ONLINE SUPPLEMENTARY MATERIAL

Alternative inert gas washout outcomes in patients with primary ciliary dyskinesia

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Methods

Lung function assessment

Nitrogen multiple breath washout

The main outcome variable to assess global VI was the LCI, first moment ratio (M_1/M_0) and second moment ratio (M_2/M_0). Standard LCI was calculated from the ratio of cumulative expired volume (CEV) divided by functional residual capacity (FRC), determined at $1/40^{th}$ washout which equals 2.5% of normalized starting N_2 end-tidal concentration ($LCI_{2.5\%}$). M_1/M_0 and M_2/M_0 were calculated as described (1): The normalized end-tidal N_2 concentration is plotted against the number of consecutive lung turn overs (TO = CEV/FRC), the area under this washout curve depicts the Moment (M) 0. Multiplying the N_2 concentration values by TO and TO^2 gives M_1 and M_2, respectively. The higher the moment the more weight is given to the tail of the washout. Ratios of MR_1 and MR_2 over M_0 were calculated, M_1/M_0 and M_2/M_0, which thus relate overall VI to VI from slowly ventilated lung regions (tail of the washout curve).

$LCI_{5\%}$ was calculated from $1/20^{th}$ or 5% of the normalized starting N_2 concentration(2). Scond* was calculated between the 0^{th} and 3^{rd} TO excluding the slope III value of the first breath (3). Sacin and Sacin*, estimates of acinary VI, were derived from the first slope III value accounting for conductive VI as recommended (3). The Scond* and Sacin* indices were proposed in more advanced disease where the progression of SIII values may form a plateau early after the 3^{rd} TO and not after the 6^{th} TO as usual. Besides this physiological consideration, the number of required breaths is decreased.

Single breath washout

The tidal SBW tests were performed using the same setup (Exhalyzer D). According to the ERS/ATS consensus, we repeated the measurement three times to obtain the average slope III values. After relaxed tidal breathing was established, measurements took one tidal in- and expiration from and back to FRC while the tracer gases were washed in and out. The double tracer gas (DTG) mixture contained 26.3% He, 5% SF6, 21% oxygen (O2) and balanced N2 (Singer, Stern et al. 2013). The total molar

mass of this gas mixture is equal to air; therefore molar mass changes during washout reflect ventilation distribution of the tracer gases. During the DTG in/expiration and the following inspiration, we recorded molar mass with a side-stream USFM, tidal flows with a mainstream USFM, and carbon dioxide and O2 signals. The molar mass signal measured in side-stream mode (MMss) during ambient air breathing was used to calibrate the pre-test CO2 signal, i.e. to transform the pre-test CO2 signal by linear regression modelling into an additional molar mass signal (calculated molar mass signal (MMcalc)). By "subtracting" MMcalc from the raw MMss during expiration of the DTG, the molar mass test signal reflecting the agglomerated SF6 and He washout pattern was obtained. The DTG expirogram (MMss-MMcalc) was plotted against expired volume. Duration of SBW testing was calculated by summing testing time with waiting time (10 breath) between tests. One measurement required a maximum of 60 seconds. During one breath the patient in- and exhaled the double-tracer gas (SF_6 and He) from FRC and back to FRC without any forced manoeuvre. Between DTG-SBW tests, at least 10 breaths of room air were required. The S_{III}-DTG was multiplied with tidal volume to normalize for physiological differences in breaths (7). We used the software Spiroware 3.1.6 (Eco Medics AG, Duernten, Switzerland) and LungSim 4.10.3 (NM GmbH, Thalwil, Switzerland) for SBW analysis.

Results

Success rate of gas washout measurements

Two patients had to be excluded from the study, because they performed only one valid N_2-MBW measurement. Thirty patients with PCD performed two and 17 performed three successful N_2-MBW measurements. Seven patients with PCD performed two and 28 at least three successful DTG-SBW. One patient was excluded from Scond and Sacin calculation, another patient was excluded only from Sacin calculation due to irregular breathing, diminishing S_{III} calculation quality. Due to software problems we had to exclude 10 patients from the Scond*, 10 patients from the Sacin* calculation. Fourteen patients from S_{III}-DTG calculation (figure E1) had to be excluded due inadequate DTG gas mixture. In the group of healthy controls 24 children performed three and 13 performed two successful

N_2-MBW measurements. Twenty-nine children performed three and eight at least two successful DTG-SBW. No child had to be excluded from the DTG-SBW measurements. Due to irregular breathing we had to exclude nine children for the analyses of Scond, Sacin, Scond* and Sacin*, respectively.

