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Original Research Article

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Natural variability of lung function in primary ciliary dyskinesia: longitudinal analysis from the PROVALF-PCD cohort

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Abstract

Background: The extent to which changes in lung function are due to natural variability in patients with primary ciliary dyskinesia (PCD) is unknown. We aimed to assess intra-individual variability in forced expiratory volume in 1s (FEV_1) derived from spirometry to define the extent to which the observed changes were due to test variability in clinically stable PCD patients.

Methods: PROVALF-PCD is a large international prospective cohort conducted in 2017-2019. We included patients ≥5 years who were clinically stable in ≥2 consecutive visits and provided spirometry-derived lung function measurements. To calculate the upper limit of normal (ULN), we fitted an unadjusted multilevel mixed effect model, and to determine the absolute change in FEV_1 z-scores, we calculated the coefficient of repeatability (CR). We performed sensitivity analyses by stratifying relative change by age (adults versus children), number of measurements (≥4), and time between measurements (<4 months apart).

Results: We included 252 participants from 12 countries with confirmed or highly likely PCD. We included 1028 FEV_1 measurements from patients in stable state. The ULN for relative change between two measurements of FEV_1 was 25%. Test variability remained high in all sensitivity analyses. The CR was 1.88 FEV_1 z-score.

Conclusions: Changes in intra-individual FEV_1 between visits greater than 25% in stable PCD patients lie beyond the expected test variability and therefore could be considered physiologically relevant. These findings inform the selection of endpoints for pulmonary intervention trials in PCD, as they suggest FEV_1 is not a sensitive test for monitoring lung health in PCD.

Keywords

Primary ciliary dyskinesia, Paediatric Lung Disease, Clinical Epidemiology, Rare lung diseases, Respiratory Measurement

Introduction

Spirometry is frequently used to monitor disease progression and response to treatment in those with lung disease. Spirometry is generally recommended every 3 to 6 months in individuals with primary ciliary dyskinesia (PCD), with results compared to reference data and previous measurements from the same patient (1, 2). For meaningful interpretation of whether a change in lung function is likely clinically relevant, we need to understand the extent of intra-individual variability between clinic measurements, in addition to the normal age-dependent increase in absolute lung function during childhood and the normal age-dependent decline in adulthood. Previous studies in healthy children assessing within-individual variability have shown a between-measures variability mean of 0.05 z-scores (standard deviation 0.6), with 95% of all the children achieving a between-measure variability within ±1.3 z-scores over the course of one year (3).

Determining whether a measured change reflects a physiologically meaningful alteration or test variability can be challenging, and this distinction is important in both clinical and research settings (4, 5). Anecdotally, there is significant variability within-individual between measures in forced expiratory volume in 1s (FEV_1) derived from spirometry in stable adults and children with PCD, perhaps due to the volume and consistency of mucus secretions, even when adhering to standards for measurement, analysis and reporting, and when using reference equations (e.g. Global Lung Function Initiative (GLI)) (6). However, there are limited longitudinal data on FEV_1 changes in patients with PCD (7-9). Furthermore, questions have been raised over the sensitivity of FEV_1 to monitor disease progression for people with PCD, particularly its ability to detect early deterioration of lung function compared to other methods, such as the lung clearance index (LCI) from multiple breath washouts (MBW) (10, 11). Additionally, FEV_1 derived from spirometry is being used as a primary endpoint in clinical trials assessing the efficacy of drugs in improving lung function or preventing the progression of lung disease in PCD patients (12, 13).

We aimed to prospectively investigate intra-individual variations in the lung function parameter FEV_1 between consecutive spirometry measurements, to define the extent to which the observed changes were due to expected test variability in clinically stable patients with PCD.

Methods

Study population and design

PROVALF-PCD (**PR**ospective **O**bser**va**tional Multicentre Study on **VA**riability of **L**ung **F**unction in Stable **PCD** Patients) is a large, international cohort initiated by the Better Experimental Approaches to Treat PCD (BEAT-PCD) network (14-17). The inclusion criteria was the following: a) confirmed or highly likely diagnosis of PCD according to European Respiratory Society (ERS) guidelines (18, 19), b) age of 5 years old or above, c) clinically stable (i.e. not experiencing a clinician-defined pulmonary exacerbation at the time of the clinical appointment) in at least two consecutive appointments, and d) had at least two consecutive appointments 6 months apart or less. We excluded those where the interval between routine spirometry measurements was more than 6 months, or where the diagnostic status was inconclusive (i.e. did not have sufficient test results to be classified as either ‘PCD highly unlikely’ or ‘PCD highly likely’).

Information on patients' demographics, clinical history, diagnostics tests, appointment data, and spirometry measurements were collected during routine clinical visits every 3 to 6 months between August 2017 and December 2019. Participants should have had a minimum of two clinical appointments during the study period but could have had up to 10. Pseudo-anonymised data were captured using a study-specific case record form (CRF). They were entered by each participating centre into a dedicated 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 (20, 21). Centres only had access to their own data. Additional information on the PROVALF-PCD cohort has been published elsewhere (22).

Ethics

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

Appropriate ethics, informed consent, and other approvals were obtained locally by each centre. Centres provided a signed paper-based or scanned copy of the agreement, to be kept at the University of Southampton. Data were pseudonymised locally before transfer to the University of Southampton for analysis, adhering to international data transfer agreements signed by all parties. The study protocol was registered in Clinical Trials (NCT03704896).

Study outcome

The outcome variable was FEV_1 derived from spirometry, obtained during clinical visits from patients in stable state. Stable state was defined as not experiencing an episode of pulmonary exacerbation when attending a clinical appointment. Presence of pulmonary exacerbation was assessed and determined by the clinician(s) responsible for the patient's clinical care, during the routine clinical appointment. Spirometry data obtained from patients experiencing a clinician-defined pulmonary exacerbation were excluded from the analyses.

Lung function measurements were carried out by qualified technicians and clinicians at each centre during routine clinical appointments, using their clinic's equipment. Centre were provided with a study-specific standard operation procedure (SOP) document, adapted from Miller et al (23), to ensure measurements and data collection were standardised. The document contained detailed information on equipment use and calibration, test procedures, and quality control (see Supplementary material).

We calculated FEV_1 z-scores using the Global Lung Function Initiative (GLI) 2012 references (24), which are adjusted for sex, age, height, and ethnicity (determined by clinicians or self-declared by the participant) on the day of the lung function test. We calculated FVC z-scores using the same method (results in the supplementary material).

Study covariates

Body mass index (BMI) z-scores were calculated based on the body weight and height collected at clinical visits, using the World Health Organisation references (WHO) (www.who.int/tools/growth-reference-data-

for-5to19-years/indicators/bmi-for-age). As there are no international references for those over 19 years of age, values for adults were calculated based on the assumption that they were 19 years old.

