ONLINE SUPPLEMENT

Lung clearance index predicts pulmonary exacerbations in individuals with primary ciliary dyskinesia - a multicentre cohort study

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Study population

Characteristics of the enrolled study population and diagnostic features for PCD are given in **OLS Tables 1-3.** All individuals had a compatible history and clinical findings of PCD, and abnormal ciliary beat frequency and pattern in high-speed video-microscopy analysis (HSVA). The majority had low nasal nitric oxide (nNO < 77 nl/min, **OLS Table 1**). The frequencies of certain diagnostic tests performed, *i.e.* transmission electron microscopy (TEM) of ciliary ultrastructure and immunofluorescence staining (IF) of ciliary proteins, differed between centres due to previously different strategies. In Center 1 and 2, eleven and two individuals had a highly likely diagnosis of PCD [1]. All eleven individuals of Centre 1 had a compatible history and clinical findings of PCD, low nNO, and abnormal ciliary beat frequency and pattern in HSVA. In addition, five out of these individuals had heterotaxy and two had abnormal IF of ciliary proteins. In Centre 2, both individuals had a compatible history and clinical findings of PCD. One patient had abnormal ciliary beat frequency and pattern in HSVA in the respiratory cell culture but normal nNO and normal ciliary ultrastructure in TEM. The second patient had abnormal ciliary beat frequency and pattern in HSVA in the respiratory cell culture confirmed twice but normal nNO and normal ciliary ultrastructure in TEM.

In Center 1 and 2, six had a diagnosis of congenital heart disease, eight had gastroesophageal reflux disease, and 15 were atopic, *i.e.* sensitized to any tested allergen. None of the individuals experienced an event, for example, clinical deterioration or treatment, which we considered competing with the occurrence of the specific event of interest, pulmonary exacerbation (PEX), in this study.

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Multiple-Breath Washout

Nitrogen multiple-breath washout (MBW) was performed using an available setup (Exhalyzer D, Eco Medics AG, Duernten, Switzerland) according to current recommendations [2,3]. Both centres used the same equipment and protocol. Lung clearance index (LCI) was calculated as recommended [2,3]. We used the latest Spiroware software (Eco Medics AG) available at that time to measure MBW and to apply quality control and analysis of LCI. The software version 3.1 remained unchanged during the study. Manufacturers and experienced personnel from Bern provided training, offline quality control, and feedback on measurements.

OLS Table 1. Clinical characteristics and diagnostic features.

	Centre 1	Centre 2
N	49	41
Heterotaxy	20 (41%)	16 (39%)
Diagnostic tests performed to diagnose PCD		
nNO	49 (100%)	38 (93%)
HSVA	49 (100%)	41 (100%)
TEM	35 (71%)	41 (100%)
IF	28 (57%)	n/a
Genotyping	31 (63%)	39 (95%)
Diagnostic findings suggestive of PCD		
nNO < 77 nl/min	41 (84%)	33 (87%)
HSVA	49 (100%)	41 (100%)
TEM	29 (83%)	25 (61%)
IF	22 (79%)	n/a
Genotyping	31 (100%)	35 (90%)

Data are given as mean (SD) or n(%) as appropriate. Diagnostic tests (%) is based on the number of individuals, diagnostic findings (%) is based on the number of diagnostic tests. HSVA - high-speed video-microscopy analysis; nNO - nasal nitric oxide; TEM - transmission electron microscopy; IF - immunofluorescence staining of ciliary proteins.

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OLS Table 2. Lung function categories.

Variable	Tertile	Category	N	Min.	25th Quart.	Median	75th Quart.	Max.
LCI	1	*best*	144	5.59	7.11	7.69	8.26	8.89
LCI	2	*moderate*	143	8.92	9.41	10.03	10.75	11.49
LCI	3	*worst*	143	11.49	12.58	13.97	16.26	23.44
FEV_1	1	*worst*	143	-5.89	-3.68	-2.68	-2.13	-1.78
FEV_1	2	*moderate*	142	-1.76	-1.45	-1.09	-0.84	-0.54
FEV_1	3	*best*	142	-0.53	-0.20	0.12	0.50	2.09

Data are given in LCI units and FEV_1 z-score. Categories "worst" comprise abnormal LCI and FEV1 values, "moderate" abnormal LCI but normal FEV_1 values, and "best" normal or nearly normal LCI and normal FEV_1 values. FEV_1 - forced expiratory volume in one second; LCI - lung clearance index. Min - Minimum; Quart. - Quartile; Max - Maximum.

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OLS Table 3. Characteristics of the study population.

