Alternate gas washout indices: Assessment of ventilation inhomogeneity in mild to moderate pediatric cystic fibrosis lung disease

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Abstract

Introduction: Normalized phase III slope (SnIII) indices from multiple breath washout (MBW) estimate ventilation inhomogeneity. Alternate (*) protocols for SnIII indices exist, however the utility of these outcomes in children with mild-to-moderate cystic fibrosis (CF) is unknown.

Methods: We measured nitrogen MBW and spirometry in 135 children (43 controls) aged 4-18 years. We assessed validity, practicability, and reliability of SnIII protocols. Outcomes included the ability to detect abnormal lung function, test agreement, measurement duration, intra-test repeatability, and quality.

Results: Lung clearance index (LCI) was abnormal in 80 (87%), Scond in 55 (60%), Scond* in 17 (19%), Sacin in 10 (11%), Sacin* in 11 (12%), and FEV1 in 28 (30%). Alternate protocols reduced measurement duration. Agreement of indices to detect abnormal lung function was poor. The quality of analysis and repeatability deteriorated with the alternate technique compared to standard.

Conclusion: In children with mild-to-moderate CF lung disease, alternate protocols seem practical but clinimetric properties of standard SnIII protocols are preferable.

KEYWORDS
child, cystic fibrosis, lung function tests

INTRODUCTION

Ventilation inhomogeneity is a widely recognized biomarker of both central and peripheral airway dysfunction.1 Children and adults with chronic lung diseases such as cystic fibrosis (CF) have varying degrees of small airways disease. Nitrogen multiple-breath washout (N2MBW) has been identified as a sensitive and feasible method to assess small airway disease that is applicable across different age-2,3 and disease-groups.4 One of the main N2MBW indices reflecting overall ventilation inhomogeneity is the lung clearance index (LCI).3 Whereas standard LCI is measured at 1/40th of the starting N2 concentration, abbreviated LCI protocols stop at 1/20th (LCI2.5% and LCI5%, respectively). The abbreviated LCI protocol can be applied in patients

Abbreviations: CDI, convection dependent inhomogeneity; CF, cystic fibrosis; DCDI, diffusion convection dependent inhomogeneity; FEV1, forced expiratory volume in one second; FRC, functional residual capacity; LCI, lung clearance index; N2MBW, nitrogen multiple breath washout; SaO2, Slope of phase II; SnIII, Slope of the normalized phase III; TO, Turn over; VT, tidal volume.
with CF to shorten the test duration, however, the sensitivity to detect abnormal ventilation inhomogeneity may decrease.\textsuperscript{5,6}

Analysis of the alveolar phase III slopes (Sn) from N\textsubscript{2}MBW provides information on more specific aspects of ventilation inhomogeneity in contrast to the LCI. Scond reflects convection-dependent inhomogeneity (CDI) arising proximal to acinar airways,\textsuperscript{7} whereas Sacin reflects diffusion-convection-dependent inhomogeneity (DCDI) close to acinar airways.\textsuperscript{8} Scond and Sacin therefore may provide additional information on responses to airway challenges or treatment effects in the lung periphery.\textsuperscript{9-12} Alternate protocols (\textdagger) for Scond\textsuperscript{*} and Sacin\textsuperscript{*} calculated from less breaths during an earlier phase of N\textsubscript{2}MBW are available. Scond\textsuperscript{*} and Sacin\textsuperscript{*} have been reported to better capture CDI and DCDI in adult patients with severe CF lung disease compared with the standard protocol.\textsuperscript{9} These alternate protocols could theoretically increase MBW success rate in children. Obtaining MBW tests in young children can be difficult, especially in the clinical setting with limited time for lung function testing. The Scond\textsuperscript{*} provides the possibility to obtain data on CDI in intentionally abbreviated trials or in trials without a complete washout, due to premature test termination or leaks. However, important clinimetric properties of Scond\textsuperscript{*} and Sacin\textsuperscript{*} have not yet been systematically studied.\textsuperscript{9}

We hypothesized that properties of Scond\textsuperscript{*} and Sacin\textsuperscript{*} are comparable to Scond and Sacin. Our aim was to assess validity, practicability, and reliability of Scond\textsuperscript{*} and Sacin\textsuperscript{*} in children with CF and healthy controls. Primary outcomes were (i) validity — ability to detect abnormal lung function and agreement between indices, (ii) practicability — test duration, and (iii) reliability — SnIII fitting quality and intra-test repeatability. Secondary outcomes were associations between Scond, FEV\textsubscript{1}, and LCI\textsubscript{2.5\%}.

