Physiological phenotyping of pediatric chronic obstructive airway diseases

Sylvia Nyilas,1,2 Florian Singer,1,3 Nitin Kumar,2 Sophie Yammine,1,2 Delphine Meier-Girard,2 Cordula Koerner-Rettberg,4 Carmen Casaulta,1 Urs Frey,2 and Philipp Latzin1,2

1Division of Respiratory Medicine, Department of Paediatrics, University Children’s Hospital of Bern, University of Bern, Bern, Switzerland; 2Department of Paediatric Pulmonology, University Children’s Hospital Basel (UKBB), Basel, Switzerland; 3Division of Respiratory Medicine, University Children’s Hospital Zurich, Zurich, Switzerland; and 4Department of Paediatric Pulmonology, University Children’s Hospital of Ruhr University Bochum at St. Josef-Hospital, Bochum, Germany

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Nyilas S, Singer F, Kumar N, Yammine S, Meier-Girard D, Koerner-Rettberg C, Casaulta C, Frey U, Latzin P. Physiological phenotyping of pediatric chronic obstructive airway diseases. J Appl Physiol 121: 324–332, 2016. First published May 26, 2016; doi:10.1152/japplphysiol.00086.2016.—Inert tracer gas washout (IGW) measurements detect increased ventilation inhomogeneity (VI) in chronic lung diseases. Their suitability for different diseases, such as cystic fibrosis (CF) and primary ciliary dyskinesia (PCD), has already been shown. However, it is still unclear if physiological phenotypes based on different IGW variables can be defined independently of underlying disease. Eighty school-age children, 20 with CF, 20 with PCD, 20 former preterm children, and 20 healthy children, performed nitrogen multiple-breath washout, double-tracer gas (DTG) single-breath washout, and spirometry. Our primary outcome was the definition of physiological phenotypes based on IGW variables. We applied principal component analysis, hierarchical Ward’s clustering, and enrichment analysis to compare clinical characteristics between the clusters. IGW variables used for clustering were lung clearance index (LCI) and convection-dependent [conductive ventilation heterogeneity index (LCI) and convection-dependent [conductive ventilation heterogeneity index (LCI) and convection-dependent [conductive ventilation heterogeneity index (LCI) and convection-dependent] and diffusion-convection-dependent variables [acinar ventilation heterogeneity index (Sacin) and carbon dioxide and DTG phase III slopes]. Three main phenotypes were identified. Phenotype I (n = 38) showed normal values in all IGW outcome variables. Phenotype II (n = 21) was characterized by pronounced global and convection-dependent VI while diffusion-dependent VI was normal. Phenotype III (n = 21) was characterized by increased global and diffusion- and convection-dependent VI. Enrichment analysis revealed an overrepresentation of healthy children and former preterm children in phenotype I and of CF and PCD in phenotypes II and III. Patients in phenotype III showed the highest proportion and frequency of exacerbations and hospitalization in the year prior to the measurement. IGW techniques allow identification of clinically meaningful, disease-independent physiological clusters. Their predictive value of future disease outcomes remains to be determined.

gas washout; spirometry; phenotypes; clustering; lung disease

NEW & NOTEWORTHY

Clustering signals from different single- and multiple-breath gas washout tests in children with various lung diseases (e.g., cystic fibrosis and primary ciliary dyskinesia) results in the identification of three different physiological phenotypes. This novel application of the hierarchical Ward’s clustering method allows the characterization of lung disease independent of the underlying disease entity and thus seems a promising tool for personalized medicine.

THE FRACTAL ARCHITECTURE OF SMALL Airways enables homogeneous ventilation with gas transport into the gas-exchanging acinar compartments by convection and diffusion (44, 45). Depending on the generation and distribution of affected airways, respective function is impaired to a varying degree and often subclinically in many chronic lung diseases. Chronic lung diseases such as cystic fibrosis (CF) and primary ciliary dyskinesia (PCD) significantly differ in etiology but may share similar physiological characteristics. In both diseases, inhomogeneous patchy distribution of airway obstruction is associated with inhomogeneous ventilation distribution (16, 25). During early stages of lung diseases, small airways in particular are most affected. This is best captured by inert gas washout (IGW) tests while ventilation capacity measured by spirometry is usually less affected (8, 17, 27). During the disease course, alterations in airway structure occur and ultimately lead to a changing picture of functional limitations. Despite possible similarities between diseases and changes in physiological properties over the disease course, treatment usually depends on underlying disease entity.