Baseline	Total	Centre 1	Centre 2	P
N (females)	90 (53)	49 (26)	41 (27)	0.22*
Age (years) at baseline	12.8 [9.1 to 17.8]	12.6 [9.6 to 16.4]	14.3 [8.3 to 19.3]	0.58#
BMI at baseline	18.7 [16.1 to 21.6]	18.8 [16.9 to 21.2]	18.3 [15.6 to 21.6]	0.43#
FEV_1 (z-score) at baseline	-1.00 [-1.92 to -0.18]	-0.89 [-1.81 to -0.13]	-1.39 [-2.10 to -0.34]	0.43#
LCI (units) at baseline	9.70 [8.24 to 11.74]	10.38 [8.54 to 12.02]	9.15 [7.94 to 10.77]	0.10#
LCI (z-score) at baseline	5.90 [2.66 to 10.44]	7.43 [3.33 to 11.09]	4.68 [2.00 to 8.29]	0.10#
FRC (L) at baseline	1.98 [1.38 to 2.62]	2.05 [1.46 to 2.56]	1.81 [1.17 to 2.69]	0.73#
Hypertonic saline at baseline	34 (42.0%)	24 (50.0%)	10 (30.3%)	0.11**
Antibiotics at baseline	26 (28.9%)	16 (32.7%)	10 (24.4%)	0.49**
Follow-up				
Total visits (n)	436	235	201	n/a
Visits per participant during follow-up	4 [2 to 7]	4 [2 to 7]	4 [2 to 7]	0.89#
PEX n (% from total visits)	39 (9.1%)	23 (9.8%)	16 (8.3%)	0.62*
Pseudomonas positive samples, n (% from total respiratory samples, N)	21 (5.8%, 365)	6 (2.6%, 234)	15 (11.5%, 131)	0.001**
Time intervals (days) between visits	120 [77 to 182]	96 [55 to 145]	182 [98 to 227]	<0.001#
Total time (days) in study	337 [93 to 583]	237 [77 to 475]	428 [183 to 883]	<0.001#

Data are given as median [interquartile range] or n (%). Data on current treatment with inhaled hypertonic saline were available in 81 individuals. Antibiotics - current treatment with oral or inhaled antibiotics; BMI - body mass index; FEV_1 - forced expiratory volume in one second; FRC - functional residual capacity from multiple-breath washout (MBW), LCI - lung clearance index from MBW; PEX - pulmonary exacerbation; P - P value: * Pearson chi2 ** Fisher's exact test # Two-sample Wilcoxon rank-sum test; *Pseudomonas* - *Pseudomonas aeruginosa*. None of the individuals had *Mycobacteria spp* or *Stenotrophomonas spp* positive respiratory samples.

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OLS Table 4. Specific changes in lung function during pulmonary exacerbation.

	Lung clearance index (LCI)				Forced expiratory volume in 1 s (FEV_1)			
	N	Coefficient	95% CI	P	N	Coefficient	95% CI	P
Model I	338	12.6	1.5 to 23.8	0.026	336	-10.3	-15.6 to -5.0	<0.001
Model II	338	13.6	2.5 to 24.7	0.016	336	-10.8	-16.1 to -5.5	<0.001
Model III	288	16.0	4.0 to 27.9	0.009	287	-11.1	-16.8 to -5.5	<0.001

Random effects multi-level linear regression coefficients given as percentage change (%) in LCI during a pulmonary exacerbation compared to LCI when clinically stable. We report unadjusted estimates and confounder-adjusted estimates. Model I is adjusted for multiple observations and centre. Model II is adjusted for age, days since last visit, multiple observations, and centre. Model III is adjusted for age, days since last visit, number of visits, *Pseudomonas* infection, multiple observations, and centre.

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OLS Table 5. Performance of LCI and FEV1 to predict pulmonary exacerbation.