2 | MATERIALS AND METHODS

2.1 | Study design

We conducted a cross-sectional multi-center study at the University Children’s Hospitals Zurich, Bern, and Lausanne (Switzerland). Children and adolescents with confirmed CF, aged between 4 and 18 years, were eligible for this study. Exclusion criteria included the need for supplemental oxygen therapy.

Between January 2011 and January 2016 subjects were enrolled in an outpatient setting in Zurich (n = 14), Bern (n = 69), and Lausanne (n = 19). Healthy controls, recruited from local schools and playgroups in Bern, had no history of chronic lung disease and no acute respiratory infections within the 3 weeks prior to the measurements. Some data from healthy controls have been published previously.\textsuperscript{13} All study participants performed N\textsubscript{2}MBW. In addition, patients with CF performed spirometry following N\textsubscript{2}MBW. The local Ethics Committees approved the study. The children’s assent was obtained and parents or their legal guardian provided written informed consent for the study.

2.2 | Nitrogen multiple-breath washout

All study participants performed N\textsubscript{2}MBW with a commercially available, unmodified device (Exhalizer D, Eco Medics AG, Duernnten, Switzerland).\textsuperscript{14} N\textsubscript{2}MBW tests were performed, according to ERS consensus and the international standard operating procedures shared between the centers.\textsuperscript{8} Briefly, study participants performed N\textsubscript{2}MBW in an upright position, while tidally breathing through a snorkel mouthpiece with a dead space reducer; a bacterial filter and a nose clip in place. Study participants were encouraged to engage in regular, relaxed breathing while watching a DVD for distraction. N\textsubscript{2}MBW was performed through tidal breathing of 100\% oxygen (O\textsubscript{2}) until the end-tidal N\textsubscript{2} concentration values fell below 1/40th of its starting concentration for at least three consecutive breaths. Between each trial, participants relaxed and were breathing room air to return the N\textsubscript{2} concentration back to baseline. N\textsubscript{2}MBW trials were accepted if there was no evidence of cough, sighs, or leaks and FRC varied less than 25\%.\textsuperscript{15} We aimed for three, but required at least two, technically acceptable N\textsubscript{2}MBW trials per test.

2.3 | Estimates of ventilation inhomogeneity

According to the current consensus (details are given below) we calculated the following indices from N\textsubscript{2}MBW using available customized in-house software (LungSim, Matlab Version 4.7.6): LCI as a marker of global ventilation inhomogeneity and Scond, Scond*, Sacin, and Sacin* as markers of CDI and DCDI. LCI\textsubscript{2.5\%} and LCI\textsubscript{5\%} were calculated from the ratio of cumulative expired volume divided over functional residual capacity (FRC) determined at 1/40th and 1/20th of the N\textsubscript{2} starting concentration. To assess CDI and DCDI, Sn\textsubscript{III} values were determined semi-automatically from N\textsubscript{2}MBW traces.\textsuperscript{8,16} The software (i) determined the alveolar plateau for calculation of Sn\textsubscript{III} between 65\% and 95\% of expired volume, (ii) normalized Sn\textsubscript{III} values for mean N\textsubscript{2} dilution across phase III and multiplied it by the tidal volume (VT) of respective washout breaths (Sn\textsubscript{III}).\textsuperscript{8} Breath-by-breath quality control and breath selection was performed by the operator as previously described.\textsuperscript{8} Only trials in which at least six breaths remained for linear regression to estimate CDI were used. The association between Sn\textsubscript{III} data and lung turnovers (TO) was assessed visually as deviation from linear association earlier than TO 6 may occur.\textsuperscript{9,11,12} and was quantified automatically by the coefficient of determination R-squared (R\textsuperscript{2}). To obtain standard Scond, the software applied and averaged slopes from linear regression of Sn\textsubscript{III} values across 4.5 lung TO (range 1.5-6.0 TO). The new Scond\textsuperscript{*} was calculated earlier and across a shorter washout period (3 TO) during N\textsubscript{2}MBW, that is, over lung TO 0-3 and excluding the first Sn\textsubscript{III} value (Sacin).\textsuperscript{9} To measure DCDI, Sacin was derived semi-automated as recommended: the averaged Sn\textsubscript{III} of the first breath subtracting the CDI contribution\textsuperscript{9} estimated between lung TO 1.5 and 6.0 or alternate for Sacin\textsuperscript{*} between 0 and 3.\textsuperscript{9} TO was calculated automated breath-by-breath with increasing cumulative expired volume over FRC. Test duration for each outcome was assessed per trial.

