Evaluation of the Forced Oscillation Technique for Clinical Assessment of Young Children with Cystic Fibrosis

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Declaration for thesis containing published work and/or work prepared for publication

This thesis contains **published work and/or work prepared for publication, some of which has been co-authored**. The bibliographic details of the works and where they appear in the thesis are set out below:

Chapter Three

Published Work:

Gangell CL (35%), Horak F (35%), Patterson H (5%), Sly PD (5%), Stick SM (10%), Hall GL (10%) (2007) Respiratory impedance in children with cystic fibrosis using forced oscillations in clinic. Eur Resp J: 30: 892-897.

Thamrin C (40%), **Gangell CL (10%)**, Udomittipong K (5%), Kusel M (5%), Patterson H (5%), Fukushima T (5%), Schultz A (5%), Hall GL (5%), Stick SM (5%), Sly PD (15%) (2007) Assessment of bronchodilator responsiveness in young children using forced oscillations. Thorax: 62; 813-818.

Each author has given permission for work arising from the above publications to be included in this thesis.

Ms Catherine Gangell PhD Candidate	Professor Peter Sly Principal Supervisor

Abstract

Background

Measurements of lung function are routinely used in patients with cystic fibrosis (CF) to provide information that may be clinically relevant. Spirometry is the conventional lung function measurement used, however young children find spirometry difficult to perform and often cannot achieve the strict acceptability criteria for the test. The forced oscillation technique (FOT) is a lung function measurement that only requires tidal breathing and is easy for young children to perform. However, there is limited information about the utility of this technique in the clinical assessment of young children with CF who are unable to perform spirometry.

Aims

The aim of this project was to evaluate the FOT for clinical assessment in 2 to 7 year old children with CF. Specifically this involved:

1. Technical assessment of the FOT in children with CF;
2. Comparisons of lung function using the FOT in children with CF and healthy children;
3. Evaluation of associations with factors known to be associated with lung disease including:
    i) inflammation
    ii) infection and
    iii) structural damage.

Methods

Lung function was measured in a cohort of 59 children between the ages of 2 and 7 years with CF at the time of quarterly clinic visits. Resistance and reactance at 6, 8 and 10Hz (Rrs6, Rrs8, Rrs10, Xrs6, Xrs8, Xrs10, respectively) were reported and expressed as Z scores. Children were classified as asymptomatic or symptomatic based on a respiratory questionnaire and physical examination at the time of testing. Bronchoalveolar lavage and high resolution computed tomography (HRCT) were performed annually under general anaesthesia annually. BAL fluid was assessed for the presence of micro-organisms and quantification of a range of inflammatory markers and HRCT used to determine the extent of structural abnormalities.

Results

The between test repeatability (n=25) for lung function was within limits previously described in healthy children. No systematic bias was observed and repeatability was not affected by the presence of respiratory symptoms. Children with CF (n=57) had significantly increased Rrs6-10 ($p<0.0001$) and decreased Xrs6-10 ($p<0.004$) compared to healthy children. Rrs6 and Xrs6-10 were significantly worse in the presence of respiratory symptoms, and Rrs6-10 progressively worsened from an asymptomatic to a symptomatic clinic visit. Children with CF (n=48) had no greater bronchodilator response (BDR) compared to healthy children. BDR was not influenced by the presence of an infection or respiratory symptoms.

No relationships between inflammatory markers and lung function (n=39) were identified when the presence of an infection was adjusted for. Children with a current infection (n=20) had increased Rrs6-10 ($p<0.01$) and decreased Xrs6-10 ($p<0.04$) compared to children who were uninfected (n=23). These relationships were most marked for children infected with *Pseudomonas aeruginosa,* with children having a reduced lung function between 0.95 and 1.47 of a Z score. No relationships with the presence or absence of mild structural abnormalities (bronchiectasis, bronchial wall thickening and air trapping) and lung function at the time of HRCT were identified (n=34).

Conclusion

The FOT is a repeatable measurement of lung function in children with CF and reliable results can be obtained in children as young as 2 years old. Young children with CF exhibit altered respiratory function which was affected by the presence of factors known to be associated with lung disease. The FOT has the potential to provide useful information about changes in clinical status in young children with CF and may be used to direct management of patient lung disease.

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Publications arising from this thesis

1. **Gangell CL**, Horak F, Patterson HJ, Sly PD, Stick SM, Hall GL. (2007) Respiratory impedance in children with cystic fibrosis using forced oscillations in clinic. Eur Respir J: 30: 892-897.

2. Thamrin C, **Gangell CL**, Udomittipong K, Kusel MMH, Patteron HJ, Fukushima T, Schultz A, Hall GL, Stick SM, Sly PD (2007) Assessment of bronchodilator responsiveness in preschool children using forced oscillations. Thorax; 62:813-818.

Published abstracts arising from this thesis

1. **Gangell CL**, Hall GL, Stick SM, Brennan S, Sly PD (2008) Lung function using forced oscillations is worse in young children with cystic fibrosis infected with *Pseudomonas aeruginosa*. Respirology. 13 (Suppl. 2): A56.

2. **Gangell CL**, Murray C, Hall GL, Sly PD, Stick SM (2007) Forced oscillations do not directly correlate with HRCT in preschoolers with cystic fibrosis. Respirology. 12 (Suppl. 4): A236.

3. **Gangell CL,** Hall GL, Stick SM, Brennan S, Sly PD (2007) Forced oscillation technique reflects lung disease in young children with cystic fibrosis. Eur Respir J. 30 (Suppl. 51): 33s.

4. **Gangell CL,** Murray C, Hall GL, Sly PD, Stick SM (2007) Increased variability in forced oscillations may predict abnormal lung structure on HRCT in young children with cystic fibrosis. Am J Respir Crit Care Med. 175: A933

5. **Gangell CL,** Hall GL, Sly PD, Brennan S, Stick SM (2007) Assessment of the forced oscillation technique in young children with cystic fibrosis. Respirology. 12 (Suppl. 1): 10.

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10. **Gangell CL**, Hall GL, Sly PD, Stick SM (2006) Changes in forced oscillatory indices during hospital admission in young children with cystic fibrosis. Respirology. 11(Suppl. 2): 59.

11. **Gangell CL**, Hall GL, Brennan S, Patterson H, Udomittipong K, Sly PD, Stick SM (2005) Increased respiratory resistance using forced oscillations in symptomatic young children with cystic fibrosis. Eur Respir J. 26(Suppl. 49): 728.

12. **Gangell CL**, Sly PD, Stick SM, Patterson HJ, Udomittipong K, Hall GL (2005) Short term repeatability of forced oscillatory indices in young children with cystic fibrosis. Eur Respir J. 26(Suppl. 49): 676.

13. **Gangell CL**, Stick SM, Sly PD, Hall GL (2005) Increased respiratory resistance using forced oscillations in symptomatic young children with cystic fibrosis. Respirology. 10(Suppl. A): 17.

Statement of Contribution

All work arising from this thesis was the sole work of the author unless otherwise stated below:

Bronchoalveolar Lavage: Under the co-ordination of Dr Friedrich Horak Jr (2003-2004), Dr Andrew Martin (2004-2005) and Dr Tonia Douglas (2005–2007), bronchoscopies were performed on a rotating roster by respiratory fellows and consultants.

Microbiological assessment of bronchoalveolar lavage fluid: Under the control of Dr Tony Keil, head of Department of Microbiology, Princess Margaret Hospital for Children.

Quantification of inflammatory markers: Under the control of Professor Peter Sly, Head of Division of Clinical Sciences, and Dr Siobhain Brennan, Cystic Fibrosis Program Co-ordinator, Telethon Institute for Child Health Research. Laboratory staff included Ms Kay Winfield, Ms Sam Gard, Ms Nina Sturges, Mr Luke Garrett and Mr Luke Berry.

High Resolution Computed Tomography: Performed by Radiology Department, Princess Margaret Hospital for Children. Scans scored by Dr Connor Murray, radiologist, Princess Margaret Hospital for Children.

Abbreviations

%	Percent
BAL	Bronchoalveolar lavage
BDR	Bronchodilator response
cfu/ml	Colony forming units per ml
CLED	Cystine lactose electrolyte deficient
cm	Centimetres
CR	Coefficient of Repeatability
CV	Coefficient of variation
ELISA	Enzyme linked immunosorbent assay
FCS	Foetal calf serum
$FEV_{0.5}$	Forced expiratory volume in 0.5 seconds
FEV_1	Forced expiratory volume in one second
FOT	Forced oscillation technique
FRC	Functional residual capacity
FVC	Forced vital capacity
Grs	Coefficient of tissue damping
η	Hysteresivity (ratio of Grs:Hrs)
hPa.s/L	Hectapascal per second per Litre
HRCT	High resolution computed tomography
Hrs	Coefficient of tissue elastance
Hz	Hertz
ICC	Intraclass correlation coefficient
IOS	Impulse oscillometry
IL-1β	Interleukin-1β
IL-8	Interleukin-8
IV	Intravenous
kg	Kilogram
kV	Kilovolts
L	Litre
LCI	Lung clearance index
LFOT	Low-frequency forced oscillation technique
LTB4	Leukotriene β4
mA	Milliamps

MBW	Multiple breath washout
MEM	Minimal Essential Medium
mins	Minutes
ml	Millilitre
NE	Free neutrophil elastase
NIH	National Institute of Health
ng	Nanograms
P	Pressure
Pao	Pressure at the airway opening
PRN	Pseudorandom noise
P/S	Penicillin/Streptomycin
Raw	Airway resistance
R_{int}	Interrupter technique resistance
$Rrs_{(Hz)}$	Respiratory system resistance (at a specific frequency)
RVRTC	Raised volume rapid thoracoabdominal compression
s	Seconds
S_{acin}	Acinar ventilation heterogeneity
S_{cond}	Conductive ventilation heterogeneity
Sn_{III}	Normalised phase III slope
sRaw	Specific airway resistance
TCC	Total cell count
TLC	Total lung capacity
µl	Microlitre
µm	Micrometre
V’	Flow
V’ao	Flow at the airway opening
$Xrs_{(Hz)}$	Respiratory system reactance (at a specific frequency)
Zrs	Respiratory system impedance

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1

A Review of Lung Function Techniques and Abnormalities in Young Children with Cystic Fibrosis

1. A review of lung function techniques and abnormalities in young children with cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that affects 1 in 2500 live births amongst Caucasians and affects multiple organs including the lungs, pancreas, liver and male reproductive organs. While the median survival rates have increased from 8 years in 1974 to 36.5 years in 2005 (1, 2) lung disease remains the most significant cause of morbidity and mortality in patients with CF. Inflammation and infection in the airways leads to damage of the respiratory epithelium causing airway obstruction and structural damage to the lungs. Pathological changes of the tracheal mucosal epithelium and its submucosal glands in aborted (19-23 weeks gestation) foetuses with suspected CF demonstrated changes to the lung begin antenatally (3). Manifestations of lung disease are observed early in infancy and progressively worsen with age (4).

Current techniques used to identify the presence of lung disease such as inflammation and infection by bronchoalveolar lavage (BAL) and structural lung abnormalities by high resolution computed tomography (HRCT) in young children cannot be performed frequently. This is due to the general anaesthesia required for BAL and concerns with exposure to radiation dose with HRCT. While spirometry is used routinely in older children and adults with CF to provide information for clinical assessment, young children find it difficult to perform acceptable measurements (5-8). The preschool years are therefore often referred to as the 'silent years' in CF as regular measurements of lung function and assessment of infection through the production of sputum are difficult. What is urgently required is a practical measurement of lung function that is sensitive to the presence of lung disease and that can be used when needed to provide information that is useful for the clinical management of young children with CF. Such a test would also have great utility as an outcome measure for intervention studies in young children with CF.

Within the clinical setting, techniques available to assess lung function in young children include the interrupter technique (9-30), plethysmography (19-21, 24-29, 31, 32), spirometry (6, 11-13, 16-18, 26-28, 32-41) and the forced oscillation technique (11, 13, 16, 19-21, 23, 25, 26, 30, 32, 35, 42-52). These techniques have been investigated in

a number of disease groups including asthma (10, 12-14, 19, 21, 24, 25, 27, 28, 31, 37, 41, 43, 49-52), CF (15, 26, 27, 36, 42, 50) and neonatal lung disease (29, 30, 32, 46, 47), and have been applied in bronchial challenge testing and to test bronchodilator responsiveness (10, 12, 14, 15, 17, 18, 21, 23-26, 29, 31, 41, 43, 49, 51, 52). This thesis focuses on the clinical use of the FOT in young children with CF and associations with FOT variables and markers of lung disease.

