A new double-tracer gas single-breath washout to assess early cystic fibrosis lung disease

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ABSTRACT

In cystic fibrosis (CF) lung disease, tests for ventilation inhomogeneity are sensitive but not established for clinical routine. We assessed feasibility of a new double-tracer gas single-breath washout (SBW) in school-aged children with CF and controls, and compared SBW between groups and with nitrogen multiple-breath washout (MBNW).

Three SBW and MBNW were done in 118 children (66 with CF) using a side-stream ultrasonic flowmeter setup. The double-tracer gas containing 5% sulfur hexafluoride and 26.3% helium was applied during one tidal breath. Outcomes were SBW phase III slope (SIII_{DTG}), MBNW derived lung clearance index (LCI), and indices of acinar (Sacin) and conductive (Scond) ventilation inhomogeneity.

SBW took significantly less time to perform than MBNW. SBW and MBNW were feasible in 109 (92.4%) and 98 (83.0%) children, respectively. SIII_{DTG} differed between children with CF and controls, mean (SD) was -456.7 (492.8) and -88.4 (129.1) mg/mol.L, respectively. Abnormal SIII_{DTG} was present in 36 (59%) of CF children. SIII_{DTG} was associated with LCI (r = -0.58), Sacin (r = -0.58), but not with Scond.

In CF, steeply sloping SIII_{DTG} potentially reflects ventilation inhomogeneity near the acinus entrance. This tidal SBW is a promising test to assess ventilation inhomogeneity in an easy and fast way.

Key words: Children, efficiency, helium, respiratory function tests, small airway remodeling, sulfur hexafluoride.
INTRODUCTION

Conventional measurements of airways obstruction are inadequate to characterize early cystic fibrosis (CF) lung disease. Compared to spirometry, the lung clearance index (LCI), a measure of overall ventilation inhomogeneity derived from inert tracer gas multiple-breath washout (MBW), is more sensitive for peripheral structural airway pathology detected in computer tomography [1-4]. LCI is associated with peripheral airways infection and seems to be a relevant end point in clinical CF trials [5-8]. Despite this, MBW has not been applied to routine clinical care due to the long duration and the need for unavailable or customized equipment such as mass spectrometer and photo-acoustic setups [3;9]. Alternatively, tidal phase III slope (SIII) analyses from tracer gas single-breath washout (SBW) tests provide insight into overall and regional ventilation inhomogeneity [10-13]. Simultaneous use of different tracer gases, e.g. helium (He) and sulfur hexafluoride (SF₆), potentially yields information regarding the site at which ventilation inhomogeneity arises [10;14]. However, SBW is also not routinely established as most SBW procedures require vital capacity (VC SBW) manoeuvres at preset flow rates, and also relied on mass spectrometers [12;15].

A more practical lung function test for ventilation inhomogeneity based on available equipment could overcome previous drawbacks. We recently developed a new and easy SBW test requiring tidal breathing only (tidal SBW) and involving a double-tracer gas mixture containing He and SF₆ to assess regional ventilation inhomogeneity in adults [16]. We were able to show that the ultrasonic flowmeter setup can accurately trace He and SF₆ washout as validated against mass spectrometry [16].

In this study we determined whether the double-tracer gas SBW derived tidal phase III slope (SIII_{DTG}) detects early lung disease in school-aged children with CF. Study outcomes were (i) feasibility, (ii) ability to detect increased ventilation inhomogeneity in CF, and (iii) the association of SIII_{DTG} with indices from nitrogen MBW and spirometry.
METHODS

Study participants
We prospectively enrolled 121 children aged between 5 and 16 years at the outpatient clinics, Children's University Hospital Bern, Switzerland, as detailed in the online supplement (OLS). Tidal SBW and nitrogen MBW measurements (MBNW) were performed in triplicate, with children with CF additionally performing spirometry [17] in that order. The study was approved by the Ethics Committee of the Canton of Bern, Switzerland. The children’s assent was obtained and parents or caregivers provided written informed consent for this study.

Double-tracer gas single-breath washout
We used an available setup (Exhalyzer D, Eco Medics AG, Duernten, Switzerland) described in the OLS. The double-tracer gas mixture contained 26.3% He, 5% SF₆, 21% oxygen, and balance nitrogen (Carbagas, Bern, Switzerland). This double-tracer gas mixture has the same molar mass as air, such that any detectable changes compared to normally expired molar mass can be attributed to relative changes in He and SF₆ concentrations [16]. Molar mass is measured by a side-stream ultrasonic flowmeter, tidal flows by a mainstream ultrasonic flowmeter.