2.4 | Spirometry

Spirometry was always performed after N\textsubscript{2}MBW using the Jaeger MasterScreen (CareFusion, Hochberg, Germany) according to current
guidelines. Outcome variables were forced expiratory volume in one second (FEV₁) and forced expiratory flow between 25% and 75% of forced expiratory volume (FEF₂₅₋₇₅).

2.5 Statistical analysis

We estimated the required sample size assuming an agreement, that is, the mean of differences between Scond and Scond*, of 0.05 z-score, a standard deviation (SD) of differences of 0.1 z-score, and a maximum allowed difference between Scond and Scond* of 0.3 z-score. Setting the two-sided alpha at 0.05 and power at 0.800, the estimated required sample size is 83 children. A power of 0.900 would require 110 children.

The distribution of data was assessed using scatter plots. N₂ MBW indices were expressed as mean ± SD (range) and compared between the groups by unpaired Student's t-test. Categorical variables with chi-square test, as appropriate. N₂ MBW standard deviation score (z-score) was calculated from the healthy population's mean value and standard deviation (SD), that is, observed-predicted/standard deviation. Spirometry z-scores were calculated from recommended reference equations. Upper and lower limit of normal (ULN, LLN) were defined as ±1.64 z-scores. Association and agreement between lung function indices were assessed by linear regression analysis, Bland Altman plots, and kappa statistics, respectively. Quality of Scond fit was estimated by visual inspection and the R² from the Scond linear regression model. We assessed intra-test repeatability by the intraclass correlation coefficient (ICC). The ICC relates between-subject variance to total variance, an ICC >0.8 suggests good repeatability. Additionally a subgroup analysis was performed between patients stratified by normal versus abnormal ventilation inhomogeneity indices. P-values < 0.05 were considered statistically significant. Sample size was estimated using MedCalc (Statistical Software version 17.5.5, MedCalc Software bvba, Ostend, Belgium). Statistical analysis was performed with Stata (Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

3 RESULTS

Following quality control, 334 N₂ MBW measurements from 135 study participants (43 controls and 92 subjects with CF) remained for final analysis. The study participants' mean age (range) was 11.1 (4.6-18.2) years. Clinical and lung function characteristics are outlined in Tables 1-3. Additional data are provided in the online supplement.

3.1 Validity of Scond* and Sacin*

The ability to detect pathological lung function (>1.64 z-scores) varied between indices (Table 2, Figure 1). The global ventilation inhomogeneity indices LCI₂₅% and LCI₅% were elevated in 87% and 66% of patients with CF. In contrast, Smᵢᵢ indices were less sensitive. Scond was pathological in 55 patients with CF (60%) but Sacin in only 10 out of 92 patients (11%) (Figure 1). FEV₁ was abnormal in 28 (30%) patients.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Characteristics of the study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>HC (n = 43)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.8 (4.4)</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>22/21</td>
</tr>
<tr>
<td>Weight kg</td>
<td>36.5 (19.6)</td>
</tr>
<tr>
<td>Weight, z-score</td>
<td>0.2 (0.8)</td>
</tr>
<tr>
<td>Length cm</td>
<td>138.9 (24.6)</td>
</tr>
<tr>
<td>Length, z-score</td>
<td>0.5 (0.8)</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>17.5 (3.7)</td>
</tr>
<tr>
<td>BMI, z-score</td>
<td>−0.1 (1.0)</td>
</tr>
<tr>
<td>LCI₂₅%</td>
<td>6.98 (0.57)</td>
</tr>
<tr>
<td>LCI₅%</td>
<td>5.27 (0.47)</td>
</tr>
<tr>
<td>Scond</td>
<td>0.02 (0.03)</td>
</tr>
<tr>
<td>Sacin</td>
<td>0.11 (0.09)</td>
</tr>
<tr>
<td>Sacin*</td>
<td>0.11 (0.08)</td>
</tr>
</tbody>
</table>