Microbiology cultures were prospectively collected during routine clinical appointments and tested at each centre's microbiology laboratory. Type of respiratory tract sample and presence (or absence) of each pathogen were recorded. The health score was comprised of the participant's perceived self-reported health compared to their usual health, and was classified as very well, well, somewhat well, ill, or very ill.

We defined adult participants as those 18 years and over and children as those under 18.

Statistical methods

We described continuous variables as median and interquartile range (IQR), and categorical variables as total numbers and proportions. We calculated relative change in FEV_1 between consecutive visits and the upper limit of normal (ULN) as detailed in the supplementary material. We fitted an unadjusted multilevel model with random intercept at patient level to calculate the ULN for relative change, as detailed in the supplementary material.

To investigate the determinants of individual variability of lung function, we investigated the association between ULN for relative change of FEV_1 and sex, ultrastructural defect by transmission electron microscopy (TEM), presence of respiratory pathogens, and FEV_1 at baseline in multivariate models. To investigate the contribution by each co-variate, we ran univariate models, stratified by children and adults, and to assess whether an initially low or high FEV_1 z-score would impact on our findings, we also adjusted these models by FEV_1 at baseline. We reported the estimates (β coefficients), with 95% confidence intervals (95%CI), and the adjusted R^2.

We constructed unadjusted multilevel models with random intercepts at patient and country levels to estimate the absolute change of FEV_1 z-scores between consecutive visits. We also calculated the coefficient of repeatability (CR) as detailed in the supplementary material. The CR is used to determine short-term variability or test measurement noise, where changes that lie outside the test's CR parameter can be considered beyond test variability (25).

To investigate long-term trends for lung function progression, we constructed multilevel linear models with random intercepts at patient and country level, and FEV_1 z-score values as outcome. We fitted unadjusted and adjusted models (adjusted for the following covariates: time since initial appointment, BMI z-scores, presence of respiratory pathogens, health score, number of antibiotics used since the last visit, the use of antibiotic prophylaxis, and the use of inhaled corticosteroid at the time of their clinical appointment), with FEV_1 z-scores from measurements obtained from patients in stable state as outcome. We fitted the same model with relative change in FEV_1 as outcome. Intra-class correlation coefficient (ICC) was calculated based on these models, to assess the proportion of the overall variation explained by clustering within people and within country. The latter can be used to indicate whether there are considerable differences between measurements obtained from different countries.

We conducted four sensitivity analyses. To determine the extent of intra-individual variability in children and adults separately, we stratified the relative and absolute change in FEV_1 by children and adults. To assess

the impact of having more than two measurements of FEV_1 per participant, we conducted two subgroup analyses: a) including only those who had at least 3 measurements, and b) including only those who had at least four measurements during the follow-up period. Lastly, we restricted our analyses to only those who had between-measurement intervals of less than four months, since test variability is higher when time between measurements increases (4, 6).

Data coding, cleaning, analyses, and plotting were conducted in Rstudio version 4.4.0, using the broom.mixed, jtools, lme4, and tidyverse packages.

Results

We analysed data from 252 participants from 19 PCD centres in 12 countries (Table S1). There were 1178 visits where lung function was assessed between August 2017 and December 2019 (median 3 visits per participant, IQR 2 to 4 visits, Table S1), of which 419 measurements were from 84 adults and 759 measurements were from 168 children. From those, 1028 measurements (87.3%) were taken when the participant was in stable state (i.e. not experiencing an episode of pulmonary exacerbation). All measurements obtained during an episode of pulmonary exacerbation were excluded from our analyses.

Median age at recruitment was 14.7 years (IQR 11.2 to 19.8 years) (Table1). Median FEV_1 z-score at baseline was -1.66 (IQR -2.74 to 0.48). Longitudinal trends for FEV_1 z-score varied between countries, but we did observe larger variations or any clear patterns in any particular country (e.g. only decreases) (Figure S1). Most lower airway microbiology samples were from sputum (92%), and a respiratory pathogen was isolated in 62.6% of samples (Table S2).

Of the 252 participants, 181 (72%) were classified as confirmed PCD based on TEM and/ or genetics, and the others were highly likely PCD based on clinical history, nNO and high-speed video analysis (HSVA) (18). There were 130 (52%) participants with abnormal ciliary ultrastructure detected by TEM, and biallelic mutations were identified in PCD-causative genes in 114 (45%) participants (Figure S2, Table 2).

Relative change in FEV_1

The ULN between two measurements of FEV_1 obtained 6 months apart or less was 25% in stable patients. Between-measures changes in FEV_1 for individual patients, expressed as relative change, varied between 1% and 81% (Figure 1). Twenty-two participants had a between-measures relative change >40%, of which in 15 the observed changes were an increase and in 7 these were a decrease in FEV_1 z-scores. In the later, the relative change of FEV_1 z-score decreased between 43% and 77%. The participant with the highest decrease in relative change (i.e. 77%) had a baseline FEV_1 z-score of -2.54 at the age of 14 years, followed by -2.45 FEV_1 z-score at the second appointment, and -4.47 at the third appointment despite reportedly not having a pulmonary exacerbation.

When stratifying the analyses by children and adults, the ULN between two measurements of FEV_1 was 20.5% in adults and 26.9% in children ($p = 0.77$). In the sensitivity analyses, the ULN when considering only those with at least three measurements was 25%, and 24% when considering only those with at least four measurements. When restricting our analyses to participants who had measurements obtained less than four months apart, the ULN was 24%.

Absolute change in FEV1 z-score

The mean absolute difference in intra-individual FEV_1 z-score was relatively small (-0.013) but had a large standard deviation (0.68). The CR was 1.88, meaning that 95% of absolute differences in FEV_1 z-score between measurements were expected to fall below 1.88. When stratifying the analyses by children and adults, the CR for children was 2.05 and for adults was 1.58.

Individual relative change in FEV_1, expressed as 95% ULN for each individual, was slightly lower in females (β coefficient = -0.04, 95%CI -0.08 to 0.00), in those with central pair defect by TEM (β coefficient = -0.12, 95%CI -0.23 to 0.00), and in those with lower FEV_1 at baseline (β coefficient = -0.06, 95%CI -0.09 to -0.04) in the fully adjusted models (Figure 2). In the univariate models, participants with ODA defect had FEV_1 variability of 0.05 (95%CI 0.00 to 0.10), while those with central pair defect had variability of -0.13 (95%CI -0.26 to -0.02), when adjusted for FEV_1 values at baseline (Table 3). Children with ODA defect also had slightly higher variability when adjusted for FEV_1 values at baseline (β coefficient = 0.06, 95%CI 0.00 to 0.13) (Table 3). The adjusted R^2 was 0.19, which means that these factors explained less than 20% of the total individual variability observed. Most of the variability was explained by FEV_1 value at baseline (adjusted R^2 from the univariate model with FEV_1 at baseline as single exposure = 0.14).

