TOPICAL REVIEW

Pulmonary function testing in children and infants

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Topical Review

Pulmonary function testing in children and infants

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Abstract

Pulmonary function testing is performed in children and infants with the aim of documenting lung development with age and making diagnoses of lung diseases. In children and infants with an established lung disease, pulmonary function is tested to assess the disease progression and the efficacy of therapy. It is difficult to carry out the measurements in this age group without disturbances, so obtaining results of good quality and reproducibility is challenging. Young children are often uncooperative during the examinations. This is partly related to their young age but also due to the long testing duration and the unpopular equipment. We address a variety of examination techniques for lung function assessment in children and infants in this review. We describe the measuring principles, examination procedures, clinical findings and their interpretation, as well as advantages and limitations of these methods. The comparability between devices and centres as well as the availability of reference values are still considered a challenge in many of these techniques. In recent years, new technologies have emerged allowing the assessment of lung function not only on the global level but also on the regional level. This opens new possibilities for detecting regional lung function heterogeneity that might lead to a better understanding of respiratory pathophysiology in children.

Keywords: lung function testing, respiratory disease, respiratory mechanics, ventilation inhomogeneity, paediatrics, obstructive lung disease
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>BTPS</td>
<td>Body conditions (body temperature, ambient pressure, saturated with water vapour at these conditions)</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>$D_{L,CO}$</td>
<td>Diffusing capacity of the lung for carbon monoxide</td>
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<tr>
<td>EIT</td>
<td>Electrical impedance tomography</td>
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<td>ERS</td>
<td>European Respiratory Society</td>
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<tr>
<td>ERV</td>
<td>Expiratory reserve volume</td>
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<tr>
<td>EVC</td>
<td>Expiratory vital capacity</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25&lt;/sub&gt;</td>
<td>Forced expiratory flow when 25% of FVC has been exhaled</td>
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<tr>
<td>FEF&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Forced expiratory flow when 50% of FVC has been exhaled</td>
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<tr>
<td>FEF&lt;sub&gt;75&lt;/sub&gt;</td>
<td>Forced expiratory flow when 75% of FVC has been exhaled</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25-75&lt;/sub&gt;</td>
<td>Forced expiratory flow between 25% and 75% of FVC</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.5&lt;/sub&gt;</td>
<td>Forced expiratory volume in 0.5 s</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.75&lt;/sub&gt;</td>
<td>Forced expiratory volume in 0.75 s</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Forced expiratory volume in 1 s</td>
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<tr>
<td>FOT</td>
<td>Forced oscillation technique</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>He</td>
<td>Helium</td>
</tr>
<tr>
<td>IC</td>
<td>Inspiratory capacity</td>
</tr>
<tr>
<td>IRV</td>
<td>Inspiratory reserve volume</td>
</tr>
<tr>
<td>ITGV</td>
<td>Intrathoracic gas volume</td>
</tr>
<tr>
<td>IVC</td>
<td>Inspiratory vital capacity</td>
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<tr>
<td>LCI</td>
<td>Lung clearance index</td>
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<tr>
<td>MOT</td>
<td>Multiple occlusion technique</td>
</tr>
<tr>
<td>N₂</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>$P_{0.1}$</td>
<td>Pressure at the airway opening 0.1 s after airway occlusion during tidal inspiration</td>
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<tr>
<td>$P_{\text{max}}$</td>
<td>Pressure at the airway opening during maximum inspiration after airway occlusion</td>
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<tr>
<td>PEF</td>
<td>Peak expiratory flow</td>
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<tr>
<td>PIF</td>
<td>Peak inspiratory flow</td>
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<tr>
<td>Raw</td>
<td>Airway resistance</td>
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<td>RIP</td>
<td>Respiratory inductive plethysmography</td>
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<tr>
<td>$R_{rs}$</td>
<td>Respiratory system resistance</td>
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<tr>
<td>RV</td>
<td>Residual volume</td>
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<tr>
<td>SOT</td>
<td>Single occlusion technique</td>
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<tr>
<td>sRaw</td>
<td>Specific airway resistance</td>
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<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>VC</td>
<td>Vital capacity</td>
</tr>
<tr>
<td>$V_T$</td>
<td>Tidal volume</td>
</tr>
<tr>
<td>$X_{rs}$</td>
<td>Respiratory system reactance</td>
</tr>
<tr>
<td>Z</td>
<td>Impedance</td>
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</table>
1. Introduction

The examination of lung function in children is not an easy task. The successful accomplishment of specific lung function tests often depends on the child’s age. From the age of six, children are usually able to undergo the same examinations as adults (Enright et al. 2000). The assessment of lung function in infants and younger children is more challenging and it is usually not included in routine clinical examinations as it may require sedation (Godfrey et al. 2003). Although conventional pulmonary function testing can already be attempted in children aged two to five years, the correct performance and reliable interpretation may not be obtained (Kanengiser and Dozor 1994). According to the common statement of the ATS and the ERS it would not be ethically acceptable to sedate preschool children for pulmonary function testing (Beydon et al. 2007).

The results of lung function testing in infancy and childhood are affected by the age-dependent changes in chest and lung anatomy and physiology which take place from birth until adulthood as the respiratory system grows and matures. Besides age, sex, body size and ethnic origin also impact lung function and have to be accounted for. In recent time, efforts have been undertaken to optimize lung function testing with regard to the inter-individual variability in order to improve the quality of the examinations and their results (Kirkby et al. 2013, Quanjer et al. 2012).

In this review, we describe several measurement techniques that are applied for the assessment of lung function in infants and children. Some of them were developed for use in both adults and children but many techniques had to be modified for a specific period of childhood. Pulmonary function testing in this population pursues the following major goals: (1) detection and identification of lung disease (diagnosis), (2) assessment of disease progress (disease monitoring and staging) and (3) analysis of therapy effectiveness (therapy monitoring and optimization).

Pulmonary function testing is also performed in clinical research fostering the development of new measuring methods and analyses. The implementation of novel techniques in addition to established ones might potentially lead to better and earlier clinical diagnosis and therapeutic intervention. Such new techniques have already accelerated our understanding of the spatial and temporal inhomogeneity of ventilation in infants and children (Altes et al. 2006, Frerichs et al. 2001, Gustafsson et al. 2008, Heinrich et al. 2006, Narayanan et al. 2012, Pham et al. 2011, Pillow et al. 2006, Riedel and Frerichs 2010).

In this review we present non-invasive methods that are currently used for lung function testing in infancy and childhood; however, we only address methods that do not require deep sedation, i.e., those techniques that are applied in awake, spontaneously breathing infants and children. We do not describe other examination approaches that can only be applied during mechanical ventilation. For each technique the following issues are addressed: (1) the measuring principle, (2) examination procedure, (3) clinical findings and interpretation, and (4) advantages and limitations.

2. General characteristics of pulmonary function testing in different age groups

Children should always be instructed in detail before the examination is started. School children are usually able to cooperatively follow these instructions. To improve the success of examination and to secure high performance, visual interactive computer animations may be used, particularly in preschool children (Neve et al. 2006, Vilozni et al. 2001, Vilozni et al. 2005).
The examinations are usually carried out with the child in a sitting position with their feet on the floor, sitting quietly without any sudden movements. Several techniques require the use of a mouthpiece which is taken between the teeth and enclosed tightly with the lips, with the tongue below the mouthpiece. The nose is often covered with a nose clip. The child must not wear clothing that restricts chest movements. During the examinations, the child usually breathes quietly at a stable end-expiratory level (corresponding to FRC). Sometimes specific ventilation manoeuvres are performed. No flexion or rotation of the head should occur during the measurements.

Infants and small children are not able to actively cooperate. The examinations are frequently performed during sleep or even under sedation whilst lying down. In contrast to the examinations of preschool and school children where devices developed for adults may often be used, pulmonary function testing in smaller children requires miniaturized devices adapted for this age group. A face mask is often needed instead of a mouthpiece. The dead space and airflow resistance of the apparatus must be as low as possible, air leaks should be prevented. Since infants and small children are preferential nose breathers pulmonary function testing is affected by the high Raw of the upper airways. Therefore, examinations should not be performed during or after upper airway infections.

