Early Detection of Lung Disease in Children with Cystic Fibrosis Using Lung Function

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Abstract

Measurement of lung function is routine in older children and adults with cystic fibrosis (CF) but not in infants and pre-school children. Pulmonary infection, neutrophil-dominated inflammation and clinical exacerbations in young children similar to that seen in older subjects have been identified and highlight the urgent need to evaluate lung function in early life. Mounting evidence suggests lung function techniques sensitive to changes in peripheral lung function may be required to detect the early functional abnormalities in infants and preschool children with CF. In addition, the majority of studies in young children with CF to date have not reported longitudinal data and therefore the prognostic potential of existing lung function methods to track disease progression is poorly understood. This review aims to describe recent research findings in infants and pre-school children, outlines currently available lung function techniques, issues around their standardisation and their relative advantages and disadvantages in young children with CF.
Background (Heading Level A)

Measurement of lung function is routine in older children and adults with cystic fibrosis (CF) but not in infants and pre-school children who are too young to do spirometry. Neutrophil-dominated inflammation similar to that seen in older subjects has been identified in the lungs of affected infants [1-3] indicating that pulmonary disease starts in early infancy and highlights the need to evaluate lung function at a younger age than currently routine. Infant lung function testing is technically difficult to perform, labour intensive, time-consuming and requires sedation. Pre-schoolers, in contrast, do not generally need to be sedated but are often strong-willed, moody, playful and uncooperative so that lung function testing at this age needs to incorporate special techniques that require minimal cooperation only and laboratory scientists with suitable communication skills.

Techniques to measure lung function in infants with CF have developed over the past four decades [4] and such testing is now on the cusp of entering the realm of clinical practice. This review aims to describe recent research findings in infants and pre-school children, outlines currently available lung function techniques, issues around their standardisation and their relative advantages and disadvantages in young children with CF.

Recent studies in infants (B)

Currently, it is unclear whether lung function is diminished in pre-symptomatic infants with CF diagnosed by newborn screening. The best available evidence has been gathered in those diagnosed clinically. Several independent research groups employing differing lung function techniques have demonstrated lower lung function in infants with CF and significant associations with inflammation and/or infection [1, 3, 5, 6]. Importantly, reduced lung function has also been reported in infants without clinical evidence of prior lower respiratory
illness [7]. Historically, comparisons of lung function in infants with CF to healthy infants have faced difficulties in accurately defining matched controls, with length being the most commonly used matching method (a necessity as length is the strongest predictor of forced expired volumes in healthy children), at the cost of poor matching in terms of age because of significantly poorer growth in those diagnosed with CF following a clinical presentation. This difficulty is likely to be less significant in future studies of children diagnosed following detection by newborn screening who are relatively well-nourished so that both length and age can be matched. This is important as there may be unrecognized contributions to lung growth by somatic growth especially during the first few months of life.

The clinical significance of these early findings depend on: how repeatable they are; whether or not they persist or ‘track’ to later years; whether early functional abnormalities predict subsequent rate of decline of conventional spirometry values in later life; how they relate to subsequent clinical progress such as frequency of exacerbations and quality of life; how they reflect the underlying pathophysiology in terms of pulmonary infection, inflammation and changes occurring in lung structure.

Recent evidence suggest that findings in infancy do indeed ‘track’ so that an individual’s relative lung function ranking within a group persists until school-age [8] but further data are awaited as to how this relates to clinical progress. There are few studies that have assessed lung structure and function at this age but the study of Martinez et al [9] provides initial evidence that diminished forced expiration in infants is related to altered lung structure as a reduction in FEV$_{0.5}$ was significantly inversely associated with airways identified by computerised tomography to have relatively thickened airway walls. Thus, bronchial wall thickening was associated with diminished lung function. Conflicting evidence on the effects
of pulmonary inflammation and/or infection on lung function exist with little of the variability in lung function explained by either inflammation or infection [1, 5, 6, 10]. It is likely that studies of infants with CF diagnosed by newborn screening will need to employ techniques sensitive to changes in either the very peripheral airways or even the lung parenchyma.