1.1 The Forced Oscillation Technique

The FOT was first described in 1956 by DuBois *et al.* (53) and is based upon the ability to measure the mechanical response of the chest wall and lung to pressure oscillations. Since its initial development the FOT has undergone modification in relation to oscillation waveform signal, frequencies and analysis of outputs that has lead to the production of commercial devices.

1.1.2 The Mechanism of the Forced Oscillation Technique

The FOT is a measure of respiratory system function. System functions are characterised by the ratio of an output variable to an input variable. The input variable is an external influence, and the output is the response of the system to the external influence (54). There are many types of system functions, one being impedance where the input is pressure at the airway opening (Pao) and output is the resulting flow at the airway opening (V'ao) (54). When the pressure difference and flow are measured at the same terminal (e.g. the mouth), input impedance (Zrs) is measured so that:

$$Zrs = Pao / V'ao \quad (54)$$

Impedance can then be divided into two parts: the resistance (Rrs) and reactance (Xrs) components of the respiratory system:

$$Zrs = Rrs + jXrs \qquad \text{where } j=\sqrt{(-1)} \quad (54)$$

When the change in pressure is in-phase with flow, Rrs of the respiratory system is reflected (54). When the change in pressure is in-phase with volume, Xrs of the respiratory system is reflected (54).

Measurement variables of the FOT

Measurement of Rrs using the FOT is dominated by the resistance of the conducting airways but also includes to a lesser extent, the Newtonian resistance of the lung tissue, chest wall and the upper airway (the oropharynx and the larynx) (55). At low frequencies oscillations are transmitted more distally in the lung and so Rrs reflects the more distal conducting airways while at higher frequencies Rrs reflects more proximal conducting airways (56). In this context, conducting airways are defined as those through which gas moves by bulk flow.

The Xrs component of Zrs measures the elastic and inertive properties of the respiratory system. The elastic component represents the elastic properties of the lung periphery and is the relationship between Pao and volume. The inertive component incorporates the inertive forces of the moving air column in the respiratory system and is the relationship between Pao and volume acceleration (57). The balance between elastic and inertive forces depends on oscillation frequency. As oscillation frequency increases the inertive component of Xrs dominates and the magnitude of inertive pressure dissipation increases (58). Inertive pressure "leads" the change in volume and has a "positive" sign. At lower frequencies, where elastic components are predominant, the magnitude of elastic pressure dissipation increases (58). Elastic pressure "lags" the change in volume and has a "negative" sign. The frequency where inertive and elastic components of Xrs are equal and opposite (i.e. the frequency where Xrs=0) is the resonant frequency. At frequencies below the resonant frequency Xrs has negative values and at frequencies above the resonant frequency it has positive values. At resonant frequency Zrs measures flow-resistance, and is the frequency point used to separate low and high oscillation frequencies.

A limitation with calculations using the FOT is the assumption of linear behaviours of the respiratory system (59). In the respiratory system elastance, inertance and resistance are all non-linear. However, provided small amplitude oscillations are used a linear approximation can be made. Due to these assumptions, small errors are introduced and non-linear properties that may be functionally important are not evaluated (60).

Equipment

In the classical set-up that is most widely used in commercial equipment, the oscillatory signal is generated by a loud speaker and delivered to the subject via a pneumotachograph and a mouthpiece (Figure 1.1). The mouthpiece contains a low resistance bacterial filter to limit any possibility of cross-infection between patients. The presence of a bacterial filter minimally affects Zrs, and is easily corrected for by subtracting the impedance of the filter from any measurements. A bias flow is maintained to minimise the effects of dead space (61).

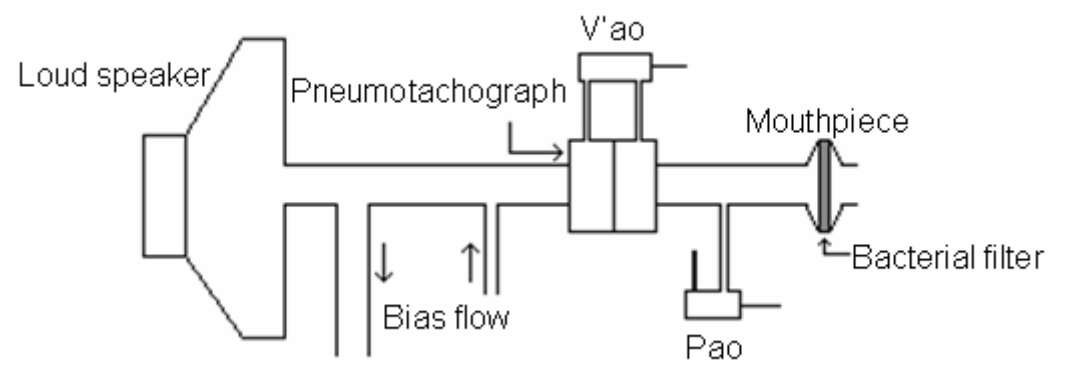

Figure 1.1: Schematic of the forced oscillation technique equipment.

The upper airway shunt effect is potentially important within the frequency ranges employed for clinical measurements and increases as Zrs increases, e.g. in the presence of lung disease (62). The upper airway shunt can contribute to frequency dependence of Rrs and an increase in resonant frequency (63). The contribution of upper airway shunt on Zrs cannot be completely eliminated, although it can be reduced. The standard method of limiting upper airway shunt is to uniformly support the upper airway walls by supporting the patient's cheeks and lower jaw. This technique is practical and well tolerated in preschool children. One potential limitation of this method however is the lack of uniformity of support that could result in increased variability between measurements.

Upper airway shunt and artefacts

An alternative method used to limit upper airway shunt is the head generator technique. First described in 1985, it differs from other FOTs as the pressure signal is applied at the mouth and around the head via an enclosed chamber to reduce transmural pressures between the mouth and the external head/neck (63). Studies in healthy children have

suggested the head generator is a superior method compared to cheek support to minimise upper airway wall shunt (62, 63). While not completely eliminated, contribution of the shunt artefact to the resulting Zrs from the head generator technique is minimised, albeit slightly overcorrected. However this method is not practical in a clinic environment and not well tolerated by young children. Also previous studies have been unable to demonstrate that this technique can discriminate between health and respiratory diseases (64, 65).

Other potential artefacts may occur during a measurement using FOT and consequently may affect Zrs results. These include occlusion of the mouthpiece by the tongue and physical artefacts such as cough, breath holds etc. The occlusion of the mouthpiece by the tongue increases Rrs, and other artefacts alter Zrs. While these artefacts are a potential limitation to this technique, these changes are generally identifiable through observations of anomalies on the pressure and flow trace.

Oscillation signals

In the original FOT study conducted by DuBois a sinusoidal pressure wave was applied at the airway opening. At the time, this was the most practical implementation based on calculation limitations (53). The sinusoidal wave was modified to form complex composite sine-waves including random noise and pseudorandom noise. These waveforms use a series of optimised sinusoidal waves applied simultaneously over a frequency range. The advantage of the pseudorandom noise model is the amplitude and energy content of the signal can be adjusted at different frequencies to optimise the signal to noise ratio across the whole frequency range (61).

A variation of oscillation mechanics, impulse oscillometry (IOS) uses square wave signals to perturb the respiratory system. Whilst square waves, and their reciprocal impulse functions contain multiple frequencies the energy content at any given frequency cannot be controlled (61). In the commercial application of IOS, Zrs is only available at multiples of 5Hz and data at other frequencies are not available. Impulses may induce non-linear flow effects and patients may have a reflex response to the impulse which may cause discomfort (61). This thesis focuses on the pseudorandom noise FOT and will be referred to as FOT for the rest of the thesis.

Frequency range

The frequency range of the oscillation signal can range between <1Hz and up to >100Hz. These frequencies are defined as low (<2Hz), mid-range (2–50Hz) and high (>100Hz). At low frequencies the frequency dependant behaviour of Rrs and Xrs is related to the mechanical properties of the respiratory tissues (66). However, this technique is limited as measurements cannot be imposed over tidal breathing at these low frequencies using input impedance. In infants this requires measurements to be performed during an apnoeic period, typically at raised volumes, which is impractical for frequent use in clinic. The routinely used oscillatory signals fall within the 'mid-range' of frequencies.

Oscillatory signals over the mid-range frequencies, delivered with small peak to peak amplitude (between 0.1 – 0.2kPa), can be imposed over tidal breathing without interfering with spontaneous breathing or patient comfort, and should not affect the assumed linear behaviour of the system (67). During spontaneous breathing the mechanical properties of the lung may change, introducing variability into the measurements. However, measurements are recorded as an average of inspiration and expiration over a number of breaths which reduces this variability.

The optimisation of amplitude and the bias of energy toward the lower range of this frequency ensure a high signal to noise ratio (60). In this frequency range the lowest frequency applied is 2Hz for FOT or 5Hz for IOS.

Coherence

The coherence of a measurement at any given frequency determines validity and acceptability of the obtained measurements. The coherence function is a measure of the relationship between Pao and V'ao calculated for each frequency separately, which is degraded by the presence of noise (68). For FOT, a measurement with coherence of 0.95 or greater is considered acceptable as the error of the measurement at the given frequency is less than 10% (68). Coherence is both frequency and age dependant (20). Reduced coherence is more pronounced in obstructive diseases in the presence of noise and alinearity of Pao and V'ao (68).

1.1.2 Feasibility and reproducibility

Feasibility

The feasibility of obtaining measurements using FOT in young children has been demonstrated and compared with alternative measures of lung function in young children (table 1.1). In general, methods of lung function requiring tidal breathing have superior feasibility compared to spirometry in a young age group (35), although incentive techniques can increase the success rate of spirometry to between 62 and 78% (69, 70). New guidelines for spirometry in young children have been proposed to standardise the use and interpretation of this technique in young children who are unable to produce acceptable measurements under the current guidelines (67). New guidelines will be valuable to centres without alternative measures of lung function in young children. However, results from the implementation of these guidelines have yet to be interpreted in a clinically meaningful way.

Feasibility studies using the FOT have reported acceptable measurements in up to 91% of naïve healthy children aged 2 to 7 years (48). Feasibility studies in naïve healthy children using alternative lung function techniques have been reported for the interrupter technique (56-100%) (14, 22), plethysmography (17-93%) (71), multiple breath washout (MBW) (50-87%) (71) and spirometry (41-87%) (5, 7, 8, 33, 70, 71) with success increasing with age (Table 1.1). All these lung function techniques are used clinically with the exception of MBW that is at present not standardised, with reference data unavailable. In all studies the number of children between 2 and 3 years that lung function was attempted on is low, so success rates are not accurately represented in that age group. A feasibility study in acutely ill asthmatic children aged 2 to 17 years who presented to an emergency department compared the rates of success of FOT and spirometry (72). Up to 65% of children could perform FOT while only 43% could perform spirometry (72). This was particularly evident in the younger children where those aged 4 and 5 years could perform FOT (40% and 80%, respectively) but not spirometry (0% and 17%, respectively) (72). The rates of success in acutely ill children are lower compared to healthy children, although the higher success rates using FOT demonstrates its use in monitoring lung function in acutely ill children.

Table 1.1: Feasibility of lung function techniques by age group in young children.

Technique \ Age (years)	2	3	4	5	6	Reference
Forced oscillation technique	(✓)	✓	✓	✓	✓	(16, 43, 48, 73)
Impulse oscillation	(✓)	✓	✓	✓	✓	(11, 20, 74)
Interrupter technique	(✓)	(✓)	✓	✓	✓	(14, 22)
Specific Raw (Plethysmography)	×	(✓)	✓	✓	—	(71)
Multiple breath washout	(✓)	(✓)	✓	✓	—	(71)
Spirometry	×	(✓)†	(✓)‡	(✓)ᶠ	(✓)	(5-8, 70, 71)

✓ Feasibility ≥ 80%;
(✓) Feasibility ≥ 50% but <80%;
× Feasibility <50%;
— Feasibility not reported.
†One study <50%;‡One study ≥ 80%; ᶠTwo studies ≥ 80%.

Reproducibility

Within-test variability

Coefficient of variation (CV) is an index of intra-test reliability and repeatability (60). Measures of variability in healthy populations are reported for most lung function techniques as the information they provide gives definitions for clinically relevant changes. Shown in table 1.2 the FOT, compared to other lung function techniques used in young children, have similar variability with the exception of reactance. Variability is larger in Xrs than Rrs, due to both physiological and numerical characteristics (57).

Table 1.2: Lower and upper ranges of coefficient of variation in healthy children for pre-school lung function techniques.