The testing of infants and small children usually takes place during quiet tidal breathing. Because children of this age are not able to perform any specific ventilation manoeuvres voluntarily, external compression of the chest and abdomen is applied when forced expiration needs to be examined.

During the measurements in infants and small children, the full resuscitation unit has to be available and all safety precautions have to be taken. The staff must be able to carry out emergency procedures at any time (Bates et al 2000, Frey et al 2000). Pulse oximetry monitoring is recommended (Merkus et al 2005).

In all age groups, pulmonary function testing is performed mainly because of the presence of obstructive lung diseases. Restrictive diseases are less frequent in children than in adults. Therefore, the primary aim of the examinations is to characterize the patency of the airways, for instance by measuring the Raw or maximum airflow rates. Other measures like static lung volumes or diffusing lung capacity, relevant especially in restrictive pulmonary diseases, are less often examined. Airway inflammation plays a major role in the pathogenesis of lung disease, therefore, the metabolic function of the lung is increasingly being assessed, e.g. by the measurement of exhaled NO or the breath condensate analysis. (Main clinical issues where the methods presented in this review may be applied are given in table 1.)

The quality of the findings obtained by pulmonary function testing in infants and children is very much dependent on the person performing the test. Only fully dedicated investigators guarantee good quality and reproducible results. The children need to be trained and motivated. However, they can be easily distracted and several attempts may be needed until the measurement is performed adequately. Experienced and patient personnel are required to fulfil this task.

3. Methods of pulmonary function testing

3.1. Spirometry

Spirometry is the basic examination in lung function testing (Miller et al 2005), especially in school children. Today’s spiroimeters are based on pneumotachometers or ultrasonic flowmeters and they measure the airflow and the volume at the airway opening. Two types of spirometry, static and dynamic, exist.
Table 1. Examples of clinical information derived from pulmonary function testing using the methods presented in the review.

<table>
<thead>
<tr>
<th>Clinical issue</th>
<th>Method</th>
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<td>Air trapping</td>
<td>Whole body plethysmography, RIP, inert gas washout techniques</td>
</tr>
<tr>
<td>Airway obstruction, response to challenge</td>
<td>Spirometry, whole body plethysmography, airway occlusion techniques (SOT, MOT), FOT, impulse oscillometry</td>
</tr>
<tr>
<td>Apnoea detection</td>
<td>RIP, EIT</td>
</tr>
<tr>
<td>Inflammation, effect of medication</td>
<td>Exhaled NO, breath condensate analysis</td>
</tr>
<tr>
<td>Location of obstruction</td>
<td>FOT, impulse oscillometry, EIT, spirometry, whole body plethysmography</td>
</tr>
<tr>
<td>Pleural disease</td>
<td>EIT, whole body plethysmography</td>
</tr>
<tr>
<td>Pulmonary respiratory gas transfer</td>
<td>$D_{L,CO}$</td>
</tr>
<tr>
<td>Respiratory drive, respiratory muscle strength</td>
<td>$P_{th}, P_{max}$ measurement</td>
</tr>
<tr>
<td>Reversibility of obstruction, effect of medication</td>
<td>Whole body plethysmography, $D_{L,CO}$</td>
</tr>
<tr>
<td>Staging of disease, risk assessment of interventions</td>
<td>Spirometry, whole body plethysmography, exhaled NO</td>
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<td>Thoraco-abdominal asynchrony, work of breathing</td>
<td>RIP</td>
</tr>
<tr>
<td>Ventilation inhomogeneity</td>
<td>Inert gas washout techniques, EIT</td>
</tr>
<tr>
<td>Ventilation-perfusion mismatch</td>
<td>$D_{L,CO}$, EIT</td>
</tr>
</tbody>
</table>

RIP, respiratory inductive plethysmography; FOT, forced oscillation technique; SOT, single occlusion technique; MOT, multiple occlusion technique; EIT, electrical impedance tomography; $D_{L,CO}$, diffusing capacity of the lung for carbon monoxide.

Static spirometry detects lung volumes involved in the process of ventilation. The following static volumes are measured: $V_T$, IRV, IC which consists of $V_T$ and IRV, IVC, ERV and EVC.

Dynamic spirometry describes the relationship between volume and time, and the relationship between flow and volume. The examination is carried out during forced respiratory manoeuvres and provides the following parameters: FVC, FEV1, FEV1/FVC ratio, PEF, PIF, FEF25, FEF50 and FEF75 and maximum mid-expiratory flow between 25% and 50% of FVC (FEF25–75) (Miller et al 2005, Pellegrino et al 2005, American Thoracic Society 1995b). The parameters are normalized to BTPS conditions, i.e. the values are given at 37 °C, ambient pressure, saturated with water vapour (Miller et al 2005).

RV, TLC and FRC are not detected by spirometry. Whole body plethysmography or the multiple-breath washout technique are required to determine them.

3.1.1. Measuring principle. A pneumotachometer consists of a tube with a built-in flow resistor with known airflow resistance under laminar flow conditions. Upstream and downstream of the resistor, the pressures are measured and the pressure difference is converted into electrical voltage by means of a pressure transducer. By integration of voltage (flow) over time, volume is determined.

Spirometry using an ultrasound flowmeter has established itself as an alternative method to pneumotachometer within recent years. Two ultrasonic transducers are mounted transversely in a tube through which the tested subject breathes. The transducers transmit and receive the ultrasonic waves that are accelerated or decelerated by the airflow. The difference in the transit times of the ultrasonic waves correlates with the airflow (Buess et al 1986, Fuchs et al 2006).
Spirometers based on pneumotachs must be carefully calibrated every day (Miller et al 2005), whereas ultrasonic spirometers need not be calibrated (Walters et al 2006).

3.1.2. Examination procedure. The child sits quietly and performs tidal breathing until stable end-expiratory level is achieved. At first, the slow VC manoeuvre is carried out: The child exhales slowly as much as possible (ERV), then maximally inhales (IVC) and, finally, exhales slowly and fully (EVC). (The respective lung volumes derived from each phase of this manoeuvre are given in the parentheses.) Thereafter, tidal breathing is resumed. Then the manoeuvre for expiratory FVC is carried out. After full exhalation and inhalation, the child is asked to exhale all the air as quickly and as long as possible. The last phase renders FVC, FEV₁, FEF₂₅, FEF₅₀, FEF₇₅. Three acceptable manoeuvres can usually be performed (Merkus et al 2005). The child must be informed, motivated and exhibit full commitment particularly during the forced expiratory manoeuvre.

In contrast to children of preschool and school age who are able to perform the forced manoeuvres after appropriate training, smaller children and infants cannot accomplish them. Therefore, spirometry is only performed during tidal breathing. A regular breathing pattern can best be achieved during natural sleep or in a state of sedation (Latzin et al 2009, Lodrup Carlsen and Carlsen 1993). Forced expiration can be studied by means of inflatable jackets placed around the trunk. The rapid compression of the chest and abdomen is initiated either after a tidal or an induced full inspiration up to the TLC level. The respective methods are called tidal and raised volume thoracoabdominal compression and they require sedation. The ERS and the ATS have published several articles with recommendations for equipment requirements, standardized procedures and reporting of data (Bates et al 2000, Frey et al 2000, Rosenfeld et al 2013a, 2013b, Sly et al 2000).

3.1.3. Clinical findings and interpretation. The qualitative and quantitative results of spirometry allow the first assessment of the nature of respiratory disorder. If an obstructive disorder is found, it can be distinguished whether it is of thoracic or extrathoracic origin. Spirometry mostly reflects pathological changes located in the central airways, peripheral changes are less well represented. The first interpretation of the findings should be visual at first with a qualitative analysis of the volume–time and flow–volume curves. Only when these curves are adequate should the quantitative results be considered.

Tidal flow–volume loops are mainly examined in infants and small children. They allow the analysis of their shape and the generation of simple measures, like the time to peak tidal expiratory flow to expiratory time ratio. This index has been used as a measure of lung function in healthy infants and in infants with asthma and severe chronic lung disease of prematurity (Clarke et al 1994). Healthy children were distinguished from children with asthma using this approach and the reversibility of airway obstruction identified (Carlsen and Lodrup Carlsen 1994). Tidal flow–volume loop analysis was also found to be useful in children with cystic fibrosis and other obstructive airway diseases (van der Ent et al 1996).