Long-term change in FEV_1 z-score

We found a mean annual absolute decrease of 0.06 (95%CI -0.14 to 0.00) and 0.11 FEV_1 z-scores (95%CI -0.20 to -0.01) in the unadjusted and adjusted models, respectively (e-Figure 3). In the adjusted models, there was a high similarity of measurements within individual patients (ICC = 0.84) and the variance was mainly explained by differences between patients (ICC = 0.81) rather than countries (ICC = 0.03). The relative annual decrease was 0.18% (95%CI -1.90% to 2.27%) in an unadjusted model.

Discussion

In this large multicentre cohort with over 250 PCD patients from 12 countries, we found high intra-individual variability of FEV_1 z-scores in patients in stable state. We found that relative changes in FEV_1 above 25% compared to the previous measurement should be considered above test variability, and therefore physiologically relevant in PCD patients. Since relative changes are related to the patients' previous measurement, previous measurements will influence the magnitude of this change in absolute terms e.g. a relative decrease of 25% in a patient with FEV_1 % predicted of 40 would mean an observed FEV_1 % predicted of 30, while for a patient with preserved lung function (100% predicted) this would translate as greater drop to 75% predicted. Previous studies in healthy children have found a decrease in the correlation between repeated measurements when there is an increase in time between measurements; however, we found that test variability remained high even when restricted to patients who had more than three measurements and when measurements were taken less than four months apart (26-28). Between-measurement variability was slightly higher in children (26.9%) than adults (20.5%) but remained considerably high in both groups. For comparison, year-to-year changes in FEV_1 above 15% over one year and relative change of 12% from baseline are considered to be physiologically meaningful in healthy adults, while in children an absolute change within ±1.3 z-scores is considered meaningful (3, 4, 29). In CF, absolute changes in FEV_1 greater than 10% compared to baseline are considered meaningful (30).

Individual relative changes of FEV_1 ranged from 1% to 81% compared to the previous measurement. This high variability could be due to differences in mucus secretions in the larger airways, which in turn may depend on when the measurements occurred e.g. before or after physiotherapy. When assessing the potential determinants of individual lung function variability, we found that patients with central pair defect and lower FEV_1 values at baseline had decreased variability, while those with ODA defect had higher variability. However, these factors could only account for a relatively small proportion of the total observed variability and therefore larger studies are needed to confirm these findings and to further explore the potential reasons for the differences observed. Additionally, our analyses were based on small numbers of participants per TEM defect and therefore we could not draw conclusions on whether different ultrastructural defects have any effect on FEV_1 variability. The impact of high individual variability on disease severity and long-term survival has been explored in CF, with some reports of an association with worse lung function decline (31).

The CR quantifies the absolute reliability measurement error (including both random and systematic errors) and sets the boundary of the minimally detectable true change that can be measured by an instrument. Since it is in the same unit as the measurement obtained (i.e. FEV_1 z-scores, in this case), the CR is adjusted for sex, age, height, and ethnicity (24). We found that changes in FEV_1 z-score less than 1.88 between measurements might simply be due to test variability i.e. changes between -0.88 and 2.88 FEV_1 z-scores. This large absolute reliability measurement error suggests that spirometry would only be able to reliably detect large changes in FEV_1 z-scores and would potentially miss early signs of lung function deterioration.

Spirometry is the most commonly used test to assess lung function due to its wide availability in clinical settings, affordability of equipment (even in resource-limited settings), ease of application, standardisation in measurements and reporting, and interpretability of results. Importantly, FEV_1 is one of the proposed core outcomes set measures that should be consistently measured and reported in PCD studies, particularly in randomised controlled trials (RCTs) and prospective cohorts (32). However, spirometric indices might not be sufficiently sensitive to monitor disease in PCD, as patients with structural and functional lung impairment can have FEV_1 values within the normal range (33). In RCTs, investigating the efficacy of new drugs in PCD, using FEV_1 as the main endpoint could lead to physiologically meaningful changes not being detected due to high test variability. (10, 11). Furthermore, studies in patients with CF have shown that day-to-day variations in FEV_1 from home spirometry are common and intra-individual variations can be as high as 16.3% (33-38). Our results, which show extremely high variability of FEV_1 in PCD, should raise concerns for the use of FEV_1 as outcome parameter in RCTs, especially if used as the primary outcome.

Various studies investigated the variability of lung clearance index derived from MBW in children with CF. Studies reported a CV of 7.4%-8.2%, with an ULN between visits ranging from 19% to 24% (39, 40). Within-individual variability of LCI measurements was 10% compared to 16% for FEV_1 % predicted (41). Large prospective cohorts comparing lung function in PCD patients, measured by spirometry and MBW, with structural and functional abnormalities, detected through high-resolution computerised tomography (HRCT) and magnetic resonance imaging, are still lacking (42).

Our study is the first to prospectively assess intra-individual variability of FEV_1 from spirometry in stable PCD patients, performed by clinicians during routine clinical appointments. Our findings enable the interpretation of FEV_1 in routine clinical surveillance of lung disease in PCD and highlight the potential limitations of using FEV_1 values to guide therapeutic decisions, both clinically and as an endpoint for RCTs. Findings can be generalised to the wider PCD community, as we included both children (over the age of 5 years old) and adults from 19 PCD centres in 12 countries, with different clinical and genetic backgrounds and lung disease severity. The study's long follow-up time allowed us to explore if having more than two lung function measures reduced FEV_1 variability. We prospectively collected data using a standard form, and lung function was measured and reported following a pre-defined study SOP. Additionally, we checked the quality of spirometry measurements obtained at different centres by checking the volume-time and flow-volume curves on test reports for the first five consecutive patients recruited into the study for each centre. We also controlled for data quality by inspecting individual trajectories in FEV_1 z-scores stratified by country, and by applying multilevel models to adjust for clustering by country so that potential differences in spirometry equipment and how measures were obtained could be taken into account.

However, results must be interpreted considering study limitations. We were unable to apply the criteria based on the expert consensus statement to define pulmonary exacerbation in PCD, as we did not collect all the necessary data since the consensus was published while our study was ongoing (43). The consensus does not include changes in FEV_1 as a criterion to define exacerbation, whereas, in our study, clinicians managing the patients might have considered a drop in FEV_1 as a sign of pulmonary exacerbation. Results based on the consensus statement may have been different. Only 45% of patients included in our study had a confirmed diagnosis of PCD based on genetic testing. This study contains data from 2017 to 2019, when genetic testing was less routinely done as part of the diagnostic work-up, particularly in centres with limited resources where a diagnosis was often based on TEM hallmark defects.