Especially with regard to precision and individualized medicine a better characterization of the underlying physiology seems worthwhile to establish; this would enable tailored diagnosis and therapy. One possibility to do so would be to cluster the compound information from different outcome variables from various IGW tests. Statistical methods such as hierarchical Ward’s clustering can then be used to combine different physiological variables in an unsupervised way (12, 35).

In our study we hypothesized that clustering may identify physiological phenotypes that do not necessarily relate to different disease etiologies but rather to common physiological information and that resulting clusters show differences in enrichment of other characteristics.

METHODS

Study Design

In this proof-of-principle study we used IGW data from 80 children with different lung diseases. We first performed conventional (biased) analyses comparing IGW outcome variables between disease groups. Second, we performed principal component analysis (PCA) to determine whether or not all IGW outcome variables are necessary for clustering. In a third step, we performed Ward’s hierarchical clustering using the outcome variables from IGW. Primary outcome was the definition of physiological phenotypes based on IGW variables calculated by hierarchical Ward’s clustering. Secondary outcome was
differences between clusters assessed by enrichment analysis. For this analysis we compared disease etiology, demographics, IGW outcomes, and disease course previous to the measurement between the groups (7, 28).

Study population. We included 80 children from 3 disease groups (CF, PCD, and former preterm children) as well as healthy children with 20 children from each group. Children were 7-18 yr old and consecutively recruited at the outpatient clinics at the University Children’s Hospital Bern, Switzerland, and Children’s Hospital Bochum, Germany, between January 2012 and May 2015 independent of disease activity or medication. PCD patients were enrolled at University Children’s Hospital Bochum (n = 17) and Bern (n = 3); all other children were enrolled at the University Children’s Hospital Bern. Exclusion criteria for all children were respiratory infection within the last 3 wk, acute pulmonary exacerbation at the time of measurements (marked increase in cough, fever, or malaise), or history of lung disease in healthy children. All children underwent two different established gas washout techniques, single- and multiple-breath washout tests (SBW or MBW). All except healthy children underwent subsequent spirometry. Measurements were performed on the same day.

Ethics Statement

The study was approved by the Ethics Committee of the Canton of Bern, Bern, Switzerland, and Ethics Committee of the Ruhr University, Bochum, Germany. We obtained written informed consent from parents and participants older than 16 yr.

Lung Function Measurements

Multiple-breath washout. Tidal N2-MBW tests were performed in triplicate with a validated setup (Exhalyzer D; Eco Medics, Duernten, Switzerland) (32) according to the guidelines (31). The main outcome variable was the lung clearance index (LCI) calculated from the ratio of cumulative expired volume divided by functional residual capacity (FRC). Resulting LCI units are lung turnovers. LCI reflects the lung turnover measured at one-fortieth of initial starting N2 concentration as recommended. Alveolar phase III slopes (SIII) from washout breaths were automatically calculated between 65 and 95% of the expired volume with manual adjustment as appropriate. Standard corrections for tracer gas concentration and tidal volume were done automatically. Conductive ventilation heterogeneity index (Scond) was calculated from the evolution of SIII between lung turnovers 1.5 and 6. The first washout breath’s SIII was used to derive acinar ventilation heterogeneity index (Sacin; 18). Please see below for detailed physiological explanations.

Single-breath washout. The tidal SBW tests were performed in triplicate using the same setup (Exhalyzer D). After established relaxed tidal breathing, measurements took one tidal inspiration 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 (32). The total molar mass of this gas mixture was equal to air; therefore molar mass changes during washout reflected ventilation distribution of the tracer gases. The SIII quantified from the molar mass expirogram (SIII-DTG) was the primary outcome variable (34). Capnography was derived from the DTG-SBW, with SIII quantified from the CO2 expirogram (SIII-CO2) as outcome variable. We used LungSim 4.6.0 (NM Numerical Modelling, Thalwil, Switzerland) for signal processing and analyses as described (1, 29, 33).