Overview of clinical characteristics of all groups. Lung function indices are given in raw values; the unit of LCI is lung turnover, Smᵢᵢ indices are dimensionless. Data are given as mean and SD. Statistically significant differences (P < 0.05) between groups are marked in bold. For comparison between the groups, we used the unpaired t-test. For sensitivity analysis, we repeated analyses using the Wilcoxon-Mann-Whitney test, which confirmed the original analyses using the unpaired t-test. Categorical variables were compared by the chi-square test. HC, healthy controls; CF, Cystic Fibrosis.

The ability to detect abnormal CDI and DCDI from the alternate protocols (Scond* and Sacin*) was low (19% and 12%, respectively; see Table 2). The association of CDI and DCDI indices from both protocols with global ventilation inhomogeneity (LCI) and spirometry indices (FEV₁) reached statistical significance, however, the strength of these associations was generally low (Table S1). The association between Scond and Scond* was poor both in controls and CF patients (R² = 0.01 and R² = 0.08; Figure 2, Table S1, respectively). In 61 (48 with CF) the difference between Scond* and Scond raw values exceeded 10%. In CF the mean difference was 1.1 z-score between Scond and Scond*, and upper and lower limits of agreement were 3.0 and −1.1 z-score, respectively (Figure 2 and 3). Compared to controls, the association between Scond and LCI₂₅% was stronger in CF (R² = 0.28) and both indices had an agreement of 69% (kappa = 0.3) to LCI₂₅%. Scond was moderately associated with FEV₁ (Table S1). Scond* poorly associated with LCI₂₅%: R² was 0.07 in CF and 0.06 in controls. In CF, we found poor agreement of Sacin and Sacin* to LCI₂₅% and for both the kappa was 0.03 (agreement of 23%, Table S2). The mean difference between both indices was 0.002 z-score, upper and lower limit of agreement were 0.7 and −0.7 z-score, respectively (Figure S1).

3.2 Practicability and reliability of Scond* and Sacin*

All alternate washout indices took a shorter measurement duration compared with the commonly used LCI₂₅%. The washout duration to
measure $S_{\text{cond}}^*$ and $S_{\text{sacin}}^*$ was more than the half of the duration required to measure $L_{\text{CI}2.5\%}$ in children with CF (Table 2). Compared to $L_{\text{CI}2.5\%}$, $S_{\text{nIII}}$ derived indices were more variable within testing occasions (Table 3). The ICC of the $L_{\text{CI}2.5\%}$ was 0.9, whereas $S_{\text{cond}}$ and $S_{\text{cond}}^*$ had an ICC of 0.6 and 0.1 in CF, respectively. For the most of the washout indices ICC tended to be higher in patients with CF than in controls. A horizontal $S_{\text{nIII}}$ plateau earlier than TO 6 was not observed in CF; fitting quality ($R^2$) of $S_{\text{cond}}$ was slightly better than $S_{\text{cond}}^*$ in both CF and controls (Table 3, Figure S2). The association between $S_{\text{cond}}$ and $L_{\text{CI}}$ is displayed in Figure S3.

### 4 | DISCUSSION

#### 4.1 Summary

This large, systematic study assessed the clinimetric properties of two SnIII protocols in children with mild to moderate CF lung disease. We found that the standard and alternate protocols differ in validity (the ability to detect abnormal lung function), reliability (fitting quality and intra-test repeatability), and practicability (measurement duration) of $S_{\text{cond}}$ and $S_{\text{sacin}}$. Alternate protocols seem practical but the validity and reliability of the standard SnIII protocol is more reliable and sensitive compared to the alternate protocol in children with mild to moderate CF lung disease. The degree of ventilation inhomogeneity is underestimated by alternate $S_{\text{nIII}}$ protocols. While measurement duration decreases, intra-test variability increases with decreasing number of washout breaths to estimate CDI and DCDI. Our data further suggest that increased global ventilation inhomogeneity and CDI were highly prevalent but not necessarily coexistent in patients with mild to moderate CF lung disease. Elevated DCDI was present only in a minority of patients.