The forced flow–volume curves provide detailed information about the maximum possible flows at different volume levels. The expiratory part of this curve, generated either by the raised volume rapid thoracoabdominal compression technique in infants and small children or voluntarily in older children, exhibits an initial peak corresponding to PEF. In the latter age group, its value is highly variable and particularly influenced by the patients’ cooperation and effort (Bateman et al 2008, Brand et al 1997, Pedersen et al 1997). PEF is a simple but very rough control measure used in follow-up of respiratory diseases, above all in asthma (Bateman et al 2008, Grad et al 2009). Tidal rapid thoracoabdominal compression only allows
Figure 1. Flow–volume loops determined by spirometry (MasterScope, CareFusion, Höchberg, Germany) in a six year old boy with asthma before (left) and after inhaled bronchodilator administration (right).

the assessment of expiratory flow at low lung volumes and, thus, the acquisition of only partial flow–volume loops. The most popular measure derived from this examination is the maximum flow at FRC enabling the estimation of intrathoracic airway obstruction (Modl et al 2000, Morgan et al 1988).

Another important measure derived from the forced full expiration manoeuvre is FEV$_1$ which mainly reflects the function of airways with an inner diameter greater than 2 mm. FEV$_1$ is highly reproducible but of limited sensitivity to detect changes in lung periphery (Hutchison et al 1981). Because of the smaller overall volume of lungs in children compared with adults, forced expiration is sometimes completed within less than 1 s, especially in young children without airway obstruction. In this case, the FEV$_{0.5}$ or FEV$_{0.75}$ can be used (Aurora et al 2004b). MEF$_{50}$ seems to be a more appropriate index than FEV$_1$ in identifying small airways disease (Bar-Yishay et al 2003, McFadden and Linden 1972).

The shape of the flow–volume curve exhibits typical characteristics in certain ventilation disorders. The expiratory part of the curve is concave in obstructive lung diseases (figure 1). Intrathoracic tracheal stenosis results in a plateau-shaped expiratory part of the flow–volume curve, extrathoracic stenosis affects the inspiratory part, and fixed stenosis affects both parts of the curve. In restrictive disorders, the shape of the curve is without pathological changes, however, with overall reduced values. The diagnosis of restriction is best established in combination with whole body plethysmography allowing the measurement of TLC and RV.

Reference values of spirometric indices have been published by the ERS Global Lung Function Initiative (Quanjer et al 2012). Multiethnic age-dependent prediction equations and the lower limits of normal spirometric values were derived for children older than three years for FEV$_1$, FVC, FEV$_1$/FVC, FEV$_{0.75}$/FVC, FEV$_{0.75}$/FEF$_{25-75}$ and FEV$_{0.75}$/FEF$_{25-75}$. Older equations are still in use for other values (Zapletal et al 1976, Zapletal and Samanek 1977).

3.1.4. Advantages and limitations. Spirometry is one of the fundamental methods in pulmonary function testing. Modern spirometers are portable and examinations can be performed anywhere (Brouwer et al 2008). Spirometry is easily feasible with trained staff
but its use is limited to school children who can perform the complex breathing manoeuvres. To achieve good measurement quality, the full forced ventilation manoeuvres often need to be repeated. This may affect the airway diameter and impact the derived measures (Milanese et al 2000). The pneumotach equipment must be regularly maintained, calibrated and cleaned to secure the necessary standards. Tidal and raised volume rapid thoracoabdominal compression techniques require well-trained personnel and are only applied in a few centres, mostly in clinical research.

In summary, spirometry is an established method to study lung function in children. It allows good assessment of obstructive disease, however, the severity of peripheral obstruction may be underestimated. It can be effectively applied to assess bronchial responsiveness (Goldstein et al 2001). The presence of restrictive diseases can merely be indirectly implied from some of the findings. Combined diseases must be verified using further diagnostic tools. The results of spirometry are effort-dependent.

3.2. Respiratory inductive plethysmography

RIP is a non-invasive method to determine respiratory rate and estimate lung volumes, typically VT. Other measures of respiratory timing and airflow can additionally be determined. This method also allows the detection of thoracoabdominal asynchrony during ventilation. RIP is not routinely used in a clinical setting.

3.2.1. Measuring principle. RIP measures the ventilation-related changes in the cross-section of the ribcage and abdomen. Two elastic bands are placed around the chest and abdomen. Each band contains an isolated wire through which an alternating electrical current is passed. The wire is sewn into the band in a zig-zag fashion. Each wire forms a coil and generates a weak magnetic field. The changing cross-sectional areas of the thorax and abdomen during breathing modify the magnetic field and the inductance of the coil. The changes in inductance can be measured. Because they are proportional to the change of the coil area they reflect the changes in lung volume. For further details, see Cohen et al (1994) or Cohn et al (1982).

3.2.2. Examination procedure. The two elastic inductive bands are placed around the ribcage and the abdomen of the child. The examination is typically performed during quiet tidal breathing. Infants are examined in the supine position, older children are sitting. The results are posture-dependent (Mayer et al 2003), therefore body position should always be documented. The RIP signals may be calibrated using spirometry or so-called qualitative diagnostic calibration (Sackner et al 1989). Face masks or mouthpieces are otherwise not needed (Tabachnik et al 1981).

3.2.3. Clinical findings and interpretation. The magnitude, the timing and the synchrony between the chest and abdominal inductance can be determined. Both uncalibrated (Nosal et al 2008) and calibrated signals (Kohler et al 2008) have been utilized in RIP examinations of infants and children. The phase shift between the chest and abdominal signals is a measure of thoracoabdominal asynchrony. Increased values may be a sign of increased work of breathing. Thoracoabdominal asynchrony is found in neuromuscular or obstructive lung diseases. In a longitudinal study of children with cystic fibrosis, an increase in thoracoabdominal asynchrony accompanied the progression of the disease (Ren et al 2012). RIP has also been applied for apnoea detection in sleep disorders or after surgery (Brown et al 2008).
3.2.4. Advantages and limitations. The great advantage of RIP is that it can be performed without applying a face mask which often has a frightening effect in small children. The method is fully non-invasive, without any known risks and requires only minimum cooperation of the child. It is primarily used in clinical research.

3.3. Whole body plethysmography

Whole body plethysmography is the gold standard for the determination of Raw and lung volumes (Nicolai and Griese 2011). It allows the measurement of all static and dynamic lung volumes, including RV, FRC and TLC that cannot be determined using spirometry.

3.3.1. Measuring principle. A whole body plethysmograph consists of a transparent rigid box, in which the patient is examined, and a computer unit. The box is approximately the size of a telephone booth and can be closed to become airtight. Smaller plethysmographic chambers have been developed for the examination of infants (Stocks et al 2001). A pneumotachometer is mounted in the wall of the chamber allowing the measurement of airway pressure and flow. Changing thoracic dimensions with lung inflation and deflation result in periodic changes in pressure within the chamber because the air in the closed box is compressed and decompressed. The plethysmograph pressure is calibrated to yield the shift in volume; alternatively, the shift in volume can be measured directly in some devices. The simultaneously measured flow at the mouth is then plotted versus the volume shift as an X–Y diagram. The slope of the curve obtained during quiet tidal breathing equals $s_{\text{Raw}}$. The shape of the $s_{\text{Raw}}$ loop is affected by Raw and lung volume. Consequently, the calculation of Raw requires the knowledge of $V_T$ and ITGV.

The measurement of ITGV (the synonym is $FRC_{\text{pleth}}$) makes use of Boyle–Maryotte’s law which states that the product of pressure and volume is constant in a sealed box at a constant temperature (Lindemann and Leupold 2003). When the lung volume at the end of normal expiration is to be determined, then the airway is transiently occluded by closing a shutter located close to the mouth at this time point of the respiratory cycle. Thereafter, the examined child continues to breathe against the closed shutter. During these respiratory efforts, ITGV is compressed and decompressed. The airway and the plethysmograph pressures are simultaneously recorded. The pressure measured at the mouth reflects the changing alveolar pressure and the calibrated plethysmograph pressure provides the volume shifts during the respiratory efforts against the occlusion allowing the calculation of ITGV. When the shutter occlusion is executed by the end of resting tidal expiration, FRC can be calculated from ITGV by subtraction of the apparatus dead space.