During normal breathing, the double-tracer gas was applied for a single tidal inhalation prior to exhalation to functional residual capacity [16]. We used Matlab (R2006a, The Mathworks Inc., MA, USA) for signal processing and analyses. The tidal SBW derived molar mass expirogram was plotted against expired volume. Two blinded investigators applied a linear fit to the tidal phase III between 65% and 95% of expired volume. The primary outcome was the mean SIII_{DTG} of three technically acceptable tidal SBW. Further details on tidal SBW measurement and analysis are given in the OLS.
Nitrogen multiple-breath washout

The reference lung function test was MBNW performed using validated equipment [18], according to guidelines [17] and is detailed in the OLS. Primary outcome was LCI calculated as ratio of cumulative expired volume divided by functional residual capacity [19;20]. Secondary outcomes were indices of conductive (Scond) and acinar (Sacin) ventilation inhomogeneity with their values increasing with increasing ventilation inhomogeneity [21]. LCI is thought to reflect global ventilation inhomogeneity whereas Sacin derived from the first nitrogen SIII reflects regional acinar ventilation inhomogeneity. Scond calculated from the slope across multiple nitrogen SIII over lung turnovers 1.5 - 6 reflects regional conductive ventilation inhomogeneity [21].

Statistics

We computed success rates and duration for tidal SBW and MBNW test sessions, and compared lung function data between groups using unpaired t-tests. Z-scores for SIII\textsubscript{DTG}, LCI, Sacin, and Scond were calculated from the healthy study population, for forced expiratory volume in the first second (FEV\textsubscript{1}) and forced expiratory flow between 25-75% of expired volume (FEF\textsubscript{25-75}) from reference data [22]. We assessed the association of SIII\textsubscript{DTG} with these lung function outcomes using Pearson correlation and applied Bonferroni correction. We also normalized SIII\textsubscript{DTG} by multiplying SIII\textsubscript{DTG} with the tidal volume of the corresponding breath (SIII\textsubscript{DTG,VT}) and assessed intra-test variability by the coefficient of variation (CV) of SIII\textsubscript{DTG,VT} and SIII\textsubscript{DTG}. Analyses quality, i.e. linear regression R\textsuperscript{2} of SIII\textsubscript{DTG} fitting, and SIII\textsubscript{DTG} agreement between observers were determined by paired t-tests and intra-class correlation coefficient, respectively. P-values < 0.05 were considered statistically significant, and analyses were done using Stata™ (Release 11. College Station, TX: StataCorp LP).
RESULTS

Success rate and duration

From 121 children enrolled, 118 children (66 with CF and 52 controls) performed triple tidal SBW tests (Figure 1). Clinical and demographic characteristics of the study population are given in Table 1. SIII\textsubscript{DTG} success rate was high and similar between groups: 109 (92.4%) children (61 children with CF) achieved 318 acceptable tidal SBW measurements. Six children (5%) were aged below six years and achieved tidal SBW measurements only. Reasons for single test rejection were unsteady breathing, e.g. inspiratory flow exceeding bias flow, (n = 22 [6%]), lack of phase III (n = 12 [3%]), and technical errors (n = 2 [1%]). An example of typical double-tracer gas tidal SBW expirograms is given in Figure 2. MBNW measurements were achieved by 54 (82%) children with CF and 44 (85%) controls. Mean (range) duration for testing and analyses of three tidal SBW trials was 3.3 (2.5 - 4.1) minutes and 29.9 (13.9 - 44.0) minutes for three MBNW trials, p < 0.001.

Double-tracer gas phase III slope in children with CF

Compared to controls, children with CF had clearly different double-tracer gas washout patterns during phase III of the tidal SBW (Table 1, Figure 2). In children with CF, SIII\textsubscript{DTG} mean (SD) was -456.7 (492.8) mg/mol.L compared to -88.4 (129.1) mg/mol.L in healthy children. Mean difference (95% CI) of SIII\textsubscript{DTG} between children with CF and controls was 368.3 (223.4 to 513.2) mg/mol.L, p < 0.001. SIII\textsubscript{DTG,VT} similarly differed between children with CF and controls. Mean difference (95% CI) was 124.1 (68.9 to 179.4) mg/mol, p < 0.001. Additional results are given in the OLS.