Spirometry. Spirometry was performed according to the guidelines (24, 36) using the MasterScreen (Jaeger, Wurzburg, Germany). Outcome was forced expiratory volume in 1 s (FEV1).

Physiological Meaning of Gas Washout Signals

Both MBW and SBW tests are established methods to quantify the extent of impaired ventilation distribution efficiency and potential areas of the airway tree where ventilation inhomogeneity (VI) may predominantly arise. LCI from N2-MBW reflects global VI, which is a mixture of VI arising in central and peripheral airways as well as in dead space (39). To characterize more specifically the location in the airway tree at which VI arises, additional outcome variables were assessed. One is the division of MBW into Scond and Sacin. Scond represents VI generated in convection-dependent precapillary airways. Sacin estimates inhomogeneity generated in the region of the diffusion- and convection-dependent front close to the entrance of acinar airways. The third method used to more specifically characterize VI is by analyzing SIII that are simultaneously obtained from inert gases of similar convection but different diffusion properties. For a heavy gas such as SF6, the diffusion-convection front approximates to the mouth of the acinus and stretches into the proximal portion of the acinus. Concerning He, this front is more proximal. The DTG-SBW signal reflects a composite signal of He, SF6, and N2 (38). Elevated SIII-DTG seems to mainly reflect VI arising due to structural changes between the different diffusion-convection fronts of all three gases. Capnography is less specific and depends on perfusion, the blood air barrier, and diffusion- and convection-dependent VI as well as dead space.

Statistical Analysis

The z-scores for washout outcomes were calculated from healthy children. Upper limit of normal (ULN) and lower limit of normal (LLN) were defined as means ± 1.64 (SD) from healthy children. The z-scores for FEV1, height, weight, and body mass index (BMI) were derived from the current standard reference equations (10, 36). For the following outcomes, higher absolute values and z-scores indicate greater disease: LCI, Scond, Sacin, and SIII-CO2. Lower absolute values and z-scores indicate worse disease for FEV1 and SIII-DTG.

For classical biased analysis, comparisons were done using Student’s t-test, Wilcoxon rank sum tests, chi-square test, and one-way ANOVA tests, as appropriate. Post hoc tests for pairwise multiple comparisons were performed using the ANOVA with Bonferroni correction, as appropriate. All tests were two-sided with a significance level of 0.05 and performed using STATA R, Version 2.10 (Stata Statistical Software: Release 13; StataCorp, College Station, TX) (27a).

Principal component analysis (PCA) was utilized as a dimension reduction procedure to reduce the large number of variables into an interpretable combination of the data. The resulting linear combination corresponds to a principal component (42). We applied PCA to identify a combination of VI outcomes that would explain more than 80% of overall variation in data. PCA performed on correlation matrix of the five IGW outcomes was used as a dimensionality reduction technique to identify which combination of five IGW outcomes might be most relevant for diagnostic purposes. For each condition (healthy, former preterm, CF, and PCD), the obtained eigenvalues and principal components (PCs) of the matrices were considered. The first PC (PC1) accounts for the majority of the data variance; PC1 and the corresponding values from IGW outcome variables for all children and each disease group separately were used to reflect the dominant value. PC1 and its corresponding loading coefficients were evaluated to determine the dominant value in the lung function measurement. The data for 1) all children and 2) each disease group separately were plotted into the first principal axis to obtain the variance explained by PC1 for all children and each disease group separately (21, 42).

Ward’s hierarchical clustering was performed to identify physiological phenotypes based on the IGW outcome variables. We performed the clustering until the next number of clusters resulted in less than five patients in one of the clusters (43). An enrichment analysis was performed using the hypergeometric test (2) to assess overrepresentation of clinical variables within any phenotype.
RESULTS

Eighty children were enrolled (Table 1). We used 400 IGW outcomes to define different clusters.

Classical (Biased) Analysis

We performed classical analysis of pulmonary function outcome variables for the different disease groups on the group level (details in Table 2) and on an individual level (Table 3).