Importantly, the increasingly widespread use of newborn screening offers the opportunity for multi-centre longitudinal studies in infants with CF in which standardised lung function techniques matched with assessments of pulmonary inflammation and lung structure can be applied to allow outcome measures for prediction of clinically relevant disease in the preschool age range to be developed.

**Recent studies in pre-school children (B)**

The years between two and six provide several challenges to those wishing to measure lung function as reflected by the absence of standardised guidelines lung function measurements in this age group until 2007 [11]. In many centres preschool children with CF do not undergo lung function until they are able to perform reproducible forced expiratory manoeuvres but many children become infected with *Pseudomonas aeruginosa* before this is feasible [12]. As infected children are subjected to more aggressive treatment interventions the ability to monitor progress objectively assumes increasing importance. A few studies in this age-group using tidal breathing techniques and even spirometry have been published recently.

The resistance interrupter technique (R_{int}) has potential in the ambulatory setting in pre-school children [13] but has been assessed only recently in CF with conflicting evidence of its discriminatory power [14, 15]. Gangell et al [16] demonstrated that the forced oscillation technique (FOT) is also feasible in the ambulatory setting in children with CF and that
measurements of resistance ($R_{ns}$) and reactance ($X_{ns}$) were more abnormal in the presence of respiratory symptoms. Studies by Neilsen et al. [15] demonstrated that specific airway resistance measured by plethysmography ($sR_{aw}$) was significantly increased in young children with CF. While a number of studies have reported diminished forced expiratory volumes and flows [8, 17, 18] the level of abnormality is smaller than that reported in infancy and could either suggest that airway function improves through the pre-school years or alternatively that incentive spirometry is less able to identify diminished airway function in pre-school subjects than the raised volume technique in infants. It is likely that preschoolers may not be able to take full inspirations resulting in less reliable data. The recent application of multiple breath washout (MBW) methods to assess ventilation inhomogeneities in this age group has demonstrated the technique is feasible in young children. Aurora and colleagues reported up to three quarters of a cohort of children with CF had increased ventilation inhomogeneities compared with only 13% of the same children identified as having diminished forced expiratory variables [19]

Considered as a whole these studies suggest that techniques insensitive to changes in peripheral lung function may not be adequate for the detection of early functional abnormalities in infants and preschool children with CF. To date the majority of studies in young children with CF have reported cross sectional data only and thus the prognostic capability of existing lung function methods to track disease progression is not well understood. Further longitudinal studies of lung function in preschool children with CF following infant cohorts diagnosed by newborn screening are needed urgently so that the progress of early lung disease can be monitored, and more importantly, its response to therapeutic interventions can be assessed acutely.
Techniques for measuring lung function in infants and young children with Cystic Fibrosis (A)

Numerous techniques are available for the assessment of respiratory function in infants and preschool children. The application of these techniques has been limited by inconsistent methodologies and poor access to commercially available equipment, restricting research to a small numbers of specialised research centres. The introduction of standardisation guidelines for lung function techniques by the joint ATS/ERS Infant and Preschool Lung Function Testing Task Force has greatly advanced the consistent application of lung function methods in these age groups. Coupled with the improved availability of commercially available equipment, an increasing number of centres are applying objective measures of lung function in this young age group, with some clinics introducing these into routine clinical practice.

Techniques for measuring lung function in infants with Cystic Fibrosis (B)

Measurements of Forced expiration (C)

Forced expiration is performed from tidal breathing or from raised lung volumes. Practice guidelines [20], commercial equipment and reference data [21, 22] are available for either technique. While the tidal technique is easier to perform the measured variable (maximal flow at functional residual capacity) depends on a variable volume landmark and therefore has decreased sensitivity compared with forced expiratory volumes and flows from the raised volume technique [23]. Although z-scores for FVC and forced expiratory flows tracked over a six month period [24], the longer term repeatability of parameters measured by forced expiration is unknown as acquiring such data is limited by the need to sedate the subject. Further work in longitudinal studies is required to determine the role and interpretation of forced expiratory flows and volumes in the clinical setting.
Measurements of Lung Volumes (C)