3.3.2. Examination procedure. The child sits upright in the sealed plethysmography box and breathes quietly until resting FRC level is achieved. At first, $s_{\text{Raw}}$ is determined during uniform tidal breathing without any active effort by the child. Preferably at least three similar $s_{\text{Raw}}$ loops are recorded. For the measurement of ITGV ($FRC_{\text{pleth}}$), the shutter is automatically closed at the end of a normal expiration and opened after 3 s. In this short period, the child should continue to breathe against the shutter with the lips closed tightly at the mouthpiece and not blow. After the determination of FRC, the child is instructed to fully exhale and then to fully inhale which allows the assessment of RV and TLC.

Finally, spirometry is performed as described in 3.1.2. During this part of the examination the door of the chamber is open.

In infants, the examination is executed in the supine position using a face mask, and air leaks must be omitted. In this age group, plethysmographic testing is performed during
Figure 2. Specific airway resistance loops determined by whole body plethysmography (MasterScreen PFT Pro, CareFusion, Höchberg, Germany) in an eight year old girl with cystic fibrosis. The examinations were performed at the beginning (top) and by the end of a three week stay in a department of rehabilitation (bottom). sRaw was 2.35 kPa·s (460.8% predicted) and 1.40 kPa·s (275.2% predicted), respectively. The corresponding RAW values were 0.93 kPa·s·l$^{-1}$ (232.0% predicted) and 0.63 kPa·s·l$^{-1}$ (157.9% predicted).

sleep and aims at establishing Raw and FRC as well as other parameters derived from these measures.

3.3.3. Clinical findings and interpretation. The clinical evaluation is both qualitative and quantitative. The shape and the slope of the sRaw loop are inspected visually at first. In healthy children, the loop is closed, its slope during inspiration and expiration is almost the same with no hysteresis and its shape is minimally nonlinear (Nicolai and Griese 2011). A slight opening and flattening of the loop with clockwise tilting implies narrowing of central airways with no obstruction of small airways. An open resistance loop especially during expiration (visually resembling a golf club) is typical for an inhomogeneous small airway obstruction with expiratory flow limitation (figure 2). The inspiratory part of the loop is closed. Dynamic
airway obstruction additionally tends to tilt the loop clockwise. Increased curvilinearity of the sRaw loop with a pronounced ‘S’ shape is typical of extrathoracic airway obstruction. A V-shaped loop is observed in overweight patients or in patients with diaphragmatic paresis. The absolute values of sRaw can distinguish healthy children from diseased children and assess the degree of airway obstruction and the severity of the lung disease (Leben and von der Hardt 1983).

If the FRC value detected by the whole body plethysmography is not within the reference range then additional determination of FRC using inert gas washout (see 3.5) may be helpful. For instance, high FRC values may be found by whole body plethysmography if regions with little or no communication with the airways exist in the lungs. These regions are not identified by inert gas washout because they are not involved in ventilation (Castile et al 2004, Tantucci et al 2011, Tierney and Nadel 1962). Consequently, FRC measured by whole body plethysmography will be higher than the value determined by inert gas washout.

Whole body plethysmography is not frequently used in infants due to its complexity and the necessity of highly trained staff. Nonetheless, it has been shown to be able to determine the severity of respiratory disorder in infancy (Kjaer et al 2008, Pelkonen et al 2010, Robin et al 2004) and to identify air trapping.

3.3.4. Advantages and limitations. Whole body plethysmography is only used by specialists in respiratory medicine in certain centres, due to the high purchase and maintenance costs of the equipment. Compared to spirometry, the examinations are less effort-dependent; however, many children are scared of sitting in the sealed box and they do not correctly follow the instructions. The duration of the examination is short and only slightly longer than for spirometry. Whole body plethysmography is the standard method for the determination of FRC and Raw. However, this method alone is not sufficient for the detection of mild and moderate ventilatory disorders in the small peripheral airways. Since the calculation of Raw as well as of RV and TLC depends on the correct measurement of ITGV it is essential that this volume is adequately measured.

3.4. Airway occlusion techniques

Several methods utilize the procedure of short occlusion of airways to obtain information on different aspects of lung function. The occlusion is performed at different time points of the respiratory cycle.

Occlusion of the airways by the end of tidal inspiration allows the assessment of the mechanics of the passive respiratory system (i.e., respiratory system compliance, resistance and time constant) from a single breath. This method is called the SOT. The MOT performs the occlusions at different time points of tidal expiration.

The interrupter technique uses the approach of airway occlusion initiated typically during peak tidal expiratory flow or mid-expiration. This method is applied to determine the resistance of the passive respiratory system, also called interrupter resistance. Occlusion can also be executed during inspiration rendering only slightly different values (Bridge and McKenzie 2001).

Finally, airway occlusion at the FRC level before tidal or voluntary forced inspiration allows the assessment of respiratory drive or respiratory muscle strength, respectively. The inspiratory muscle strength can alternatively be tested by starting at the RV level.

The described occlusion techniques have not gained broad clinical acceptance yet, they are mostly used in clinical research.
3.4.1. Measuring principle. SOT utilizes the fact that a brief occlusion of the airway taking place above the FRC level elicits the Hering–Breuer reflex. This leads to a short apnoea and relaxation of the respiratory muscles. Provided that no flow is present during the relaxation, the pressure at the airway opening corresponds to the pressure in the alveoli (Gappa et al. 2001, Merkus et al. 2005, Rabbette et al. 1994). It equals the recoil pressure of the whole chest–lung system. If the volume change during the tidal inspiration preceding the occlusion is related to this pressure then compliance of the passive respiratory system can be determined. Provided the respiratory muscles remain relaxed during the subsequent passive expiration, the registered expiratory flow–volume curve allows the calculation of the expiratory time constant from the linear part of the flow–volume relationship. The prerequisite is that the respiratory system behaves as a single compartment. The knowledge of the respiratory system compliance and time constant allows a simple calculation of the $R_{rs}$. Alternatively, resistance can be calculated from the pressure measured at the airway opening at the time of occlusion divided by the extrapolated flow at the beginning of expiration.

MOT also relies on the Hering–Breuer reflex that is repeatedly evoked by several short airway occlusions during expiration. The corresponding pressures at the airway opening are related to the respective volumes above FRC at all time points of occlusions. The slope of the resulting pressure–volume relationship equals the respiratory system compliance.

The interrupter technique simultaneously measures the pressure and flow at the airway opening. Rapid and brief airway occlusion leads to an instantaneous change in pressure. The ratio of the pressure change and the flow directly before the occlusion renders the value of $R_{rs}$.

An airway occlusion at the end of tidal inspiration and the measurement of the pressure at the airway opening generated by the child during the subsequent tidal inspiratory effort may provide information on the respiratory drive. If the occlusion lasts 100 ms (0.1 s) then the pressure fall during this interval is called $P_{0.1}$ and it is the measure of the child’s respiratory neural output. The respiratory muscle strength can be assessed using the maximum static inspiratory pressure ($P_{imax}$) an infant or a child develops during a longer period of airway occlusion.

3.4.2. Examination procedure. The infant is in a supine position, the head is stabilized in a special head ring, and the neck is gently extended. The face mask encloses nose and mouth (Child et al. 2001). Older children are examined in a sitting posture using a mouthpiece. The occlusion must be airtight, quick and preferably quiet.