Lung Function Technique	Lung Function Variable	Age (years)[§]	Coefficient of Variation (%)		Reference
			Lower	Upper	
Forced Oscillation Technique	Rrs	2 - 18	<5	15	(20, 35, 48, 74)
	Xrs	2 - 7	16	21	(11, 48)[†]
Impulse Oscillation	Rrs	2 - 7	6	10	(11, 20, 21, 74)[†]
	Xrs	2 - 7	16	17	(11, 20, 21, 74)
Interrupter Technique	R_{int}	2 - 10	7	12	(20, 22, 75-77)[†]
Plethysmography	sRaw	2 - 7	8	13	(11, 20, 23, 71)[†]
Multiple Breath Washout	LCI	2 - 16	4	5	(71, 78, 79)
Spirometry	FEV_1	3 - 7	2	7	(6, 33, 34, 36, 70)[†]
	FVC	3 - 7	2	9	(6, 33, 34, 36, 70)[†]

[§]The age range of all studies combined; [†]Includes some children with respiratory disease. Rrs=resistance; Xrs=reactance; R_{int}=resistance; sRaw=specific airway resistance; LCI=lung clearance index; FEV_1=forced expiratory volume in 1 second; FVC=forced vital capacity.

Short-term repeatability

Short-term repeatability gives validity to outcome variables for identifying clinically relevant changes in lung function. This is useful for valid interpretation of intervention therapies, assessment of bronchodilator response or response to challenge testing. As shown in table 1.3, the standard deviation of FOT variables are within the reported range of other lung function methods in same age group (14, 20, 48, 74, 75). Even though the repeatability of FOT is consistent with other measures of lung function in this age group, large changes are required to achieve clinical significance which may lead to a possible reduction in sensitivity(50). This possible limitation however, is not unique to the FOT with similar repeatability observed in other preschool lung function techniques.

Table 1.3: Standard deviation of short term repeatability for pre-school lung function techniques in healthy children.

Lung Function Technique	Lung Function Variable (units)	Age (years)[§]	Repeatability Time (Minutes)	Standard Deviation	Percent repeatability (%)*	Reference
Forced Oscillation Technique	Rrs ($hPa.s.L^{-1}$)	2 - 7	15	1.02	1[†]	(48)
	Xrs ($hPa.s.L^{-1}$)	2 - 7	15	0.85	4[†]	(48)
Impulse Oscillation	Rrs ($hPa.s.L^{-1}$)	2 - 7	15 - 20	0.55 – 1.84	9 - 11	(11, 20, 49, 74)
	Xrs ($hPa.s.L^{-1}$)	2 - 7	15 - 20	0.57 – 1.41	17 - 26	(11, 20, 49, 74)
Interrupter Technique	R_{int} ($hPa.s.L^{-1}$)	2 - 7	15 – 30	0.95 – 1.98	8 - 12	(11, 14, 20, 75)[‡]
Plethysmography	sRaw (hPa.s)	2 - 7	15 – 20	1.56 – 1.98	11 - 13	(11, 20)
Spirometry	FEV_1 (L)	4 - 6	15	0.05	5	(11)

[§]Age range of all studies combined; *SD/baseline; [†]mean difference/baseline; Rrs=resistance; Xrs=reactance; R_{int}=resistance; sRaw=specific airway resistance; [‡]one study included wheezy children.

1.1.3 Reference values

Reference equations are required for measurements of lung function to enable values to be expressed in relation to healthy children of similar age, height, weight, sex and ethnicity. Appropriate healthy controls are essential so respiratory dysfunction in children with respiratory illness can be compared. Reference equations should never be extrapolated for any dependant variables due to inaccuracies this may create.

Numerous reference ranges for the FOT have been published in the last 20 years for Rrs (35, 45, 48, 73, 80-83) and Xrs (48, 82, 83) and are shown in figure 1.2. The differences in these reference regressions are likely due to differences in equipment and methodology. Differences in frequency reported may affect impedance. The Soylmar

(83) and Hantos (80) studies reported data at 4Hz; Hall (48), Mazurek (82), Duiverman (45) and Hordvick (81) reported data at 6Hz; Ducharme (73) reported data at 8Hz; and Lebecque (35) reported data at 10Hz. These differences in reported frequency will have an effect on Rrs and Xrs values as shown in the reference graph (Figure 1.2). All studies, with the exception of the Mazurek study (82), used hand support to limit upper airway shunt. As reported earlier, the use of the head generator technique, as in the case of the Mazurek study (82) may have an effect on Rrs and Xrs values. Recently, in a joint statement the American Thoracic Society and The European Respiratory Society published guidelines for application and interpretation of pulmonary function testing in children (67).

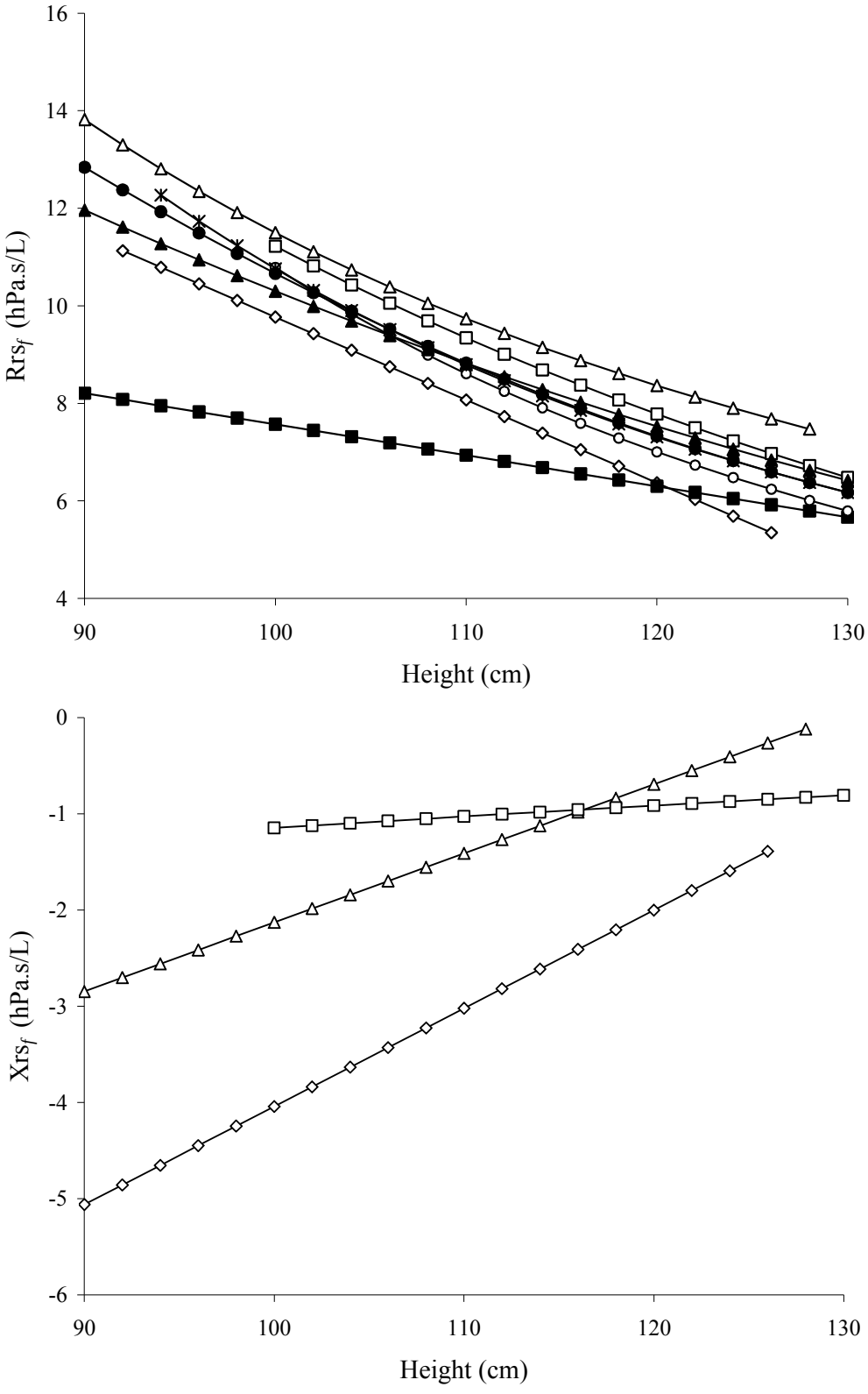

Figure 1.2: Published reference data for respiratory resistance (Rrs) and reactance (Xrs) data using forced oscillations in healthy young children.

—◇—(48); —■—(35); —□—(83); —△—(82); —○—(73); —●—(45); —✳—(80); —▲—(81)

1.2 Lung Function in Children with Cystic Fibrosis

Studies investigating lung function in young children with CF compared to a reference group are mainly cross-sectional studies (26, 36, 50, 71, 76, 83-89) with only one longitudinal study (26) currently reported in the literature. Studies reporting associations with lung function techniques and respiratory disease demonstrate the sensitivity of the lung function test. However, lung function is most relevant clinically if relationships between it and markers of lung disease are present.

1.2.1 Lung function compared to a reference population

Studies relating to the difference in lung function between a healthy reference group of children and children with CF have reported mixed results. These differences are generally based on the age of children studied and the severity of lung disease.

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 an early study, Solymar *et al*. described frequency pattern and dependence in children with respiratory disease using the FOT. In this study it was noted that of the 13 children with CF (no age range provided), Rrs and Xrs were abnormal in 4 and 6 children, respectively(83). In children with a mean age of 14 years, Lebecque *et al*. compared Rrs in children with CF and reported 32/45 children had Rrs outside normal limits(50). In a more recent study by Hellinckx *et al*. in children over 7 years the 20 patients in the study had normal Rrs, but abnormal Xrs(87).

A longitudinal study by Nieslen *et al*. measured Rrs and Xrs by IOS in 30 children with CF between 2 and 7 years (26). In this study, Rrs was abnormal in the CF group in 2 out of 5 follow-up visits, while Xrs was normal for all visits with the exception of the first visit (26). This difference in Xrs can be attributed to one outlier and so 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 (26). Neither was periods of exacerbation between visits reported, although children had no reported exacerbation in the 3 weeks prior to lung function testing (26). This may have a significant impact on the number of children with abnormal lung function at any one time.

In the same longitudinal study by Nielsen *et al*. Resistance (Rint) using the interrupter technique was also recorded over the follow-up period (26). The mean Rint was normal at all visits during the follow-up, except at visit 4 where mean Rint was abnormal in 40% of children (26). At that point, 24 months into the study, there was shift for increased Rint for all children which was not explained by the authors(26). A study by Beydon *et al*. on 39 children with a mean age of 5.2 years, reported children with CF had a significantly increased Rint with 23% of children outside normal Z score limits (76). Most children's lung function was within the normal limits and 2 children had Z score of >2 which may possibly contributed to the overall increase in Z-score (76). One child fell outside the height range of the reference population (76). In both studies, information on recent infections was not recorded. Rint is a measure of overall airway dimensions and therefore may not be sensitive to the peripheral airways (26, 76).

Measurement of specific airway resistance (sRaw) using plethysmography in children with CF under the age of 7 years has demonstrated significantly increased sRaw in up to 57% of children with CF. The Nieslen longitudinal study demonstrated sRaw to be the only variable in the study of multiple lung function techniques to be consistently increased over the 2 year assessment period (26). Consistent with this, Aurora *et al*. reported in their study 52% of children with CF had abnormal sRaw (71). In both studies measurements of sRaw were highly variable, especially in comparison to other lung function methods used in the same studies (26, 71). Measurements of sRaw are related to lung volume, therefore changes in volume between visits and children will affect results.

Studies investigating MBW measurements in children older than 2 years with CF have shown lung clearance index (LCI) to be significantly greater in CF than in a reference population. Aurora *et al*. reported 73% (22/30) of 2-6 year olds with CF had abnormal LCI (71). Variability of LCI measurements was high and was significantly higher in CF compared to the healthy population(71). A study by Gustafsson in 11 subjects with CF between the ages of 7 and 29 years also demonstrated LCI to be significantly increased compared to controls. However the study population fell outside the age range of the healthy reference population (12 to 19 years) and so data in the CF population for children outside these age ranges were extrapolated (86). In older children and adults with more severe lung disease (as defined by FEV_1) LCI was significantly increased in

patients with CF (78, 86, 88). Measurements of LCI represent ventilation inhomogeneity of the lung, and relevance of LCI to biological mechanisms of the lung needs to be investigated.