Legend to the figures

Figure E1 Flow-diagram of inclusion of patients in the gas washout measurements.
49 patients with PCD performed N_2-MBW and DTG-SBW. Two patients had to be excluded from the study, because they performed only one valid N_2-MBW measurement. Due to technical issues we had to further exclude the same 10 patients from the Scond* and Sacin* calculation and 14 patients from the S_{III}-DTG calculation. After breath quality control two patients were excluded from the Sacin and one from the Scond calculation due to strong irregular breathing pattern and infeasible S_{III} calculation. Graph was drawn by using CONSORT diagram (8).

Figure E2

Association between $LCI_{2.5\%}$ and Scond in 46 patients (black points) with primary ciliary dyskinesia (PCD) given in z-scores. Fitted values are shown by the black line. The grey background denotes the 95% confidence interval.

Figure E3

Association between $LCI_{2.5\%}$ [z-score] and S_{III}-DTG [z-score] in 35 patients (black points) with primary ciliary dyskinesia (PCD). Fitted values are belonging to the black line. The grey background denotes the 95% confidence interval.

Figure E4

Association between Scond [z-score] and FEV_1 [z-score] in 46 patients (black points) with primary ciliary dyskinesia (PCD). Fitted values are belonging to the black line. The grey background denotes the 95% confidence interval.

Figure E5

Association between Scond* [z-score] and FEV_1 [z-score] in 38 patients (black points) with primary ciliary dyskinesia (PCD). Fitted values are belonging to the black line. The grey background denotes the 95% confidence interval.

References

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Table E1 Demographic parameters of healthy controls and patients with PCD

Characteristics	**Healthy controls (n = 37)**	**PCD (n = 49)**
Male/Female	18/19	19/30
Age (years)	14.3 ± 1.4, range (4 to 42)	14.7 ± 6.6, range (11 to18)
Height (cm)	163 ± 9.73^{+}	152 ± 19.6^{+}
Weight (kg)	54 ± 12.3	48 ± 17.5
BMI (kg/m^2)	20 ± 3.4	20 ± 3.7

Baseline characteristics of healthy controls and primary ciliary dyskinesia (PCD) patients.

Values are expressed as absolute values (mean ± SD), except male/female. $^{+}$Only the height differed significantly between healthy controls and PCD, $p<0.05$. P-values are calculated by Student's t test.

Table E2 Detailed description of PCD diagnostic data of the ten PCD patients with normal TEM results

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
normal TEM	x	x	x	x	x	x	x	x	x	x
typical PCD phenotype[a]	x	x	x	x	x	x	x	x	x	x
situs inversus			x	x						
situs inversus in a sibling	x	x				x				
abnormal IF	lack of DNAH5	lack of DNAH5	lack of DNAH5	lack of CCDC11, DNALI1	abnormal DNAH5					
HVM										
- dyskinestic	x	x		x	x		x	x	x	x
- immotile			x			x				
genetic analysis with PCD defect identified				CCDC11 homozygoes	DNAI1 homozygous	DNAH5 homozygous +DNAH11 heterozygous				
positive family history of PCD (in a sibling)	x	x				x		x		
nasal NO below cut off (<77nl/min)	x	x	x		x	x	x		x	x
nasal NO value (nl/min)	29,30	29,54	18,48	251,53	16,40	12,21	8,90	185,46	2,3	7,69

[a] Typical symptoms of PCD were defined as neonatal onset airway symptoms, recurrent lower respiratory tract infections, chronic productive cough, blocked nose, recurrent sinusitis, recurrent otitis media.

Table E3 Detailed description of diagnostic data of the ten PCD patients without available TEM

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
typical PCD phenotype	x	x	x	x	x	x	x	x	x	x
situs inversus	x	x	x					x	x	
situs inversus in a sibling									x	
abnormal IF									x	
HVM (nearly) immotile*	x	x	x	x	x	x	x	x	x	
HVM hyperkinetic*										x
genetic analysis with PCD defect identified							DNAH5	DNAH5	ARMC4	
positive PCD family history				x	x					
nasal NO below cutoff of 77nl/min	x	x	x	x	x	x	x	x	x	x

*HVM showed congruent results on at least two occasions (for immotile cilia) or three occasions (for hyperkinetic ciliary beating pattern).