All techniques are used during regular tidal breathing at a stable end-expiratory level. (They have been applied in awake or sedated infants and children.) If measurements are repeated, which is recommended to obtain a greater number of reliable data, enough time should pass between them to allow the end-expiratory level to stabilize. The airway occlusions are performed at different phases of the respiratory cycle depending on the technique. The duration of the occlusions varies among the techniques. In SOT, an occlusion of at least 400 ms is started at the end of tidal inspiration. The occlusion is released if a pressure plateau at the airway opening of 100 ms duration is detected. In MOT, the requirements are similar to for SOT; ideally, the occlusions should be performed at different time points of expiration (Gappa et al. 2001). The $R_{rs}$ measurement using the interrupter technique requires brief occlusions of less than 100 ms (Merkus et al. 2005, Wojsyk-Banaszak and Breborowicz 2010). $P_{0.1}$ is determined after an occlusion at the FRC level. Occlusion at this lung volume level or at RV is also applied to measure $P_{imax}$. In this case, the child is asked to perform a maximum inspiratory effort against the occlusion and to maintain it for at least 1 s (Fauroux and Aubertin 2007, Keochkerian et al. 2005).
3.4.3. Clinical findings and interpretation. Determination of respiratory system mechanics by SOT, MOT and the interrupter technique is useful in the monitoring of disease and its progression (van der Gugten et al. 2013). SOT has been used to evaluate the bronchodilator response (Brundage et al. 1990) and airway responsiveness (Malmberg et al. 2011). The interrupter technique was successfully applied to assess the bronchodilator effect in healthy children (Beydon et al. 2012, Merkus et al. 2010) as well as in children with cystic fibrosis, bronchopulmonary dysplasia, and recurrent wheezing (Rosenfeld et al. 2013a). The respiratory timing and breathing strategy were determined in children with cystic fibrosis using $P_{0.1}$ and $P_{\text{imax}}$ (Keochkerian et al. 2005).

3.4.4. Advantages and limitations. All occlusion techniques are fairly simple. They are usually portable, non-invasive and feasible (Katier et al. 2005). The methods and their application are partly not well standardized and they are not routinely used in a clinical setting. However, recent progress associated with the establishment of reference values of respiratory system mechanics in infants and children is promising (Koopman et al. 2011, Merkus et al. 2002, 2010, Nguyen et al. 2013). Except for the measurement of respiratory muscle strength, where the child has to perform a voluntary forced maximum inspiration that is effort-dependent, all other examinations are performed during regular tidal breathing and require no effort. Sniff nasal inspiratory pressure was suggested as an alternative to the $P_{\text{imax}}$ measurement (Anderson et al. 2012).

The prerequisite for successful examination using all occlusion techniques is that the respiratory pattern of the child is stable and there exists no air leak compromising the airway pressure and flow measurements. It is essential that muscle relaxation is present during the assessment of respiratory system mechanics. The equilibration of the alveolar pressure with the measured pressure at the mouth must be rapid. This may be problematic in children with severe airway obstruction or in infants, where the largest share of Raw is in the upper respiratory tract. A reliable determination of the expiratory time constant under passive conditions requires that the lung behaves as a single compartment.

3.5. Multiple-breath helium dilution

The multiple-breath He dilution technique is a relatively simple method for the determination of lung volumes, mostly FRC (Castile et al. 2004, Tantucci et al. 2011, Tierney and Nadel 1962). It is frequently applied during the measurement of the diffusing capacity of the lungs (see 3.10.)

3.5.1. Measuring principle. The method is based on the washin of the inert gas He into the lungs in a closed circuit. A gas container of a known volume is filled with a He mixture and the initial concentration of He is measured. Then the examined child is connected with the container and rebreathes the gas mixture during multiple breaths whereby the He concentration falls until equilibrium is reached. The measured concentrations of He before and after equilibration and the known volume of the container allow the calculation of the lung volume at the beginning of the examination, i.e. at the point when the child was connected with the He gas mixture (Meneely and Kaltreider 1949).

3.5.2. Examination procedure. The child is connected to the measuring system via a mouthpiece and instructed to perform tidal breathing until a stable breathing pattern is achieved. Then, at the end of normal expiration, a special valve opens and the child is connected with
the He mixture and the washin begins. The child continues to breathe quietly and the He concentration equilibrates between the gas container and the lungs. During this period CO₂ is removed by a lime absorber and the consumed oxygen (O₂) is supplied continuously. The equilibration process lasts 2–10 min and requires a different number of breaths in individual patients, depending on lung disease. The He washin is terminated when the continuously measured He concentration remains constant as indicated by a plateau of the multiple-breath washin curve. The results must be converted to BTPS conditions (Wanger et al. 2005). (If the rebreathing procedure starts after full and not after tidal expiration, RV can be determined instead of FRC.)

3.5.3. Clinical findings and interpretation. Differences between the FRC values found by the He dilution method and whole body plethysmography may imply ventilation distribution disorders. The difference between the two values equals the trapped gas not participating in ventilation which may exist in the lungs with an obstructive disorder (Cutrera and Helms 1988, O’Donnell et al. 2010).

3.5.4. Advantages and limitations. The multiple-breath He dilution method can be easily executed. The child has to perform just tidal breathing. The equipment is relatively simple and inexpensive. The measured volume represents only the ventilated part of the total lung volume, trapped gas cannot be detected.

3.6. Multiple-breath inert gas washout

The multiple-breath inert gas washout (or dilution) method allows the assessment of global inhomogeneity of ventilation and the determination of FRC (Gustafsson et al. 1994, Hulskamp et al. 2006, Pillow et al. 2006). The focus of the examination is the detection of ventilation disorders in small peripheral airways which are not identified or are underestimated by spirometry or whole body plethysmography (Aurora et al. 2004a, 2005, Gustafsson et al. 2003, 2008, Kraemer et al. 2005). Detection of early pulmonary disease in children without or with minimum clinical symptoms is another field where multiple-breath inert gas washout is used.

3.6.1. Measuring principle. The typical inert tracer gases used during the multiple-breath washout are He and sulphur hexafluoride (Aurora et al. 2004a, 2005, Fuchs et al. 2006, Gustafsson et al. 2003, Latzin et al. 2008, Ostlund et al. 1992). An alternative indicator gas is N₂, a naturally resident gas in the air (Jensen et al. 2013, Yammine et al. 2012). The measurement is performed in an open circuit. The necessary equipment consists of a flowmeter, a gas analyser (e.g., respiratory mass spectrometer or ultrasonic sensors), and a computer unit with the software for data acquisition. (The ultrasonic sensors determine the tracer concentrations from the measurement of molecular mass derived from the absolute transit times of ultrasonic pulses.) The examinations are carried out during tidal breathing. The inert gas washout is achieved by the breathing of room air after the previous washin. When N₂ is used, the washout is performed by pure oxygen. During the washout, the airflow and the respective tracer concentration are continuously measured at the airway opening. If the initial and final tracer concentrations are known and the total volume of the expired tracer is calculated from the continuously registered flow and tracer concentration signals then FRC can be determined. The global gas mixing efficiency in the lungs and the degree of ventilation inhomogeneity can
be characterized by different indices that are derived from the normalized plots of end-tidal tracer gas concentrations versus lung turnover (Fuchs and Gappa 2011, Pillow et al 2006).

3.6.2. Examination procedure. The patient sits upright and a nose clip closes the nostrils. All phases of the examination are performed during quiet tidal breathing through a face mask or a mouthpiece. At first, the child breathes room air until a constant respiratory pattern is achieved. Thereafter, the inert gas (He or sulphur hexafluoride) is washed in until the tracer gas concentration achieves a steady state. Finally, the tracer gas supply is interrupted and the washout process begins while airflow and tracer concentration are recorded.


The most frequently used index is the LCI which is particularly sensitive in detecting global ventilation inhomogeneity. LCI is the number of lung turnovers necessary to reduce the concentration of the washed-out inert gas to 1/40 of the concentration at the beginning of the washout process. The lung turnover is defined as the ratio between the cumulative expired gas volume and FRC. A high value of LCI (>7.0) implies disturbed ventilation distribution (Aurora et al 2005, Becklake 1952, Gustafsson et al 2003, Horsley 2009).

Many studies have shown pathologically increased LCI in patients with still normal FEV₁ (Aurora et al 2004a, 2005, Gustafsson et al 2003). This finding is typical in the early stages of lung disease initially affecting the small airways (Tiddens 2002).

The assessment of FRC using multiple-breath inert gas washout allows the determination of trapped gas in obstructive diseases when compared with FRC determined by whole body plethysmography.

3.6.4. Advantages and limitations. The advantage of the multiple-breath inert gas washout method is its feasibility. The examination can be performed at any age (Aurora et al 2005, Gustafsson et al 2003) without active effort of the patient. Forced ventilation manoeuvres are not required. LCI can detect global ventilation inhomogeneity in the very early stages of a lung disease which is not possible in conventional lung function testing, like spirometry (Stocks et al 2012).