	Sensitivity (%)	Specificity (%)	ROC area	PPV (%)	NPV (%)
(i) Lung function categories					
LCI > ULN	89.7 (75.8 to 97.1)	20.9 (16.9 to 25.3)	0.55 (0.50 to 0.61)	10.4 (7.3 to 14.1)	95.2 (88.3 to 98.7)
FEV1 < LLN	64.1 (47.2 to 78.8	73.9 (69.2 to 78.3)	0.69 (0.61 to 0.77)	20.2 (13.5 to 28.3)	95.3 (92.2 to 97.4)
(ii) Lung function change					
LCI ≥10%	46.9 (29.1 to 65.3)	65.0 (59.4 to 70.4)	0.56 (0.47 to 0.65)	12.3 (7.0 to 19.5)	92.1 (87.7 to 95.3)
LCI ≥20%	43.8 (26.4 to 62.3)	76.8 (71.7 to 81.4)	0.60 (0.51 to 0.69)	16.5 (9.3 to 26.1)	92.9 (89.0 to 95.7)
LCI ≥30%	31.3 (16.1 to 50.0)	85.9 (81.5 to 89.6)	0.59 (0.50 to 0.67)	18.9 (9.4 to 32.0)	92.3 (88.5 to 95.1)
LCI ≥40%	18.8 (7.2 to 36.4)	91.2 (87.4 to 94.1)	0.55 (0.48 to 0.62)	18.2 (7.0 to 35.5)	91.5 (87.8 to 94.4)
LCI ≥1.0 unit	46.9 (29.1 to 65.3)	69.6 (64.1 to 74.7)	0.58 (0.49 to 0.67)	13.9 (8.0 to 21.9)	92.6 (88.4 to 95.6)
LCI ≥2.0 unit	34.4 (18.6 to 53.2)	85.0 (80.5 to 88.8)	0.60 (0.51 to 0.68)	19.3 (10.0 to 31.9)	92.5 (88.8 to 95.3)
LCI ≥3.0 unit	15.6 (5.3 to 32.8)	91.8 (88.2 to 94.6)	0.54 (0.47 to 0.60)	16.7 (5.6 to 34.7)	91.2 (87.5 to 94.1)
LCI ≥4.0 unit	3.1 (0.1 to 16.2)	96.1 (93.3 to 98.0)	0.50 (0.46 to 0.53)	7.7 (0.2 to 36.0)	90.5 (86.7 to 93.4)

Data are given as mean (95% confidence intervals). Sensitivity is the proportion of individuals with primary ciliary dyskinesia (PCD) correctly identified that will have a pulmonary exacerbation (PEX). Specificity is the proportion of individuals with PCD correctly identified to remain clinically stable. The Receiver Operating Characteristic curve (ROC) area is the average of sensitivity and specificity. The positive and negative predictive values (PPV & NPV) take the prevalence of PEX [9.5 (6.6 to 13.1)%] into account and show the probability of the individuals having a PEX with (i) a lung function test above or below the upper or lower limit of normal (ULN, LLN; ±1.96 z-score) or (ii) following certain changes in lung function. FEV1 - forced expiratory volume in one second; LCI - lung clearance index.

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Lung function and risk of pulmonary exacerbation

In our time-to-occurrence study, 39 out of 436 visits were classified as pulmonary exacerbation (PEX) in 23 out of 90 individuals with PCD. Two PEX occurred in seven individuals, and three, four and five PEX in one individual, respectively. Because the time variable was time in the study, we did not adjust for time intervals between visits in the Cox proportional regression models.

Analysis of LCI categories based on the upper limit of normal LCI (< 1.96 z-score) is provided below (OLS Table 6) [4].

OLS Table 6. Lung function impairment and risk of pulmonary exacerbation.

		Univariable Model				Multivariable Model			
	Unit	N	Hazard Ratio	P	K	N	Hazard Ratio	P	K
LCI	category	3	2.37 (1.16 to 4.83)	0.018	0.65	3	2.97 (1.35 to 6.52)	0.007	0.69

Hazard Ratios (95% confidence interval) and postestimation concordance coefficients from the univariable Cox proportional regression adjusted for multiple observations and multivariable Cox proportional regression adjusted for multiple observations, age (tertiles), number of visits, and *Pseudomonas* positive respiratory samples (21 (5.8%) out of 365 samples). FEV_1 - forced expiratory volume in one second; K - *Gönen* and *Heller's K* concordance coefficient; LCI - lung clearance index; P - P value. Alternative lung function categories were z-score categories: normal LCI < 1.96 z-score, moderately elevated LCI ≥1.96 and ≤10 z-score and highly elevated LCI > 10 z-score)

We replicated univariable analysis with the same sample (n = 360 and 358, respectively) as the multivariable analysis (OLS Table 7) and confirmed risk estimates derived from the original univariable analysis in the whole population (Table 2, n = 430).

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OLS Table 7. Lung function impairment and risk of pulmonary exacerbation.

		Univariable Model			
	Unit	N	Hazard Ratio	P	K
LCI	unit	360	1.13 (1.05 to 1.23)	0.002	0.61
	category	3	2.03 (1.12 to 3.68)	0.020	0.64
FEV_1	z-score	358	0.63 (0.51 to 0.78)	<0.001	0.68
	category	3	0.34 (0.19 to 0.62)	<0.001	0.70

Hazard Ratios (95% confidence interval) and postestimation concordance coefficients from the univariable Cox proportional regression adjusted for multiple observations within the same sample as for the multivariable analysis (Table 2). FEV_1 - forced expiratory volume in one second; K - *Gönen* and *Heller's K* concordance coefficient; LCI - lung clearance index; P - P value. Lung function categories were tertiles.

