Sensitivity of Bronchial Responsiveness Measurements in Young Infants*

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Objectives: There is limited evidence on the preferred methods for evaluating lung function in infancy. The objective of this study was to compare sensitivity and repeatability of indexes of lung function in young infants during induced airway obstruction.

Methods: The study population consisted of 402 infants (median age, 6 weeks). Forced flow-volume measurements were obtained by the raised volume rapid thoracoabdominal compression technique and were compared with indexes of tidal breathing, measurements of transcutaneous oxygen (PtcO₂), and auscultation during methacholine challenge testing.

Results: PtcO₂ was the most sensitive parameter to detect increasing airway obstruction during methacholine challenge, followed by forced expiratory volume at 0.5 s (FEV₀.₅). Both were superior to other indexes of forced spirometry as well as tidal breathing indexes and auscultation. Coefficients of variations for PtcO₂ and FEV₀.₅ were 4% and 7%, respectively.

Conclusions: PtcO₂ and FEV₀.₅ are the most sensitive parameters for measurement of bronchial responsiveness in young infants. Measurements of baseline lung function should preferably be made using FEV₀.₅. Measurements of bronchial responsiveness are best assessed using PtcO₂, which may be performed in nonsedated infants and improve feasibility of future studies on lung function in infancy.

Key words: bronchial provocation tests; reproducibility of results; respiratory function tests

Abbreviations: COPSAC = Copenhagen Studies on Asthma in Childhood; CV = coefficient of variation; FEV₀.₅ = forced expiratory volume at 0.5 s; FEF₂₀ = forced expiratory flows at 50% of FVC; FEF₇₅ = forced expiratory flow at 75% of FVC; PIF = inflation pressure; PtcO₂ = transcutaneous oxygen pressure; PTEF = peak tidal expiratory flow; PTRANS = transmitted jacket pressure; RVRTC = raised volume rapid thoracoabdominal compression; SD-index = ratio between the change in lung function from post-saline solution measurement and the within-subject SD; SDw = within-subject SD; TPEF = time to reach peak tidal expiratory flow; TPEF/TE = time to reach peak tidal expiratory flow/expiratory time ratio; VT = tidal volume

Infant lung function testing is increasingly applied in research and tertiary clinical centers, but the choice of lung function indexes applied in infants is more varied compared with older children,¹ with no consensus on the preferred methods. The choice of the method should consider the sensitivity and repeatability of the methods in addition to the specificity and

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feasibility, including the demand of resources, all in relation to the clinical question being asked. The aim of this study was to define the order of sensitivity and repeatability for measurement of changes in lung function during bronchial responsiveness testing with a view to choose the preferred method for lung function measurement in young infants.

Lung function was measured by spirometry using the raised volume rapid thoracoabdominal compression (RVRTC) technique and by tidal breath analysis. In addition, lung function was estimated by transcutaneous oxygen (PtcO2) and auscultation. These techniques reflect different aspects of lung function. Forced-flow maneuvers reflect a global assessment of airway resistance and respiratory viscoelastic recoil. Tidal flow measures and auscultation are related to flow limitation and tidal breathing and also to other factors, such as control of breathing. PtcO2 provides information on gas exchange, i.e., balance between ventilation and perfusion, and reflects the integrated output of the respiratory and vascular system. The sensitivity of these methods was compared by the ability to detect a significant dose-related change in lung function after inhalation of methacholine.

Materials and Methods

The study included infants from the Copenhagen Studies on Asthma in Childhood (COPSAC) birth cohort study. COPSAC includes 411 infants of mothers with doctor-diagnosed asthma, excluding infants with gestational age <36 weeks, congenital abnormality, systemic illness, or a history of mechanical ventilation or lower airway infection. Parents gave written informed consent. The study was approved by the local ethics committee (KF 01–289/96, KF 01–227/97), and the Danish Data Protection Agency (1996–1200-360).

Methods

Additional details are provided in a Web-only data supplement. Lung function measurements were collected at baseline and after each dose step of the challenge protocol in the sequence: auscultation, tidal breathing, and RVRTC measurements. PtcO2 was measured continuously.

