Associations between respiratory pathogens and lung function in primary ciliary dyskinesia: cross-sectional analysis from the PROVALF-PCD cohort

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Shareable abstract (@ERSpublications)
This large international study found that PCD patients with *Pseudomonas aeruginosa* lung infection have impaired lung function, particularly adults. The findings suggest that close monitoring and eradication of the pathogen are needed. https://bit.ly/4cNM6ts

Cite this article as: Rubbo B, Kant A, Zhang K, *et al*. Associations between respiratory pathogens and lung function in primary ciliary dyskinesia: cross-sectional analysis from the PROVALF-PCD cohort. *ERJ Open Res* 2024; 10: 00253-2024 [DOI: 10.1183/23120541.00253-2024].

Received: 22 March 2024
Accepted: 3 June 2024

Abstract

Introduction Respiratory pathogens are frequently isolated from airway samples in primary ciliary dyskinesia (PCD) patients. Few studies have investigated associations between these pathogens and lung function, with current management based on evidence from cystic fibrosis. We investigated the association between commonly isolated respiratory pathogens and lung function in PCD patients.

Methods Using a cross-sectional design, we prospectively collected clinical and concurrent microbiology data from 408 participants with probable or confirmed PCD, aged ⩾5 years, from 12 countries. We used Global Lung Function Initiative 2012 references to calculate forced expiratory volume in 1 s (FEV_1) z-scores. For 351 patients (86%) with complete data, we assessed the association of the four most frequently isolated pathogens with lung function by fitting multilevel linear models with country as random intercept, adjusted for age at diagnosis, age at lung function, use of antibiotic prophylaxis and body mass index z-scores.

Results Individuals with *Pseudomonas aeruginosa* growth in culture had significantly lower FEV_1 z-scores (β= −0.87, 95% CI −1.40– −0.34), adjusted for presence of *Haemophilus influenzae*, methicillin-sensitive *Staphylococcus aureus* and *Streptococcus pneumoniae*, and for covariates. When stratified by age, associations remained strong for adults but not for children. Results were similar when ciliary defects by transmission electron microscopy were included in the models and when restricting analysis to only confirmed PCD cases.

Conclusions We found that *P. aeruginosa* was associated with worse lung function in individuals with PCD, particularly adults. These findings suggest that it is prudent to aim for *P. aeruginosa* eradication in the first instance, and to treat exacerbations promptly in colonised patients.

Introduction

Primary ciliary dyskinesia (PCD) is a rare genetic disease characterised by abnormality of motile cilia movement. Asynchronous, impaired or absent ciliary movement in people with PCD leads to the accumulation of mucus, bacteria and cell debris in lower airways, which can result in repeated airway infections from birth.

There are no evidence-based guidelines for PCD management, with most consensus strategies derived from cystic fibrosis (CF), despite the fact that the two diseases have different pathophysiology [1–3]. In CF, infections with certain respiratory pathogens are linked to worse lung function, particularly in patients with chronic infection with *Pseudomonas aeruginosa*. Some of the pathogens found in the lower airway microbiology of patients with CF can also be found in patients with PCD and the current recommendation for both CF and PCD patients is early eradication of *P. aeruginosa* upon initial detection [1, 4–6]. However, there is limited evidence on the effect of different respiratory pathogens on lung function, most of which was based on retrospectively collected observational data [7, 8].

We aimed to investigate the association between commonly isolated respiratory pathogens and lung function in a large international cohort of participants with PCD.

Methods

Study population and design

The Prospective Observational Multicentre Study on Variability of Lung Function in Stable PCD patients (PROVALF-PCD) is a large, international cohort initiated during the COST-Action Better Experimental Approaches to Treat PCD (BEAT-PCD) Clinical Research Collaboration [9–12]. We prospectively collected information on patient demographics, clinical history, diagnostic tests, appointment data, microbiology and spirometry results from participating PCD centres. We included individuals that were being treated as PCD, with either a confirmed, highly likely or inconclusive (pending additional confirmatory tests) diagnosis of PCD according to the European Respiratory Society (ERS) diagnostic guidelines, *i.e.* those with bi-allelic causative mutations in PCD-related genes or hallmark defects on transmission electron microscopy (TEM) were considered confirmed cases [13]. Only participants with supportive diagnostic data (PCD positive or PCD highly likely) were included in the statistical modelling.

We analysed cross-sectional data from baseline visits from all participants recruited into the PROVALF-PCD cohort between August 2017 and July 2018. All contributors were provided with a data dictionary prior to the start of the study, which contained definitions of the variables to be collected in the PROVALF-PCD study (supplementary materials).

Data collection platform

Pseudonymised data were collected by participating centres using a paper-based case report form (see supplementary material) and entered into an online database, available through Research Electronic Data

Capture (REDCap). REDCap is a secure, web-based software platform designed to support data capture for research studies, with audit trails for tracking data management. Each contributor has access only to their own centre's data [14, 15].