Multiple-breath inert gas washout is capable of detecting functional changes resulting from structural alterations of the lung tissue. Therefore, it might be regarded as an alternative method to high resolution computed tomography because it does not expose children to radiation (Gustafsson et al 2008). Another potential alternative technique is EIT (see section 3.11).

3.7. Forced oscillation technique and impulse oscillometry

The FOT and impulse oscillometry determine the mechanics of the respiratory system by measuring its response to an external excitation. The physiological basis for the measurement of oscillation mechanics of the respiratory system originates from DuBois (DuBois et al 1956).

3.7.1. Measuring principle. The excitation of the respiratory system is usually executed by means of external pressure signals applied at the airway opening. These pressure signals contain
one or more oscillation frequencies and are applied either continuously or as discrete pulses. The simultaneous measurement of the resulting flow allows the determination of mechanical respiratory system impedance and its components, the $R_{rs}$ and the $X_{rs}$. Multi-frequency excitation allows more detailed characterization of the respiratory system mechanics than the single frequency technique by generating frequency spectra of $R_{rs}$ and $X_{rs}$. The continuous (periodic or pseudorandom) and impulse oscillations are generated by a loudspeaker or a pulse generator and are superimposed onto normal tidal breathing through a mouthpiece (Beydon *et al* 2007, Nussbaum and Galant 1984, Oostveen *et al* 2003, Smith *et al* 2005). The excitation frequencies usually range from about 4–35 Hz. At lower oscillation frequencies, $R_{rs}$ mainly reflects the tissue resistance, whereas at higher frequencies, $X_{rs}$ dominates. In the case of $X_{rs}$, elastic properties of the respiratory system are mainly characterized at lower and inertial properties at higher frequencies.

### 3.7.2. Examination procedure

The child sits upright and performs quiet tidal breathing through a mouthpiece whilst wearing a nose clip. The cheeks need to be supported, usually by another person, to minimize the possible upper airway shunt. The duration of the examination is short: it lasts approximately 15–30 s in the case of impulse oscillometry.

### 3.7.3. Clinical findings and interpretation

FOT and impulse oscillometry are mostly used in the diagnostics of obstructive lung diseases both in the acute and chronic stages, especially in asthma (Beydon *et al* 2007) or in neonatal chronic lung disease (Udomittipong *et al* 2008). Healthy children may not always be distinguished from the diseased ones, as shown in children with wheezing or asthma (Harrison *et al* 2010, Hellinckx *et al* 1998). However, the recent study by Oostveen *et al* (2010) was able to find significant differences between preschool healthy children and children with a wheezing history using FOT, and to determine the bronchodilator response.

The results are presented in the form of diagrams of $R_{rs}$ and $X_{rs}$, plotted as functions of the oscillation frequency (figure 3). The $X_{rs}$ plots allow the determination of the resonant frequency of the respiratory system. If $R_{rs}$ and $X_{rs}$ are within their normal ranges with little frequency-dependency then this implies normal lung function. Obstruction of the central airways is present when the $R_{rs}$ spectrum shows higher values than the pathological threshold with an almost linear and frequency-independent course (i.e. parallel with the predicted one) and $X_{rs}$ is within the normal range. In peripheral airway obstruction, both $R_{rs}$ and $X_{rs}$ show pathological values ($R_{rs}$ values are higher and $X_{rs}$ lower than the predicted ones). The course of $R_{rs}$ is clearly frequency-dependent: $R_{rs}$ values are high at low frequencies and decrease with increasing frequency). The resonant frequency is high.

### 3.7.4. Advantages and limitations

FOT and impulse oscillometry are non-invasive methods that can be used at any age. The equipment is often portable. The patient must only perform quiet tidal breathing; no specific ventilation manoeuvres are required. Only in the case of single frequency oscillations are the measurements executed during apnoea (Sly *et al* 1996). The results are effort independent. A limitation is the potential influence of the upper airway compliance on the determined respiratory system mechanics.

FOT and impulse oscillometry can be regarded as complementary methods to spirometry. The determined $R_{rs}$ and $X_{rs}$ values can be applied for severity classification of airway obstruction (Frei *et al* 2005). They can distinguish healthy children from children with asthma and assess the effect of bronchodilation (Marotta *et al* 2003). In restrictive lung diseases, spirometry is needed for the additional determination of VC.
3.8. Exhaled nitric oxide fraction measurement

The expired air contains several biological substances that may serve as markers of airway inflammation. The most studied substance is exhaled NO, particularly in asthma (Buchvald et al. 2005, Jones et al. 2001, Kharitonov et al. 1994, Kharitonov and Barnes 2000). However, NO is not a marker of any specific disease (Chatkin et al. 1999, Kharitonov and Barnes 2000). NO originates from both the upper and the lower respiratory tract (Kharitonov et al. 1996).

3.8.1. Measuring principle. NO diffuses into the exhaled gas from the upper and lower airways. There exist two types of measurements by which the NO concentration can be determined in the exhaled air.

In the on-line measurement, exhaled NO is continuously measured (e.g. by the chemoluminescence technique) along with airflow during a single-breath exhalation. The advantage of the single-breath approach is that the fractional NO concentration is determined during all phases of the expiration. The airflow during the exhalation must be constant within a required limit to eliminate possible contamination by the nasal gas with its high NO concentration (Deykin et al. 2002, Pedroletti et al. 2002). The single-breath method is the gold standard for the measurement of the exhaled NO fraction in children (American Thoracic Society and European Respiratory Society 2005). The examination is easy to perform and less invasive than alternative methods like bronchoscopy with lavage or biopsy (Payne et al. 2001, Wilson et al. 2000).

In the off-line measurement, the exhaled VC gas is collected in a gas-impermeable bag which does not react with NO. The exhaled gas sample is analysed later (American Thoracic Society and European Respiratory Society 2005).

In the on-line and off-line measurements of exhaled NO the nasal gas is considered to be a contamination. However, nasal NO can be measured separately and used as a biological marker; however, not of allergic disease but mainly in primary ciliary dyskinesia and cystic
fibrosis where low values are typically found (American Thoracic Society and European Respiratory Society 2005, Konradsen et al 2011).

3.8.2. Examination procedure. The measurement of the exhaled NO concentration is fast when the child is cooperative. Before the measurement, the child has to sit and breathe quietly for about 5 min. A nose clip is not used. The child is asked to take the mouthpiece into their mouth and to inhale NO-free air quickly and deeply to TLC. Afterwards, the child continuously exhales without breath-holding at a constant flow of $50 \text{ ml} \cdot \text{s}^{-1}$ for at least 4 s until a NO concentration plateau of at least 2 s duration is identified. This plateau corresponds to the NO concentration from the lower airways. Visual aids supporting the constant flow expiration are helpful (American Thoracic Society and European Respiratory Society 2005).

The child should exhale against an expiratory resistance during the examination. The pressure at the mouthpiece should be between 5 and 20 cm H$_2$O. This prevents contamination of the gas originating from the lungs by the nasal gas with high NO concentration. Otherwise, the data quality would be compromised (Imada et al 1996, Kimberly et al 1996). The nasal origin of NO is characterized by an initial NO peak during expiration before the later NO plateau sets in. It is recommended that two to three appropriate measurements are performed. The mean NO concentration is calculated afterwards (American Thoracic Society and European Respiratory Society 2005). Young children aged 2–5 years are not able to generate a stable NO concentration plateau, therefore, analysis of exhaled NO is executed during tidal breathing with a steady breathing pattern that needs to be monitored (Buchvald and Bisgaard 2001).

For determining the separate nasal NO concentration, two olives are placed in the nostrils of the child at first. The child inhales through a mouthpiece to TLC and then exhales against an expiratory resistance in order to close the velum (American Thoracic Society and European Respiratory Society 2005). At the same time, air is insufflated through one nostril and fed out through the other one where the samples for NO analysis are taken.