RVRTC Measurements. We used a "squeeze" jacket consisting of an inflatable “balloon” inside a nonexpandable outer coat. This jacket was wrapped around the infant’s chest and abdomen, with the arms outside the jacket. The equipment was custom built as described by Hayden et al. The method is standardized by fixed inflation and transmission pressures for forced expiration. Prior to forced expiration, the infant’s lung volume was raised above the tidal range, inflating air through the pneumotachograph. The infant was administered three inflations reaching a transrespiratory pressure of 2 kPa with passive deflations between each. This slight hyperventilation adds to the Hering-Breuer reflex induced by the raised volume before expiration, and results in a short respiratory pause, allowing the performance of a full forced expiratory maneuver. A compression force transmitting an additional pressure of 2 kPa was then applied via the squeeze jacket to the thorax and abdomen at the end of the third inspiration, leading to an airway opening pressure of 4 kPa for the forced expirations. Transmitted jacket pressure (PTRANS) was calculated as the difference between the pressure at airway opening after inflation of the jacket measured at the mouth inside the mask and the inflation pressure (PINF).

Parameters comprised forced expiratory volume at 0.5 s (FEV0.5), FVC, FEV0.5/FVC, forced expiratory flow at 25% of FVC, forced expiratory flow at 50% of FVC (FEF50%), forced expiratory flow at 75% of FVC (FEF75%), PINF, and PTRANS. All parameters were measured five times at baseline and three times after each challenge step, using the median value for FEV0.5 to decide on the continued provocation.

Tidal flow was measured by the pneumotachograph of the RVRTC equipment. Indexes comprised tidal volume (VT), respiratory frequency, peak tidal expiratory flow (PTFE), expiratory time as ratio of respiratory cycle time, time to reach PTEF (TPTEF), and PTEF/expiratory time ratio (PTPEF/TE). Tidal breathing was recorded in 20-s periods attempting to get at least five consecutive, technically acceptable breath cycles at baseline and 2 min after each challenge step.

PtcO2. The electrode (TCGM; Radiometer; Copenhagen, Denmark) was heated to 44°C and positioned on the flexor side of the lower arm 15 min before measurements. PtcO2 was determined as mean value over 1 min immediately before the lung function testing. Auscultation was performed by the investigator using a stethoscope attached to the front of thorax inside the jacket.

Methacholine Challenge Testing: The aerosol was administered with a dosimeter attached to a nebulizer (SPIRA 08 TSM 133; Respiratory Care Center; Hämeenlinna, Finland) and inhaled from a custom-made spherical metal spacer. Mass median aerodynamic diameter was 1.52 μm. After inhalation of saline solution, methacholine chloride was administered in quadrupling dose steps from 0.04 to 16.67 μmol/L (Table 3 in the Web-only data supplement). The test was stopped after a 20% fall in FEV0.5 or after administration of the maximum dose; 1 mg of terbutaline via a pressurized metered-dose inhaler was administered via spacer at the end of the test.

Statistical Analysis

Repeatability: Within-subject SD (SDw) was calculated as the SD of the difference between the median values of pre-saline solution and post-saline measurements, divided by the square root of 2. A coefficient of variation (CV) was computed as SDw in percentage of the average of the mean of the pre-saline solution and post-saline solution measurements.

Sensitivity: The change in lung function during challenge was transformed into an index: the ratio between the change in lung function from post-saline solution measurement and the SDw (SD-index). This allows direct comparison of techniques despite different repeatability. Dose-response curves were constructed plotting SD-index against dose step, and sensitivity was evaluated by comparing such curves. The binary variable auscultation was compared with the most sensitive indexes by dichotomizing them as the percentage of measurements exceeding a change of 3 × CV at each dose step.

Summary statistics of lung function measurements are described by mean ± SD. In addition, demographic data are described by median, quartiles, and range. SD was calculated by one-way analysis of variance with patient as factor. We used the measurement containing the median value of FEV0.5 for analysis of the other RVRTC indexes.

Results

Lung function tests were completed by 402 infants (205 girls) of the cohort of 411; 392 were white. Nine
infants from the COPSAC cohort were not included due to respiratory infection or refusal by parents. Demographic data are described in Table 1. Mean Pinf was 1.8 kPa (SD, 0.2), and mean Ptrans was 2.2 kPa (SD, 0.3). Regression coefficients for FEV0.5 on Pinf and Ptrans were 31.1 and −9.8, respectively.

The sensitivity of lung function measurements was compared in the 252 infants in whom Pinf and Ptrans reached within-group means ± 1 SD, and who also had complete data for tidal measurements as well as PtcO2. Auscultation was completed in a subgroup of 198 of these infants. Table 2 compares mean, SD, CV, and SDw (calculations based on pre-saline solution and post-saline solution measurements), and maximal change in lung function during challenge for all indexes. FEV0.5 was equal to FVC in 27% of measurements.