Infant lung volume estimations fall into two broad categories; multiple breath gas dilution and whole body plethysmographic techniques and each has their strengths and weaknesses. FRC by gas dilution measures gas communicating with the mouth and potentially misses trapped gas, whereas plethysmographic FRC (FRC_{pleth}) measures all gas in the body at the time of airway occlusion including any that may be in the gut or “trapped” in the chest. FRC is the most common lung volume measured in infants [25] with standardization guidelines available for lung volume estimation by nitrogen washout using bias flow [26] and plethysmography [25]. FRC is known to increase with increasing height. However in CF, where failure to thrive is common, this becomes problematic as at a given height an infant may be significantly undernourished [27]. Resultant “poor” lung function may simply be due to mismatched reference data. Additionally, healthy reference data, needed to correlate with testing at diagnosis in infants with CF, are difficult to obtain in those < 3 months. There is certainly room for more work in this area particularly now that techniques and equipment have in general become more standardized [28].

Plethysmography (D)

During whole body plethysmography, calculation of the intrathoracic gas volume during occluded breathing efforts against a closed shutter is made using Boyle’s law. However, a number of potential problems arise when applying this to infants, including gas compression within the box and underestimation of alveolar pressure changes at the airway opening, particularly in the presence of obstructive airways disease. The use of plethysmography derived airway resistance in infants has also been questioned [29]. In addition, the end-expiratory level used to define and calculate functional residual capacity is actively determined early in life and may vary considerably over time. The availability of commercial
devices and guidelines [25] is likely to increase the uptake of this technique. As gas-trapping is likely one of the first features of lung disease in infants with CF, the use of plethysmography in combination with the raised volume technique to allow calculation of \( FRC_{\text{pleth}} \), residual volume (RV) and total lung capacity (TLC) may provide a role for this technique in future studies [30]. However, reference data, both cross-sectional and longitudinal, in healthy subjects using the new equipment is still lacking potentially limiting its role in the clinical setting.

**Multiple breath inert gas washout (D)**

Whilst multiple breath gas dilution has been used as a simple technique to apply in infants for almost 30 years now it is only in recent times that standardized methods [26] have been developed. The standards stipulate criteria for equipment design, manufacture and calibration, test technique, variables measured, indications and contra-indications for the technique. These standards improve comparability between centres; vital if multi-centre clinical trials in early CF are to be a reality in the future [31]. A bias flow technique with careful attention to minimizing system dead-space is the most practical to apply and commonly one of two trace gases, nitrogen or SF6, are used in this technique. Closed circuit helium dilution commonly used in the adult world is compromised in infants by a long time constant of equilibration and large system dead-space that, whilst acceptable with adult tidal volume, would elevate FRC in infants. The bias flow technique also negates the need for a re-breathing circuit with \( CO_2 \) scrubbers and reduces issues related to infection control.

The addition of the assessment of ventilation inhomogeneity using MBW methods offer significant insight into peripheral airway disease, describing the efficiency with which the
alveoli are ventilated. Global indices of gas mixing such as lung clearance index (LCI), alveolar mean dilution, and mixing ratio variables can be obtained. Such measurements appear to provide sensitive indicators of early inflammation and infection in these infants [32]. Recent work in older children with CF suggests LCI is significantly related to structural changes [33]. A more detailed assessment of ventilation inhomogeneities includes the analysis of the slope of changing gas concentrations on a breath by breath basis and hence the calculation of the ventilation efficiencies from the conducting and acinar airways[34]. Early work suggests the assessment of acinar ventilation may provide a technique sensitive to alterations in peripheral lung function in children with CF [35], although data in infants are not yet available. MBW methods have the advantage that the technique can be applied in a relatively similar manner across all ages and therefore lends itself to longitudinal studies from early infancy through school aged children and into adulthood. The primary disadvantages currently are the lack of reference data from healthy children and consensus on the most appropriate analysis methods.