Respiratory pathogens

Microbiology cultures were prospectively routinely collected during the clinical appointment and tested at each institution's microbiology laboratory. The type of respiratory tract sample and presence or absence of each pathogen in culture sample was recorded. Each participant could have multiple pathogens detected per sample.

Clinical outcomes

Our main outcome was spirometry-derived lung function. Measurements were prospectively taken at the time of recruitment at each centre, using their institution's spirometry equipment and following a study-specific standard operating procedure (SOP), adapted from Miller *et al.* [16]. The SOP included information for the use and calibration of spirometry equipment, test procedures, and quality control. Centres submitted the spirometry report including flow–volume curves for five consecutive participants recruited from their centre in order to enable quality control and ensure standardisation of measuring and reporting across centres. We excluded individuals aged <5 years at the time of recruitment, as younger children might not be able to perform spirometry reliably.

We calculated forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$) z-scores using the Global Lung Function Initiative (GLI) 2012 references [17]. These values are adjusted for sex, age, height and ethnicity, all of which were determined by clinicians or self-declared by the participant on the day of the lung function test.

Research ethics and data protection

This study was approved by the Ethics and Research Governance Online service provided by the University of Southampton (identifier 27420), under wider approval from the Research Ethics Service for collection of clinical data for use in research (NRES Committee South Central Hampshire A Ethics 06/Q1702/109).

Appropriate ethics and research development approvals were obtained locally by each of the participating centres. Data originating from participating centres were pseudonymised locally before transfer to the University of Southampton for analysis, adhering to international data transfer agreements signed by all parties involved.

Study data were handled under the Data Protection Act of 1998 and the European Union General Data Protection Regulation. Confidential data from the University of Southampton were kept securely in password-protected spreadsheets, separate from clinical data.

Statistical analysis

We described the full cohort of participants recruited into PROVALF-PCD as all were being managed in PCD centres where the clinician believed the diagnosis was PCD. Continuous variables were described as median and interquartile range. Categorical variables were described as total numbers and proportions.

To assess the association between different pathogens and lung function, we fitted random intercept multilevel linear models to account for unobserved country-level differences. We constructed unadjusted models with the four most commonly isolated pathogens and an adjusted model, to assess the simultaneous impact of multiple pathogens on lung function. Potential confounders were selected using a directed acyclic graph, based on knowledge derived from the literature (figure 1). Covariates for the adjusted models were age at diagnosis (continuous), age at lung function (continuous), use of antibiotic prophylaxis (binary) and body mass index (BMI) z-scores (continuous). We conducted complete case analysis by case-wise deletion. We calculated the Akaike Information Criterion and the Bayesian Information Criterion to assess model fit. The same models were fitted for the following outcome measures: FEV_1 z-scores, FVC z-scores and $FEF_{25–75\%}$ z-scores. We only included individuals with a confirmed or highly likely diagnosis of PCD, based on the diagnostic data provided to us by the centres, in these models.

We performed several sensitivity analyses to assess the robustness of our findings. First, we included only participants with confirmed diagnosis of PCD according to the ERS diagnostic guidelines [13]. Secondly, we repeated our analyses without including antibiotic prophylaxis in the model, because this could have led to collider bias. Thirdly, we included only patients who were in stable conditions. Fourthly, we divided