An early study by Corey *et al*. that pre-dated new-born screening for CF, found that children 5 to 10 years old had spirometry within normal ranges (84). Likewise in a later study, where new born screening for CF was introduced, Farrell *et al*., found children with CF had spirometry measurements within normal limits (>80%) from the age of 5 until 10 years of age (85). Conversely in a small (n=21) study in 3 to 6 year olds, Marostica *et al*. demonstrated lung function was significantly worse in children with CF compared to a healthy reference group (36). The mean age of participants in the Marostica study was 5.3 years, and so the range of ages was slightly skewed towards the older age group (36). In a recent study of 3 to 6 year old children (again with a mean age of 5.1 years and so a larger number of children in the older age group) Vilozni *et al*. found Z score FEV_1 was abnormal in children with CF compared to the reference group (89). As spirometry has been shown to be sensitive to changes with disease, the conflicting results in these cases are more than likely due to patient effort and protocols used by the different groups, rather than a representation of disease. The literature consistency reports that lung function is abnormal in children with CF on grouped data whereas relatively few individual children have values that fall outside "normal limits" based on Z scores being greater or less than 2.0 of the variability of healthy children. This makes individual patient assessment difficult.

Worse lung function in older children with more severe lung disease has been well reported and described. Young children (<7 years) with CF also have worse mean lung function compared to a reference population although this has not been described using the FOT. The FOT provides information about the respiratory system and may report early changes in lung physiology in children with CF compared to a reference group. To be a useful clinical tool in CF, lung function measurements need to be reflective of changes in pulmonary status, and for use in young children it needs to be sensitive enough to identify mild physiological changes in the lung.

1.2.2 Respiratory symptoms

Onset of respiratory symptoms in CF begins early in life with 14% of children having experienced frequent cough and 3% having audible wheeze at time of diagnosis in the first weeks of life (6.7 ± 2.7 weeks) (85). At 6 months of age the incidence of cough increases with the onset of chronic cough occurring within the first 2 years of life in many children (85). Cough may persist throughout life, becoming a daily symptom often accompanied by expectoration of sputum in many older children. Both Dakin *et al.* and Brennan *et al.* have reported higher incidence of respiratory symptoms in children with an infection (86% and 80%, respectively) compared to those without (50% and 17%, respectively) (42, 90). The presence of respiratory symptoms may be reflective of lower airway infections amongst other causes. If the presence of symptoms indicates lung disease and this change is reflected with changes in lung function it adds value to the clinical significance of lung function testing.

Studies in infants, with a mean age of <6 months, with CF have demonstrated children with respiratory symptoms had decreased respiratory flow and evidence of air trapping (91, 92). Infants (<7 months) with minimal symptoms (occasional cough) had airway obstruction and those with severe respiratory symptoms also had low compliance and hyperinflation (93). In slightly older children with a median age of 1.6 years Brennan *et al*. reported no difference between children with or without respiratory symptoms (42). Children in the Brennan study also underwent BAL and were clinically stable at the time of lung function testing.

The effect of history of symptoms on lung damage and the resulting changes in lung function have also been investigated. Ranganathan *et al*. reported that in infants under 24 months lung function, using the raised volume rapid thoracoabdominal compression (RVRTC), was no different in children who did or did not have a prior lower respiratory illness (94). In a study using the interrupter technique in preschoolers, Beydon *et al*. reported an association between patients with a history of CF-related respiratory symptoms and worsening lung function (76). However, the number of children with no history of CF-related respiratory symptom was considerably less than the group with a history, 8 patients compared to 31 patients (76).

Changes in respiratory symptoms are clinical indicators in CF and may be associated with progressive lung disease. If a lung function method accurately reflects the presence of disease or damage in the lung, the clinical significance of symptoms can be better interpreted. As a measurement of lung physiology the FOT has the potential to add information about relationships with symptoms and the presence of lung disease.

Reporting of respiratory symptoms

In epidemiological studies, the reporting of symptoms is usually conducted through a questionnaire. Questionnaires concentrating on the quality of life of patients are generally used; however they focus on a range of factors affecting individuals and are not specific to respiratory symptoms. Questionnaires previously developed have been designed for adults with few studies incorporating modifications for younger children. For example, spirometry values may contribute to the overall score, and so therefore are aimed for children 7 years and older. Currently no questionnaire specific to respiratory symptoms in young children with CF has been developed and validated.

Currently the Shwachman-Kulcyzcki score (95), Taussig modified National Institute of Health score (NIH) (96), and Kanga scores (97) are routinely used questionnaires in CF. While all these questionnaires contain a respiratory component, they are not specific to identify changes in respiratory health alone. Kanga scores are specific to evaluate acute pulmonary exacerbations in CF. The questionnaire contains a large respiratory component including cough, sputum production, wheezing and crackles as well as incorporating systemic symptoms that can be associated with a pulmonary exacerbation (97). Correlations between pulmonary function tests and Kanga score demonstrated significant association with FEV_1 and FVC (97). This scoring system was validated in children older than 5 years who were able to complete acceptable pulmonary function tests (97). This system was developed specifically to be independent of pulmonary function score to provide an alternative to pulmonary function test during a time of acute exacerbation (97). While effective at identifying changes over the course of treatment and may be useful to measure acute day to day changes, this questionnaire may not be able to demonstrate changes in children with mild lung disease or in young children.

Other questionnaires developed to evaluate disease severity and predict prognosis, such as the Shwachman-Kulcyzcki (95) and NIH scores (96), lack specificity for changes in respiratory symptom status. Both questionnaires include X-ray findings as an outcome measure, with the NIH also including spirometry variables (95). As such, frequent evaluation in young children is not feasible using these scoring methods.

To address the misgivings of previously published questionnaires we need a simple questionnaire that allows accurate determination of the presence of respiratory symptoms, that can be routinely used in a clinical setting, does not require the use of any additional procedures, and is developed for and validated in preschoolers. This will allow standardisation of respiratory symptom evaluation in preschool children with CF and would ideally be used as an adjunct to methods that are currently used for clinical evaluation.

1.2.3 Bronchodilator response

Bronchodilators are used in CF as a therapeutic tool to facilitate mucocilary clearance by rehydrating the mucus and to provide protection against bronchoconstrictors that are used in treatment, such as DNase, hypertonic saline and some antibiotics. As shown in table 1.4, studies investigating bronchodilator responses (BDR) in patients with CF have reported variable pulmonary function outcomes including improvement, worsening and no change (26, 87, 98-101). The one outcome that is consistent between studies is that bronchodilator response is highly variable, between 16 and 49% of children responding in single visit studies (26, 87, 98, 102, 103), and up to 95% of patients (7 to 45 years) responding at least once in a one-year longitudinal study (103).

An overview of bronchodilator studies published in children with CF is summarised in table 1.4. All studies reported a mean statistically significant improvement in lung function in children with CF following inhalation of bronchodilator, although this improvement did not necessarily shift lung function results into the normal range (26, 76, 100, 101).

Table 1.4: Reported bronchodilator response in children with cystic fibrosis.

Technique	Variable	Age range (years)	n	Mean improvement in lung function (% of baseline)	Cut off limits	Larger BDR compared to healthy children	No. of children with CF with significant BDR (%)	No. of children with CF with paradoxical BDR (%)	Reference
Forced oscillation technique	Rrs6	6 - 18	20	16%	12%	Not assessed	13 (65)	0 (0)	(88)
	Xrs6	6 - 18	20	21%	25%	Not assessed	Not reported	Not reported	(88)
Impulse oscillation	Rrs5	2 – 7	30	12%	30%	No	3 (10)	5 (17)	(26)
	Xrs5	2 – 7	30	25%	40%	Yes	6 (20)	5 (17)	(26)
Interrupter technique	R_{int}	2 – 7	30	12%	34%	No	0 (0)	4 (13)	(26)
		3 – 8	38	17%	24%	No	0 (0)	3 (8)	(77)
Plethysmography	sRaw	2 – 7	30	23%	40%	No	9 (30)	2 (7)	(26)
		6 – 18	20	16%	12%	Not assessed	11 (55)	0 (0)	(88)
Spirometry	FEV_1	7 – 45	24	8.1%	15%	Not assessed	12 (49)	2 (10)	(99)
		7 – 26	50	3.4%	-	Not assessed	5 (10)	4 (8)	(96)
		6 - 18	20	4.3%	15%	Not assessed	4 (20)	0 (0)	(97)

Rrs=resistance; Xrs=reactance; Rint=resistance; sRaw=specific airway resistance. Nielsen *et al.*, study the first visit is reported(26).

Few studies have assessed BDR in young children with CF using the FOT. Nielsen *et al*. evaluated BDR in 2 to 7 year olds with CF on two separate occasions one month apart using IOS (26). Compared to healthy children, a greater BDR was observed for Xrs5 in CF on the first, but not the second visit. This observed difference was not attributed to a real difference by the authors but a type I statistical error (26). More likely the difference was caused by two outliers with a BDR of around 100% of predicted values for only Xrs5 and no other variables (26). Also 6 children were excluded from the follow-up visit one month later because β_2-antagonists were not withheld prior to lung function test. This reduction in numbers from an original group of 30 children may have affected statistical outcomes.

Also using IOS, Hellinckx *et al*. assessed BDR on older children aged between 6 and 18 years with CF (87). Inappropriate values to determine significant bronchodilator response were used in this study and so the number of children classified as responders may be overestimated. These cut-off values of 12% for Rrs6 and 25% for Xrs6 were the calculated CV in healthy children from a study 20 years prior using a similar FOT method.

Similar cut-off values for Rrs5 have been reported as the 95% confidence interval of healthy children's response to bronchodilator and stand at 41% (49) and 37% (74) and 42% for Rrs6 (104), although the Nielsen and Bisgaard study determined a lower response of 28% (24). Other studies have reported a change of 30% at Rrs10 by calculating twice the standard deviation (105), while Hellinckx *et al*. may have underestimated change in Rrs6 by calculating the response as the CV at 12% (87, 105). Only two studies have reported a significant change in Xrs at the 95^{th} percent confidence interval of 45% at 5Hz and 61% at 6Hz (24, 104). Significant changes in lung function using FOT appear much larger than spirometric parameters, although variability of the FOT is higher than spirometry, and cut-offs are similar to other preschool measures of lung function.

Bronchodilator response in the presence of respiratory symptoms

Few studies have investigated the effect of exacerbation or respiratory symptoms on magnitude of BDR (76, 102, 103). During periods of exacerbation Hordvick *et al*. reported in the 1 to 2 weeks after admission, a significant BDR using spirometry (103). In the Hordvick *et al*., study the ages of the 20 patients ranged between 7 and 45 years, mainly with severe and moderate lung disease (FEV_1 < 69%) (103). Comparison of BDR in children with CF using RVRTC in infants (102) and the interrupter technique in preschool children (76) found BDR did not reflect respiratory symptom status. In the preschool study the children were classified as having a history of respiratory symptoms rather than current symptoms (76). In the infant study half of the CF group had a history of wheeze, which may have adversely affected results. Airway obstruction in CF is primarily caused by accumulation of secretions, not bronchospasm as with asthma (106). As such the most frequent symptom experienced by CF is cough with fewer children having wheeze. Because of this, the action of bronchodilator to dilate the airway through a decrease in bronchospasm does not always occur in CF as it does in asthma.

Evaluation of the significance of a BDR as a clinical outcome in the young CF population using FOT is lacking. Relationships between the effect bronchodilators have on lung function and markers of lung disease in CF have not been clearly addressed. By investigating changes in response, the efficacy of administration of bronchodilator during clinic visits and any predictive value which BDR has on clinical status can be determined.

1.3 Pulmonary Inflammation in Cystic Fibrosis

A predominant characteristic of the CF lung is the presence of pro-inflammatory cytokines and inflammatory cells. The recruitment of inflammatory cells and production of inflammatory mediators are part of the body's natural immune response to rid the body of bacterial infection. In healthy individuals, after the offending pathogen is removed, the immune response is 'switched off' and normal functioning resumes. In CF however, a persistent inflammatory response ensues, contributing to lung damage.

As reviewed by de Rose, high levels of chemoattractant interleukin-8 (IL-8), leads to an influx of neutrophils (107). Neutrophils play a major role in lung damage through the production of noxious mediators including neutrophil elastase (NE), reactive oxygen species and proteolytic enzymes (107). Neutrophil elastase degrades structural proteins in the lung and induces production of IL-8 from epithelial cells (107). The stimulation of production of more IL-8 activates a positive feedback loop to induce accumulation of more neutrophils (107).

Debate over whether inflammation precedes infection, or is secondary to infection in CF continues. Hubeau *et al*. assessed pulmonary cell and cytokine profiles of CF and non-CF foetuses aborted (induced) between 10 and 36 postmenstrual weeks, representing three different developmental stages of the lung – pseudoglandular, canalicular and saccular/alveolar (108). At all stages of development, presence of interleukin-8 (IL-8) in tracheal and lung tissues was similar in both groups (108). As IL-8 is associated with the differentiation and migration of immune cells, this similarity between groups is probably a function of foetal development with no direct association with CF. The number of neutrophils remained low during foetal development in CF, while the number of macrophages in the distal lung and number of mast cells in the mesenchyma of the trachea was increased. This increased presence of macrophages and mast cells is representative of the proinflammatory state of naïve CF tissue and may contribute to recruitment of neutrophils in the airways of infants (108).