Conclusions

Our findings suggest that changes above 25% in intra-individual measurements of FEV_1 derived from spirometry in stable PCD patients should be considered beyond the expected test variability. However, it is important to consider that all test results, including FEV_1, must be interpreted by clinicians with knowledge of the individual patient's clinical condition, history, and disease status, as statistically insignificant (or significant) changes can be meaningful (or meaningless) depending on the individual's clinical situation, particularly as we have shown a large range of individual FEV_1 relative change in our population.

This study provides important information for ongoing and future RCTs, particularly those focused on developing and testing new drugs designed to improve lung function and/or impede progression of lung disease in PCD, as our findings suggest that, when used in isolation, FEV_1 is not a sensitive test for monitoring lung health in PCD (12). More sensitive markers of lung function in PCD, such as LCI derived from MBW, should be considered when selecting outcome measures for future pulmonary intervention studies.

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Author contributions

KZ, CK, NB, PL, JL, BR contributed to the conception and design of the work.

KZ, AV, MB, CC, SCC, RC, SD, SEP, EE, NF, AH, CH, CKR, PK, EL, NL, JKM, VM, AMG, LM, KM, HO, UO, PP, PR, SRA, FS, AS, AT, GT, DAT, NU, WTW, PY, JL, BR made substantial contributions to the acquisition and provided contextual evidence for the interpretation of the data.

KZ, CP, BF, BR made substantial contributions towards the analyses of the data.

KZ, BF, CK, NB, PL, JL, BR made substantial contributions to the interpretation of the data and the findings from the analyses.

KZ and BR drafted the work, all authors reviewing it critically for important intellectual content. All authors had provided final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

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Tables and Figures

Table 1. Characteristics of participants included in the study.

Participants' characteristics		**n (%)**
Subjects		252 (100)
Visits		1178 (100)
Stable visits		1028 (87.3)
Female		117 (46.0)
Age at baseline in years, median (IQR)		14.7 (11.2 to 19.8)
Age at diagnosis in years, median (IQR)		6 (1.1 to 11.5)
BMI z-score at baseline, median (IQR)		0.12 (-0.68 to 0.85)
FEV_1 z-score at baseline, median (IQR)		-1.66 (-2.74 to -0.48)
FEV1 values in L at baseline, median (IQR)		2.24 (1.7 to 2.9)
Ethnicity (n)	White	233 (92.5)
	Black	3 (1.2)
	North-East Asian	0 (0)
	South-East Asian	1 (0.4)
	Other/mixed	15 (6.0)
Cardiac situs (n)	Situs solitus	131 (52.0)
	Situs inversus	113 (44.8)
	Situs ambiguous	7 (2.8)
	Unknown	1 (0.4)

n = numbers, IQR = interquartile range, BMI = body mass index, FEV_1 = forced expiratory volume in 1s.

Table 2. Diagnostics for the 252 patients included in the study.

Diagnostic tests	n (%)	Children n = 168	Adults n = 84
Transmission electron microscopy (TEM)			
ODA defect*	59 (23.4)	41 (24.4)	18 (21.4)
IDA and ODA defect*	45 (17.9)	29 (17.3)	16 (19.0)
Microtubular disorganisation with IDA defect*	21 (8.3)	15 (8.9)	6 (7.1)
IDA defect	7 (2.8)	6 (3.6)	1 (1.2)
Central pair defect	6 (2.4)	1 (0.6)	5 (6.0)
Absent/completely reduced cilia	5 (2.0)	4 (2.4)	1 (1.2)
Other	3 (1.2)	2 (1.2)	1 (1.2)
Normal TEM test	23 (9.1)	15 (8.9)	8 (9.5)
Inconclusive	19 (7.5)	16 (9.5)	3 (3.6)
Not done	64 (25.3)	39 (23.2)	25 (29.8)
Genetic testing			
Positive genetic test	113 (44.8)	83 (49.4)	30 (35.7)
No genetic diagnosis found	9 (3.6)	5 (3.0)	4 (4.8)
Not done	130 (51.6)	80 (47.6)	50 (59.5)
Nasal nitric oxide (nNO) testing			
Low nNO (<77nL/min)	148 (58.7)	95 (68.8)	53 (63.1)
Normal nNO (>77nL/min)	13 (5.2)	11 (6.5)	2 (2.4)
Not done	91 (36.1)	62 (36.9)	29 (34.5)
High-speed video microscopy analysis (HSVA)			
Static or static with residual movement	165 (65.5)	113 (67.3)	52 (61.9)
Rotating/circular movement	3 (1.2)	2 (1.2)	1 (1.2)
Abnormal CBP likely secondary (e.g. infection, mucus impedance)	3 (1.2)	3 (1.8)	0 (0)
Other abnormal CBP	6 (2.4)	3 (1.8)	3 (3.6)
Normal	1 (0.4)	0 (0)	1 (1.2)
Inconclusive	26 (10.3)	15 (8.9)	11 (13.1)
Not done	48 (19)	32 (19.0)	16 (19.0)

*Class I defects, according to Shoemark et al (19).

Table 3. Determinants of individual relative variability (95% upper limit of normal) for forced expiratory volume in 1s (FEV_1) in 201 patients with primary ciliary dyskinesia (PCD) with complete data available (141 children and 60 adults). Results are presented as beta coefficients for univariate or adjusted models, with 95% confidence intervals.