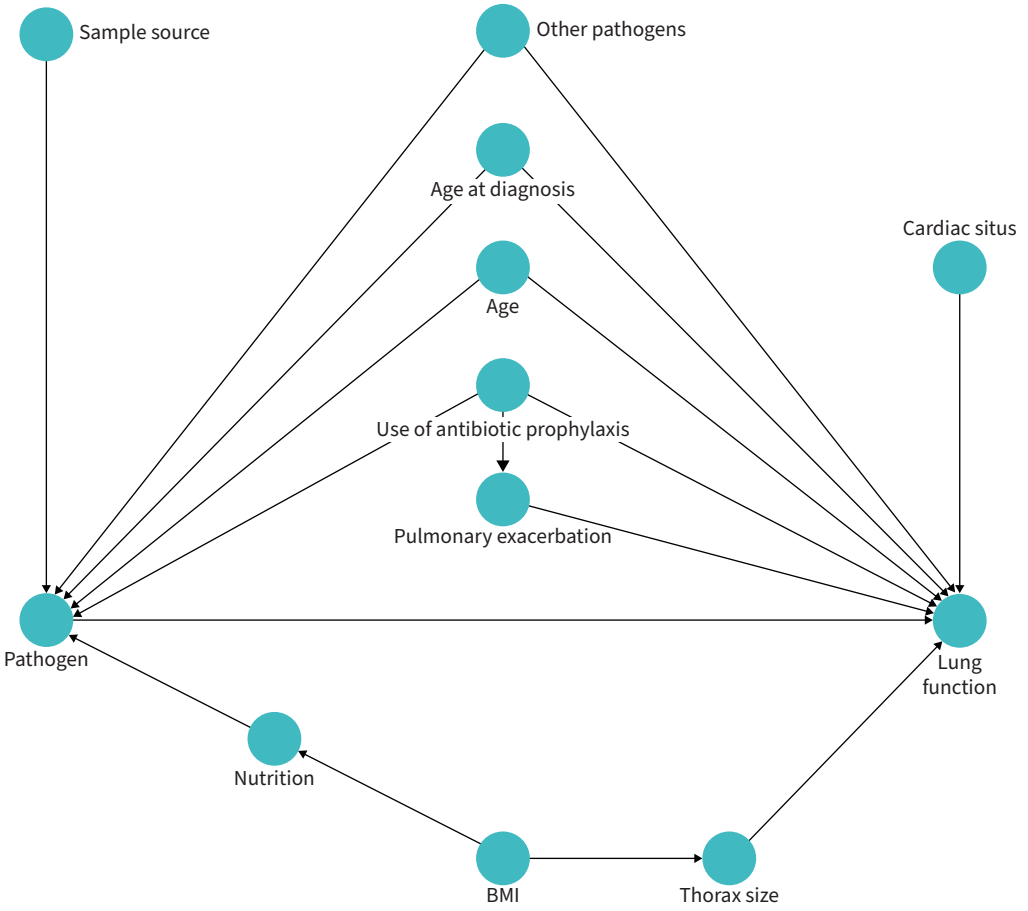

FIGURE 1 Directed acyclic graph for the association between respiratory pathogens and lung function at baseline, with potential confounders and mediators. BMI: body mass index.

our cohort into paediatric and adult participants and applied a linear regression model to each population separately, adjusted for country (as fixed effects) and covariates. Fifthly, we included interaction terms between each pathogen and age categorised as adult or children. Lastly, we included TEM defects in our main model as a covariate, in order to assess if there were any significant differences in the association of pathogens and lung function.

We used R version 4.2.1 (www.r-project.org) for all statistical analyses. The packages tidyverse, lme4, sjPlot, jtools and ggpubr were used to construct our models and plot our results.

Results

Population characteristics

We included baseline data collected at the first clinical assessment for 408 participants from 19 PCD centres in 12 countries (table 1). There were 280 paediatric (69%) and 128 adult (31%) cases at baseline. Most individuals from Australia, France, Italy, Portugal, Spain, Turkey and England were children, while the majority from Cyprus and Denmark were adults.

Most individuals were White (88%) and half were male. In terms of past medical history, 27% were from consanguineous parents, 55% had neonatal chest symptoms, 42% had been admitted to neonatal intensive care units, and 6% were born pre-term. Most reported a history of wet cough (85%) and rhinosinusitis (76%), and approximately half reported chronic ear symptoms (*i.e.* chronic otitis media with effusion or hearing loss). *Situs inversus* was present in 43% of cases, *situs ambiguous* in 2%, and congenital heart defect in 11%. The median age at diagnosis was 6 years (range 0–63 years) and the median age at measurement of lung function was 15 years (range 5–71 years) (table 1 and supplementary table S1).

TABLE 1 Demographics, clinical characteristics and history of participants

	Total	Children	Adults
Participants	408	280	128
Age at lung function, years	15 (11–20)	13 (9–15)	25 (21–36)
Age at diagnosis, years	6 (2–11)	5 (0–8)	11 (6–18)
Country			
Australia	16 (4)	13 (5)	3 (2)
Belgium	29 (7)	15 (5)	14 (11)
Cyprus	24 (6)	5 (2)	19 (15)
Czech Republic	19 (5)	13 (5)	6 (5)
Denmark	40 (10)	14 (5)	26 (20)
England	63 (15)	59 (21)	4 (3)
France	21 (5)	19 (7)	2 (2)
Germany	40 (10)	25 (9)	15 (12)
Italy	56 (14)	41 (15)	15 (12)
Portugal	8 (2)	7 (3)	1 (1)
Spain	27 (7)	22 (8)	5 (4)
Turkey	65 (16)	47 (17)	18 (14)
Female	206 (50.5)	136 (49)	70 (55)
Ethnicity			
White	359 (88)	240 (86)	119 (93)
Black	4 (1)	4 (1)	0 (0)
North-East Asian	1 (0.2)	1 (0.5)	0 (0)
South-East Asian	3 (1)	3 (1)	0 (0)
Other/mixed	41 (10)	32 (11)	9 (7)
BMI z-score	0.1 (−0.6–1.0)	0.04 (−0.7–0.8)	0.2 (−0.5–1.3)
Presence of persistent cough			
No	122 (30)	86 (31)	36 (28)
Yes, wet cough	265 (66)	179 (64)	86 (67)
Yes, dry cough	6 (1)	5 (2)	1 (1)
Yes, unspecified	11 (3)	6 (2)	5 (4)
Increase in sputum volume since last clinic appointment	72 (26)	46 (16)	26 (20)
Change in sputum colour since last clinic appointment	36 (13)	20 (7)	16 (13)
Shortness of breath	35 (9)	20 (7)	15 (12)
Pulmonary exacerbation at clinic appointment	66 (16)	44 (16)	22 (17)
Antibiotic prophylaxis	51 (13)	33 (12)	18 (14)

Data are presented as n, median (interquartile range) or n (%). BMI: body mass index.