1.3.1 Inflammation in CF compared to controls

In infancy, the majority of studies in children with CF report increased levels of IL-8 in the BAL fluid of children with CF compared to controls (109-111). Results from studies to determine whether children with CF have increased numbers of neutrophils compared to control children are not consistent. Two groups, Marguet *et al.* and Khan *et al.*, observed the percent neutrophils in children with CF (26% and 57%) was significantly increased compared to the presence of neutrophils in control children (2.7% and 2.2%) (110, 112). Both studies were confounded by the presence of infection in some of the children with CF, possibly over representing the number of neutrophils in children with CF. Armstong *et al*. compared percentage of neutrophils between children with CF with no detectable infection following BAL and no history of respiratory symptoms or antibiotic use, to control children. While the children with CF were significantly

younger (1.5 – 6.0 months) compared to the control group (2.0 – 48 months), there was no difference in the percentage of neutrophils (CF=8.4%, controls=8.2%) (113). The controls in the Armstrong *et al.* study (113) had a high percentage of neutrophils (8.2%), compared to those in the Marguet *et al.*, and Khan *et al.*, studies (2.2% and 2.7%, respectively) (110, 112). In the Armstrong study 40% of children in the control group were infected, which may account for the high percentage of neutrophils reported (113).

1.3.2 Inflammation and infection in CF

The observation of differences in inflammatory profiles of children with CF infected or uninfected at the time of BAL is more complex. During BAL aliquots are taken from limited areas of the lung and may not provide an accurate profile of infection throughout the whole lung. Patients may have a regional infection, undetected by BAL, but a generalised inflammatory response. Regional variability of inflammatory profiles also exists. In a recent study by Davis *et al.,* HRCT was performed before BAL to determine two areas of the lung that had "greatest" disease and "least" disease (114). In this small sample of 16 children younger than 4 years of age, IL-8 and neutrophils were significantly higher in the lobe with "greatest" disease ($p<0.001$ and $p=0.04$, respectively) (114). This study highlights the regional variability of inflammatory markers in the lungs. Variability in cell profiles is also influenced by the BAL aliquot used for analysis. It has been suggested analysis of aliquots subsequent to the first may underestimate the amount of inflammatory cells detected (115). Finally, as BAL requires patients to undergo general anaesthesia, children need to be relatively well at the time of their lavage.

A number of studies have investigated differences in inflammatory profiles of children with and without a significant pathogen infection. An increased amount of IL-8 in BAL fluid is reported in children with CF with an identified pathogen, compared to children with no detectable pathogen (90, 110, 111, 113, 116) in all but one study (42). IL-8 is a chemoattractant, and presence of IL-8 leads to an influx of neutrophils. Children (< 6 years) with an infection had between 50% and 91% neutrophils recovered in BAL fluid, significantly more than children with no detected infection (8% to 28%) (42, 90, 111, 113, 116, 117). Neutrophils produce noxious mediators, including neutrophil elastase, which degrades structural proteins of the lung.

Previous studies have reported that uninfected children had no free NE activity, while between 38% and 80% of infected children had free NE activity (42, 110, 113, 117). Contrary to this, Nixon *et al*. reported no statistical difference in the amount of free NE between children who were uninfected (5.6 μg/ml) and children who were infected (8.3 μg/ml), although a trend was present (p=0.09) (116). However, the mean level of NE activity in the uninfected group included values below the lower limits of detection of the assay (<5.0 μg/ml) (116). As discussed previously, the regional variability of infection and inflammation may affect results (114). Also there is no way of identifying whether 'uninfected' children had a recent infection that may be cleared, but still have signs of inflammation.

1.3.3 Inflammation and lung function

Relationships between inflammatory markers and lung function in children with CF are less well defined. The few studies that exist report conflicting results and are difficult to compare due to different age ranges, levels of infections, bacteria isolated and lung function measurements used.

Brennan *et al*. reported in a study of preschoolers (up to 5 years old) relationships with low-frequency FOT (LFOT) and inflammation (42). Lung function was performed immediately prior to BAL under the same general anaesthesia. Relationships with lung function was observed with number of neutrophils, total cell counts, IL-8 and leukotriene β4 (42). LFOT measurements are representative of the peripheral non-conducting airways and suggest lung damage in young children begins peripherally. These differences would be difficult to detect using techniques that were not capable of partitioning lung function between conducting airways and the lung periphery.

The only other study in young children that has identified relationships between lung function and inflammation was in children up to 4 years old.(90) In this study relationship between specific compliance and air trapping (functional residual capacity / total lung capacity: FRC/TLC) was associated with levels of IL-8 and neutrophils (90). No other relationships were identified with IL-10 and IL-8 and neutrophils, and Rrs or TLC or FRC alone (90). Again these changes reflect disease in the peripheral lungs.

Other studies in young children have not reported relationships with FRC or using RVRTC (111, 116). These studies include a three year follow-up by Rosenfeld *et al*. that identified no relationships between FRC and IL-8 or neutrophils (111). Lung function was assessed 28 days prior to or following BAL and so may not be an accurate profile of relationships between these two markers (111). Nixon *et al*., reported no relationships with $FEV_{0.5}$, $FEV_{0.75}$ and FEF_{25-75} (using RVRTC) and neutrophils, IL-8 or free NE (116). Lung function measures using forced expiration does not clearly separate changes in the conducting airways from those in the peripheral lung. Where both are included in the same measurement, sensitivity may be lost.

The presence of inflammation is a characteristic of CF lung disease and may be present even before birth (108). Inflammation in the lung is associated with worse clinical outcomes and structural damage to the tissue and airways. The FOT does not require the use of anaesthesia and can be used in a clinical setting. Information on lung function can therefore be obtained frequently whether a child is well or not. If FOT outcomes reflect lung damage due to inflammation, the FOT could be used to identify children at high risk of worsening lung disease and who might respond to therapeutic interventions.

1.4 Infection in Cystic Fibrosis

Infections in the lung are characteristic of CF and are generally associated with worse respiratory outcomes. The presence of infection has been detected in infants at the time of newborn screening, with the incidence of infections increasing with age.

The body initially mounts an innate immunological response to the presence of infecting organisms. In healthy individuals this involves mechanical clearance of the pathogenic particles by entrapment of mucus that is cleared through movement of cilia and assisted by cough and sneezing (118). A host of cellular components directly affect the pathogen or contribute to host response to the infection. These cellular components include cells (macrophages, neutrophils and epithelial cells), secretions from the large (gland and goblet cells) and distal airways (Clara cells), the lung parenchyma (type II pneumocytes), and components of the airway surface fluid and proteins (complement, surfactant proteins and Clara-cell protein) (118).

A number of theories have been proposed to explain the inability of the CF lung to effectively eradicate a microbial infection. One proposes an alteration of water and/or salt content of the airway surface fluid resulting in ciliary dysfunction and/or inhibition of antibacterial substances (118). Another suggests increased binding of micro-organisms to the airway cells or its secretions, directly interferes with the hosts defence system (118). Whatever the underlying fundamental reason why the CF lung is vulnerable to chronic bacterial infection, the resultant inflammatory process further contributes to tissue destruction and impairment of mucocilary function through proteases, oxidants and defensins from cells, and DNA from lysed cells and bacteria (118). The reduced presence of nitric oxide in CF lungs may also play a role in the inflammatory process as nitric oxide regulates inflammatory and immune cells as well as smooth muscle tone (118).

From the 2002 Annual Report from the Australasian CF Data Registry (119), less than 10% of children under 9 years had no reported pathogen growth. The incidence of no growth and normal flora decreased with increasing age. Shown in figure 1.3, the most prevalent micro-organisms recorded in the data registry in children under 9 years were *Staphylococcus aereus* and *Pseudomonas aeruginosa*, followed by *Escherichia coli* and *Haemophilus influenzae*. However, these data do not come from a systematic surveillance of infection as specimens are more likely to be obtained during respiratory exacerbations.

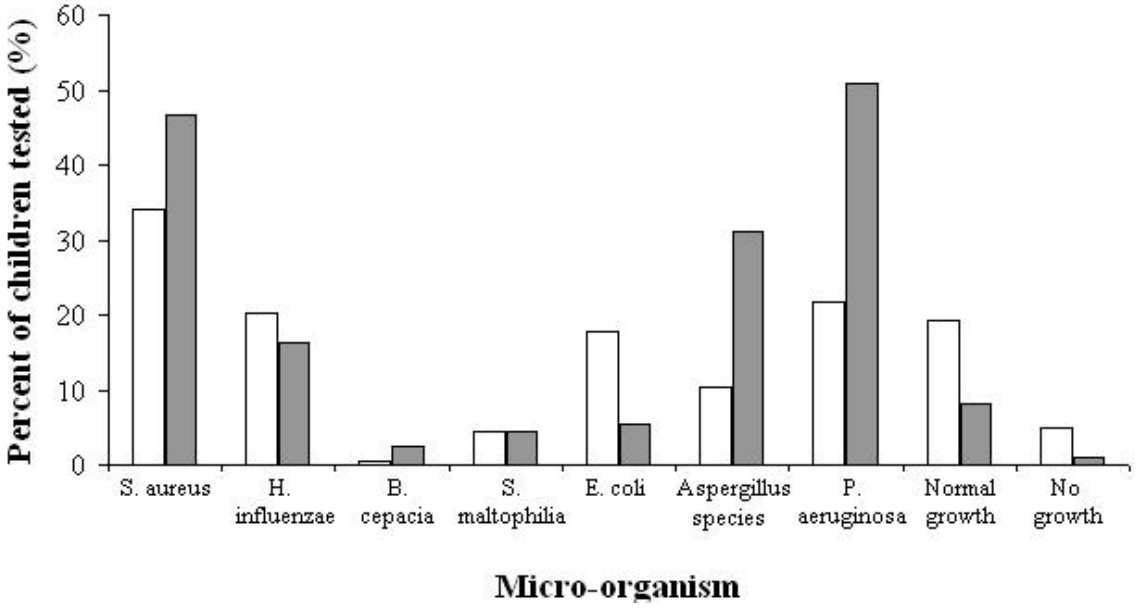

Figure 1.3: Infection status of children 0-4 years (○) and 5-9 years (●) recorded on the Australasian CF Data Registry in 2002

The registry data confirm general observations worldwide that *P. aeruginosa* is a common respiratory pathogen in CF and published data suggest that the prevalence increases with age (119). Infections with *P. aeruginosa* are associated with increased levels of morbidity and mortality, particularly following persistent infection with the mucoid type (120). Longitudinal studies assessing clinical outcome until school age demonstrate mortality is increased in children infected with *P. aeruginosa* (121, 122). Infections with this micro-organism are also associated with increased rates and duration of hospitalisation (121, 122). Consequences of infections with other micro-organisms in young children are less well understood. *S. aureus* is a major cause of nosocomial infections and produces major virulence and adherence factors, although alone this pathogen does not appear to affect survival or lung function (123-125).

1.4.1 Infection and lung function

The effects of infection on lung function have been well documented in older children and adults, primarily because these patients are able to expectorate sputum and perform spirometry. BAL has previously been validated for detecting lower respiratory tract infections and is the gold standard method for pathogen detection in the lower airways in younger children with CF who are unable to expectorate (126, 127). There are limitations with the BAL technique that include: the need for a general anaesthetic (this might not be possible when children are unwell) and regional heterogeneity of pathogen infection in the lung. Some studies in young children use oropharyngeal cultures (OPC) for the detection of upper respiratory tract infections, although this method of assessment is insensitive to lower airway pathogens (126, 128).

Few studies have investigated the impact of pulmonary infection on lung function in preschool aged children with CF (26, 71, 76, 89, 111, 129). Nielsen *et al*., reported lung function using IOS, interrupter technique, plethysmography and spirometry was not worse in the presence of *P. aeruginosa* infection (26). These children were between 3 and 8 years old, and presence of infection was confirmed through assessment of nasopharyngeal suctions. Other studies in young children that used spirometry as an outcome measure found no relationship with $FEV_{0.5}$ or FEF_{25-75} and *P. aeruginosa* infection (71, 89). These studies used sputum (in older children) and OPC or BAL (in young children) to detect infection (26, 71, 89). A study in older children with a mean age of 7.9 ± 0.8 years reported no difference in FEV_1 and FEF_{25-75} in children with a *P.*

aeruginosa infection (129). However, the authors report FVC was significantly worse in the presence of an infection (129). While these data appear contradictory, different techniques were used to identify the presence of an infection, which may over- or underestimate the presence of a true lower respiratory tract infection. Studies in the younger cohorts used either OPC exclusively or with a combination of BAL and sputum, while in the Bodini study sputum only was used. Previous reports in the literature define the insensitivity of OPC to detect lower respiratory tract infections; hence these studies in younger children may be under-reporting presence of lower respiratory tract infections (126, 128).