		All	Children	Adults	Adjusted* all	Adjusted* children	Adjusted* adults
Sex							
	Female (reference: male)	-0.01 (-0.05 to 0.03)	-0.01 (-0.06 to 0.04)	0 (-0.06 to 0.06)	-0.03 (-0.07 to 0)	-0.02 (-0.07 to 0.02)	-0.03 (-0.09 to 0.04)
TEM							
	Normal	-0.01 (-0.09 to 0.07)	0 (-0.10 to 0.09)	-0.04 (-0.18 to 0.09)	-0.02 (-0.10 to 0.05)	-0.03 (-0.12 to 0.06)	-0.04 (-0.17 to 0.09)
	ODA	0.05 (-0.01 to 0.10)	0.06 (-0.01 to 0.13)	0.01 (-0.08 to 0.10)	0.05 (0 to 0.10)	0.06 (0.00 to 0.13)	0.02 (-0.07 to 0.11)
	ODA & IDA	-0.02 (-0.08 to 0.04)	-0.02 (-0.10 to 0.06)	-0.01 (-0.10 to 0.08)	-0.01 (-0.07 to 0.04)	-0.02 (-0.09 to 0.05)	-0.01 (-0.10 to 0.08)
	IDA & MTD	-0.02 (-0.11 to 0.06)	-0.04 (-0.15 to 0.07)	0.02 (-0.10 to 0.13)	-0.03 (-0.10 to 0.05)	-0.02 (-0.12 to 0.08)	0.01 (-0.11 to 0.12)
	IDA	0.03 (-0.11 to 0.16)	0.01 (-0.13 to 0.16)	N/A	0.01 (-0.12 to 0.13)	0 (-0.14 to 0.13)	N/A
	Central pair	-0.14 (-0.26 to -0.02)	-0.12 (-0.42 to 0.19)	-0.11 (-0.23 to 0.01)	-0.13 (-0.24 to -0.01)	-0.09 (-0.37 to 0.18)	-0.11 (-0.23 to 0.01)
	Absent or reduced cilia	0.01 (-0.12 to 0.15)	-0.03 (-0.19 to 0.13)	0.19 (-0.05 to 0.43)	0.01 (-0.12 to 0.13)	-0.06 (-0.20 to 0.09)	0.20 (-0.04 to 0.44)
	Other	-0.10 (-0.27 to 0.07)	-0.13 (-0.35 to 0.09)	-0.04 (-0.27 to 0.20)	-0.06 (-0.22 to 0.10)	-0.09 (-0.29 to 0.11)	-0.02 (-0.26 to 0.21)
	Inconclusive	0.03 (-0.05 to 0.11)	0.04 (-0.06 to 0.13)	-0.03 (-0.18 to 0.12)	0.02 (-0.05 to 0.09)	0.02 (-0.10 to 0.10)	-0.02 (-0.17 to 0.13)

Presence of Pseudomonas aeruginosa	0 (-0.08 to 0.09)	0 (-0.12 to 0.13)	0.03 (-0.07 to 0.14)	-0.01 (-0.09 to 0.07)	0 (-0.11 to 0.12)	0.02 (-0.09 to 0.12)
Presence of Haemophilus influenzae	0.05 (0.01 to 0.09)	0.03 (-0.02 to 0.08)	0.01 (-0.08 to 0.10)	0.03 (-0.01 to 0.07)	0.03 (-0.02 to 0.08)	0.00 (-0.08 to 0.09)
Time between visits (in years)	0.01 (0 to 0.02)	0.01 (0 to 0.02)	0.01 (-0.01 to 0.02)	0.01 (0 to 0.02)	0.01 (0 to 0.02)	0 (-0.01 to 0.02)
FEV_1 at baseline	-0.06 (-0.09 to -0.04)	-0.07 (-0.10 to -0.05)	-0.03 (-0.06 to 0.01)	N/A	N/A	N/A

* Models were adjusted for FEV1 values at baseline. TEM = transmission electron microscopy, ODA = outer dynein arm defect, IDA = inner dynein arm defect, MTD = microtubular disarrangement.

Figure 1. Forced expiratory volume in 1s (FEV_1) relative change upper limit of normal (ULN) for individuals in stable state between two consecutive clinic appointments (n = 252 patients). The red line shows the ULN for patients in stable status. Changes above this value would be considered physiologically relevant (i.e. not due to the test variability).

Figure 2. Estimates (β coefficients) for covariates included in fully adjusted model, with individual relative variability (95% upper limit of normal) of forced expiratory volume in 1s (FEV_1) as outcome.

Model was adjusted by all covariates shown in the figure. Estimates are represented by the circles, and the 95% confidence intervals by the whiskers.

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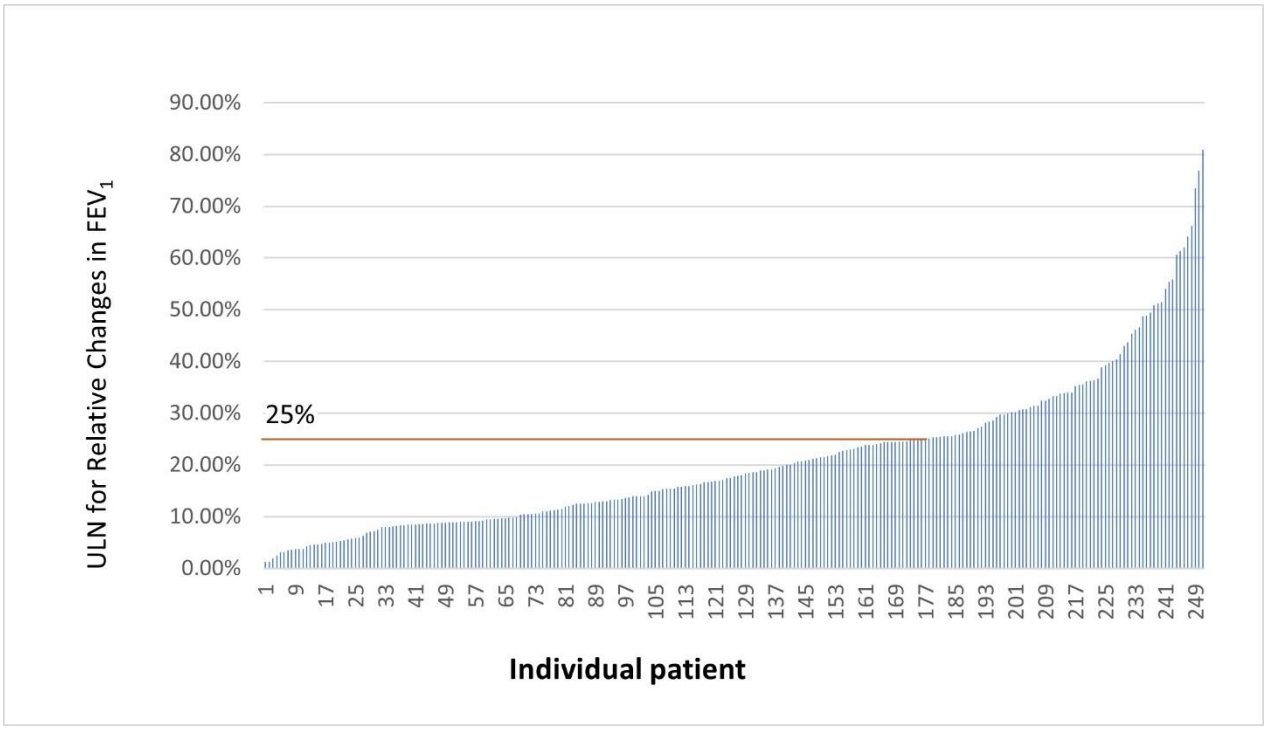