#### 4.2 Physiological considerations

In the original description of $S_{\text{cond}}^*$ (9), the proposed outcomes were intended to better describe CDI and DCDI in a more severe CF cohort where $S_{\text{cond}}$ reaches a plateau. To reliably estimate CDI in advanced lung disease, the modified protocol proposed by Verbanck may be appropriate also in children (9). Severe ventilation inhomogeneity may lead to large $S_{\text{nIII}}$ increases in the initial portions of the MBW test and level off as the MBW test progresses. If severe ventilation inhomogeneity is present, the classical protocol, the regression of $S_{\text{nIII}}$ versus TO between TO 1.5 and 6 ($S_{\text{cond}}$), can be replaced by $S_{\text{cond}}^*$, the regression of $S_{\text{nIII}}$ versus TO 0 and 3 while excluding the first breath to minimize contribution of acinar ventilation. Prevalence of abnormal CDI assessed by $S_{\text{cond}}$ (60%) was comparable to other studies in CF children and plausibly lower compared to adult CF patients (91%).

### TABLE 2 Validity and practicability, ability to detect abnormal lung function in CF and test duration for the population

<table>
<thead>
<tr>
<th>Indices</th>
<th>Mean (SD)</th>
<th>Abnormal values</th>
<th>Duration HC</th>
<th>Duration CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{\text{CI}2.5%}$</td>
<td>5.0 (3.2)</td>
<td>80/92 (87%)</td>
<td>107.7 (48.7)</td>
<td>133.4 (57.6)</td>
</tr>
<tr>
<td>$L_{\text{CI}5%}$</td>
<td>3.2 (2.3)</td>
<td>61/92 (66%)</td>
<td>77.9 (35.5)</td>
<td>81.5 (34.3)</td>
</tr>
<tr>
<td>$S_{\text{cond}}$</td>
<td>2.0 (1.3)</td>
<td>55/92 (60%)</td>
<td>90.2 (45.0)</td>
<td>75.9 (30.1)</td>
</tr>
<tr>
<td>$S_{\text{cond}}^*$</td>
<td>1.1 (1.2)</td>
<td>17/92 (19%)</td>
<td>45.1 (22.5)</td>
<td>38.0 (15.0)</td>
</tr>
<tr>
<td>$S_{\text{sacin}}$</td>
<td>0.12 (1.3)</td>
<td>10/92 (11%)</td>
<td>90.2 (45.0)</td>
<td>75.9 (30.1)</td>
</tr>
<tr>
<td>$S_{\text{sacin}}^*$</td>
<td>0.05 (1.7)</td>
<td>11/92 (12%)</td>
<td>45.1 (22.5)</td>
<td>38.0 (15.0)</td>
</tr>
<tr>
<td>$F_{\text{EFV1}}$</td>
<td>-1.17 (1.3)</td>
<td>28/92 (30%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>$F_{\text{EF25-75}}$</td>
<td>-1.27 (1.3)</td>
<td>32/92 (35%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Lung function indices are given in z-scores, test duration per trial is given in seconds. Data are displayed as mean (SD); absolute and relative numbers of CF patients with normal versus abnormal values (upper and lower limits of normal (ULN/LLN = $\pm 1.64/1.64$ z-scores). HC, healthy controls, CF, Cystic Fibrosis.