Aurora *et al*. reported a significantly increased LCI using MBW in children with a *P. aeruginosa* infection, although sRaw using body plethysmography was not affected (71). Beydon *et al*. reported no difference in lung function using the interrupter technique or helium dilution in children with a chronic *P. aeruginosa* infection (76). This study classified children as chronically infected with *P. aeruginosa*, although analysis of infections was only completed on those children with respiratory symptoms (76). Previous studies have reported the presence of an infection in the absence of symptoms (42).

A three year longitudinal study by Rosenfeld *et al*. reported percent predicted FRC using helium or nitrogen dilution was elevated in children with an infection at 2 years of age (111). This was a comprehensive three year follow-up, with a relatively large patient cohort. Lung function was taken 28 days prior to or following BAL and therefore may not represent what is happening in the lungs at the time of lung function testing. No other lung function parameters were significantly increased in the presence of infection, and the difference between the two infection groups was only apparent in children at their 2 year old follow-up and not at 1 or 3 years (111). This observed difference is more than likely a statistical error due to the fact that there were considerable fewer patients with a pathogen density of $<10^5$ cfu/ml. At this follow-up age no other factors such as Brasfield score or Shwachman score were increased (111).

Current techniques for routine microbiological surveillance are suboptimal and newer, more sensitive and specific techniques suitable for detecting lower airway infection are needed.

1.5 Structure Abnormalities in Cystic Fibrosis

Bacterial infections and subsequent immune response lead to structural damage to the airways and parenchyma. Imaging of the airways and parenchyma illustrates the presence, severity and the extent of lung disease. This has previously been limited to chest x-ray, but advances in imaging techniques now enable direct assessment of airways and parenchyma. High resolution computed tomography (HRCT) is more sensitive than chest radiographs in providing information on structural abnormalities, especially in the very young (130, 131). Concerns about increased exposure to radiation using HRCT which may have limited its use in the past have been resolved through modification of procedures to minimise radiation (132).

HRCT provides imaging of the peripheral and parenchymal airways, as well as a quantitative measurement of airway abnormalities. Airway wall thickening and loss of respiratory epithelium in the peripheral rather than central airways is characteristic of early CF lung disease and these changes are most likely to be attributed to past or present infection. Studies in children and adolescents ranging from 5 months to 18 years have reported variable findings on structural abnormalities identified by HRCT (133-137). All studies have low numbers and a large age range and therefore it is not surprising there are differences in the extent of lung damage observed. It has been shown previously that structural abnormalities identified by HRCT progressively worsen with age (138).

Peribronchial thickening was observed in the majority patients in two studies (134, 135) and not in the others (133, 139), although the location of thickening (bronchial, central, peripheral) was not identified. Bronchiectasis was a common finding amongst all studies (133-135, 139). Evidence of consolidation was consistent among studies at between 44% and 51% (133-135). Mucoid impaction was more common in one study (79% (134)) compared to the others (<50% (133, 135, 139)), with one study reporting presence was mainly peripheral (139). Air trapping was prevalent in two studies (135, 139), but not another (134). Finally, in a small study of 14 patients, the 7 children who had HRCT performed who were under 7 years old had evidence of structural abnormalities confined to the lower lobes (154). In a study in children under 4 years old, Davis *et al*. reported the lobes with the greatest structural damage on HRCT was the right upper and right lower lobes (140).

There have been a few studies in children exclusively under the age of 5 years with CF that have investigated the presence of structural damage in early life (136, 137, 140). Both the Long and Martinez studies reported children with CF had thicker airway walls compared to control children and Martinez *et al*. reported smaller lumen size while Long *et al*. reported increased lumen size compared to controls (136, 137). While both studies were in children younger than 5 years, children in the Long study were on average older. At this early stage of lung development and disease, this may affect the structural abnormalities observed. Also differences in techniques, largely inflation pressures and protocols for airway measurement, may explain the differences in results of lumen size between the studies. However, all studies demonstrate the presence of structural abnormalities in children with CF within the first 5 years of life.

1.5.1 Lung function and structural abnormalities

Analysis of HRCT scans appears to be a sensitive measure of lung damage as scans are able to detect small changes in airway calibre. Previous reports in patients with CF have demonstrated relationships with spirometry measures and extent of structural abnormalities and are discussed below.

Studies reporting relationships with HRCT score and spirometry measures have been conducted in children over the age of 6 years as these children are able to perform acceptable measurements (133-135, 138, 139). However the studies generally have a large age range incorporating patients up to 18 years old. In all studies there was a significant association with FEV_1 and HRCT score. Relationships between FVC and HRCT scores were observed in some studies (133-135) but not others (138, 139). Associations with FEF_{25-75} were reported in two studies (134, 139) but not others. In cases where a significant relationship was reported, associations were often driven by one factor, for example in de Jong's study relationships were driven by bronchiectasis and in Brody's study FEF_{25-75} was dominated by air trapping. Also, no study, with the exception of Brody *et al*. took age into account when analysing results. In the three studies that reported it, structural abnormalities were present in children with normal lung function (133, 138, 139).

Only one longitudinal study by de Jong *et al*., has reported changes in lung function and HRCT over a period of 2 years (138). This study demonstrated progressive changes in lung abnormalities while spirometry remained unchanged (138). Significant relationships between change in lung function and change in HRCT were observed although associations were weak (138). This supports the current thinking in the literature that structural changes in the lungs occur before children are able to produce acceptable and reproducible measurements of spirometry for the use of clinical management of lung disease (133, 136).

A recent study published by Gustafsson *et al.,* in a group in 6 to 20 year olds with CF, demonstrated significant relationships with HRCT score and measurements of LCI (141). While relationships with LCI demonstrated high sensitivity, specificity was low with 50% of children having abnormal LCI but no bronchiectasis (141). Therefore while normal LCI values indicate absence of structural abnormalities, an abnormal LCI does not necessarily mean structural abnormalities will be observed on HRCT (141). From these results, the authors suggest an increased LCI could be used as a method to decide when HRCT should be performed (141). However, due to the low specificity of LCI, up to half of the scans performed may show no structural alterations. Secondly, the authors state that LCI may be abnormal due to airway wall thickening or mucus accumulation in the very small airways, abnormalities that may not be detected by HRCT (141).

Studies by de Jong *et al*. investigated the associations between HRCT and lung function using body plethsmography (134, 138). An initial study of 23 patients (mean age 10.7±3.6 years) reported significant relationships with Raw and HRCT (134). However in a later study of 33 patients (mean age 11.1±3.3 years) no relationship between Raw and HRCT was observed (138). The later study was a longitudinal investigation and did report weak but significant changes in Raw with HRCT over the follow-up (138). These studies did not mention if Raw was influenced by a single structural abnormality, or if associations observed were distributed evenly amongst reported abnormalities (138).

Finally, in study in children under the age of 5 years old, Martinez *et al*. reported associations with structural abnormalities and lung function using RVRTC (137). This method of lung function is similar to spirometry in older children and adults and provides information on forced expiratory flows. This study compared the airway lumen

and the outer airway wall perimeters in 11 children with CF to a non-CF control group (137). The study reported negative correlations with lung function and ratios of wall to lumen area in children with CF (137). However, associations are generally weak (r^2<0.4) with the exception of wall area to lumen area and $FEV_{0.5}$ (r^2=0.66). In general the distribution of lung function and ratio's of airway to lumen size were small with a few children possibly driving the relationship. However, this study is the first to report the presence of structural airway abnormalities and associations with lung function in children this young.

As early lung disease is mainly peripheral, lung function techniques that are specific to the peripheral airways would be most sensitive to these changes. This was demonstrated in Gustafsson's study where LCI was related to structural abnormalities. Spirometry measures ventilation of the respiratory system and is not sensitive to pathological changes in the small airways although it can reflect the consequence of small airway abnormalities. Spirometry values may underestimate early lung destruction in the peripheral airways, as effects of small airway plugging may not lead to loss of volume until later in life.

Spirometry has been shown to be sensitive to structural abnormalities; however these studies have been conducted in older children and adults where lung damage is likely to have already extended to the central airways. Martinez *et al*. and Long *et al*. have demonstrated structural damage begins early in life with the presence of structural abnormalities in children with CF under the age of five (136, 137). The location of these abnormalities was typically in the lower lobes in the Martinez study and in both the smaller and larger airways in the Long study (136, 137). While Martinez *et al*. demonstrated an association with the airway and lumen size and lung function using RVRTC no studies have investigated whether a measurement of lung physiology that reflects the peripheral airways like the FOT is likely to reflect these early changes in lung structure.

1.6 Aims of this thesis

In CF, lung function forms part of routine clinical assessment in older children and adults for the clinical management of lung disease. Techniques are available to measure lung function in young children, however evidence of the utility of these techniques in the clinical evaluation of lung disease is lacking. Investigation into the relationships between respiratory function and lung disease and changes in lung physiology may aid in the clinical evaluation of young children with CF. Validation of the use of the FOT in young children with CF and the clinical utility of this lung function technique in this group has yet to be investigated.

The specific aims of the studies that comprise this thesis were to:

1. Describe the repeatability of the FOT in children with CF and compare to a healthy reference population;
2. Describe the respiratory function of children with CF in a clinical setting and in the presence and absence of respiratory symptoms;
3. Characterise BDR in young children with CF and investigate relationships with markers of lung disease;
4. Investigate associations with FOT and gold-standard measures of lung damage including inflammation and infection identified by BAL, and structural abnormalities identified by HRCT.

2

General Methods

2. General Methods

This section describes:

- The techniques commonly used in this thesis;
- The children with CF routinely seen at Princess Margaret Hospital for Children as part of their ongoing assessment;
- The development and validation of a respiratory symptom questionnaire for 2 to 7 year old children with CF.

2.1 Data Collection

2.1.1 Patients

Children between the ages of 2 and 7 years with CF who attended Princess Margaret Hospital for Children respiratory clinic during the period March 2004 to December 2006 participated in this study. This cohort included a total of 59 children. The diagnosis of CF was confirmed following identification by newborn screening in 37 children, meconium ileus in 3 children, failure to thrive in 11 children, presence of respiratory symptoms in 5 children and 4 children whom had siblings with CF. All patients underwent genotype analysis for identification of common CF genotypes. The genotypes of children were classified as follows: 32 were homozygous for mutation ΔF508 (deletion which causes the loss of the amino acid phenylalanine at position 508), 25 were heterozygous for ΔF508 and 2 children were classified as 'other' in the absence of ΔF508 mutation. In these 2 children one was homozygous for G542X mutation (glycine at position 542 is replaced by a stop codon) and the other child had 2 unidentified mutations. Children heterozygous for ΔF508 had diagnosis confirmed by sweat test (142).

2.1.2 Data Collection Protocol

The following data were collected on the following occasions:

Table 2.1: Time points for collection of research and clinical data in children with CF.

	FOT	BAL	HRCT	Questionnaire
3 month clinic visit	✓	×	×	✓
Annually at review	✓	✓	✓	✓
Hospitalisation	✓	×	×	✓
✓=data collected; ×=data not collected				

Children attended clinic routinely 3 monthly, however if they were required for medical reasons, to attend clinic more frequently, respiratory function was measured and a questionnaire administered. If children were infected with *P. aeruginosa* at the time of BAL they were offered a *P. aeruginosa* eradication programme, and had a follow-up BAL approximately three months after original BAL. At this time, respiratory function was measured and a questionnaire administered. When children were admitted to hospital for intravenous antibiotic treatment, data were collected at admission and again at discharge. After a child's 5th birthday spirometry was attempted and was repeated, with FOT, at every clinic visit until the age of 7 years. On these occasions, FOT was obtained prior to spirometry so full inspirations and forced expiration would not adversely influence FOT results.

2.2. Forced Oscillation Technique

2.2.1 Apparatus

The FOT measurements were performed with a commercially available device (I2M, Chess Medical Technology, Gent, Belgium) based on the research equipment prototype described by Landser *et al* (68). MS Windows designed software for this device was used. Calibration of the I2M was performed daily, with a commercial device supplied by the manufacturers, using known impedance and measured Zrs spectra. Impedance (Zrs) spectra were calculated from both inspiratory and expiratory signals and corrected for the impedance characteristics of the mouthpiece and bacterial filter.