Figure 1 depicts dose-response curves for PtcO2 and the most sensitive indexes representing the RVRTC technique (FEV0.5 and FEF50) and tidal breathing technique (VR) in addition to the traditional indexes FEV0.5/FVC from the RVRTC and TPTEF/TE from the tidal breathing technique. PtcO2 was the most sensitive parameter for detecting changes in lung function, followed by FEV0.5 obtained by the RVRTC technique. Both were superior to other indexes of forced spirometry as well as all tidal breathing indexes and auscultation. Likewise, maximal change during challenge estimated by PtcO2 and FEV0.5 were significantly different from estimates by other measures. Auscultation was compared to PtcO2 and FEV0.5 in Figure 2, depicting the percentage of children with positive auscultation findings at each dose level together with the percentage of children with a significant change as measured by PtcO2 (15%) and FEV0.5 (20%).

Three operators examined 80 infants, 153 infants, and 15 infants, respectively. Sensitivity and repeatability were comparable between operators for FEV0.5. For PtcO2, there was a difference for sensitivity but not for repeatability between operators (see Web-only data supplement).

**Discussion**

We compared indexes of RVRTC, tidal breathing, PtcO2, and auscultation during methacholine challenge testing in young infants with median age of 6 weeks. PtcO2 was the most sensitive parameter detecting increasing airway obstruction, followed by FEV0.5, both being clearly superior to the all other measurements. Additional indexes of forced flow, tidal breathing indexes, and auscultation changed in parallel but were notably less sensitive. All infants included had a family history of asthma; therefore, the absolute level of lung function and bronchial responsiveness may not be representative of the general population. However, this does not affect the

**Table 1—Demographic Data for 402 Infants**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>25th Quartile</th>
<th>75th Quartile</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, wk</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>3–32</td>
</tr>
<tr>
<td>Height, cm</td>
<td>57</td>
<td>3</td>
<td>56</td>
<td>54</td>
<td>58</td>
<td>48–74</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>5.0</td>
<td>0.9</td>
<td>5.0</td>
<td>4.5</td>
<td>5.5</td>
<td>2.6–9.7</td>
</tr>
</tbody>
</table>

**Table 2—Lung Function Indexes: Mean, SD, CV, SDw (Based on Pre-Saline Solution and Post-Saline Solution Measurements), and Maximal Change During Methacholine Challenge Testing in 252 Infants**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline Mean</th>
<th>SD</th>
<th>CV</th>
<th>SDw</th>
<th>Maximal Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV0.5, mL</td>
<td>67.8</td>
<td>14.92</td>
<td>7</td>
<td>4.82</td>
<td>3.8</td>
</tr>
<tr>
<td>FEF25, mL/s</td>
<td>315.5</td>
<td>80.81</td>
<td>21</td>
<td>65.83</td>
<td>0.5</td>
</tr>
<tr>
<td>FEF50, mL/s</td>
<td>192.1</td>
<td>48.34</td>
<td>15</td>
<td>28.94</td>
<td>2.7</td>
</tr>
<tr>
<td>FEF75, mL/s</td>
<td>115.5</td>
<td>35.62</td>
<td>16</td>
<td>18.81</td>
<td>2.6</td>
</tr>
<tr>
<td>FVC, mL</td>
<td>71.0</td>
<td>15.96</td>
<td>8</td>
<td>5.68</td>
<td>3.2</td>
</tr>
<tr>
<td>FEV0.5/FVC</td>
<td>0.96</td>
<td>0.04</td>
<td>3</td>
<td>0.03</td>
<td>1.0</td>
</tr>
<tr>
<td>PtcO2, kPa</td>
<td>9.2</td>
<td>2.39</td>
<td>4</td>
<td>0.38</td>
<td>7.8</td>
</tr>
<tr>
<td>VR, mL</td>
<td>47.6</td>
<td>9.42</td>
<td>10</td>
<td>4.76</td>
<td>2.2</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>46</td>
<td>13</td>
<td>13</td>
<td>5.90</td>
<td>1.3</td>
</tr>
<tr>
<td>PTEF, mL/s</td>
<td>−105.4</td>
<td>23.05</td>
<td>15</td>
<td>16.69</td>
<td>0.6</td>
</tr>
<tr>
<td>Te/Tot</td>
<td>0.45</td>
<td>0.04</td>
<td>7</td>
<td>0.03</td>
<td>0.2</td>
</tr>
<tr>
<td>TPTEF, s</td>
<td>0.31</td>
<td>0.09</td>
<td>38</td>
<td>0.08</td>
<td>0.8</td>
</tr>
<tr>
<td>TPTEF/TE</td>
<td>0.51</td>
<td>0.12</td>
<td>22</td>
<td>0.11</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*FEF25 = forced expiratory flow at 25% of FVC; f = respiratory rate; Te/Tot = expiratory time as ratio of respiratory cycle time.*

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**Figure 1.** Dose-related bronchial obstruction from aerosolized methacholine in 252 infants, showing change in SD vs dose step. The number of children at each dose step is given in parentheses. ■ PtcO2; □ VR; ◆ FEV0.5; × FEV0.5/FVC; ▲ FEF50; ◆ TPTEF/TE.
Figure 2. Dose-related bronchial obstruction from aerosolized methacholine in 252 newborns. The y-axis shows the percentage of infants with significant change vs dose step. The number of children at each dose step is given in parentheses. ■ PtcO2; ○ FEV0.5; ▲ auscultation.