Lung function in former preterm children was normal on the group level apart from elevated SIII-CO2. SIII-CO2 was 0.7 ± 0.2 (mean ± SD) and thus slightly increased compared with healthy children (0.5 ± 0.2, P = 0.047). On an individual level, prevalence of pathological values did not exceed 10% of patients in any of the lung function indexes (Table 3).

CF showed significantly impaired IGW results compared with healthy children on the group level. LCI was significantly higher in CF than in healthy children (10.8 ± 2.4 vs. 7.4 ± 0.7, P < 0.001). Scond also differed significantly, with 0.07 ± 0.03 compared with 0.01 ± 0.02, P < 0.001. On an individual level, pathological values were found in 16/20 patients (80%) for LCI and Scond. Despite that, interindividual heterogeneity of VI was high, with very different z-scores for the different IGW outcome variables as shown for two specific patients with CF in Figs. 1–3.

In patients with PCD, in addition to convection-dependent VI, also diffusion- and convection-dependent VI measured by SIII-DTG was significantly elevated on the group level compared with healthy children (−0.2 ± 0.1 vs. −0.1 ± 0.09, P < 0.001). A comparable elevation was found for SIII-CO2 with 0.8 ± 0.3 vs. 0.5 ± 0.2, P = 0.004. On an individual level, up to 30% of patients with PCD showed pathological values for peripheral VI, compared with 85% of patients with pathological values for global VI (Table 3).

Unsupervised Analysis

Principal component analysis. We applied PCA to IGW outcome variables expressed in z-scores from MBW and SBW. The first three PCs explaining 87% of total variance were selected. PC1 reflected the comparison between SIII-DTG and all other outcome variables. PC2 had a higher Scond loading. PC3 reflected SIII-DTG and Sacin vs. LCI, Scond, and SIII-CO2. The presence of all five IGW outcomes in the first three principal components suggests the importance of all these outcomes to explain the variability of the data and to differentiate between diseases. Therefore we included all five IGW outcomes in the cluster analysis (Tables 4 and 5 and Fig. 4, A and B).

Hierarchical Ward’s clustering. Unsupervised analysis was feasible and identified three physiological phenotypes. The number of phenotypes was determined by a satisfying sample size in each phenotype as described above. The heat map containing the dendrogram obtained using clustering of IGW outcome variables is shown in Fig. 5. Enrichment analysis revealed an overrepresentation of healthy children and former preterm children in the first phenotype (P < 0.001) and of CF (P = 0.03) and PCD (P = 0.008) in the second phenotype. The third phenotype consisted only of patients with CF (P < 0.001) and PCD (P < 0.001). Age and other anthropometric variables were not different between phenotypes. The following IGW outcomes were different between the first and second phe-

Table 1. Demographics of the healthy, former preterm, CF, and PCD subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy (n = 20)</th>
<th>Former Preterm (n = 20)</th>
<th>CF (n = 20)</th>
<th>PCD (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>13.5 ± 2.1</td>
<td>9.0 ± 1.7*</td>
<td>11.4 ± 2.6</td>
<td>13 ± 2.7</td>
</tr>
<tr>
<td>Gender, male</td>
<td>9 (45%)</td>
<td>7 (35%)</td>
<td>8 (40%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>49.5 ± 12.9</td>
<td>27.4 ± 4.9</td>
<td>36.6 ± 11.6</td>
<td>48.2 ± 13.6</td>
</tr>
<tr>
<td>Weight, z-score</td>
<td>0.1 ± 0.1</td>
<td>−0.4 ± 1.0</td>
<td>−0.5 ± 1.4</td>
<td>0.2 ± 1.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>19.4 ± 3.0</td>
<td>15.8 ± 1.4</td>
<td>17.7 ± 2.7</td>
<td>20.0 ± 3.3</td>
</tr>
<tr>
<td>BMI, z-score</td>
<td>−0.02 ± 0.9</td>
<td>−0.4 ± 0.9</td>
<td>−0.1 ± 1.0</td>
<td>0.2 ± 1.3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>158.6 ± 12.6</td>
<td>131.5 ± 9.0</td>
<td>142.0 ± 16.0</td>
<td>153.8 ± 16.2</td>
</tr>
<tr>
<td>Height, z-score</td>
<td>0.2 ± 1.1</td>
<td>−0.2 ± 0.9</td>
<td>−0.5 ± 1.5</td>
<td>−0.08 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD or number (percentage). CF, cystic fibrosis; PCD, primary ciliary dyskinesia. *Statistically significant differences (P < 0.05) in the demographic (z-scores for weight, BMI, and height) compared with the healthy group. Comparisons were done using Student's t-test or Wilcoxon rank sum tests, as appropriate. One-way ANOVA showed no significance for group comparison.