Figure 1

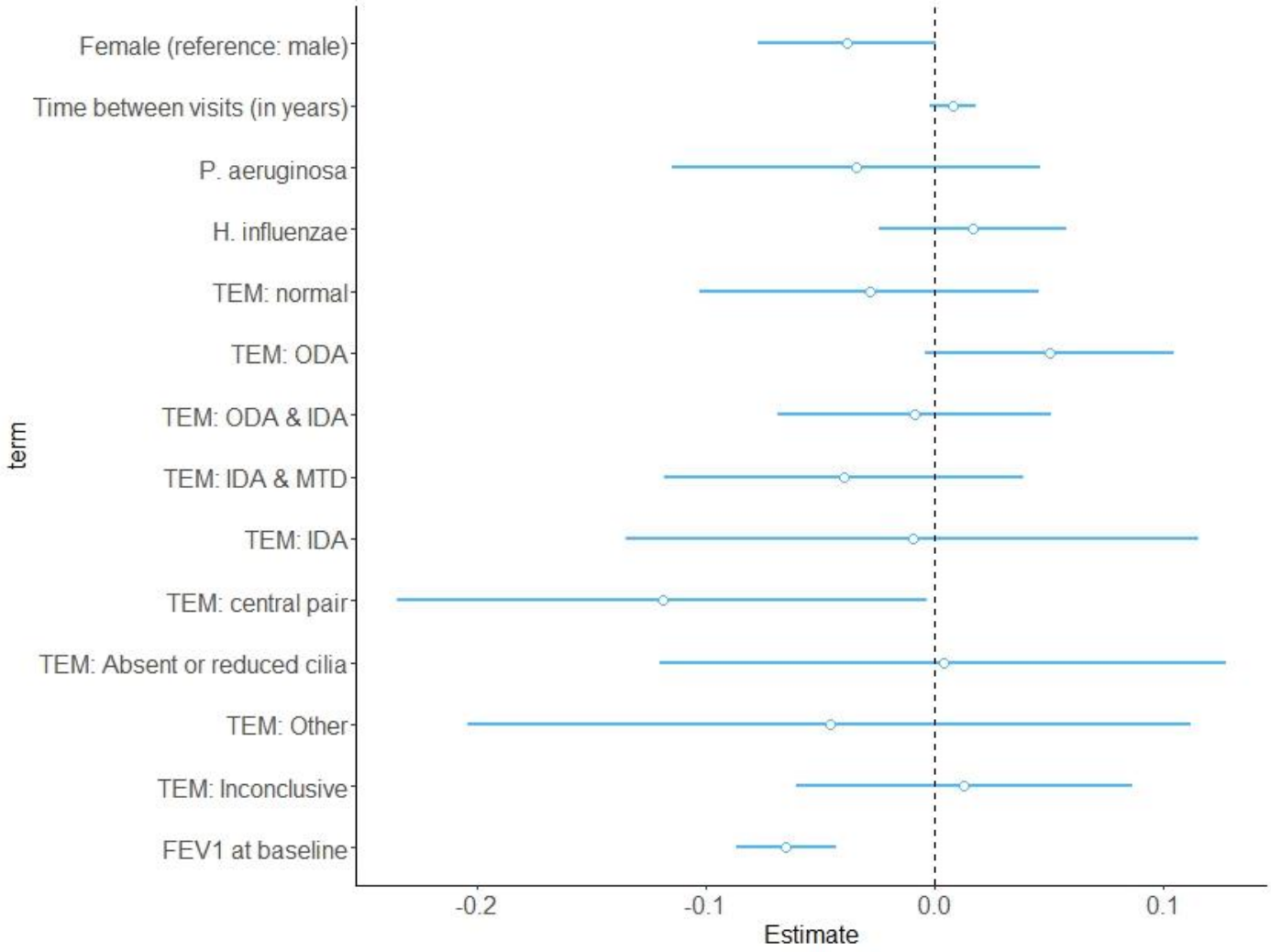


Figure 2

Supplementary

Spirometry measurements and quality control

In order to obtain an acceptable blow, the following conditions had to be met: a) satisfactory start of expiration (i.e. no excessive hesitation or false start back extrapolated volume or back extrapolated volume >5% of forced vital capacity (FVC)), b) no cough during the first second of the manoeuvre or any cough after that in which, in the operator's judgement, interferes with obtaining accurate results, c) no early termination of expiration, d) no cessation of airflow due to Valsalva manoeuvre or hesitation, e) no leak, f) no obstruction of the mouthpiece, and g) no extra breath was taken during the manoeuvre. A minimal of 3 manoeuvres with 1 minute interval between blows was required. After 3 acceptable spirograms were obtained, the 2 largest values of FVC and of forced expiratory volume in 1s (FEV_1) should be within 0.15 litres of each other to meet the repeatability criteria. The best measurements obtained that met the acceptable blow and repeatability criteria were recorded in the study CRF.

Test operators were instructed to visually inspect both flow-volume and volume-time displays during each manoeuvre before proceeding with subsequent manoeuvres to ensure measurements were of good quality. Centres were requested to communicate any changes of spirometry equipment that occurred during the study to the coordinating centre. They were instructed to upload anonymised copies of spirometry reports for the first five consecutive participants recruited into the study, which were reviewed by the coordinating centre.

Statistical analyses

Relative change in FEV_1 between consecutive visits in stable participants was calculated as follows:

$$(FEV1_{i+1} - FEV1_i)/FEV1_i * 100\%$$

, where FEV_1 is the FEV_1 raw value (in litres) and i represents the clinic appointment at which lung function was measured. To calculate the upper limit of normal (ULN), we log-transformed FEV_1 by the natural logarithm, and then fitted an unadjusted multilevel model with random intercept at patient level. Based on this model, we calculated the ULN for relative change (95% quantile of normal distribution) as below:

$$\mathrm{ULN} = e^{\sqrt{2}*1.64*\sigma} - 1$$

, where σ is the residual SD derived from the multilevel model.

To determine limits for each individual, we calculated ULN of relative changes of FEV_1 between measurements for each patient in stable condition. The formulae used is similar to the one shown above; however, σ in this instance represents the SD of log transformed FEV_1 values.

We also calculated the coefficient of repeatability (CR) using the following formula:

$$CR = 1.96 \times \sqrt{2} \times \sigma_w$$

, where σ_w is the within subject SD.

Results for patients experiencing an episode of pulmonary exacerbation

The mean absolute difference of FEV_1 z-scores between visits in patients that were stable in the first clinical appointment but had an exacerbation in the subsequent appointment was -0.23 (SD 0.56). The ULN for patients experiencing an exacerbation was 29%, marginally higher than the ULN observed for patients in stable state (i.e. 25%).

Results for FVC as outcome

Results for FVC were similar to those for FEV_1. The upper limit of normal (ULN) between two consecutive measurements of FVC obtained 6 months apart or less was 25.2% in stable patients (versus 25% for FEV1) and the coefficient of repeatability (CR) was 2.09 (versus 1.88 for FEV_1).

Table S1. Number of participants with primary ciliary dyskinesia (PCD) included in the study and number of visits per patient, stratified by country.