PCD diagnostics

There were 194 individuals (48%) classified as confirmed PCD cases according to the ERS diagnostic guidelines [13] (supplementary table S2). Bi-allelic mutations in causative PCD genes were found in 74 individuals. Only those with hallmark defects on TEM (*i.e.* isolated outer dynein arm (ODA) defect, combined ODA and inner dynein arm (IDA) defect, or IDA defect with microtubular disarrangement) were considered as having a confirmed diagnosis of PCD by TEM [13]. In those who did not have a confirmatory genetic test or where no known mutation was detected, 55 individuals had ODA defect, 42 had combined ODA and IDA defect, and 23 had IDA defect with microtubular disarrangement. 161 participants (39%) had a highly likely diagnosis according to the ERS diagnostic guidelines and 19 participants (5%) had an inconclusive diagnosis. The remaining 34 participants (8%) had missing data on diagnostic testing.

Microbiology

We obtained microbiological culture samples from 382 individuals (94% of total participants). Most samples were expectorated sputum (n=328, 86%). Other sample methods included throat swabs (n=18, 5%), cough swabs (n=10, 3%), bronchoalveolar lavage (n=3, 1%), laryngeal swabs (n=2, 0.5%) and nasal swabs (n=1, 0.5%). We did not have data on the method of sample collection for 45 individuals (14%).

At least one pathogen was detected in 234 individuals (61%) for whom we had a culture sample collected, of which 160 were children and 74 were adults. *Haemophilus influenzae* was the most commonly isolated respiratory pathogen, found in 120 individuals, which corresponds to 31% of those who had a culture

sample collected (table 2). Methicillin-sensitive *Staphylococcus aureus* (MSSA) was detected in 49 individuals (13%), *P. aeruginosa* in 30 individuals (8%) and *Streptococcus pneumoniae* in 30 individuals (8%) (table 2). Other pathogens were rarely isolated, *e.g.* only three individuals had nontuberculous mycobacteria isolated from culture.

The frequency and type of pathogens isolated from microbiological culture varied by age, with a greater proportion of *H. influenzae* and *S. pneumoniae* found in samples from participants aged <18 years, and *P. aeruginosa* in older participants (figure 2).

Lung function measurements

We obtained FEV_1 z-scores for 401 individuals (seven had missing data), FVC z-scores for 400 participants (eight had missing data) and $FEF_{25–75\%}$ z-scores for 382 participants (26 had missing data) at their clinical appointment. The median (interquartile range) FEV_1 z-score was −1.72 (−2.74– −0.65), FVC z-score was −0.88 (−1.89–0.06) and $FEF_{25–75\%}$ z-score was −1.81 (−2.84– −0.93).

Spirometry indices varied between countries (supplementary figure S1). Individuals from Australia had the highest FEV_1 z-score (median −0.84), while those from Cyprus (median −2.32), Denmark (median −2.24) and Turkey (median −2.24) had the lowest FEV_1 z-scores (supplementary table S3).

Association between respiratory pathogens and lung function

We included 351 individuals (86%) in our models. These participants had a confirmed or highly likely diagnosis of PCD (n=355) and also had complete data for all covariates (n=351; four participants with missing data were excluded from these analyses). In the adjusted models, individuals with *P. aeruginosa* isolated in their culture had significantly lower FEV_1 z-scores (β= −0.87, 95% CI −1.40– −0.34) compared to those who did not have the pathogen isolated from microbiological cultures. There were no differences in FEV_1 z-scores in those that had *H. influenzae* (β= −0.04, 95% CI −0.36–0.28), MSSA (β=0.03, 95% CI −0.40–0.46) or *S. pneumoniae* (β= −0.21, 95% CI −0.74–0.33) compared to those who did not have these pathogens isolated from culture, adjusted for covariates (table 3). We found similar results for the association between *P. aeruginosa* and FVC z-scores (supplementary table S4) and $FEF_{25–75\%}$ z-scores (supplementary table S5). We also found a positive association between FEV_1 z-scores and BMI z-scores.

