys Foundation, a non-profit organization, supported Mikkel Christian Alanin, MD as a PhD stipend. The Novo Nordisk Foundation supported Helle Krogh Johansen, MD, DMSc as a clinical research stipend. Kim Gjerum Nielsen, MD, DMSc received funding from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement n8305404 (BESTCILIA).

Acknowledgements

We thank laboratory technicians Ulla Rydal Johansen, Lena Nørregaard, Katja Bloksted and Bonnie Horsted Eriksen (Department of Clinical Microbiology) for their dedication to the project. We also wish to acknowledge Maria Philipsen (Danish PCD Centre) for her diagnostic skills and for providing and keeping data files on this cohort. Karl Bang Christensen (Section of Biostatistics, University of Copenhagen) is thanked for statistical advice.

Appendix A. Supporting information

Supporting information related to this article can be found at <http://dx.doi.org/10.1016/j.cmi.2015.08.020>.

References

- [1] Werner C, Onnebrink JG, Omran H. Diagnosis and management of primary ciliary dyskinesia. *Cilia* 2015;4:2.
- [2] Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, et al. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004;169:459–67.
- [3] Maglione M, Bush A, Nielsen KG, Hogg C, Montella S, Marthin JK, et al. Multicenter analysis of body mass index, lung function, and sputum microbiology in primary ciliary dyskinesia. *Pediatr Pulmonol* 2014;49:1243–50.
- [4] Rogers GB, Carroll MP, Zain NM, Bruce KD, Lock K, Walker W, et al. Complexity, temporal stability, and clinical correlates of airway bacterial community composition in primary ciliary dyskinesia. *J Clin Microbiol* 2013;51:4029–35.
- [5] Davis SD, Ferkol TW, Rosenfeld M, Lee HS, Dell SD, Sagel SD, et al. Clinical features of childhood primary ciliary dyskinesia by genotype and ultrastructural phenotype. *Am J Respir Crit Care Med* 2015;191:316–24.
- [6] Barbato A, Frischer T, Kuehni CE, Snijders D, Azevedo I, Baktai G, et al. Primary ciliary dyskinesia: a consensus statement on diagnostic and treatment approaches in children. *Eur Respir J* 2009;34:1264–76.
- [7] Johansen HK, Moskowitz SM, Ciofu O, Pressler T, Høiby N. Spread of colistin resistant non-mucoid *Pseudomonas aeruginosa* among chronically infected Danish cystic fibrosis patients. *J Cyst Fibros* 2008;7:391–7.
- [8] Ojeniyi B, Frederiksen B, Høiby N. *Pseudomonas aeruginosa* cross-infection among patients with cystic fibrosis during a winter camp. *Pediatr Pulmonol* 2000;29:177–81.
- [9] Johansen HK, Norregaard L, Gotzsche PC, Pressler T, Koch C, Høiby N. Antibody response to *Pseudomonas aeruginosa* in cystic fibrosis patients: a marker of therapeutic success? – a 30-year cohort study of survival in Danish CF patients after onset of chronic *P. aeruginosa* lung infection. *Pediatr Pulmonol* 2004;37:427–32.
- [10] Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *J Cyst Fibros* 2003;2:29–34.
- [11] Proesmans M, Balinska-Miskiewicz W, Dupont L, Bossuyt, Verhaegen J, Høiby N, et al. Evaluating the “Leeds criteria” for *Pseudomonas aeruginosa* infection in a cystic fibrosis centre. *Eur Respir J* 2006;27:937–43.
- [12] Standardization of Spirometry. 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;152:1107–36.
- [13] Høiby N, Koch C. Maintenance treatment of chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. *Thorax* 2000;55:349–50.
- [14] Høiby N, Jacobsen L, Jørgensen BA, Lykkegaard E, Weeke B. *Pseudomonas aeruginosa* infection in cystic fibrosis. Occurrence of precipitating antibodies against *Pseudomonas aeruginosa* in relation to the concentration of sixteen serum proteins and the clinical and radiographical status of the lungs. *Acta Paediatr Scand* 1974;63:843–8.
- [15] Høiby N, Flensburg EW, Beck B, Friis B, Jacobsen SV, Jacobsen L. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. *Scand J Resp Dis* 1977;58:65–79.
- [16] Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;33:2233–9.
- [17] Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003;168:918–51.
- [18] Goddard AF, Staudinger BJ, Dowd SE, Joshi-Datar A, Wolcott RD, Aitken ML, et al. Direct sampling of cystic fibrosis lungs indicates that DNA-based analyses of upper-airway specimens can misrepresent lung microbiota. *Proc Natl Acad Sci USA* 2012;109:13769–74.
- [19] Pasteur MC, Bilton D, Hill AT. British Thoracic Society guideline for non-CF bronchiectasis. *Thorax* 2010;65(Suppl. 1):i1–58.
- [20] Kapur N, Grimwood K, Masters IB, Morris PS, Chang AB. Lower airway microbiology and cellularity in children with newly diagnosed non-CF bronchiectasis. *Pediatr Pulmonol* 2012;47:300–7.
- [21] Marvig RL, Sommer LM, Molin S, Johansen HK. Convergent evolution and adaptation of *Pseudomonas aeruginosa* within patients with cystic fibrosis. *Nat Genet* 2015;47:57–64.
- [22] Alanin MC, Johansen HK, Aanaes K, Høiby N, Pressler T, Skov M, et al. Simultaneous sinus and lung infections in patients with primary ciliary dyskinesia. *Acta Otolaryngol* 2015;135:58–63.
- [23] Johansen HK, Aanaes K, Pressler T, Nielsen KG, Fisker J, Skov M, et al. Colonisation and infection of the paranasal sinuses in cystic fibrosis patients is accompanied by a reduced PMN response. *J Cyst Fibros* 2012;11:525–31.
- [24] Jelsbak L, Johansen HK, Frost AL, Thøgersen R, Thomsen LE, Ciofu O, et al. Molecular epidemiology and dynamics of *Pseudomonas aeruginosa*

- populations in lungs of cystic fibrosis patients. *Infect Immun* 2007;75:2214–24.
- [25] Bonestroo HJ, de Winter-de Groot KM, van der Ent CK, Arets HG. Upper and lower airway cultures in children with cystic fibrosis: do not neglect the upper airways. *J Cyst Fibros* 2010;9:130–4.
- [26] Marthin JK, Petersen N, Skovgaard LT, Nielsen KG. Lung function in patients with primary ciliary dyskinesia: a cross-sectional and 3-decade longitudinal study. *Am J Respir Crit Care Med* 2010;181:1262–8.
- [27] Hosie PH, Fitzgerald DA, Jaffe A, Birman CS, Rutland J, Morgan LC. Presentation of primary ciliary dyskinesia in children: 30 years' experience. *J Paediatr Child Health* 2015;51:722–6.
- [28] Green DM, Collaco JM, McDougal KE, Naughton KM, Blackman SM, Cutting GR. Heritability of respiratory infection with *Pseudomonas aeruginosa* in cystic fibrosis. *J Pediatr* 2012;161:290–5.