3.8.3. Clinical findings and interpretation. The determination of the NO concentration is relevant in pulmonary inflammatory diseases, especially in asthma. The exhaled NO is very sensitive to steroid treatment in asthma providing a dose-dependent response (Jatakanon et al 1998, 1999, Lim et al 1999). It can be used as a monitoring tool for asthma because the exhaled NO responds very quickly to fluctuations in the disease progression (Kharitonov et al 1996). NO concentrations depend on the type of asthma (Gratziou et al 1999); however, they seem not to predict the bronchial hyper-responsiveness to provocation by exercise or methacholine well (Ramser et al 2008).

In some diseases, like cystic fibrosis, NO concentrations are low (Dotsch et al 1996, Grasemann et al 1997). A pronounced decrease in nasal NO concentration is found in patients with primary ciliary dyskinesia (Noone et al 2004, Wodehouse et al 2003).

3.8.4. Advantages and limitations. The single-breath method for determination of the exhaled NO fraction is easily reproducible (Kharitonov et al 2003). NO concentrations are influenced by age (Avital et al 2003, Franklin et al 1999), height (Malmberg et al 2006) and race (Kovesi et al 2008). There exist many other factors that affect the exhaled NO, such as medication. Therefore, these potentially confounding effects should be considered before examination and interpretation of the results. The single-breath exhaled NO measurement can be rapidly performed, especially when the child is allowed to repeatedly practice the manoeuvre. Young children often experience difficulties in performing the very long expiration at a constant flow.
During tidal breathing, their breathing pattern is frequently irregular which may affect the findings.

3.9. Exhaled breath condensate measurement

The exhaled gas is fully saturated with water vapour and it contains small volatile and non-volatile particles of different numbers and sizes (Mutlu et al 2001, Papineni and Rosenthal 1997, Yang et al 2007). By cooling the exhaled gas, the water vapour condenses and the markers under examination can be retrieved. The origin of these substances spans from the oropharynx to the alveoli (Mutlu et al 2001). Exhaled breath condensates serve as indicators of oxidative stress and inflammation in the airways (Kazani and Israel 2012, Kharitonov and Barnes 2002).

3.9.1. Measuring principle. The exhaled breath condensate is obtained during tidal breathing. The exhaled gas is transported into a cooled, mostly Teflon-coated, collecting tube of different length and diameter or into a cooled double-wall glass chamber. The vapour condenses or freezes on the cold walls of the collecting system and can be examined after thawing (Horvath et al 2005).

3.9.2. Examination procedure. The patient sits upright and breathes quietly through the mouthpiece with or without using a nose clip. Room air is inhaled and the exhaled gas is directed into a condenser system. A valve prevents rebreathing. The examination procedure lasts about 10 min and results in 1–3 ml of condensate. It is well tolerated by patients (Horvath et al 2005). It is necessary to prevent the contamination of the condensate sample with saliva containing many inflammatory markers. Therefore, a saliva trap is installed between the mouthpiece and the collecting system and the child should swallow the saliva periodically (Horvath et al 1998).

3.9.3. Clinical findings and interpretation. The exhaled breath condensate contains a large variety of markers relevant particularly in obstructive airway diseases (Kazani and Israel 2012). The most studied markers are: hydrogen peroxide, nitrite and nitrate, adenosine, arachidonic acid metabolites, leukotrienes, 8-isoprostane, ammonia, cytokines, and pH (Horvath et al 2005). The concentrations of biomarkers are mostly analysed in asthma (Caballero Balanza et al 2010, Caffarelli et al 2012, Glowacka et al 2013) but also in cystic fibrosis (Newport et al 2009, Robroeks et al 2010) and other respiratory diseases (Goldbart et al 2006, Zihlif et al 2006).

3.9.4. Advantages and limitations. This method is non-invasive and does not require active effort. However, the duration of the examination is a limiting factor because the children tend to cooperate less with time (Horvath et al 2005). Infants are nose breathers, therefore face masks are needed, or collector systems which suck the exhaled air from the nose (Griese et al 2001, Moeller et al 2006).

3.10. Diffusing capacity measurement

The main physiological function of the lungs is the respiratory gas exchange. The effectiveness of the transport of O2 and CO2 through the alveolar–capillary membrane can be examined with the determination of the diffusing capacity for CO, abbreviated as $D_{L,CO}$. An alternative term for
$D_{L,CO}$ is the transfer factor of the lungs for CO which better reflects that not only the diffusion process is involved in the uptake of CO (Macintyre et al. 2005). However, for historical reasons the term ‘diffusing capacity’ is still widely used.

3.10.1. Measuring principle. Diffusing capacity is defined as the gas quantity exchanged between the alveoli and the capillary blood in a particular time period divided by the difference of the gas partial pressures across the alveolar–capillary membrane. It is directly proportional to the membrane surface and inversely proportional to its thickness.

Appropriate gases for this examination are O$_2$ and CO because of their chemical properties and binding with haemoglobin. CO has an affinity for haemoglobin about 250 times higher than O$_2$ (Reeves and Park 1992). Its concentration in the inspired air is extremely low, it is soluble in blood and its concentration in venous blood is negligible. For these reasons, CO is the most popular gas in this application in the clinical setting. Several types of gas analyzers (gas chromatograph, mass spectrometer, infrared analyzer) are used to measure CO concentration. The determination of $D_{L,CO}$ is usually performed using a single-breath test and requires the knowledge of the fractional CO concentrations in the inhaled test gas mixture and in the alveolar gas after a breath-hold, the breath-hold time, barometric pressure and alveolar volume. Alveolar volume is determined by the inert gas (mostly He) dilution method. The diffusing capacity for O$_2$ can be predicted from $D_{L,CO}$ (Hsia et al. 2008).

3.10.2. Examination procedure. In school children, the determination of diffusing capacity is based on the single-breath method. The examination should take place only when the child has not been physically active for about 15 min. The child sits upright and performs quiet tidal breathing through a mouthpiece. The nose is closed by a nose clip. When regular breathing has been achieved, the patient is instructed to exhale deeply to RV. Then a switch to the test gas container is performed and the patient rapidly inhales the test gas mixture up to TLC. The gas mixture consists of O$_2$, N$_2$, CO (approximately 0.3%) and an inert gas, usually He (5%–9%). Then the child has to hold their breath for about 10 s.

During this time, the gas mixture spreads in the lungs, and CO diffuses through the alveolar–capillary membrane into the erythrocytes and binds with haemoglobin. The patient then exhales slowly and evenly until RV is reached. The first part of this exhaled gas is discarded because it corresponds to the instrumental and anatomical dead space. The rest of the gas is collected in a special bag and examined for the CO concentration. $D_{L,CO}$ is usually reported as the mean value of two to three adequate measurements. The time interval between repeated examinations should be at least 4 min (Blakemore et al. 1957, Lindemann and Leupold 2003, Macintyre et al. 2005, Nicolai and Griese 2011, American Thoracic Society 1995a).

The multiple-breath method is favoured in young children because they are not able to hold their breath for 10 s nor able to perform the deep exhalation and inhalation properly. The child quietly breathes the gas mixture, consisting of room air, CO and He, in a closed system (0.3% CO, 5% He) for some minutes. CO$_2$ must be absorbed and O$_2$ supplied continuously. FRC is determined by the He dilution method, and the diffusing capacity by measuring the decay in CO concentration in a closed system (Lindemann and Leupold 2003, Nicolai and Griese 2011, Stam et al. 1998).

3.10.3. Clinical findings and interpretation. The determination of diffusing capacity in clinical practice is performed in children with expected disorders of respiratory gas exchange where low values of $D_{L,CO}$ are encountered as in patients with interstitial lung disease (Buchvald et al. 2011, Fathi et al. 2012, Filosa et al. 2001, Petermann 1985, Stam et al. 1996). In these patients,
the severity of the impaired gas transport through the blood–gas barrier and disease monitoring can be established using $DL_{CO}$. Monitoring of other diseases or therapy, e.g. chemotherapy can also be based on $DL_{CO}$ (Leiskau et al 2012).

The $DL_{CO}$ values need to be cautiously interpreted, for instance when pulmonary haemorrhage (Greening and Hughes 1981), polycythaemia (Greening et al 1982), asthma and obesity (Saydain et al 2004) are present. Reference values are available (Koopman et al 2011).