**Measurements of respiratory resistance and compliance (C)**

**Interrupter technique (D)**

\( R_{\text{int}} \) is determined from a brief occlusion of the airway opening during expiration and assumes that during the occlusion airway opening pressure equilibrates with alveolar pressure. \( R_{\text{int}} \) is calculated as the ratio of airway opening flow immediately prior to occlusion and the post-occlusion airway opening pressure [36]. \( R_{\text{int}} \) is possible in infants [37] compromised by a lack of standard technique and equipment design. Whilst it has good short term repeatability in pre-school children [11] this work has yet to be repeated in infants. The technique has the advantage in that it can be easily applied in infants; however the value of \( R_{\text{int}} \) in the tracking of disease severity in infants with CF is unknown.
**Single Occlusion Technique (D)**

The single occlusion technique uses an airway occlusion of at least 500ms during which a plateau in pressure should be seen and measures flow and volume change after opening to quantify the passive respiratory mechanics, respiratory system resistance ($R_{rs}$) and compliance ($C_{rs}$). Guidelines for the use of occlusion techniques have been published that detail quality control, standardised technique, and data handling and analysis [38]. Katier and co-workers have reported reference data from healthy neonates and young infants [39]. $C_{rs}$ has been demonstrated to be decreased with increasing respiratory pathogen load in infants with CF [5]. No longitudinal data are available using this technique in infants with CF.

**Forced oscillation technique (D)**

Forced oscillation measures input impedance of the respiratory system ($Z_{rs}$) by applying low amplitude pressure oscillations to the airway opening and guidelines for its clinical use in preschoolers [11] and older children and adults [40] are available. The measured $Z_{rs}$ can be separated into $R_{rs}$ and reactance ($X_{rs}$). $R_{rs}$ includes the Newtonian resistance of airway, lung tissue and chest wall while $X_{rs}$ represents the balance of respiratory elastance and inertance at each frequency included in the oscillatory signal. As such the technique has significant potential for the quantification of peripheral lung mechanics in infants with CF. The FOT has been applied in numerous studies in infants [41] but only one study has been published using this technique in infants with CF [1]. The use of low frequencies, encompassing the breathing frequency, allows the simultaneous assessment of airway (airway resistance ($R_{aw}$)) and parenchymal (tissue elastance ($H_{rs}$) and tissue damping ($G_{rs}$)) respiratory system mechanics by modelling[42]. Our group has demonstrated significant relationships between lung function ($R_{aw}$ and $G_{rs}$) and neutrophil number and with IL-8 in the lungs [1]. To date only a
few research laboratories have the necessary equipment and experience to apply the FOT in infants and the development of commercial systems is required to allow broader access to this technique.

**Techniques for measuring lung function in pre-school children (B)**

The most significant limiting factor for determining lung function in young children is the level of active cooperation required from the patient. As such, techniques that only require tidal breathing have consistently higher success rates than tests requiring the child to perform specific respiratory manoeuvres (such as spirometry). Recent efforts by the joint ATS/ERS Infant and Preschool Lung Function Testing Task Force have contributed greatly to the standardisation of the lung function testing in pre-school children with recommendations for methodological testing practices recently released [11]. Readers with an interest in pre-school testing are advised to consult these documents for detailed information on the performance of pre-school lung function tests. The primary advance in pre-school lung function testing has been the availability of commercial equipment. The feasibility of the various techniques available in this age group is shown in table 1.
Table 1: Feasibility of lung function techniques by age group in young children.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td></td>
<td></td>
<td></td>
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<td>✓</td>
<td>✓</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>[13, 49]</td>
</tr>
<tr>
<td>Specific Raw (Plethysmography)</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>—</td>
<td>[19]</td>
</tr>
<tr>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>—</td>
<td>[19]</td>
</tr>
<tr>
<td>Spirometry</td>
<td>×</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>[18, 19, 50, 51]</td>
</tr>
</tbody>
</table>

✓ Feasibility ≥80%;

(✓) Feasibility ≥50% but <80%;

× Feasibility <50%;

— Feasibility not reported.