2.2.2 Input signal

A loudspeaker, driven by an amplifier, generated a pseudorandom multifrequency pressure oscillation from 2 – 48Hz. Flow (V'ao) and pressure (Pao) were measured at the airway opening using a pneumotacograph and a peizoresistive pressure transducer, respectively. Fast fourier transform converted the time-domain signal into the frequency domain. Zrs was calculated from an average of inspiratory and expiratory signals. Coherence, a measurement of relationship between input and output signals, was calculated at each frequency.

2.2.3 Reporting of results

Results for Rrs and Xrs were reported at 6, 8 and 10Hz. Resistance of the respiratory system and Xrs were dependant on height. Both Rrs and Xrs were expressed as a Z score calculated using reference values derived from a local healthy population (48). This population consisted of 158 healthy preschool-children, aged 2 to 7 years, in whom respiratory impedance was measured using an identical FOT protocol (48). This group of healthy children did not have doctor diagnosed or parentally reported wheeze or asthma at any time of their life and no acute respiratory infections within the past 3 weeks and are described in detail elsewhere (48).

Z scores were calculated for children between 92 and 127cm which represent the height range of the reference population. The Z score equations are described below, where 'measured' is the respiratory function variable, 'predicted' is the respiratory function variable calculated from the equation below and 'SEE' is the standard error of the estimate of the regression equation (48).

Table 2.2: Z score regression equation for children 92 – 127 cm

Parameter	Equation	SEE
Rrs6	27.860 - (0.180 * Ht)	1.918
Rrs8	26.136 - (0.167 * Ht)	1.754
Rrs10	23.647 - (0.147 * Ht)	1.567
Xrs6	-15.345 + (0.113 * Ht)	1.212
Xrs8	-10.746 + (0.074 * Ht)	1.024
Xrs10	-9.716 + (0.063 * Ht)	1.069
Z score = (Measured – Predicted) / SEE		

2.2.4 Measurement conditions

Measurements were performed according to European Respiratory Society recommendations (60) and are described below.

Subjects position

Measurements were taken with the patient sitting in an upright position facing forward with a straight back. The patient was required to wear nose pegs and breathe quietly through a mouthpiece containing a 0.1μm bacterial filter (SureGard, BirdHealthcare, Melbourne, Australia). The child's cheeks and lower jaw were supported by a technician to minimise upper airway shunting. Each single measurement was recorded over an 8 second acquisition period.

Measurement acceptance criteria

Measurements were excluded if during testing the patient had cough, leak, glottis closure, irregular breathing (including breath hold or hyperventilation) or any other factors that may have caused a visible disruption of the trace. A measurement was considered technically unacceptable if the coherence of three or more individual frequencies was less than 0.95. We aimed to obtain a within-test variability of Rrs of less than 10%. However we would emphasise that individual measurements and the subsequent averaged Zrs data were not excluded if this criterion was not met.

Number of measurements

A minimum of three technically acceptable measurements were taken and a fitted curve for the frequency range was calculated from the average of measurements recorded. Although infrequent, some children had up to 7 measurements recorded to ensure accuracy of the measurements.

2.2.5 Training

Pulmonary function was first attempted at 2 years of age and at every subsequent clinic visit in order to familiarise the children with the FOT. Measurements were recorded and retained once children were comfortable with the equipment and could produce at least three technically acceptable measurements in a single test session.

2.3 Bronchoalveolar Lavage

2.3.1 Bronchoscopy

Bronchoscopy and BAL were conducted at Princess Margaret Hospital by an experienced respiratory paediatrician. The procedure was performed under general anaesthesia using a standard general intravenous anaesthesia protocol using propofol (3-4mg/kg) and size 2.8-3.2mm flexible paediatric bronchoscopes. Suction through the bronchoscope was delayed where possible until the tip of the scope reached the carina. Three aliquots of warmed saline (1ml/kg) were instilled into the right middle or right lower lobe and retrieved using low pressure suction.

2.3.2 Microbiology

The first aliquot from the BAL was processed by the Department of Microbiology at Princess Margaret Hospital for Children. Identification and quantification of bacterial pathogens were conducted through culture on blood, CLED and Fildes agar. Bacterial density of between 10 and 10^4 cfu/ml were recorded as isolated colonies, while densities of $\geq 10^5$ cfu/ml were classified as an infection. Fungal pathogens were cultured on Sarabouds agar with chloramphenicol, and viruses were detected using direct immunofluorescence and/or rapid viral tissue culture.

2.3.3 Processing of BAL fluid

Both the second and third aliquots of BAL fluid were processed on ice at the Clinical Sciences Department at The Telethon Institute for Children Health Research. The samples were pooled and centrifuged 405g for 5 mins (Sigma 3-15, Germany). The supernatant was divided into 500µl aliquots and stored at -80˚C for future use. The cell pellet was washed in sterile 1% PBS if required. The pellet was resuspended in 1000µl of sterile MEM 10%FCS P/S media. The cell suspension was diluted 1:1 with tryphan

blue and a total viable cell count was performed using a Neubaur haemocytometer (Hirschmann, EM Technicolor, Erberstadt, Germany). Cells were diluted to a suspension of 1 x 10^6 cells/ml. Two cytospins were performed on 70µl of cell suspension for 5 minutes at 900 rpm (Sigma 3-15, Germany). Cytospins were stained with Leishmans Eosin-Methylene blue solution (Merck, Darmstadt, Germany), dried and ultra mounted. Differential cell counts were conducted on 300 consecutive cells identifying macrophages, neutrophils, epithelial, lymphocytes, eosinophils and other identifiable cells.

2.3.4 Inflammation factor analysis

Inflammation factor analysis was conducted at the Clinical Sciences Department at The Telethon Institute for Child Health Research. Frozen supernatant from BAL fluid was thawed and IL-1b, IL-8 and NE levels were measured. Levels of IL-1β were measured using a commercially available cytometric bead array (BD Cytometric Bead Array (CBA) Human Inflammation Kit, BD Biosciences, San Diego, CA) with a working range between 20 to 5000 pg/ml. Levels of IL-8 were measured using a commercially available ELISA (BD Opt EIA, BD Biosciences, San Diego, CA) with a working range of 0.01 to 6.40ng/ml. Samples that exceeded the maximum range were diluted in PBS containing 10% FBS and re-tested. Activity of NE was measured using an adapted ELISA from Delacourt *et al.* (143) and have been previously described (42). The lower limit of detection using this assay was 0.2µg/ml.

2.4 High Resolution Computed Tomography

HRCT was conducted immediately prior to BAL under general intravenous anaesthesia in the Radiology Department of Princess Margaret Hospital for Children. A three slice scan was performed (Philips Brilliance 64 CT Scanner) at full inspiratory and relaxed lung volume. Three 0.625 collimation scans were taken from above the bifurcation, just above the diaphragm and one midway between. Exposure time was limited to 0.5s rotation time. Recorded voltage for three scans was 20kV with 70mA tube current. Images were analysed using a 768 x 768 matrix. Radiation dose for combined inspiratory and expiratory scan was 0.08mSv.

HRCT scans were reported by an experienced paediatric respiratory radiologist. The presence or absence of bronchiectasis, bronchial wall thickening and/or air trapping was recorded. Other structural abnormalities that are commonly scored for in older children and adults, such consolidation, cysts, bullae etc were not recorded as these abnormalities are rarely seen in infants and young children. All HRCT data were presented as binary variables as there is yet no validated scoring system in young children and infants.

2.5 Development and Validation of Respiratory Symptom Questionnaire

Development and validation of the Respiratory Symptom Questionnaire for Children with Cystic Fibrosis (RSQCCF) were conducted in three stages; item generation, preliminary testing and validity tests.

2.5.1 Item generation

Items for the questionnaire were generated from previous questionnaires and consultation with health professionals with expertise in CF. From previous questionnaires, respiratory symptoms and signs including cough, sputum production, wheeze, crackles and respiratory tract infections were identified as appropriate variables (95-97). Following interviews with four respiratory fellows and consultants it was decided varying degrees of severity for respiratory signs was appropriate. Information on symptoms during the months preceding clinical consultation was considered important to determine if current symptoms were persistent or transient. As such it was decided information from the month preceding clinic would provide the most accurate information from parental memory.

Discussions with other health professionals (a CF liaison nurse, a respiratory technician and two research scientists) contributed to cough and sputum production being classified into varying degrees of production and frequency. It was also decided lethargy should be included into the questionnaire to provide information on current health status. To avoid confusion among parents with the use of medical jargon, lethargy was assessed in the form of the child's exercise abilities. It was decided clinicians would provide details on current wheeze, crackles and respiratory tract

infections while parents would be asked the other sections of the questionnaire. The clinician's section was not assessed as this section was objectively assessed based on detection of respiratory signs. See appendix one for the complete questionnaire.

2.5.2 Preliminary testing of questionnaire

Preliminary testing addressed the assessment of clarity, apparent internal consistency and content validity of the questionnaire, and was conducted using methods described by Imle and Atwood (144) where parents served at the rating panel (145). Preliminary testing of the questionnaire was conducted on six parents whose children were aged between 4 and 6 years. Parents were given instruction and response sheets and asked to rate the clarity, apparent internal consistency and content validity of each question in the RSQCCF. All questions were completed without any missing data. Of the six panel members recruited for this study, a minimum of five raters should agree to attain a percent agreement of at least the required 80% (146).

To assess item clarity, panel members received instructions, rating scales (binary "yes/no"), and a response format that asked whether each item was clear or unclear, with space for comments provided beside each item (144). Apparent internal consistency (144) of the items were tested so they could be revised if there was evidence of inadequate domain sampling (147). Panel members were asked two questions: "Do these questions "generally" belong together in this survey?", and "Does each question belong in the survey?". Space was also provided for panel members to comment on items. Content validity was assessed by asking parents to read the label and definition of the RSQCCF, and then indicate whether or not the label and definition fit the set of items. The label used was the title of the survey: "Respiratory Symptom Questionnaire for Children with Cystic Fibrosis". The definition used was: "The questionnaire is intended to measure the respiratory symptoms of CF in children aged 2 to 7 years." Raters were then asked to answer "yes/no" to indicate whether each individual item belonged to the label and definition. The question of redundancy was addressed by asking raters to indicate if each item was unique (144). Space was provided for comments. A final question asked raters to add any items they considered to be missing from the questionnaire.

All items in the questionnaire achieved a minimum of 83% agreement (Table 2.3). Some discrepancies between parents were identified; with one parent (parent C) consistently stating questions were repetitious even for the first question in the series of questions asked. It is also noted that parent C would consider questions about cough and sputum production in the last month repetitious if the child had current symptoms.

Parents were also invited to add any questions they would feel appropriate for inclusion in the questionnaire. Suggestions included the type of cough, how much and the type of exercise and amount of physiotherapy in the last 3 months. After consultation with clinicians it was decided these additions would not add any clinical value to the questionnaire. Parent C suggested that questions about health in the last month and current health could be merged. This was decided against to keep the questionnaire simple and to clearly separate categories to limit confusion. Parent B suggested that symptoms over the last 3 months, rather than last month be included. This also was rejected as it was thought results may be confounded with lapses in parent's memory over such a period of time. Parent F questioned the relevance of sputum production in this age group. However, some children under 7 years do produce sputum and this will provide information on possible colonisation with a micro-organism after sputum is cultured.

Table 2.3: Total number of parents who answered positively to questionnaires on the validity, clarity and consistency of the RSQCCF[†].

	Question	Validity		Clarity	Consistency
		Label and definition	Uniqueness		
1a	Cough in last month	6	5	6	6
1b	Cough produced with	6	6	6	6
1c	Cough frequency	6	6	6	6
2a	Current cough	6	5	6	6
2b	Current cough different to last month	6	6	6	6
3a	Sputum production in last month	6	5	6	6
3b	Sputum produced with	6	6	6	6
3c	Sputum description	6	6	5	5
4a	Current sputum production	6	4[§]	6	6
4b	Current sputum production different to last month	6	5	6	6
5	Cold in the last month	6	5	6	6
6	Current cold	6	5	6	6
7a	Admission to hospital in the last three months	6	6	6	6
7b	Admission for respiratory reasons	6	6	6	6
8	Current medications and in the last month	6	6	6	6
9	Current exercise	6	6	6	6

[†]All questions achieved ≥83% agreement; [§]≥83% agreement for combined validity score.

2.5.3 Validity of questionnaire

Conventional test-retest (148) of the questionnaire to assess validity was deemed unsuitable because assessment of test-retest in a disease population such as CF has

limitations with disease progression potentially impacting on observed results. Gold standards of assessment of disease severity in CF include spirometry, HRCT/BAL or consult with respiratory specialist. As children in the age group studied were unable to complete spirometry, and assessment of manifestations of lung disease were not conducted at the time of testing, validity of the RSQCCF was conducted through consultation with a respiratory clinician.