The purpose of comparing sensitivity and repeatability of lung function measurements within infants.

CVs for PtcO2 and FEV0.5 were 4% and 7%, respectively. Studies using similar equipment and technique (irrespective of inflation pressure) reported CVs (FEV0.5) of 4%11,12 to 5%.4,13 The CV (PtcO2) was 1%14 to 3%10 for PtcO2 in previous reports. The young age of the infants in our study may contribute to the higher CVs. We based CV on measurements before and after saline solution inhalation, while others based the calculation on two or more repeat measurements with no intervention between, which is probably the main reason for the different CV reported.

Forced expiratory maneuvers are widely used in the clinic and studies of pulmonary disease in children and adults. Techniques are also available ensuring such information on lung function in infants. The most widely applied technique to date is the Vt forced expiration technique, the rapid thoracic compression technique, which has been applied in large cohort studies.15–22 The parameter commonly calculated from this technique is maximal flow at functional residual capacity. However, flow measurements are volume dependent, and functional residual capacity is not a stable volume anchor point, as it is being dynamically determined by factors such as airway caliber, sleep state, and changes of dead space. Therefore, maximal flow at functional residual capacity has a poor reproducibility, with CVs reported from 11 to 36%.3 The RVRTC is better standardized for volume by applying an initial inflation pressure and raising the lung volume of the infant toward total lung capacity prior to the external compression. This ensures a better-defined anchor point at which to initiate the forced exhalation and thereby better standardized volume for measurements of forced expiratory volume and flow. This improved standardization is reflected in significantly reduced CV.4,12 In addition, FEV0.5 and forced expiratory flows obtained with RVRTC have been reported to be more sensitive than rapid thoracic compression parameters in discriminating between respiratory diseased and healthy infants3,23–25 as well as detecting changes in lung function within an infant.26,27

Far from ideal, the RVRTC is still sensitive to variations in inflation pressure and Ptrans,28–31 as shown in this study, with regression coefficients for FEV0.5 on PINF and Ptrans of 31 and −10, respectively, ie, changes of the target inflation pressure by 0.1 kPa for unchanged Ptrans leads to changes in FEV0.5 of 3 mL, ie, approx 5% of baseline. Changes in Ptrans of 0.1 kPa for unchanged PINF leads to a reduction of 1 mL of FEV0.5, ie, approx 1% of baseline.

Due to this sensitivity of the RVRTC indexes to the pressures applied, we chose to standardize the method by only accepting measurements in which inflation pressure and transmitted pressure both were within the mean ± 1 SD of the population, ie, minimizing variability of pressure at the airway opening measured at the mouth inside the mask within and between infants. Despite our standardization of pressures, FEV0.5 could vary up to 12 mL (18% of mean post-saline solution value) simply from changes in inflation pressure within such standardization limits. Alternatively to our choice of standardization between children, one may chose standardization of pressures achieved within children. This is just one example of the need for international recommendation on standardization of this method.

There is no general agreement on the optimal inflation pressure for the RVRTC technique; some centers aim at 2 kPa,11,24 while others aim at 3 kPa.12,13,31 The centers using 3 kPa for inflation pressure uses variable transmission pressures to obtain maximal values for flow and volume. It is possible that higher inflation pressure increase the sensitivity of the RVRTC technique. However, there are no studies comparing the two techniques.

Jones et al32 using inflation pressure of 3 kPa, found a higher sensitivity for baseline forced expiratory flow parameters (FEF50, FEF75, and forced expiratory flow, midexpiratory phase) than for FEV0.5; while Ranganathan et al23 using a similar inflation pressure, found FEV0.5 more sensitive than forced expiratory flows to discriminate between respiratory diseased and healthy infants. The discrepancy of the relative sensitivity between volume and flow parameters between our study and the study of
Jones et al. could be due to different definition of sensitivity. In our study, it was defined as the ability to detect induced changes in lung function within an individual, and in their study as the ability to discriminate between disease and health. We chose the former, as the latter hinge on strict criteria for defining health and disease, which are inaccurate in young infants. As another difference between the studies, we compared the indexes after adjustment for differences in the repeatability of the parameters, whereas Jones et al. compared indexes irrespective of different repeatability. Thus, the lower variability of the forced volume parameters contributes to the better sensitivity reported in our study.