The unadjusted models showed a similar pattern. Patients with *P. aeruginosa* detected in culture sample had considerably lower FEV_1 z-scores (−1.19, 95% CI −1.75– −0.63) compared to those without the pathogen, while those with MSSA and *S. pneumoniae* had slightly lower FEV_1 z-scores (−0.14 (95% CI −0.59–0.32) and −0.23 (95% CI −0.79–0.33), respectively) compared to those without the pathogens. However, participants with *H. influenzae* isolated from culture sample had slightly higher FEV_1 z-scores (0.12, 95% CI −0.22–0.45) in the unadjusted models.

When we included only confirmed PCD cases in the sensitivity analysis (n=194), presence of *P. aeruginosa* remained significantly associated with lower FEV_1 and FVC z-scores. Results were also

TABLE 2 Presence of pathogens isolated by microbiology

	Total	Children	Adults
Participants with culture sample	382	266	116
Aspergillus fumigatus	10 (3)	6 (2)	4 (4)
Candida	24 (6)	17 (6)	7 (6)
Haemophilus influenzae	120 (31)	101 (38)	19 (16)
Pseudomonas aeruginosa	30 (8)	9 (3)	21 (18)
Streptococcus pneumoniae	30 (8)	23 (9)	7 (6)
Streptococcus pyogenes	4 (1)	3 (1)	1 (1)
Moraxella catarrhalis	18 (5)	12 (5)	6 (5)
MSSA	49 (13)	35 (13)	14 (12)
MRSA	3 (1)	2 (1)	1 (1)
Other pathogens	33 (9)	16 (6)	17 (15)
No growth	108 (28)	70 (26)	38 (33)

Data are presented as n or n (%). More than one pathogen could have been isolated for each individual. MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *S. aureus*.

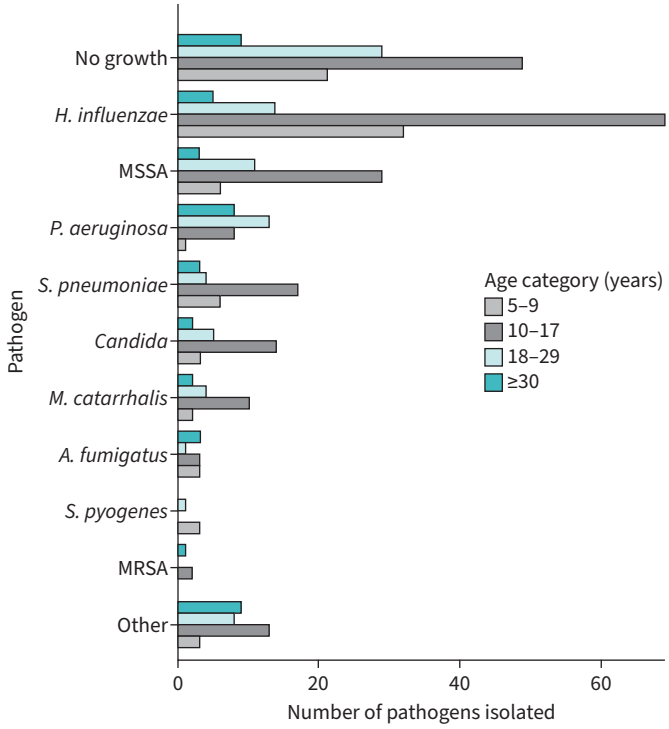

FIGURE 2 Pathogens isolated from 382 participants, collected at the baseline assessment and stratified by age group. More than one pathogen was isolated from some individuals. *H. influenzae*: *Haemophilus influenzae*; MSSA: methicillin-sensitive *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. pneumoniae*: *Streptococcus pneumoniae*; *M. catarrhalis*: *Moraxella catarrhalis*; *A. fumigatus*: *Aspergillus fumigatus*; *S. pyogenes*: *Streptococcus pyogenes*; MRSA: methicillin-resistant *S. aureus*.

similar when including only patients in stable conditions, when excluding antibiotic prophylaxis, and when including ultrastructural defect by TEM (figure 3).

We found the same results for the model that included adults only when stratifying our analysis into paediatric and adult cases, and when adding an interaction term for adults/children and each pathogen (supplementary table S6 and figure 4). Presence of *P. aeruginosa* was no longer significantly associated with lower lung function when only children were included but the direction of the association remained the same (supplementary table S6). We also found that adult participants on antibiotic prophylaxis had significantly lower FEV_1 z-scores.

TABLE 3 Estimates for forced expiratory volume in 1 s z-scores for the full model, adjusted for the most common pathogens and covariates (n=351)

Predictors	Estimate (95% CI)	p-value
Intercept	−0.74 (−1.13– −0.36)	<0.001
Age at diagnosis, years	0.03 (0.01–0.05)	0.010
Respiratory pathogen isolated		
Haemophilus influenzae	−0.04 (−0.36–0.28)	0.813
Pseudomonas aeruginosa	−0.87 (−1.40– −0.34)	0.001
MSSA	0.03 (−0.40–0.46)	0.905
Streptococcus pneumoniae	−0.21 (−0.74–0.33)	0.451
Antibiotic prophylaxis	−0.28 (−0.59–0.03)	0.081
BMI z-scores	0.43 (0.32–0.55)	<0.001
Age at lung function, years	−0.06 (−0.08– −0.04)	<0.001

MSSA: methicillin-sensitive *Staphylococcus aureus*; BMI: body mass index.