In our CF population, sensitivity and specificity for abnormal lung function (> 2 z-scores) was 59% and 93% for SIII\textsubscript{DTG}, 46% and 96% for SIII\textsubscript{DTG,VT}, 83% and 98% for LCI, 55% and 93% for Sacin, and 12% and 94% for Scond, respectively. Sensitivity for FEV\textsubscript{1} was 34%. In those
40 (66%) children with CF and normal FEV$_1$, SIII$_{DTG}$ was abnormal in 18 (47%) children (Figure 4), LCI was abnormal in 25 (73%), Sacin in 13 (41%), and Scond in one child.

**Double-tracer gas phase III slope and other lung function indices**

SIII$_{DTG}$ was strongly associated with ventilation inhomogeneity measured by LCI and Sacin but not Scond (Table 2, Figure 3). SIII$_{DTG}$ decreased with increasing LCI ($r = -0.58$) and Sacin ($r = -0.58$). SIII$_{DTG}$ was weakly associated with airways obstruction measured by FEV$_1$ ($r = 0.26$, Figure 4) but not with FEF$_{25-75}$.

**Quality control of double-tracer gas phase III slope**

Intra-test repeatability of SIII$_{DTG}$ was acceptable and comparable between children with CF and controls. Comparing children with CF and controls, intra-test CV was 23.9% vs. 18.5%. Mean difference (95% CI) of CV was 5.4 (-3.8 to 14.5)%, $p = 0.243$. Mean intra-test variability of SIII$_{DTG}$ (CV = 22.5%) was comparable to SIII$_{DTG,VT}$ (CV = 20.8%). Mean difference (95% CI) was 3.0 (-2.7; 8.8)%. Variability of SIII$_{DTG}$ (CV) was higher than CV of LCI (7.4%), but not associated with the primary outcome SIII$_{DTG}$, age or breathing pattern (OLS). SIII$_{DTG}$ data ($n = 318$) were reliably calculated by two blinded, independent investigators (CA, FS). Inter-observer variability of fitting quality (linear regression $R^2$) was negligible: Mean (SD) linear regression fit $R^2$ was 0.65 (0.22) and 0.63 (0.23), respectively. Inter-observer agreement for the outcome SIII$_{DTG}$ was strong, intra-class correlation coefficient = 0.92.
DISCUSSION

Summary

This new double-tracer gas tidal SBW detects early CF lung disease and is easy, fast, and safe to perform. We obtained technically acceptable SIII_{DTG} in 92% of untrained school-aged children. To identify abnormal lung function in children with CF, the tidal SBW was more sensitive than FEV\textsubscript{1}. Compared to indices derived from the much longer MBNW tests, SIII_{DTG} was comparably sensitive and specific as Sacin, but less sensitive than LCI. SIII_{DTG} was associated with acinar ventilation inhomogeneity (Sacin) and global ventilation inhomogeneity (LCI), but not with ventilation inhomogeneity in conductive airways (Scond). SIII_{DTG} was repeatable within tests and reproducible between investigators. These data suggest that the double-tracer gas tidal SBW is a promising lung function test to assess ventilation inhomogeneity.

Classical VC SBW has previously been used to report steeper SIII for He than SF\textsubscript{6} in patients with CF [12]. However, this result was deemed to have limited applicability to non-specialised laboratories, due to the need for a mass spectrometer [15]. We now report a new SBW technique which dispenses with this need. Compared to the clinical applicability of previous MBW and VC SBW techniques, the advantages of the new tidal SBW from our study lie within three major aspects: (i) an easy measurement protocol, (ii) fast testing and analysis procedures, and (iii) a setup being available. This new double-tracer gas tidal SBW technique seems to have important basic requirements for clinical application. SIII_{DTG} was by nature obtained significantly faster and in a greater proportion of children as compared to LCI or Sacin. This is important as the number of feasible lung function tests in preschool- and early school-age years is clearly limited by their test duration and protocol complexity. Interestingly, success rate and repeatability of tidal SBW in our study also compare preferably.
with previous VC SBW studies: In healthy children and adults, success rates of VC SBW ranged from 74% to 89%, and intra-test variability of nitrogen SIII ranged between 13% to 24% [23;24]. Previous MBW success rates ranged between 50% to 100% in children [20;25;26]. The need for complete tracer gas washin and leak free washout, with e.g. coughing or sighs leading to MBW rejection, led to higher failure rates. However, LCI seems more repeatable than SIII DTG [6;25]. This may be explained by measuring expired tracer gas volumes over a ten times greater test duration as compared to the double-tracer gas tidal SBW. Further studies are needed to assess protocols reducing SIII DTG variability which would also be applicable in children.