TABLE E4 Intra-test variability (CV) in patients with PCD and healthy controls who performed three trials.

Indices	Healthy controls	PCD	p-value for comparison PCD and healthy controls
$LCI_{2.5\%}$	4.6 (0.4 to 10.7)	5.5 (1.4 to 15.4)	0.44
$LCI_{5\%}$	3.7 (0.7 to 9.6)	4.4 (0.8 to 10.9)	0.40
M_1/M_0	3.7 (0.3 to 8.4)	4.5 (0.9 to 11)	0.29
M_2/M_0	8.6 (0.5 to 20.5)	9.9 (0.4 to 27.3)	0.47
Scond	55.7 (2.5 to 113.9)	24.2 (2.7 to 96.4)	**0.002**
Scond *	75.5 (2.8 to 134.4)	37.9 (2.0 to 111.5)	**0.004**
Sacin	61 (6.1 to 268.7)	32.6 (2.1 to 138.5)	0.14
Sacin*	48.6 (0.8 to 120.6)	38.3 (0.3 to 121)	0.07
S_{III}-DTG	41.9 (6.9 to 238)	23.9 (4.3 to 76.4)	0.08

Coefficient of variation data are expressed in mean (range). PCD (primary ciliary dyskinesia). $LCI_{2.5\%}$, lung clearance index 2.5%; $LCI_{5\%}$, lung clearance index 5%; M_1/M_0, M_2/M_0, moment ratio; Scond/Sacin, Scond*/Sacin* and S_{III}-DTG, normalized phase III slope indices (see text for explanation). Significant differences are marked as bold. Student's t test was used , as appropriate.

TABLE E5 Intra-test variability in patients with PCD and healthy controls who performed two trials.

Indices	Healthy controls	PCD
$LCI_{2.5\%}$	5.2 (2.8 to 7.6)	6.8 (3.4 to 10.1)
$LCI_{5\%}$	4.2 (1.6 to 6.7)	6.4 (4.3 to 8.6)
M_1/M_0	4.2 (1.3 to 7.2)	5.9 (3.1 to 8.8)
M_2/M_0	8.8 (3.1 to 14.6)	11 (5.9 to 16)
Scond	56 (29.1 to 83)	42.6 (33.5 to 52)
Scond *	300.4 (74 to 534)	49 (37.9 to 60.2)
Sacin	28.6 (9.7 to 47.4)	29.7 (18.5 to 40.8)
Sacin*	71.4 (2.5 to 58.5)	37.6 (26.9 to 48.3)
S_{III}-DTG	17.6 (4.3 to 30.9)	44.8 (-1.8 to 91.3)

Intra-test variability data are expressed in mean (95% Conf. Interval). PCD (primary ciliary dyskinesia). $LCI_{2.5\%}$, lung clearance index 2.5%; $LCI_{5\%}$, lung clearance index 5%; M_1/M_0, M_2/M_0, moment ratio; Scond/Sacin, Scond*/Sacin* and S_{III}-DTG, normalized phase III slope indices (see text for explanation).

Table E6 Association between FEF_{25-75} and different gas washout variables in z-scores

Indices	Coefficient	95 % CI	R^2	p-value
$LCI_{2.5\%}$	-0.2	-0.3 to -0.2	0.5	**<0.001**
$LCI_{5\%}$	-0.3	-0.4 to -0.2	0.4	**<0.001**
M_1/M_0	-0.3	-0.4 to -0.2	0.4	**<0.001**
M_2/M_0	-0.2	-0.2 to -0.09	0.4	**<0.001**
Scond	-0.2	-0.5 to 0.04	0.06	0.088
Scond*	-1.0	-1.3 to -0.6	0.5	**<0.001**
Sacin	-0.1	-0.3 to -0.02	0.1	**0.020**
Sacin*	-0.5	-0.9 to -0.1	0.2	**0.014**
S_{III}-DTG	0.6	0.2 to 1.0	0.2	**0.006**

Linear regression between FEF_{25-75} (forced expiratory flow 25–75%) and different gas washout indices: : lung clearance index, $LCI_{2.5\%}$; 2.5%; lung clearance index 5%, $LCI_{5\%}$; moment ratio, M_1/M_0, M_2/M_0; Scond/Sacin, Scond*/Sacin* and S_{III}-DTG, normalized phase III slope indices (see text for explanation). Significant values are marked in bold. Results are derived from linear regression analysis.