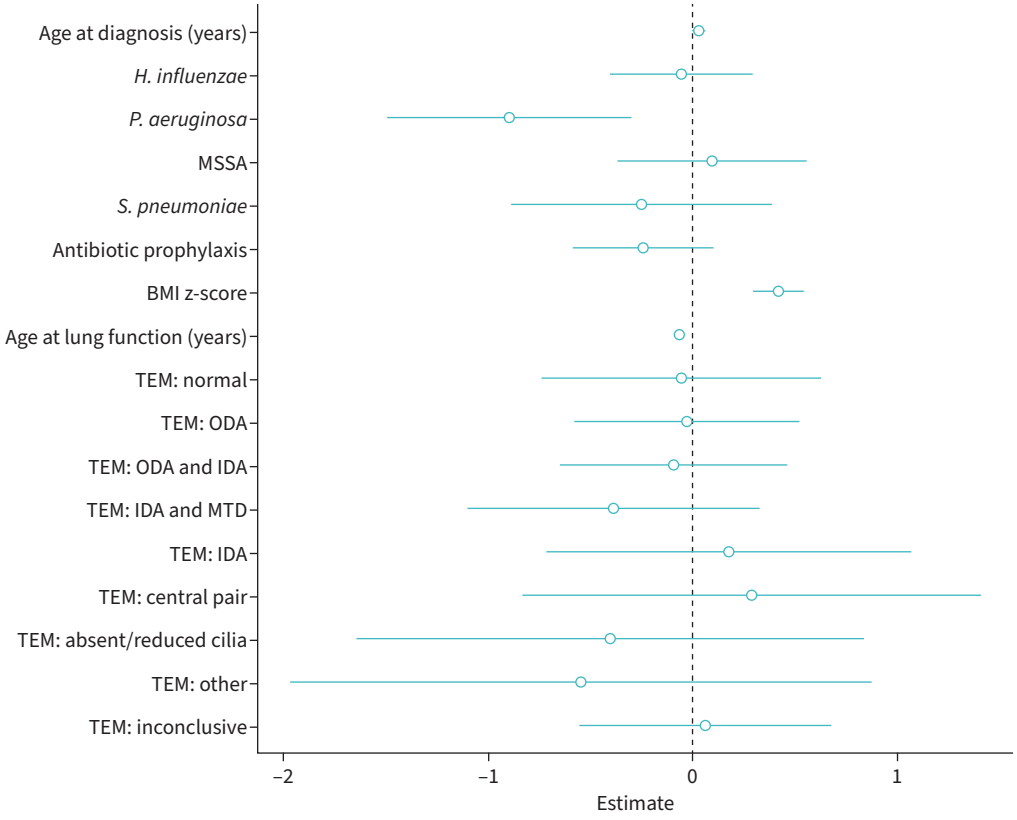

FIGURE 3 Estimates for forced expiratory volume in 1 s z-scores in the full model, adjusted for transmission electron microscopy (TEM) defects (n=351; reference category: normal TEM). *H. influenzae*: *Haemophilus influenzae*; *P. aeruginosa*: *Pseudomonas aeruginosa*; MSSA: methicillin-sensitive *Staphylococcus aureus*; *S. pneumoniae*: *Streptococcus pneumoniae*; BMI: body mass index; ODA: outer dynein arm; IDA: inner dynein arm; MTD: microtubular disarrangement.

Discussion

This is the first study to examine the association between pathogens and lung function in both paediatric and adult PCD populations using prospectively collected data. In this international study containing data from over 400 participants from 12 countries, we showed that adults with *P. aeruginosa* isolated from airway samples had significantly lower FEV_1, FVC and $FEF_{25–75\%}$ z-scores, adjusted for clustering on