### TABLE 3 Reliability—$S_{\text{nIII}}$ fitting quality and intra-test repeatability

<table>
<thead>
<tr>
<th>Indices</th>
<th>Groups</th>
<th>ICC (95%CI)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{\text{CI}2.5%}$</td>
<td>HC CF</td>
<td>0.5 (0.4 to 0.7)</td>
<td>0.9 (0.9 to 0.9)</td>
</tr>
<tr>
<td>$L_{\text{CI}5%}$</td>
<td>HC CF</td>
<td>0.7 (0.5 to 0.8)</td>
<td>0.9 (0.8 to 0.9)</td>
</tr>
<tr>
<td>$S_{\text{cond}}$</td>
<td>HC CF</td>
<td>0.1 (0.0 to 0.5)</td>
<td>0.6 (0.5 to 0.7)</td>
</tr>
<tr>
<td>$S_{\text{cond}}^*$</td>
<td>HC CF</td>
<td>0.2 (0.1 to 0.5)</td>
<td>0.1 (0.0 to 0.5)</td>
</tr>
<tr>
<td>$S_{\text{sacin}}$</td>
<td>HC CF</td>
<td>0.3 (0.1 to 0.5)</td>
<td>0.4 (0.2 to 0.6)</td>
</tr>
<tr>
<td>$S_{\text{sacin}}^*$</td>
<td>HC CF</td>
<td>0.1 (0.0 to 0.5)</td>
<td>0.3 (0.1 to 0.5)</td>
</tr>
</tbody>
</table>

Lung function indices are given in raw values; the unit of $L_{\text{CI}}$ is lung turnover, $S_{\text{nIII}}$ indices are dimensionless. Intra-class correlation (ICC) with 95%CI; coefficient of determination R-squared ($R^2$) from the linear regression fit to calculate $S_{\text{cond}}$ and $S_{\text{cond}}^*$. N/A, not applicable; HC, healthy controls; CF, Cystic Fibrosis.
Yet, agreement between standard and alternate SnIII indices was much lower compared with adult CF patients. We assume that alveolar gas fractions from mainly convection-dependent and possibly fast ventilated lung compartments were being washed much more homogeneously in the beginning of MBW in our study as compared to MBW in adults with CF. Phase III slopes during MBW remained low but steadily increased and then leveled out at the sixth TO. In severe disease, SnIII was already elevated early in the washout, then rose steeply, possibly suggesting distorted fractional structure and function of the small airways.\textsuperscript{7} In these previously described patients, the association between SnIII and TO showed a plateau-ceiling effect beyond TO 3. Previous studies described this plateau effect if Scond was greater than 0.150 [L\textsuperscript{-1}].\textsuperscript{19,20} In our study, Scond did not exceed 0.167 in CF. We found no deviation from linearity in the SnIII versus TO regression models used to calculate Scond.

We only included measurements after strict quality control, yet variability in breaths may be naturally larger during free tidal breathing. Variability appears to be a feature of healthy lungs with efficient, fractional washout behavior of alveolar gas fractions from convection-dependent lung zones.\textsuperscript{21} While normal values for LCI and Scond are comparable between cohorts, Sacin seems to vary more between studies.\textsuperscript{4,22-24} We however accounted for tidal volume variability to some extent by multiplying SIII with tidal volume as recommended.\textsuperscript{8} Only a few CF patients had elevated Sacin values.\textsuperscript{23} In CF, cross-sectional studies suggest that the rise of Sacin seemed to occur later in life when ventilation inhomogeneity may be more progressed.\textsuperscript{19} This seems the case in adults with chronic lung diseases such as bronchiolitis obliterans or COPD.\textsuperscript{25,26} In our study, agreement of LCI2.5% and LCI5% was lower than expected.\textsuperscript{27} Yet data from Green et al\textsuperscript{5} support our recent findings and question the use of LCI5% in mild-to-moderate lung disease.

4.3 Methodological considerations

The Scond and Scond* algorithms are simple linear regression models. Only the range of data differs between those models. The range where a linear model is fitted to data is however irrelevant given a linear relationship. This is the basic assumption of Scond and Scond*. Therefore Scond* could contain the same information as the standard Scond. This was not case in our study presumably because the signal to noise ratio is lower in children with mild disease. Measurement of SnIII indices is more susceptible to breathing pattern variations compared to LCI2.5% and LCI5%, especially in healthy young children.\textsuperscript{28} Smaller breaths in children may influence the expirogram’s phase III

