Comparison of different analysis algorithms to calculate multiple breath washout outcomes

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Online supplement
METHODS

Linear interpolation analysis

Data from N₂ concentration/CEV curve and N₂ concentration/FRC curve were linearly interpolated between the standard LCI breath and the previous breath (Figure E1). Thus, CEV, FRC, and LCI at a certain N₂ concentration were calculated according to equations 1 and 2. LCI was then calculated according to equation 3.

\[
CEV_{2.5} = CEV_0 + (C_{2.5} - C_0) \times \frac{CEV_1 - CEV_0}{C_1 - C_0}, \text{ where}
\]

\(CEV_{2.5}: CEV \text{ at the cut-off; 2.5\% of the starting concentration}

\(CEV_0: CEV \text{ of the breath preceding the one defining the LCI according to the consensus}

\(CEV_1: CEV \text{ of the breath defining the LCI according to the consensus}

\(C_{2.5}: \text{interpolated point where the end tidal concentration would be 2.5\% of the starting concentration}

\(C_0, C_1: \text{end tidal concentrations of the breaths as defined for } CEV_0, CEV_1

Equation 1. Calculation of CEV\text{linear} at 2.5\%.cut-off.

\[
FRC_{2.5} = FRC_0 + (C_{2.5} - C_0) \times \frac{FRC_1 - FRC_0}{C_1 - C_0}, \text{ where}
\]

\(FRC_{2.5}: \text{FRC at the cut-off}

\(FRC_0: \text{FRC of the breath preceding the one defined by the consensus}

\(FRC_1: \text{FRC of the breath defining the LCI according to the consensus}
Equation 2. Calculation of $FRC_{\text{linear}}$ at 2.5% cut-off.

$$LCI_{2.5} = \frac{CEV_0 \cdot \Delta C_1 + CEV_1 \cdot \Delta C_0}{FRC_0 \cdot \Delta C_1 + FRC_1 \cdot \Delta C_0}$$

$\Delta C_0: C_{2.5} - C_0$

$\Delta C_1: C_1 - C_{2.5}$

Equation 3. Calculation of $LCI_{\text{linear}}$ at 2.5% cut-off.

**Fitting-curve Analysis**

Based on a two compartment lung model (E1) equation 4 was used in an unconstrained nonlinear optimization algorithm to find parameters to describe the development of the end-tidal tracer gas concentration (Figure E2) (https://ch.mathworks.com/matlabcentral/fileexchange/8277-fminsearchbnd--fminsearchcon).

$$f_{\text{twoCompartments}}(\alpha_1, \tau_1, \alpha_2, \tau_2, x) = \alpha_1 \cdot e^{x\tau_1} + \alpha_2 \cdot e^{x\tau_2}$$

$\alpha = \text{Initial compartment value}$

$\tau = \text{Exponential decay time constant of the compartment}$

Equation 4. Decay of the end tidal concentration as expected in a two compartment lung model.

Using the previous step the CEV for each cut-off is calculated using Equation 5.

Find $CEV_x$ where: $f_{\text{twoCompartments}}_{\text{Cet-CEV}}(CEV_x) - C_{\text{et crit}} = 0$
\[ f_{\text{two compartments}}(c_{et}, CEV) = \text{Function fitted to the measured data (} x = CEV, y = c_{et}) \]

\[ CEV_x = \text{Cumulative expired volume at cut-off } x \]

\[ c_{et \text{ crit}} = \text{Target concentration} \]

Equation 5. Equation to determine CEV for each cut-off

The corresponding FRC is then found by fitting the measured FRC over the CEV with Equation 4. With known CEV and FRC at a certain cut-off, the LCI can be calculated as described previously (E2).

**Tracer gas fluctuation around a cut-off**

In order to assess tracer gas fluctuations around a certain cut-off, we compared the washout breath number where LCI was measured (BrNr) between the standard analysis (BrNr\text{standard}) and the analysis using the first breath below the cut-off (BrNr\text{first-breath}) (\Delta \text{BrNr: BrNr}_{\text{standard}} - \text{BrNr}_{\text{first-breath}}). \Delta \text{BrNr} \geq 2 was a sign of fluctuation, and this was verified by visual control of the N\text{2} concentration curve (figure 2).
<table>
<thead>
<tr>
<th>Analysis</th>
<th>Healthy</th>
<th>CF</th>
<th>PCD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FRC&lt;sub&gt;2.5&lt;/sub&gt;</td>
<td>CEV&lt;sub&gt;2.5&lt;/sub&gt;</td>
<td>FRC&lt;sub&gt;2.5&lt;/sub&gt;</td>
</tr>
<tr>
<td>Standard</td>
<td>2.01 ± 0.63</td>
<td>14.19 ± 4.24</td>
<td>1.16 ± 0.53</td>
</tr>
<tr>
<td>Linear</td>
<td>2.01 ± 0.63*</td>
<td>13.96 ± 4.20***</td>
<td>1.16 ± 0.53</td>
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<tr>
<td>Fit-curve</td>
<td>1.91 ± 0.55</td>
<td>13.13 ± 3.74*</td>
<td>1.15 ± 0.53***</td>
</tr>
<tr>
<td>1st Breath</td>
<td>2.01 ± 0.62</td>
<td>14.06 ± 4.17</td>
<td>1.18 ± 0.53</td>
</tr>
<tr>
<td>C&lt;sub&gt;mean&lt;/sub&gt;</td>
<td>2.02 ± 0.63</td>
<td>13.94 ± 4.10***</td>
<td>1.16 ± 0.53</td>
</tr>
<tr>
<td>C&lt;sub&gt;median&lt;/sub&gt;</td>
<td>2.03 ± 0.63*</td>
<td>13.97 ± 4.13***</td>
<td>1.16 ± 0.53</td>
</tr>
</tbody>
</table>