†One study <50%;‡One study ≥80%; ‡Two studies ≥80%.

**Spirometry (C)**

Spirometry is the most common lung function technique used routinely in the older CF population and plays a key role in clinical decision making [52]. It is critical that age appropriate collection techniques, outcome variables and quality control and clinical
interpretation strategies are used; details of which can be found in the recent consensus guidelines [11].

There are a limited number of studies reporting spirometry performed in preschool children with CF. Marostica et al [17] performed spirometry in 38 children with CF aged 3 to 6 years old, demonstrating significantly reduced mean z-score for forced expiratory variables independent of age tested and pseudomonas status. Vilozni et al [18] reported spirometry in 93 children with CF aged 2.5 to 9 years and demonstrated that the mean z-scores for FVC, FEV$_1$, FEV$_{0.5}$ and FEF$_{25-75}$ were diminished compared to healthy children and that FEV$_{0.5}$ and FEF$_{25-75}$ were more sensitive than FEV$_1$ for detecting diminished lung function. Aurora et al [19] obtained spirometry and lung clearance index (LCI) data from 40 children with CF aged 2-6 years reporting reduced mean FEV$_{0.5}$ z-score in the CF cohort (-0.76) compared to controls, however only two out of 30 children had an FEV$_{0.5}$ below the normal range, compared with 22 children who had abnormal LCI. An advantage of using spirometry in young children once they are able to perform reliable forced expiratory manoeuvres is that longitudinal tracking of lung function throughout life is possible. However, as demonstrated by Aurora et al [19], spirometry may not be the most sensitive test available in this young age group. In addition, longitudinal studies will need to account for the differences in physiological meaning of FEV$_1$ at different ages.

**Measurements of Lung Volumes (C)**

**Plethysmography - Specific Airway Resistance (D)**

The use of plethysmography in young children tends to be limited to measurements of specific airway resistance ($sR_{aw}$) due to the difficulties children of this age group have with the panting required for measurements of FRC$_{pleth}$. Children under the age of 7 years with CF
have been reported to have increased \( sR_{aw} \) [15]. The longitudinal study reported by Nielson et al. [15] demonstrated \( sR_{aw} \) to be the only variable measured consistently increased over the 2 year assessment period [15]. Consistent with this, Aurora et al., reported 47% of children had abnormal \( sR_{aw} \) [19]. Infection with \( P. aeruginosa \) did not significantly influence \( sR_{aw} \) in either study. Measurements of \( sR_{aw} \) are related to lung volume; therefore intra-subject volume changes between visits and inter-subject differences will affect results.

**Multiple breath inert gas washout (D)**

Full details of technical aspects of MBW for the assessment of FRC and gas mixing indices can be found in the recent consensus guidelines [11]. Studies investigating MBW measurements in children older than 2 years with CF have focused on indices of ventilation inhomogeneities [53] with the most commonly reported index being the LCI. Aurora et al., [19] reported 73% (22/30) of 2-6 year old children with CF had abnormal LCI. In addition these authors found LCI to be more sensitive to \( P. aeruginosa \) infection than \( sR_{aw} \) or forced expiratory variables. The use of MBW has previously been limited to specialized research centres due to the lack of commercial systems and standardized guidelines. Recent guidelines for the use of MBW in this age group [11] will hopefully encourage broader application of this method. The assessment of ventilation inhomogeneities in young children with CF presents a unique opportunity to obtain an assessment of peripheral lung function with a relatively easily applied test, in particular the assessment of conducting and acinar ventilation distribution [35].