**Physiological and methodological considerations**

Increased overall ventilation inhomogeneity quantified by LCI or VC SBW using a single inert tracer gas may reflect increases in airway resistance, inhomogeneous changes in lung compliance, hyperinflation, and alterations to lung structure. Regional ventilation inhomogeneity near the diffusion-convections fronts, which are specific for diffusivity of tracer gases, is thought to be the major determinant of the sloping phase III. The site along the airway tree primarily affected by ventilation inhomogeneity can be estimated using two approaches. One is the partitioning of MBW into Sacin and Scond [21;27]. The second method for localising inhomogeneity is by analysing tidal or VC SBW derived SIII that are simultaneously obtained from two inert gases of different diffusivity, e.g. SF₆ and He [12;28;29]. For light gases such as He, the diffusion-convection front is probably proximal but close to the *acinus* [14;30]. For the heavy gas SF₆, this front may approximate to the entrance of the *acinus* and probably stretches into the proximal portion of the *acinus*. The exact anatomical correlates of these measures remain largely unknown.

This new tidal SBW method aggregates ventilation inhomogeneity of He and SF₆. The reason these gases are aggregated is because all have simultaneous impact upon the molar mass
signal. A steeply sloping $S_{III_{DTG}}$, which was significantly more common in children with CF compared to controls, means that significantly more He contributes to the phase III than $SF_6$ ($< -2$ z-scores, Figures 3, 4) [16]. In contrast, steeply rising $S_{III_{DTG}} (> 2$ z-scores) with significantly more $SF_6$ contributing to the phase III than He was present in only four children. This suggests that airway pathology may be localized rather near the *acinus* entrance in the majority of children with CF. This assumption is supported by similar findings from *Van Muylem* et al. [12] and the fact that steeper $S_{III_{DTG}}$ was associated with increased Sacin. Increased He ventilation inhomogeneity compared to $SF_6$ was also correlated to histological changes near the *acinus* entrance [29]. Recent magnetic resonance imaging studies visualized He ventilation inhomogeneity in children with CF [31]. However, we are not able to determine the exact location or nature of regional ventilation inhomogeneity leading to altered $S_{III_{DTG}}$ in CF.

Tidal SBW performed from FRC is felt to be more reflective of peripheral airway function than that performed using a VC manoeuvre from residual volume [29]. Nevertheless SIII may be influenced by breathing pattern and raised volume protocols have arguably been regarded as being more robust [24;32;33]. However, shift in vital capacity due to training effects introduces bias [24]. In younger subjects, a *net* multiplication of MBW derived SIII by tidal volume has been proposed [34]. In our study, $S_{III_{DTG,VT}}$ did not improve intra-test variability or discriminatory capacity as compared to the simpler index $S_{III_{DTG}}$ (OLS). It remains to be determined which approach to adjust for tidal breathing pattern *post-hoc* is physiologically and clinically relevant.

This is the first study reporting SIII analysis derived from molar mass signals in lung disease. Compared to mass spectrometry signals, ultrasonic flowmeter derived molar mass does not require $SF_6$ and He signal alignment and excellent agreement between molar mass and mass spectrometry signals has been shown [16]. $S_{III_{DTG}}$ analysis quality ($R^2$) and reproducibility
between observers was good. Signal resolution is also higher with a sampling rate of 200 Hz for the molar mass signal, compared to current mass spectrometry setups at 33 Hz.

Implications for clinics and research

This is the first study showing that tidal breath SIII analyses can identify abnormal lung function in CF using available equipment. A significant proportion of children with CF had normal FEV₁ but abnormal indices of ventilation inhomogeneity. The weak correlation between FEV₁ and SIII_DTG suggests that measures of distal ventilation distribution inhomogeneity (SIII_DTG) are sensitive in early CF lung disease whereas measures of proximal airways obstruction (FEV₁) are reliable rather in advanced CF lung disease. Assessing ventilation inhomogeneity in patients with normal FEV₁ but at risk of small airway disease is considered particularly important as it may allow early intervention and prevent progress of disease [34]. In clinical routine, the double-tracer gas tidal SBW has three potential practical applications: (i) as a screening and monitoring tool in patients without respiratory symptoms or airway obstruction, (ii) as an easy test for ventilation inhomogeneity in preschool children, and (iii) as an alternative test to MBW in primary care settings. Longitudinal change of He SIII as compared to SF₆ SIII seems very sensitive for early detection of bronchiolitis obliterans in patients at risk [11;35]. Similar He, SF₆, and N₂ MBW indices can detect increased ventilation inhomogeneity in patients with emphysema [36], primary ciliary dyskinesia [37], asthma [38;39] and COPD [40;41].