Country	**n patients (%)**	**Median number of visits per patient (range)**
England	26 (10.3%)	4.5 (2 to 8)
Australia	6 (2.4%)	3 (3 to 4)
Italy	34 (13.5%)	4 (2 to 6)
Denmark	18 (7.1%)	3.5 (2 to 7)
Germany	29 (11.5%)	4 (2 to 10)
Cyprus	15 (6.0%)	6 (3 to 8)
France	15 (6.0%)	4 (2 to 6)
Belgium	22 (8.7%)	4 (3 to 9)
Portugal	6 (2.4%)	4.5 (3 to 7)
Turkey	38 (15.1%)	5 (2 to 8)
Czech Republic	17 (6.7%)	5 (3 to 9)
Spain	26 (10.3%)	5 (3 to 8)
Total	**252 (100%)**	**3 (2 to 4)**

Table S2. Details of covariates adjusted for in multilevel model of longitudinal changes in forced expiratory volume in 1s (FEV_1) z-score in patients with primary ciliary dyskinesia (PCD).

Covariates	n visits (%)
Presence of respiratory pathogens	737 (62.6)
Health status (compared to baseline)	
Very well	200 (17.0)
Well	655 (55.6)
Somewhat well	220 (18.7)
Ill	70 (5.9)
Very ill	0 (0)
Courses of antibiotics since last visit (median, IQR)	0 (0 to 1)
Use of antibiotic prophylaxis	415
Use of inhaled corticosteroid	449

Figure S1. Forced expiratory volume in 1s (FEV_1) z-score individual trajectories for participants in stable state, stratified by country.

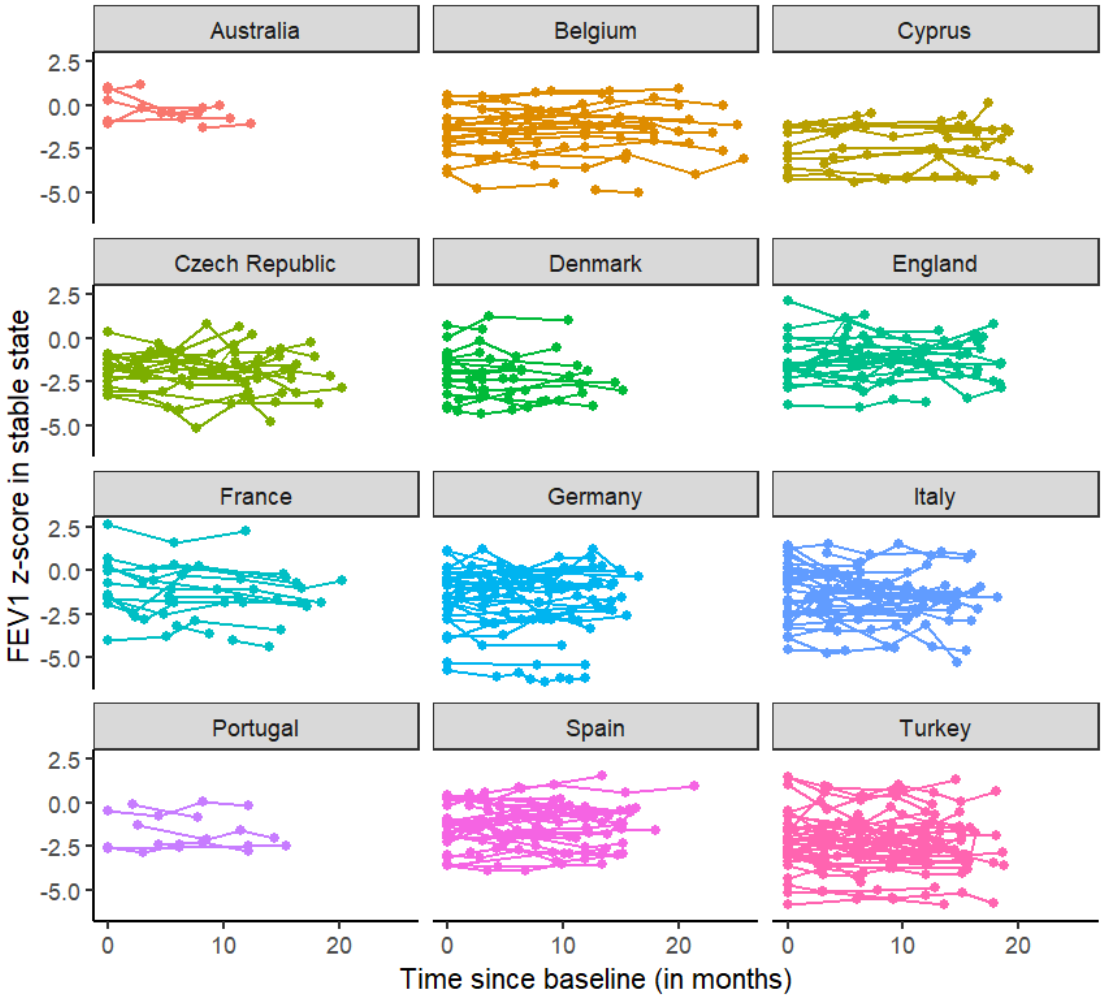


Figure S2. Venn diagram showing the numbers of abnormal diagnostic tests for each diagnostic test and for the combinations of diagnostic tests.