Table 2. Lung function outcomes

<table>
<thead>
<tr>
<th>Index</th>
<th>Healthy (n = 20)</th>
<th>Former Preterm (n = 20)</th>
<th>CF (n = 20)</th>
<th>PCD (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI, turnover</td>
<td>7.4 ± 0.7</td>
<td>7.5 ± 0.6</td>
<td>10.8 ± 2.4</td>
<td>11.3 ± 2.9</td>
</tr>
<tr>
<td>LCI, z-score</td>
<td>0.2 ± 0.9</td>
<td>5.0 ± 3.5</td>
<td>5.7 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Scond, %/turnover</td>
<td>0.01 ± 0.02</td>
<td>0.02 ± 0.02</td>
<td>0.07 ± 0.03</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Scond, z-score</td>
<td>0.5 ± 1.2</td>
<td>4.2 ± 2.3</td>
<td>3.3 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Sacin, %</td>
<td>0.09 ± 0.05</td>
<td>0.07 ± 0.05</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Sacin, z-score</td>
<td>−0.3 ± 1.0</td>
<td>0.4 ± 2.0</td>
<td>0.5 ± 2.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD for absolute values and z-scores. lung clearance index (LCI), outcome for global VI; Scond, outcome for convection-dependent VI; Sacin, outcome for the diffusion- and convection-dependent VI; SIII-DTG and SIII-CO2, diffusion- and convection-dependent VI; forced expiratory volume in 1 s (FEV1), outcome for central airway VI.

Table 3. Prevalence of abnormal lung function

<table>
<thead>
<tr>
<th>Index</th>
<th>Healthy (n = 20)</th>
<th>Former Preterm (n = 20)</th>
<th>CF (n = 20)</th>
<th>PCD (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global ventilation inhomogeneity</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td>16 (80%)</td>
<td>14 (70%)</td>
</tr>
<tr>
<td>LCI, turnover</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td>16 (80%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Convection-dependent ventilation inhomogeneity</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td>16 (80%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Scond, %/turnover</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td>16 (80%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Sacin, %</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td>16 (80%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>SIII-DTG, g/mol</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td>16 (80%)</td>
<td>17 (85%)</td>
</tr>
</tbody>
</table>

Data are presented as number (percentage). Upper limit of normality was defined as mean ± 1.96 (SD) from the healthy children. CF, cystic fibrosis; PCD, primary ciliary dyskinesia; LCI, outcome for global VI; Scond, outcome for convection-dependent VI; Sacin, outcome for diffusion- and convection-dependent VI; SIII-DTG and SIII-CO2, diffusion- and convection-dependent VI.
notypes: LCI (P < 0.001), Scond (P < 0.001), SIII-DTG (P = 0.016), and SIII-CO2 (P = 0.008). Sacin did not significantly differ between the first two phenotypes. Interestingly, FEV1 was comparable between the first and second phenotypes. All IGW outcome variables and also FEV1 were significantly different in the third phenotype compared with the other two phenotypes (Table 6 and Fig. 5). Although patients in the third phenotype showed a higher rate and frequency of exacerbations and hospitalization, differences between phenotypes did not reach statistical significance (Table 6).