This tidal SBW test may also be applicable for intervention trials in patients with small airway disease. While between-test variability of tidal SBW remains to be determined, time series measurements can be easily obtained and fluctuation over time of He and SF₆ SIII could be clinically relevant [11;35]. Compared to MBW, low exposure to dry tracer gas reduces the risk of triggering bronchial hyperreactivity. Lower consumption of inert gases and shorter test
durations for tidal SBW as compared to MBW can reduce cost and decrease greenhouse gas emission, as SF$_6$ is known to be a potent greenhouse gas.

**Conclusion**

This new double-tracer gas tidal SBW is a feasible, fast, and safe test for detecting ventilation inhomogeneity in children with CF and healthy controls. The tidal SBW is performed during tidal breathing and outcomes are obtained within four minutes. SIII$_{DTG}$ is associated with increased global and regional ventilation inhomogeneity near the *acinar zone*. The tidal SBW may be also promising for other patient groups with small airway disease. This study supports the applicability of the double-tracer gas tidal SBW as a sensitive and efficient lung function test to assess ventilation inhomogeneity.
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COMPETING INTERESTS

FS has no conflicts of interest to disclose. GS has no conflicts of interest to disclose. CT has no conflicts of interest to disclose. CA has no conflicts of interest to disclose. CC has no conflicts of interest to disclose. UF has no conflicts of interest to disclose. PL has no conflicts of interest to disclose.

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REFERENCES


## TABLES

### Table 1. Comparison between children with CF and healthy children.

<table>
<thead>
<tr>
<th></th>
<th>Cystic Fibrosis</th>
<th>Controls</th>
<th>Mean diff. (95% CI)</th>
<th>p-value</th>
</tr>
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<tr>
<td><strong>Subjects</strong></td>
<td>n (males)</td>
<td>61 (27)</td>
<td>48 (26)</td>
<td>n.a.</td>
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<tr>
<td><strong>Age</strong></td>
<td>(years)</td>
<td>11.1 (3.0)</td>
<td>11.8 (2.9)</td>
<td>0.7 (-0.4; 1.8)</td>
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<tr>
<td><strong>Height</strong></td>
<td>(z-score)</td>
<td>-0.57 (1.20)</td>
<td>0.16 (1.03)</td>
<td>0.72 (0.23; 1.21)</td>
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<tr>
<td><strong>Weight</strong></td>
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<td>-0.66 (1.07)</td>
<td>-0.05 (1.06)</td>
<td>0.61 (0.16; 1.10)</td>
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<td><strong>Double-tracer gas SBW (n)</strong></td>
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<td>61</td>
<td>48</td>
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<tr>
<td>SIII(_{DTG}) (mg/mol.L)</td>
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<td>-456.7 (492.8)</td>
<td>-88.4 (129.1)</td>
<td>368.3 (237.1; 499.5)</td>
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<tr>
<td>SIII(_{DTG.VT}) (mg/mol)</td>
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<td>-181.2 (177.8)</td>
<td>-57.1 (85.9)</td>
<td>124.1 (68.7; 179.4)</td>
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<td>Sacin</td>
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<td>0.07 (0.06)</td>
<td>0.16 (0.11; 0.21)</td>
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<tr>
<td>Scond</td>
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<td>0.04 (0.06)</td>
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<td><strong>Spirometry (n)</strong></td>
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<td>FEF(_{25-75}) (z-score)</td>
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<td>-1.86 (1.59)</td>
<td>n.a.</td>
<td>n.a.</td>
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</table>

Numeric data are displayed as mean (SD) and were compared by unpaired t-tests giving mean difference (mean diff.) with 95% confidence intervals. Gender distribution was assessed by Fisher exact test*. Double-tracer gas single-breath washout (tidal SBW) indices were phase III slope (SIII\(_{DTG}\)) and SIII\(_{DTG}\) multiplied with tidal volume (SIII\(_{DTG.VT}\)). Multiple-breath washout indices were lung clearance index (LCI), Sacin, and Scond. Z-scores for forced expiratory volume in one second (FEV\(_1\)) and forced expiratory flow between 25% - 75% of expired volume (FEF\(_{25-75}\)) were calculated from reference data [22]. n.a. = not applicable.
Table 2. Association of SIII_{DTG} with other lung function indices.