Overall changes in lung function

Mean changes in LCI were not statistically significantly greater compared to changes in FEV_1 (P = 0.087, random effects model). Mean absolute and relative (SD; 95%CI) change in LCI was 0.22 units (2.17; -0.01 to 0.45) and 3.1% (30.8; -0.2 to 6.4). Mean absolute and relative change in FEV_1 was -0.01 z-score (1.23; -0.14 to 0.12) and -0.1% (14.9; -1.7 to 1.5).

Across the study period which was one year on average, LCI changed by 0.10 (-0.24 to 0.43) units per year and 1.90 (-1.71 to 5.51) % per year, respectively. FEV_1 changed by 0.03 (-0.13 to 0.19) z-score per year and -0.46 (-2.25 to 1.33) % per year, respectively.

Association of Lung Clearance Index and FEV_1

We analysed the association between LCI and FEV1 using a linear regression model. We took the subjects` average values (n = 90) from LCI and FEV_1 from all LCI and FEV_1 measurements. Per one z-score decline in FEV_1, LCI increased by on average (95%CI) by 1.42 (1.05 to 1.79 to); p < 0.001; linear regression R-squared = 0.40 (**OLS Figure 1**)

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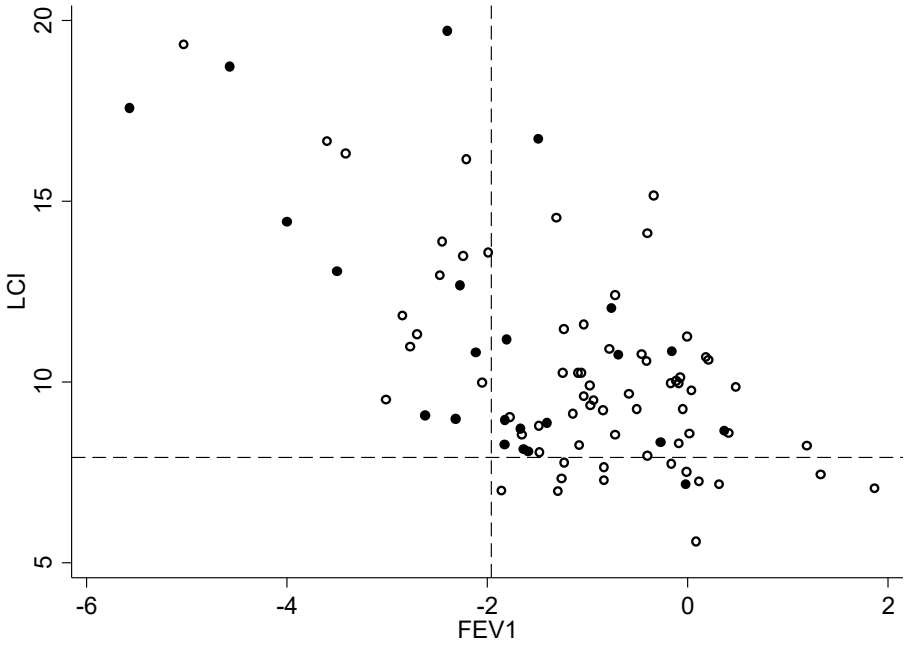
OLS Figure 1. Association of Lung Clearance Index and FEV_1.



Correlation of LCI and FEV_1 measurements averaged per individual (n = 90). LCI is given in original units, FEV_1 in z-score. Closed circles = individuals with at least one pulmonary exacerbation (PEX) during the study; open circles - individuals without any PEX during the study. Dashed lines - upper limit of normal LCI (7.91 units) and lower limit of normal FEV_1 (-1.96 z-score). FEV_1 - forced expiratory volume in one second; LCI - lung clearance index.

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References

[1] Lucas JS, Barbato A, Collins SA, Goutaki M, Behan L, Caudri D, et al. European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. vol. 49. 2017. doi:10.1183/13993003.01090-2016.

[2] Robinson PD, Latzin P, Ramsey KA, Stanojevic S, Aurora P, Davis SD, et al. Preschool multiple-breath washout testing an official American thoracic society technical statement. Am J Respir Crit Care Med 2018;197:e1–19. doi:10.1164/rccm.201801-0074ST.

[3] Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. Eur Respir J 2013;41:507–22. doi:10.1183/09031936.00069712.

[4] Anagnostopoulou P, Latzin P, Jensen R, Stahl M, Harper A, Yammine S, et al. Normative data for multiple breath washout outcomes in school-aged Caucasian children. Eur Respir J 2020;55:1901302. doi:10.1183/13993003.01302-2019.