DISCUSSION

Using different IGW outcomes, we identified three main phenotypes of peripheral airway disease in children with different lung diseases independent of the underlying disease entity. While IGW outcomes were different between those phenotypes, interestingly, spirometry did not differ between the first two phenotypes. Hierarchical Ward’s clustering is easily applicable to IGW outcomes and seems to be a suitable method to better characterize physiological lung disease to a large degree independent of the clinical diagnosis. Phenotype I showed normal VI. Phenotype II showed pronounced global and convection-dependent VI while diffusion-dependent VI was normal. Phenotype III was characterized by increased global and diffusion- and convection-dependent VI. The physiological clusters appear clinically meaningful. Comparing clinical characteristics, we found an increase in occurrence and frequency of exacerbations and hospitalization for intravenous antibiotic treatment from phenotypes I to III. The clusters’
predictive value of future disease outcomes and stability over time remain to be determined.

The first three PCs explained 87% of total variance on the basis of all five IGW outcomes. This underlines the different information obtained from all IGW outcomes. This may in part confirm previous numeric lung model work (40, 41) suggesting that indexes of global and specific VI relate to the full range of airway calibers across all generations. Classical descriptive comparison between groups did not distinguish well between individual differences in lung function parameters. This high individual heterogeneity in IGW outcome variables requires a different and independent approach to determine individual VI. We believe that unsupervised analysis using hierarchical Ward’s clustering represents such an approach, especially as it is easily feasible and provides physiologically meaningful phenotypes.

These phenotypes are not necessarily specific for disease entities in children but reflect physiological relations, which are similar in CF and PCD from a functional outcome perspective. As VI in chronic lung disease is complex and dynamic over time and current methods do not provide, for example, spatial resolution (4, 5), characterization into physiological phenotypes may indeed help to personalize diagnostic procedures and therapeutic approaches in the future. Notably, convection-dependent and diffusion- and convection-dependent VI is rather independent from airway resistance formed by larger airway spaces (22).

On the basis of hierarchical Ward’s clustering we identified three main phenotypes. While the majority of children in the first phenotype were healthy children and former preterm children, still a few patients with CF and PCD were included in this phenotype. The second cluster is mainly represented by CF and PCD patients, but also healthy children and former preterm children were included. A clear delineation between healthy children and patients with chronic lung disease depends on disease severity and phenotype. In agreement with that, previous studies in adult patients with chronic obstructive pulmonary disease (COPD) could also distinguish three phenotypes by using the hierarchical Ward’s clustering (12). They found an overlap between healthy subjects and patients with mild COPD.

FEV₁ did not differ between the first two clusters, which is no surprise as FEV₁ is rather insensitive for structural pathology in small airways or central bronchiectasis detected in CT scans (8). This may, however, prompt the question of whether previously described associations between LCI and FEV₁ were directly related or rather an epiphenomenon of advanced airway obstruction impairing both ventilatory capacity and efficiency (8, 14, 15, 17, 23, 26). Patients in the third cluster with

Table 4. Principal component analysis

<table>
<thead>
<tr>
<th>Lung Function Outcome</th>
<th>PC₁</th>
<th>PC₂</th>
<th>PC₃</th>
<th>PC₄</th>
<th>PC₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_{III}$-DTG</td>
<td>0.292</td>
<td>0.218</td>
<td>0.902</td>
<td>0.193</td>
<td>−0.128</td>
</tr>
<tr>
<td>$S_{III}$-CO₂</td>
<td>−0.513</td>
<td>−0.270</td>
<td>0.065</td>
<td>0.811</td>
<td>0.056</td>
</tr>
<tr>
<td>LCI</td>
<td>−0.559</td>
<td>0.235</td>
<td>0.065</td>
<td>−0.229</td>
<td>−0.759</td>
</tr>
<tr>
<td>Scond</td>
<td>−0.409</td>
<td>0.726</td>
<td>0.048</td>
<td>−0.059</td>
<td>0.548</td>
</tr>
<tr>
<td>Sacin</td>
<td>−0.415</td>
<td>−0.546</td>
<td>0.419</td>
<td>−0.500</td>
<td>0.323</td>
</tr>
</tbody>
</table>

The five extracted principal components (PCs) and their loading coefficients for all children. $S_{III}$-DTG and $S_{III}$-CO₂, diffusion- and convection-dependent VI; lung clearance index (LCI), outcome for global VI; Scond, outcome for convection-dependent VI; Sacin, outcome for diffusion- and convection-dependent VI.