We found FEV\textsubscript{0.5} to be the most sensitive of the RVRTC parameters investigated, despite a mean ratio of FEV\textsubscript{0.5}/FVC of 0.96 indicating that infants in this age group often reach FVC within 0.5 s. We found FEV\textsubscript{0.5} equal to FVC in 27\% of measurements. A shorter time limit such as 0.4 s may provide improve sensitivity.\(^{12}\) We found no differences between operators with respect to sensitivity and repeatability for the RVRTC technique.

Tidal breathing measurements are attractive, as they require a minimum of intervention and are easier to apply in infants during natural sleep. Tidal breathing measurements have been applied in several large cohort studies.\(^{33-37}\) A wide number of parameters have been derived from tidal data in a way to identify a parameter describing the changes in tidal breathing pattern seen during bronchoconstriction yet with no consensus on the best index.\(^{5}\) The most sensitive of the tidal parameters was simply the V\textsubscript{T}, while the more commonly used index of T\textsubscript{PTEF}/T\textsubscript{E}\(^{5}\) was less sensitive. However, we found all indexes of tidal breathing to have a very low sensitivity to airway obstruction, which is in agreement with previous observations.\(^{38,39}\) Despite our attempts to standardize tidal measurements, they were noticeably inferior to forced flow-volume measurements and measurements of P\textsubscript{te}O\textsubscript{2} for detecting increased airway obstruction.

Measurement of P\textsubscript{te}O\textsubscript{2} is easily applicable and is commonly used for monitoring infant lung function in the clinic and in research to detect changes in lung function.\(^{14,38,40,41}\) We found P\textsubscript{te}O\textsubscript{2} being superior to other lung function indexes in detecting methacholine-induced airway obstruction. This measure is highly sensitive to induced airway obstruction while of little value to gauge the baseline lung function. We found an unexplained difference between the three operators completing these measurements. Positioning of the probe on the thorax may actually further improve the sensitivity of this method.

P\textsubscript{te}O\textsubscript{2} was also found in our previous study of lung function measurements in preschool children to be more sensitive than forced exhaled flow measures and as sensitive as specific airway resistance measured by whole-body plethysmography. These findings suggest P\textsubscript{te}O\textsubscript{2} is a sensitive parameter for detecting changes in lung function during challenge test from infancy to adulthood.\(^{10,38,40,42}\) These findings open a wider use of measurement of bronchial responsiveness in infants, since P\textsubscript{te}O\textsubscript{2} can be applied to the awake infant during challenge.\(^{16}\) This has important implications for future epidemiologic studies.

Auscultation has been the most commonly used technique evaluating lung function in all age groups. In research, it has also been used to detect response to airway challenge in children and infants.\(^{43-45}\) It is easily applicable and does not require complicated or expensive equipment. In our study, auscultation was a much less sensitive indicator of airflow limitation than the forced expiratory parameters and measurement of P\textsubscript{te}O\textsubscript{2}. Our procedure was suboptimal because of the placement of the jacket and because the infant was placed in a fixed supine position. Still, the low sensitivity found in this study is supported by previous findings in children and adults.\(^{40}\) The usefulness of lung sound analysis using electronic equipment requires further investigation.\(^{46}\)

Bronchial responsiveness testing in infants is not standardized,\(^{47}\) neither with respect to the choice of bronchoconstrictor agent nor the administration of aerosol.\(^{48}\) We chose direct-acting methacholine because it has few side effects and a relatively long duration of action compared to histamine.\(^{10,49}\) The latter characteristic makes timing between administration and subsequent measurements less critical, which is important as the techniques compared were applied in sequence. We chose a modified dosimeter method to enhance reproducibility of aerosol delivery and accuracy of the dose-response relation. Dosimetry allows exact calculation of the delivered dose, as opposed to continuous nebulization that delivers unpredictable doses to the infant. We chose a relative (CV) not an absolute (SD) reduction in lung function to decide on the last dose step in individual infants in order to relate the reduction in lung function to the baseline lung function, thereby enhancing safety for the infants with the lowest baseline values.

In summary, we suggest FEV\textsubscript{0.5} measured by RVRTC for determination of baseline lung function, while P\textsubscript{te}O\textsubscript{2} measurements are recommended for subsequent measurement of bronchial responsiveness. This approach to infant lung function measurement will improve feasibility and allows a wider application of early measurements of lung function.
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