3.10.4. Advantages and limitations. Determination of $DL_{CO}$ is a relatively simple method to determine whether the passage of respiratory gases through the blood–gas barrier is impaired. In contrast to the measurement of the diffusing capacity of O$_2$ which requires a blood sample to establish the partial pressure of O$_2$ in the venous blood, the measurement of $DL_{CO}$ is non-invasive as the partial pressure of CO in the blood entering the lungs is negligible. However, the method is not applicable in children who are not able to perform the single-breath manoeuvre. The multiple-breath technique is not an ideal alternative to the single-breath method because of its long duration.

Several physiological and pathological factors may impact the measured $DL_{CO}$. Insufficient effort or thoracic deformities may limit the inhalation of the test gas mixture and reduce $DL_{CO}$. Changed binding properties of haemoglobin, anaemia or emboli may have a similar effect. Increased $DL_{CO}$ values may occur after exercise, in supine posture or in children with asthma, obesity, or left-to-right shunt. All conditions and diseases affecting $DL_{CO}$ are listed, e.g. in MacIntyre et al (2005).

3.11. Electrical impedance tomography

EIT is a functional imaging modality providing information on the distribution of ventilation in the chest cross-section. The method is fully non-invasive. EIT examinations do not require use of radiation and they are suitable for use in infants and children. EIT is not routinely used in a clinical setting yet, however, several clinical studies have utilized this technique and demonstrated its ability to identify physiological and pathological changes in regional lung ventilation depending on posture, age, lung disease or type of ventilation in neonatal and paediatric populations (Armstrong et al 2011, Frerichs et al 2001, 2003, Heinrich et al 2006, Miedema et al 2013, Pham et al 2011, Wolf et al 2007, Zhao et al 2012). Additional evidence is provided by experimental studies (Bhatia et al 2012, Gomez-Laberge et al 2013, Wolf et al 2013). The heterogeneity of lung function can be assessed by EIT even during dynamic conditions because of high scan rates of up to about 40 images per second. In contrast to the inert gas washout technique that can determine global ventilation heterogeneity, EIT is able to detect it on the regional level (Pillow et al 2006). EIT is potentially able to also examine regional pulmonary perfusion as shown in a few studies (Borges et al 2012, Carlisle et al 2010, Frerichs et al 2002).

3.11.1. Measuring principle. EIT measures electrical impedance of biological tissues (i.e., electrical bioimpedance). An array of electrodes needs to be placed around the chest in one transverse plane. Most EIT devices require 16 electrodes. Very small alternating electrical currents are repetitively applied to the body through these electrodes in a rotating pattern. The resulting voltages are measured at the same electrodes and used to calculate the distribution of electrical impedance within the studied chest slice. One complete rotation of current applications and voltage measurements renders one EIT image.
Electrical impedance of the lung tissue depends on its air content, thus, EIT images acquired during ventilation reflect regional ventilation-related changes in lung volume. Perfusion-related changes can also be traced although the amplitude of these impedance changes is much lower. EIT images typically show not absolute but relative (i.e. normalized) changes in impedance.

3.11.2. Examination procedure. Before the examination, an electrode belt or individual electrodes have to be attached on the chest circumference, preferably in the 4–6 intercostal space. One reference (ground) electrode is placed on the abdomen.

EIT scanning can be performed without any cooperation or effort from the infant or child during any type of ventilation and at any age. During tidal breathing, only a few breaths are needed to examine the tidal ventilation distribution. EIT scanning can also be carried out during ventilation manoeuvres like those used in conventional spirometry (e.g., during forced full inspiration and expiration). In this case, the child should be able to follow the instructions of the medical personnel. EIT examination can be carried out alone or in parallel with spirometry, RIP or other methods.

The measurement lasts a few seconds to minutes, depending on the requirements. Continued long-term observations or monitoring of regional lung function are also possible. The children tolerate the electrodes and the required measurement duration well.

3.11.3. Clinical findings and interpretation. Conventional lung function testing typically measures global lung function parameters at the airway opening. EIT is able to assess lung function regionally. Impedance waveforms reflecting the instantaneous changes in regional lung volumes can be acquired in several hundreds of individual image pixels. These waveforms allow the calculation of multiple measures characterizing regional pulmonary function. Such measures can be the well-known conventional parameters used in global lung function testing, such as FEV₁ or FVC. Figure 4 shows a functional EIT scan obtained in a child with asthma showing the regional distribution of FVC. Three selected waveforms highlight the differences in regional lung emptying during forced full expiration. EIT has also been used to determine regional respiratory system compliance (Dargaville et al 2010, Wolf et al 2013, Zick et al 2013).

Additionally, there exist some new EIT specific parameters that can characterize the degree of ventilation inhomogeneity, for instance in obstructive pulmonary disorders (Vogt et al 2012a, 2012b, Zhao et al 2012). The capacity of EIT to assess regional ventilation heterogeneity and respiratory system mechanics may potentially be used to determine the effects of inhalation therapy or physical therapy on regional lung function.

3.11.4. Advantages and limitations. EIT is a non-invasive radiation-free method. It is portable and relatively cheap compared with other imaging modalities. EIT allows short-term or long-term continuous examinations of regional lung function. It has a high temporal resolution, therefore, even dynamic changes of the air content in the lungs can be determined in the examined chest plane.

Regional detection of ventilation-related changes in electrical impedance allows gross location of disorders in spatially heterogeneous pulmonary disease. The limited spatial resolution of EIT precludes high-resolution assessment of lung structure as in computed tomography or magnetic resonance imaging. However, the primary goal of EIT use is the functional and not the anatomical imaging. The ongoing technological development of EIT and the increasing number of clinical studies may help to establish this method for pulmonary
Figure 4. Electrical impedance tomography (EIT) examination of a 12 year old boy with asthma during a forced full expiration manoeuvre. A 16-electrode EIT system GoeMF II (CareFusion, Höchberg, Germany) was used at a scan rate of 33 images s$^{-1}$. Functional EIT image (left) shows the distribution of regional FVC in the chest cross-section. (1) Three image pixels in the anterior, (2) medium and (3) posterior region of the left lung out of the total of 912 pixels are highlighted and the corresponding EIT waveforms are presented in the right-hand panel. The three curves of relative impedance change (rel. ΔZ) are normalized to better visualize the spatial dissimilarity of regional lung emptying among the selected image pixels.

function testing in paediatric patients. EIT has already improved our understanding of how ventilation is distributed in the lungs of healthy children (Heinrich et al 2006, Lupton-Smith et al 2014) where other methods using radiation could not be applied for ethical reasons. The routine clinical use of this technology would require standardized approaches to data acquisition and analysis, unified terminology and data interpretation schemes and meaningful combined use of EIT with other examination techniques.

The perspectives of EIT use in neonates, infants and children have been summarized in a few review articles (Pillow et al 2006, Riedel and Frerichs 2010, Frerichs et al 2008).

4. Summary

This review presents an overview of methods used for the testing of lung function in infants and children. Some of these methods, like spirometry and whole body plethysmography, are routinely used in a clinical setting and may be considered the gold standards in pulmonary function testing. Other methods like impulse oscillometry, the interrupter technique or multiple-breath washout are on their way to establish themselves in everyday clinical use because they provide useful information that is either not provided by the conventional methods or with lower sensitivity. Some of these methods benefit from the increasing availability of commercial devices. Still, in spite of the relative ease of their application, well-trained staff are needed to conduct and interpret the examinations correctly. Finally, some of the presented methods like EIT or respiratory muscle drive measurement based on $P_{0.1}$ are used only in a few centres, primarily in clinical research. These methods seem to generate valuable information in infants and children with lung disease. However, their ability to impact the clinical decision-making, the management of the patients’ treatment and the outcome will have to be established to promote their routine clinical use. The decisive factor will also be the availability of commercial equipment.
The progress in pulmonary function testing in infants and children is evident and ongoing. Standardization of examination procedures, equipment and analysis, and the availability of accurate reference values have been accomplished for some but not yet for all techniques reviewed in this review. The ERS and the ATS have promoted this process by funding expert task forces and the publication of several consensus articles. One of the major future tasks in this field will be to provide the evidence that the methods outlined here, for the testing of lung function in infants and children, enable staging and phenotyping of respiratory diseases and improve the therapy and outcome.

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