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**FIGURE 1** Washout indices in patients with CF. Lung function values for LCI2.5%, LCI5%, Scond, Sacin, Scond*, Sacin*, FEV\textsubscript{1}, and FEF\textsubscript{25-75}\textsubscript{5} are given in z-scores. Dashed lines indicate upper and lower limits of normal (ULN/LLN = 1.64/−1.64 z-scores). Mean and standard deviation are marked as solid lines. For illustration we excluded two Sacin* values (9.7 and −7.8 z-scores)

**FIGURE 2** Association between different SnIII protocols. Scatter plot of Scond versus Scond*. Children with CF (n = 91) are presented as open circles. Solid lines indicate upper limits of normal (ULN = 1.64 z-scores). For better illustration, one Scond* value exceeding 10 z-scores is not displayed (but is included in analyses)

**FIGURE 3** Bland Altman Plot of Scond and Scond*. Children with CF (n = 91) are displayed as open circles. Differences between Scond and Scond* are plotted against mean of Scond and Scond*. Mean difference (1.1 z-scores), and upper (3.2 z-scores) and lower (−1.1 z-scores) limits of agreement are given as lines. For better illustration, one Scond* value exceeding 10 z-scores is not displayed (but is included in analyses)
identification and visual inspection is required. In controls, \( S_{\text{III}} \) values plotted against TO were weakly associated per se, because normal ventilation presumably is characterized by relatively low determinism of alveolar gas clearance across time. Natural undirected signal fluctuation in \( S_{\text{III}} \) in controls suggests a more dynamic signal behavior compared to CF patients with a narrower span corresponding to nearly mono-behavior.

### 4.4 Strengths and weaknesses of the study

Our study is one of the largest studies in children with CF to assess CDI and DCDI. Clinimetric properties of Scond and Sacin have not been systematically studied previously. We used validated equipment, standardized protocols, and assessed intra-test repeatability by calculating ventilation inhomogeneity indices per trial. These estimates of reliability are often not reported. The consensus suggests pooling of \( S_{\text{III}} \) data from trials to estimate CDI and DCDI. However, this protocol would not allow assessing intra-test variability. Although controls were on average younger, group differences in lung function parameters were comparable to previous studies. All washout indices reported here were adjusted for resting lung volume (FRC) and the \( S_{\text{III}} \) indices for VT as recommended. Comparison to other studies currently seems constrained by different equipment setups. For example, in the study by Verbanck et al\(^9\) LCI\(_{2.5\%}\) was abnormal in 78% patients with severe lung disease while LCI\(_{2.5\%}\) was abnormal in 87% of patients with CF in our study. While Sacin and Scond indices were derived according to consensus in our study, Sacin and Scond were not as good as LCI at discriminating health and disease. This was presumably due to physiological but also technical reasons. The influence of dead space could be relevant especially when tidal volumes were relatively small and variable. However, fixed one-liter breathing protocols may have detrimental effects on MBW indices in children\(^30\) and are therefore not recommended in children.

Normative MBW data in children are scarce. We consider the standard deviation scores (z-scores) reported in this study adequate to compare intra-individual changes and differences between health and disease. Yet the data reported here are not generalizable or applicable for other MBW setups.

### CONCLUSIONS

A major drawback of MBW in routine clinics is its duration limiting feasibility especially in younger children. Measuring MBW to derive Scond* would be a significant abbreviation of the MBW test if terminated at TO3. While these alternate protocols for Scond* and Sacin* seem practical, the validity and reliability of these indices are significantly lower compared with standard protocols in children with mild to moderate CF lung disease. Therefore we cannot recommend to use Scond* and Sacin* in children with mild to moderate CF lung disease to estimate CDI and DCDI. More established MBW indices, such as LCI\(_{2.5\%}\) and Scond, are sensitive and robust biomarkers of pediatric CF lung disease. In children with more severe lung disease, Scond* and Sacin* require further study.

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### AUTHORS’ CONTRIBUTION

SN, AB, PL, and FS designed the study concept. SN, SY, FS, AM, and IR collected study data. SN and AB analyzed the data. All authors interpreted the data. SN, AB, and KR drafted the manuscript. All authors revised the manuscript.

### CONFLICT OF INTEREST

The authors do not have conflicts of interest, financial or otherwise, related to this work.

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### REFERENCES


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