Table E1: Functional residual capacity (FRC) and cumulative expired volume (CEV) values at 2.5% in 20 healthy controls, 20 children with cystic fibrosis (CF), and 17 children with primary ciliary dyskinesia (PCD), calculated with different analysis methods in LungSim. Linear: linear interpolation method, Fit-curve: fitting-curve method, 1st Breath: LCI calculated at the 1st Breath below a cut-off, C<sub>mean</sub>: mean expiratory N2 concentration, C<sub>median</sub>: median expiratory N2 concentration (*p<0.05, **p<0.01, ***p<0.001 paired t-test compared with standard analysis).
<table>
<thead>
<tr>
<th>Analysis</th>
<th>Healthy (n=60)</th>
<th>CF (n=60)</th>
<th>PCD (n=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># Breaths</td>
<td># Breaths</td>
<td># Breaths</td>
</tr>
<tr>
<td>standard</td>
<td>33.40 ± 12.15</td>
<td>31.92 ± 12.05</td>
<td>46.07 ± 15.91</td>
</tr>
<tr>
<td>C&lt;sub&gt;mean&lt;/sub&gt;</td>
<td>32.9 ± 12.18*</td>
<td>30.20 ± 10.84*</td>
<td>43.15 ± 13.88*</td>
</tr>
<tr>
<td>C&lt;sub&gt;median&lt;/sub&gt;</td>
<td>32.95 ± 12.19*</td>
<td>30.27 ± 10.9*</td>
<td>43.09 ±13.84*</td>
</tr>
</tbody>
</table>

Table E2: Mean breath number ± SD, at which LCI<sub>2.5</sub> is calculated using different analysis methods, in healthy, CF and PCD groups (*p<0.001, paired t-test compared with standard analysis).
Figure E1. Model of linear interpolation between the breath below 2.5% of the initial tracer gas concentration and the preceding breath. The y-axis represents the end-tidal N\textsubscript{2} concentration in log scale, the x-axis the cumulative expiratory volume (CEV). CEV\textsubscript{2.5}: CEV at 2.5%, CEV\textsubscript{1}: CEV of the breath below 2.5%, CEV\textsubscript{0}: CEV of the last breath above 2.5%, C\textsubscript{1}: N\textsubscript{2} concentration of the breath below 2.5%, C\textsubscript{0}: N\textsubscript{2} concentration of the last breath above 2.5%.

Figure E2. Visualization of the decomposition of a measurement into a fast and slow compartment in a semilog plot as described by \textit{f}_{\text{twoCompartments}}. The red curve shows the contribution of the fast compartment, the green shows the contribution of the slow compartment. The blue curve is the sum of fast and slow compartments. Both compartments have a decreasing tracer gas concentration over time.
Figure E3: Capture of end-tidal concentration (95-98% of expired volume) and mean/median N2 concentration (65-95% of expired volume) in a N2 concentration vs. expired volume plot of a single washout expiration.
Figure E4. Bland-Altman plots of relative difference between LCI_{1stBreath} and LCI_{standard} plotted versus mean of LCI_{1stBreath} and LCI_{standard} for healthy children (n= 60 measurements), children with CF (n=58 measurements, 2 excluded because of incorrect LCI calculation in a small breath) and children with PCD (n= 42 measurements, 9 excluded because of incorrect LCI calculation in a small breath) (one-way ANOVA analysis of variance, p<0.001).
Figure E5. Fluctuations around 2.5% are more frequent in measurements with higher lung clearance index (LCI) values in patients with cystic fibrosis (CF) and primary ciliary dyskinesia (PCD). LCI values analysed with the standard method at 2.5% (LCI\textsubscript{2.5}) in measurements with and without fluctuation of (a) 20 healthy children (52 measurements without fluctuation, 8 with fluctuation), (b) 20 children with CF (47 measurements without fluctuation, 13 with fluctuation) and (c) 17 children with PCD (28 measurements without fluctuation, 19 with fluctuation).

Figure E6. LCI values at 5% cut-off in measurements with and without fluctuation in (a) 20 healthy controls (56 measurements without fluctuation, 4 with fluctuation), (b) 20 children with CF (54 measurements without fluctuation, 6 with fluctuation) and (c) 17 children with PCD (45 measurements without fluctuation, 6 with fluctuation).
E-References

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