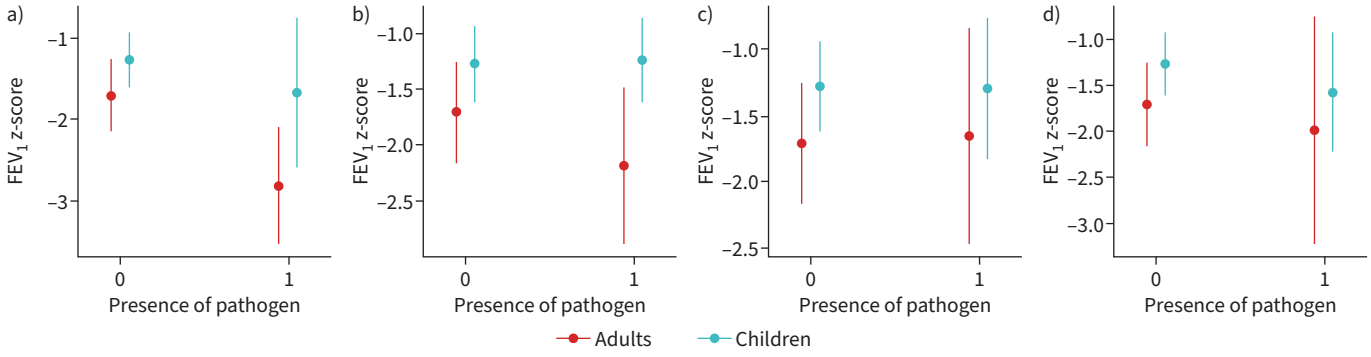

FIGURE 4 Predicted values of forced expiratory volume in 1 s (FEV_1) z-score for the full model, with interaction term between categorised age (*i.e.* adults and children) and each pathogen included in the model: a) *Pseudomonas aeruginosa*, b) *Haemophilus influenzae*, c) methicillin-sensitive *Staphylococcus aureus* and d) *Streptococcus pneumoniae*. Presence of pathogen: 0=not isolated; 1=isolated in culture.

country and for presence of other respiratory pathogens (*H. influenzae*, MSSA and *S. pneumoniae*), while results were less prominent for children. Without longitudinal data we cannot be certain that *P. aeruginosa* causes worse lung disease, but these findings indicate that it is prudent to aim for *P. aeruginosa* eradication in the first instance, and to treat exacerbations promptly in colonised patients.

In PCD, routine sputum monitoring is recommended for both children and adults [1, 3]. We found that the pathogen most frequently isolated from airways in children was *H. influenzae*, while *P. aeruginosa* was most commonly isolated in adults. These results are in line with findings from previous studies [6, 18–24]. The recently published international consensus on infection prevention and control for PCD patients recommends eradication of *P. aeruginosa* if isolated, even in asymptomatic cases, while *H. influenzae* and MSSA should only be treated if patients are symptomatic [1]. Our study adds evidence to support these recommendations.

Our findings suggest that presence of *P. aeruginosa* has a negative impact on lung disease in patients with PCD, since we have shown that presence of *P. aeruginosa* is associated with worse lung function; a prospective longitudinal study would be needed to confirm this. These findings corroborated those from studies that showed that colonisation by *P. aeruginosa* was associated with lower baseline FEV_1 [6, 19]. However, the authors described a decline in FEV_1 that was similar between those that were colonised and those that were not [6]. Similar results were observed in adults with PCD [25]. Kos *et al.* [26] reported a decline in lung function that was steeper in patients with *P. aeruginosa* infection in a geographically isolated Dutch population with a specific founding mutation in the *CCDC114* gene. Elevated concentrations of sputum proteases and cytokines have been associated with impaired lung function and chest structural damage and, importantly, were higher in patients with positive sputum cultures for common bacterial pathogens [27]. Moreover, a recent retrospective single-centre study demonstrated that early eradication antibiotic therapy for *P. aeruginosa* in 55 children with PCD is highly effective, with 70% negative culture results after 1 year of treatment, although no data were shown on the effects on lung function [28]. In our study, we could not discard the possibility that individuals with lower FEV_1 z-scores might have a higher risk of becoming infected with *P. aeruginosa*, due to the cross-sectional design.

We found a clear association between the presence of *P. aeruginosa* and lower lung function indices in the adult population. In children, the results were not statistically significant; however, the association was in the same direction. This could reflect a reduced power to detect smaller differences in the outcome, a smaller effect size, or the fact that we were not able to differentiate between acute and chronic infection. Chronic infection with *P. aeruginosa*, which is more likely in adults with PCD, might have a more detrimental effect on lung function trajectory compared to acute infections. A previous study found that PCD patients with chronic colonisation have worse spirometry and multiple-breath washout results in the short-term [24]. In adults with bronchiectasis, presence of *P. aeruginosa* has been linked to increased frequency of exacerbations and worse health-related quality of life [29]. Further studies are needed to better understand this relationship in PCD, particularly in children.

We found differences in the median lung function of individuals included from the 12 countries. These might be due to the proportion of adults and children from each country, differences in case ascertainment, treatment strategy or body dimensions, or perhaps more markedly due to differences in underlying genetic make-up of patients between countries [30]. Patients with mutations in certain genes have lower values of FEV_1 z-score and these mutations might be more or less prevalent in specific populations [26, 31–34]. Halbeisen *et al.* [8] reported significant differences in lung function trajectories between countries in a similar population to ours.