<table>
<thead>
<tr>
<th>Lung Function</th>
<th>Correlation coefficient</th>
<th>Crude p-value*</th>
<th>Adjusted p-value†</th>
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<td>LCI (z-score)</td>
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<td>&lt; 0.001</td>
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<tr>
<td>Sacin (z-score)</td>
<td>-0.58</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Scond (z-score)</td>
<td>-0.03</td>
<td>0.760</td>
<td>1.000</td>
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<td>FEV1 (z-score)</td>
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<td>0.044</td>
<td>0.653</td>
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<td>FEF_{25-75} (z-score)</td>
<td>0.18</td>
<td>0.170</td>
<td>1.000</td>
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</table>

Association of double-tracer gas single-breath washout derived phase III slope (SIII_{DTG}) with indices from nitrogen multiple-breath washout in 98 children (54 children with CF), and spirometry in 61 children with CF. Pearson correlation coefficients with their crude p-values* and Bonferroni adjusted p-values† are given. Z-scores for SIII_{DTG}, lung clearance index (LCI), Sacin, and Scond were calculated from controls, and for forced expiratory volume in one second (FEV1) and forced expiratory flow between 25% and 75% of expired volume (FEF_{25-75}) from reference data [22].
FIGURE LEGENDS

Figure 1. Success rate of double-tracer gas SBW. From 121 children enrolled, 118 children, 66 children with CF and 52 healthy children, performed 354 double-tracer gas single-breath washout (tidal SBW) tests. Of these, 330 (93%) accorded to measurement quality criteria of phase III slope (SIII\textsubscript{DTG}), and 318 (90%) accorded to analyses quality criteria (OLS). Technically acceptable SIII\textsubscript{DTG} were available in 61 (92%) children with CF and 48 (92%) controls, nitrogen multiple-breath washout indices in 54 (82%) children with CF and 44 (85%) controls. No severe adverse event occurred.

Figure 2. Typical expirograms of double-tracer gas SBW. Double-tracer gas single-breath washout (tidal SBW) giving molar mass (MM) samples (dots) and carbon dioxide (CO\textsubscript{2}) samples (dashes) plotted against expired volume in a boy with CF (Figure 2a) and a healthy boy (Figure 2b), both aged nine years. Traditionally SBW signals (e.g. N\textsubscript{2}, SF\textsubscript{6}, He) are plotted reversed with increasing tracer gas concentrations with increasing expired volume. We displayed the original MM curve with SF\textsubscript{6} leading to a positive MM deflection and He leading to a negative MM deflection. MM above zero reflects greater SF\textsubscript{6} than He contribution and, vice versa, MM below zero reflects greater He than SF\textsubscript{6} contribution (16). Investigators fitted
the slope (solid line) according to the phase III of MM and CO$_2$ between 65\% and 95\% of expired volume. SIII$_{DTG}$ was -591.6 mg/mol.L in Figure 2a and -65.8 mg/mol.L in Figure 2b.

**Figure 3. Association of SIII$_{DTG}$ with ventilation inhomogeneity.** Double-tracer gas single-breath washout derived phase III slopes (SIII$_{DTG}$) were plotted against lung clearance index (LCI; Figure 3a) and Sacin (Figure 3b) from nitrogen multiple-breath washout in 54 children with CF (closed circles) and 44 controls (open circles). The LCI reflects global ventilation inhomogeneity whereas Sacin reflects regional acinar ventilation inhomogeneity. Z-scores for SIII$_{DTG}$, LCI, and Sacin were calculated from the controls. Dashed lines reflect limits of
abnormal lung function (two z-scores). Due to the inverse relationship between $S_{III_{DTG}}$ decrease and LCI increase, the y-axis scaling is reversed.

Figure 4. Association of $S_{III_{DTG}}$ with airways obstruction. Double-tracer gas single-breath washout derived phase III slopes ($S_{III_{DTG}}$) were plotted against forced expiratory volume in one second ($FEV_1$) in 61 children with CF (closed circles). Z-scores for $S_{III_{DTG}}$ and $FEV_1$ were calculated from the controls and reference data [22], respectively. Dashed lines reflect limits of abnormal lung function (two z-scores), the y-axis scaling is reversed.