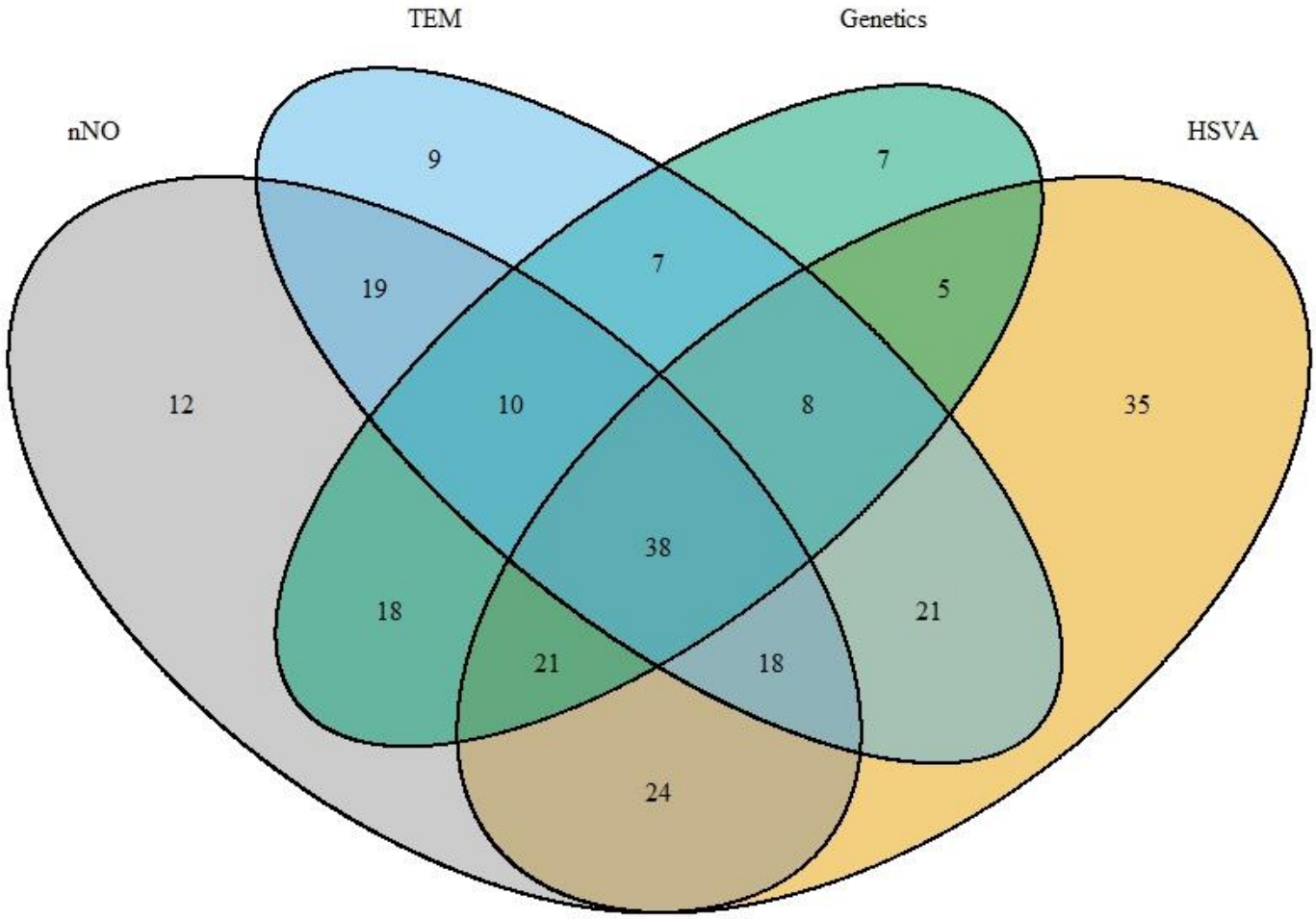
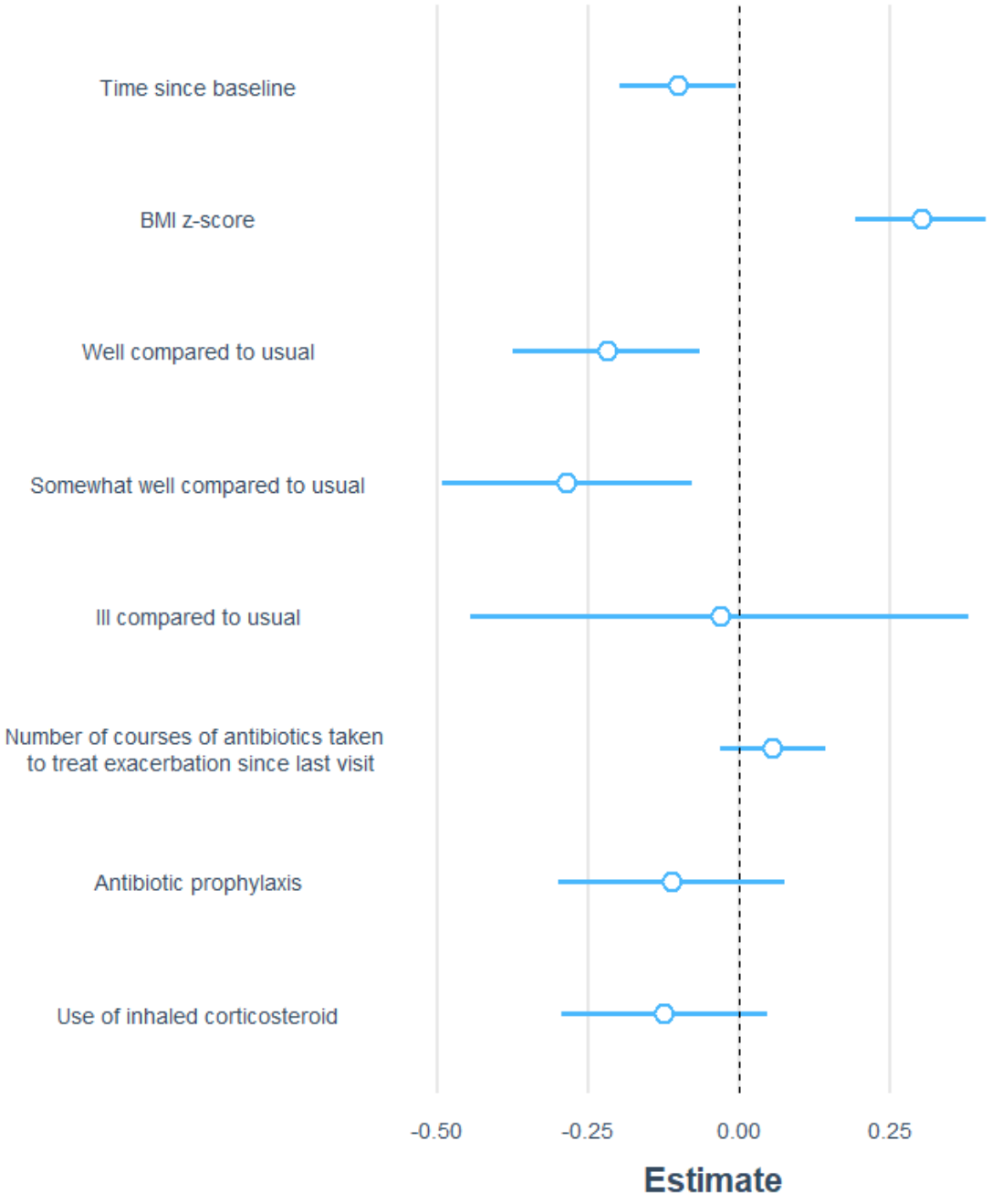


Figure S3. Coefficient estimates for forced expiratory volume in 1s (FEV_1) z-scores in adjusted multilevel model of rate of lung function decline in patients with primary ciliary dyskinesia (PCD).



Model was adjusted for time since baseline, BMI z-score, current health compared to usual (reference category: very well compared to usual), number of courses of antibiotics, use of antibiotic prophylaxis, and use of inhaled corticosteroids. Estimates are represented by the circles, and the 95% confidence intervals by the whiskers.