To our knowledge, this is the largest study on the association between respiratory pathogens and lung function in PCD. The random multilevel study design allowed us to consider differences between countries such as the frequency of management appointments, including microbiology analysis and lung function measurements and differences in expectorated sputum sample processing. Additionally, our data were collected prospectively and recorded using a standardised protocol as part of the PROVALF-PCD study. The quality of spirometry data from each centre was checked for five consecutive participants recruited into the study, in order to ensure standardisation of measurements across settings.

Our study has limitations. We restricted our models to only include the four most frequently isolated pathogens, due to limited sample size for this rare disease. We had a confirmed genetic diagnosis in only approximately a fifth of the study participants, probably because routine genetic testing was less common when the study was conducted and a smaller number of disease-causative genes had been described. We did not have access to radiological data and therefore could not ascertain presence or severity of

bronchiectasis in patients included in our study. Since this was a cross-sectional analysis, we were unable to differentiate between chronic colonisation and acute infection. Due to the cross-sectional design of these analyses, we were also unable to investigate reverse causality and we therefore could not discard the possibility that patients with impaired lung function were more susceptible to infection by respiratory pathogens such as *P. aeruginosa*. Longitudinal analyses of the association between microbiology and lung function in patients with PCD are needed.

In conclusion, this large international study suggests that patients with PCD and *P. aeruginosa* lung infection have impaired lung function. Until prospective, longitudinal confirmation is available, this study suggests that clinicians should regularly monitor for *P. aeruginosa*, aiming to eradicate infection when it occurs.

Provenance: Submitted article, peer reviewed.

Acknowledgements: This work was presented in a poster discussion session at the ERS International Congress in 2023.

Ethics statement: This study was approved by the Ethics and Research Governance Online service provided by the University of Southampton (identifier 27420), under wider approval from the Research Ethics Service for collection of clinical data for use in research (NRES Committee South Central Hampshire A Ethics 06/Q1702/109). Appropriate ethics and research development approvals were obtained locally by each of the participating centres.

Conflict of interest: M. Boon declares receiving grants or contracts from the Fund Alphonse and Jean Forton, managed by the King Baudouin Foundation, and by the Belgian Cystic Fibrosis Association (number 2020-J1810150-217926); and payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Vertex. S.B. Carr declares receiving payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Chiesi Pharmaceuticals, and participation on a Data Safety Monitoring Board or Advisory Board for Vertex Pharmaceuticals. S. Dillenhöfer declares receiving support for attending meetings and/or travel from Vertex (Advance Program 2022). B. Karadag declares receiving payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from VEM Ilac, Abdi Ibrahim and OMRON, and participating on a Data Safety Monitoring Board or Advisory Board for Abdi Ibrahim. C. Koerner-Rettberg declares receiving speaker honoraria from Berlin Chemie, and advisory board participation and being a member of the medical board of the German PCD patient organisation. V. Martinu declares having received grants or contracts from Ministry of Health of the Czech Republic (grant number NV19-07-00210) and participation on an advisory board for indication of tobramycin in noncystic fibrosis bronchiectasis for Chiesi, and receiving payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Chiesi Pharmaceuticals. A. Moreno-Galdó declares receiving personal payment for lectures from AstraZeneca, Sanofi-Pasteur and Janssen, receiving support for attending meetings or travel from Sanofi-Pasteur and Vivisol, participation on a Data Safety Monitoring Board or Advisory Board for AstraZeneca and Sanofi-Pasteur, and is President of the Spanish Society of Pediatric Pulmonology. U. Ozcelik is Head of the Turkish Respiratory Disease and Cystic Fibrosis Society (unpaid). P. Pohunek declares receiving payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from GlaxoSmithKline, AstraZeneca and Chiesi, and consulting fees, support for attending meetings and/or travel, and participation on a Data Safety Monitoring Board or Advisory Board from AstraZeneca and GlaxoSmithKline. J. Raidt declares receiving the following funds: DFG CRU326 RA3522/1. P. Latzin declares receiving grants or contracts from Vertex and OM Pharma, payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Vertex, Vifor and OM Pharma, and participating on a Data Safety Monitoring Board or Advisory Board for the following: Polyphor, Santhera (DMC), Vertex, OM Pharma, Vifor, Allecra and Sanofi Aventis. J.S. Lucas declares receiving support for the present manuscript (funds the clinical investigations (spirometry, microbiology)) from NHS England, to the institution; and grants or contracts from COST ACTION to fund the BEATPCD COST Action BM1407, which funded the network that undertook this work, and the institution managed the funding. The other authors have nothing to disclose.

Support statement: This study was supported by the Swiss National Science Foundation (SNSF 320030B_192804/1), the Ministry of Health of the Czech Republic (grant number NV19-07-00210) and the Motol University Hospital, Prague, Czech Republic (00064203, conceptual development of research). Many researchers and data contributors participate in ERN-LUNG (PCD core).

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