**Measurements of respiratory resistance and compliance (C)**

**Interrupter technique (D)**
Numerous data for the interrupter technique exist in preschool children including reference ranges in healthy children obtained using commercial devices. Details of standardised methods and interpretation have been recently published [11]. In young children with CF significantly increased $R_{\text{int}}$ has been reported by Beydon et al. [14] with 23% of children having abnormal $R_{\text{int}}$. While these authors reported an association between patients with a history of CF-related respiratory symptoms and worse lung function, few children with no history of CF-related respiratory symptom; 8 patients compared to 31 symptomatic patients. In contrast, Nieslen et al., [15] used $R_{\text{int}}$ in a longitudinal study of young children with CF and reported the mean $R_{\text{int}}$ was normal at all visits during the follow-up, except at visit 4 where mean $R_{\text{int}}$ was abnormal with 40% of having a significantly increased Z-score, a shift unable to be explained by the authors. Neither of the studies described above reported increased $R_{\text{int}}$ in children with *P. aeruginosa* infection [14, 15]. As $R_{\text{int}}$ is influenced by overall airway dimensions it may not be sensitive to the peripheral airways, potentially limiting its value in young children with CF. In addition, the influence of the upper airway compliance becomes systematically more important as resistance of the lower airways increases; potentially further limiting the sensitivity of $R_{\text{int}}$ in young children with CF.

**Forced oscillation technique (D)**

Previous studies in CF using FOT have been performed in mainly older children where the primary aims of the studies were not to determine differences from a reference group. In children with a mean age of 14, Lebecque et al., [54] compared $R_{rs}$ in children with CF and reported 32/45 children had $R_{rs}$ outside normal limits. In a more recent study Hellinckx et al., [55] reported normal $R_{rs}$, but abnormal $X_{rs}$ in 20 children aged 6 to 17 years. In younger children Nieslen et al., [15] measured $R_{rs}$ and $X_{rs}$ by using impulse oscillations in 30 children with CF between 2 and 7 years. In this study, $R_{rs}$ was abnormal in the CF group in 2 out of 5
follow-up visits, while $X_{rs}$ was normal for all visits with the exception of the first visit. This
difference may result from a single outlier and thus may not indicate a clinically significant
difference in lung function. Also, over the longitudinal follow-up comparisons to clinically
relevant indicators of lung disease, such as inflammation and infection were not included [15].
In a cross-sectional study of 56 children with CF aged between 2 to 7 years of age Gangell et
al [16] reported increased $R_{rs}$ and $X_{rs}$ compared to healthy children. Interestingly, $X_{rs}$ was also
significantly worse in the presence of mild parentally reported symptoms. Nielsen et al.,
reported lung function using IOS was not lower in the presence of $P. aeruginosa$ infection
confirmed through assessment of nasopharyngeal suctions [15]. In contrast preliminary data
from our group suggests that $R_{rs}$ worsens in the presence of $P. aeruginosa$ infection confirmed
by bronchial alveolar lavage, while both $R_{rs}$ and $X_{rs}$ worsen with increasing IL-8 [56]. Studies
utilising FOT in young children with CF are likely to provide clinically relevant information
from the peripheral lung. It is important to note that FOT data collected using mulit-
component oscillatory signals, such as those used in our studies, are not comparable to those
collected with the IOS device as the latter devices used square wave impulses to oscillate the
respiratory system.

**Summary (A)**
Increasing availability of both consensus documents for infant and preschool lung function
techniques and commercially availability of equipment suitable for testing in these age groups
with rapidly enhance the available information of lung function outcomes in early lung
disease in infants and children with cystic fibrosis. Studies using methods sensitive to
functional changes in the peripheral lung are most likely to allow the early detection of
progressive lung disease and hence clinically relevant outcomes later life. It is critical that
longitudinal, multi-centre trials using standardised protocols are embarked upon and these
should include detailed assessments of pulmonary inflammation and infection as well as lung structure and function to ensure the most appropriate test or combination of tests are identified.


8. Kozlowska, W, P Aurora, S Lum, C Saunders, S Ranganathan, R Castle, and J Stocks. Longitudinal assessment of lung function in infants and pre-school children with cystic fibrosis. *Arch Dis Child*, 2004; 89(suppl 1): A38.

9. Martinez, TM, CJ Llapur, TH Williams, C Coates, R Gunderman, MD Cohen, MS Howenstine, O Saba, HO Coxson, and RS Tepper. High-resolution computed tomography imaging of airway disease in infants with cystic fibrosis. *Am J Respir Crit Care Med*, 2005; 172(9): 1133-8.