Table 5. Percentage of total variance

<table>
<thead>
<tr>
<th></th>
<th>PC₁</th>
<th>PC₂</th>
<th>PC₃</th>
<th>PC₄</th>
<th>PC₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>54</td>
<td>19</td>
<td>18</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Healthy</td>
<td>34</td>
<td>29</td>
<td>20</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Former preterm</td>
<td>50</td>
<td>26</td>
<td>17</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>CF</td>
<td>48</td>
<td>35</td>
<td>12</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>PCD</td>
<td>53</td>
<td>21</td>
<td>17</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are given in percent. The percentages of variance accounted by each PC are derived for all subjects, healthy subjects, former preterm subjects, patients with cystic fibrosis (CF), and patients with primary ciliary dyskinesia (PCD).
CF and PCD were mainly characterized by marked overall and diffusion- and convection-dependent VI and a trend for a higher rate and frequency of exacerbations and hospitalization. This has also been reported in patients with severe CF lung disease (3, 15, 17, 18).

**Implications of the Study**

This is the first study showing that different physiological phenotypes for pediatric obstructive small airway diseases can be derived by applying established clustering methods using individual washout variables. The applied IGW seem useful for routine application because of their ease and reliability of data collection in both children and adults (20). Patients with distinct VI phenotypes may benefit from, for example, VI specific particle size of inhaled drugs to improve deposition. This seems to be a further step toward personalized medicine.

Spirometry and disease entities did not add to these phenotypes. Phenotyping the functional deficits may open up new

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**Fig. 4. Total and cumulative variance explained by principal components.**

A: percentage of total variance explained by each PC derived for the four different groups: ○, all children; ▼, only healthy children; squares with x, former preterm children; ◊, patients with CF; diamonds with cross, only patients with PCD. PC1 to PC5, the first to the fifth principal components. B: cumulative percentages of variance accounted by the PCs for all children.

**Fig. 5. Heat map representing hierarchical Ward’s clustering.**

Scord, outcome for convection-dependent VI; Sacin, outcome for the diffusion- and convection-dependent VI; lung clearance index (LCI), outcome for global VI; SIII-DTG and SIII-CO2, diffusion- and convection-dependent VI. The left color bar denotes individual subject grouping and their related cluster. Column on the left: red, healthy children; blue, former preterm children; green, cystic fibrosis; black, primary ciliary dyskinesia. Color gradients: brighter red tones indicate a higher z-score; darker red tones indicate a lower z-score.
Unanswered Questions and Outlook

We did not apply computed tomography (CT) scans and thus association with structure cannot be derived from this study. Whether or not adding data from CT scans will allow even better characterization of the phenotypes is thus unknown. Previous studies found associations between global VI and acinar VI and CT scores (13, 27). We acknowledge the relatively small sample size of each disease group, which constrained internal validation; thus further external validation is warranted.

Longitudinal data are required to assess the stability of identified clusters and their ability to differentiate specific disease-independent phenotypes from disease-dependent stages.

We did not integrate analysis of fast and slowly ventilated compartments in our study (18). Thus it is unclear whether compartment analysis would have exhibited colinearity with those VI indexes assessed in this study. Another drawback is that the upper limit of normal for all outcomes is based on a sample of only 20 healthy subjects. This limits the precision and general applicability of this upper limit. However, all measurements were performed according to current guidelines (6, 29) and use of the setup without further modification. The technical error of the setup is as low as 5%. Thus our findings can be easily reproduced. Notably, the upper limits of normal are comparable with previous studies from different groups (8).

For our proof-of-principle study we only included few children and well-defined airway diseases. However, this approach is certainly applicable to other chronic lung diseases with even greater heterogeneity and larger patient groups, such as asthma or COPD, where classical diagnosis may overlap.

Conclusions

Taken together, we can easily and precisely phenotype patients independently of underlying disease entities using established clustering methods. In our proof-of-principle study, unsupervised VI analysis identified three different physiological phenotypes. Classical comparison of groups between CF and PCD was informative in a different way but did not reveal individual physiological differences independent of the disease.
group. Longitudinal larger studies may establish the clusters’ stability over time and potential predictive value for later outcome.

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AUTHOR CONTRIBUTIONS

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