Fifteen parents who had a child with CF that was aged between 2 and 7 years were approached for this section of the study. These parents were randomly chosen from the total population of 46 families (1 family had siblings, 1 family had twins and 1 family had twins and another sibling with CF), and did not include any of the six families involved in the preliminary testing phase of the questionnaire. Initially at the time of a clinic visit parents completed the administered questionnaire with a respiratory technician before appointment with the clinician. During consultation the clinician, based on examination and discussion with parents, completed the parental section of the RSQCCF to validate parent responses to the questionnaire. The clinician was masked to the parent's original answers. In this case the clinician was the gold standard in identifying respiratory symptoms in children.

Validity testing demonstrated good reliability between binary measures (Table 2.4). This is observed with the exception of current sputum production and assessment of a current cold. In children less than 7 years of age sputum production is limited, with most children able to produce sputum but unable to expectorate. One child was reported unable to produce sputum by their parent, but able to by the clinician with a note "unable to expectorate". This was observed twice with the second incident the parent reporting sputum production but "unable to expectorate", and the clinician reporting no production. Discrepancies in the reporting of colds were also reported in three cases. In one case it was noted by the parent that it was the end stage of a cold, although the clinician reported absence.

For data that report increments, that is production and frequency of cough and sputum and current exercise, intraclass correlation coefficient (ICC) was calculated. Production and frequency of cough and sputum were only assessed if children had symptoms. Therefore, the subset of 8 children from the original 15 children assessed was

insufficient to compute results with any statistical value. Information on the child's current exercise effort demonstrated good agreement (ICC=0.683; p=0.003).

No formal statistical analysis could be performed on the section of the questionnaire regarding medications children were taking currently and in the preceding month. It is of interest to note that 29% (4 of 14 parents whose children were taking antibiotics) parents failed to identify their child was taking antibiotics currently or in the last month. It appears that parental report of antibiotic use might not be a useful indicator of change in clinical status. Of the 14 children who were pancreatic insufficient, all parents were able to identify whether their child was taking enzymes.

Table 2.4: Measurement of reliability of symptom questionnaire in children with cystic fibrosis.

Question	Cohen's kappa	Significance
Cough in last month	0.865	0.001**
Current cough	0.727	0.003**
Sputum production in last month	0.659	0.011*
Current sputum production	0.423	0.101
Cold in the last month	0.708	0.008**
Current cold	-0.098	0.685
Admission to hospital in the last 3 months	0.842	0.001**

**p<0.01; *p<0.02

This is the first questionnaire (appendix 1) developed that is specific to identify the presence respiratory symptoms in children between 2 and 7 years with CF. This questionnaire is not a scoring system but rather a tool that could be used to record changes in key symptoms at successive clinic visits. The questionnaire was not intended

for determining prognosis or as an outcome measure. The questionnaire, designed to be administered to parents of young children with CF, was brief and simple to complete with parental reported clarity and consistency rating highly. The RSQCCF will allow standardised symptom evaluation to be used as an adjunct to methods that are currently used to monitor progression of lung disease in children with CF.

3

Respiratory Impedance and Bronchodilator Responsiveness in Young Children with Cystic Fibrosis using Forced Oscillations

Based on:

Gangell CL, Horak F, Hall, GL, Sly PD, Patterson H, Udomittipong K, Stick SM (2007) Respiratory impedance in children with cystic fibrosis using forced oscillations in clinic. Eur Resp J; 30: 892-897. *(Impact Factor=5.076).*

and

Thamrin C, **Gangell CL,** Udomittipong K, Kusel M, Patterson H, Fukushima T, Schultz A, Hall GL, Stick SM, Sly PD (2007) Assessment of bronchodilator responsiveness in young children using forced oscillations. Thorax; 62: 813-818. *(Impact Factor=6.064).*

3. Respiratory impedance and bronchodilator responsiveness in young children with cystic fibrosis using forced oscillations

This section reports an investigation into the use of the FOT in young children with CF. In this section the between test repeatability measurements of the FOT are reported. Baseline lung function in children with CF are described and compared to a local healthy population. The effect of age on lung function in the presence and absence of respiratory symptoms are discussed. The BDR in children with CF is also addressed.

3.1 Methods

3.1.1 FOT collection protocol

Basic measurement protocols using the FOT have been previously described in "General Methods". Between-test repeatability describes the variability between two sets of measurements. To achieve this, two sets of lung function measurements were recorded 15 minutes apart. During this 15 minute interval children were encouraged to sit or play quietly. This 15 minute interval is the time that corresponds to a BD assessment.

During clinic baseline respiratory function was recorded. To determine BDR, Salbutamol (600 μg) was administered via a pressurised metered-dose inhaler (Ventolin, GlaxoSmithKline) and spacer device (Volumatic, GlaxoSmithKline) following the baseline lung function measurement. Respiratory function was again recorded 15 minutes following inhalation of the BD to determine BD response. Assessment of BD response was conducted at a different clinic visit to the between-test repeatability measurements.

Children were categorised into symptom groups based on the Respiratory Symptom Questionnaire for Children with Cystic Fibrosis (discussed in section two of this thesis) that was administered at the time of their lung function test. Children were categorised as symptomatic in the presence of cough, sputum production, wheeze, crackles or respiratory tract infection. Children were categorised as binary currently asymptomatic (0) or currently symptomatic (1).

A child's first acceptable lung function measurement during clinic was used for analysis (n=59). Longitudinal information on symptoms over a period of time was conducted on 39 children at routine quarterly clinic visits. Associations between lung function and age were conducted on 47 children at the time of their annual BAL. Bronchodilator measurements were performed when requested by the referring clinician, and analyses were conducted using children's first acceptable lung function test.

For baseline lung function measurements, infection data were obtained from 59 children at their most recent bronchoalveolar lavage performed as part of the routine annual assessment. Infection data were not used for 5 children as BAL was more than one year before FOT, or children had not had a BAL. The median (interquartile range) time between lung function and BAL in this section was 1.5 (0.0, 4.0) months.

3.1.2 Statistical analysis

Data are expressed as mean (± standard deviation (SD)) and were normally distributed unless otherwise stated. Coefficient of repeatability (CR) (1.96*SD of difference between 2 measurements) was calculated for tests performed 15 minutes apart according to the methods of Bland and Altman (149) and comparison between symptom groups analysed using an independent t-test. All repeatability analyses were performed with raw Rrs and Xrs scores (i.e. not Z scores).

Z scores were not calculated in children with CF who were shorter than 92 cm as this was the lower limit in the healthy population (48). Data on children shorter than 92cm was excluded from analyses requiring a Z score calculation. Bartlett's Test (150) was used to test whether children with CF had respiratory function different from the healthy reference population using Z scores. Differences in anthropometrics and differences in lung function with symptom status were analysed using an independent t-test.

Changes in respiratory function with age were analysed using generalised estimating equations to account for the representation of children in more than one age group.

Differences in respiratory function between two consecutive clinic visits were calculated using a paired t test for individual children. Changes in Z score lung function were used to avoid the influence of somatic growth. Limits of significant responses between clinic visits with changes in symptoms were calculated from the 5^{th} and 95^{th} confidence interval of changes between two consecutive asymptomatic visits. Difference in anthropometrics were analysed with an independent t-test.

The calculation of longitudinal changes in lung function in the presence/absence of symptoms was conducted using GEE. Lung function data were taken from consecutive

clinic visits from individual patients. The first clinic visit was an asymptomatic visit and designated t=0. Consecutive symptomatic visits were assigned according to the time following the asymptomatic visit. In the event of a hospital admission, or if symptom information at a clinic visit was missed, the time series for that child was stopped.

Response to BD was calculated as absolute change (ΔRrs and ΔXrs hPa.s/L), percent change (%Δ from initial), and as Z score change (ΔZ score Rrs and ΔZ score Xrs). Due to height restrictions for calculating Z scores, a Z score change was not calculated for 2 children. Multivariate modelling was conducted to determine the main effects of age, gender, weight, height and baseline respiratory function on BDR. Univariate analysis, adjusted for covariates, was used to determine differences in BDR between symptom and infection groups in CF, and between healthy children and children with CF. Difference in anthropometrics were analysed with an independent t-test.

All statistical analyses were performed using SPSS for Windows 11.5 (SPSS inc., Chicago, IL, 2002).

3.2 Results

Anthropometrics of children with CF involved in this section are described below in table 3.1. There were no significant differences in age (95% CI of difference=-0.99, 0.24; p=0.23), height (95% CI of difference=-5.56, 4.49; p=0.83), weight (95% CI of difference=-1.77, 2.11; p=0.86) or sex (p=0.22) between children classified as currently asymptomatic or currently symptomatic.

Table 3.1: Anthropometrics of children with cystic fibrosis

	Repeatability sample	**Total CF group**	**Asymptomatic**	**Symptomatic**
n	25	59	26	33
Gender (male:female)	10:15	25:34	13:13	12:21
Age (years)	4.3 (0.7)	4.2 (1.2)	4.03 (1.09)	4.41 (1.23)
Height (cm)	102.8 (7.3)	102.0 (9.50)	101.6 (9.8)	102.2 (9.37)
Weight (kg)	17.0 (3.1)	16.7 (3.7)	16.8 (3.7)	16.6 (3.7)
Symptomatic (%)	48%	56%	-	-
Genotype (n)				
ΔF508 Homozygous	12	32	15	17
ΔF508 Heterozygous	12	25	9	16
Other	1	2	2	0
Microbiology* (n)				
Pseudomonas aeruginosa	1	3	2	1
Staphylococcus aureus	4	8	2	6
Haemophilus influenzae	0	5	2	3
Mixed oral flora	6	10	3	7
Isolated colonies	3	10	4	6
No detectable bacteria	7	19	12	7
Not available	2	5	1	4

Values shown are mean ± SD; *results from last bronchoalveolar lavage, pathogen identifed as $\geq 10^5$cfu/ml., isolated pathogen identified as between 10^2-10^4cfu/ml.

3.2.1 Repeatability

Between-test repeatability of FOT measurements were calculated 15 minutes apart, the time of BD response assessment; and 3 months apart, the time between clinic visits. Repeatability of measurements over a 15 minute period was conducted on 25 children at the time of a clinic visit where children were asymptomatic (52%) or symptomatic (48%). Absolute and relative differences in lung function between tests were not dependant on mean lung function, height, weight or age using univariate modelling. No systematic bias in between-test repeatability for Rrs or Xrs was observed (figure 3.1). The CR between tests was <2.5 hPa.s/L for Rrs and <1.5 hPa.s/L for Xrs. (table 3.2). This equates to a difference of <3% for Rrs and <13% for Xrs. Repeatability was not affected by the presence of respiratory symptoms (table 3.2).

Repeatability over a 3 month period was recorded at two consecutive clinic visits 2.87±1.10 months apart in 23 children (65% female). Children were asymptomatic at both visits. Children had a mean age of 4.74±0.91 years with a mean height of 106.7±7.53cm and a mean weight of 18.63±2.80kg. Between the two visits there was a mean Z score difference of <0.1 for Rrs and <0.2 for Xrs (Table 3.3). The upper and lower confidence limits of agreement are reported in Table 3.3 and bland altman analysis of differences between visits is shown in figure 3.2. Please refer to table 5.1 in the summarising discussing for the upper 95% confidence limits for repeatability of Z score measurements for 15 minute and 3 month repeatability.

Table 3.2: Short term repeatability (15 minutes) of lung function using FOT in asymptomatic and symptomatic children with cystic fibrosis.

	Whole population			Asymptomatic		Symptomatic	
	Mean difference (SD) (hPa.s/L)	Percent difference (%)	CR	Mean difference (SD) (hPa.s/L)	CR	Mean difference (SD)(hPa.s/L)	CR
Rrs6	0.18 (1.25)	2.02 (11.24)	±2.46	0.21 (1.07)	±2.10	0.15 (1.47)	±2.89
Rrs8	0.18 (1.08)	1.99 (10.13)	±2.12	0.31 (0.75)	±1.47	0.04 (1.37)	±2.69
Rrs10	0.27 (1.07)	2.55 (10.60)	±2.10	0.17 (0.63)	±1.24	0.38 (1.43)	±2.80
Xrs6	-0.16 (0.70)	4.73 (18.11)	±1.36	-0.21 (0.73)	±1.43	-0.11 (0.69)	±1.35
Xrs8	-0.28 (0.67)	9.46 (27.18)	±1.31	-0.27 (0.71)	±1.39	-0.29 (0.65)	±1.28
Xrs10	-0.34 (0.74)	12.65 (29.75)	±1.45	-0.34 (0.73)	±1.44	-0.33 (0.78)	±1.52

Asymptomatic v symptomatic = p value >0.05 for Rrs and Xrs at all reported frequencies

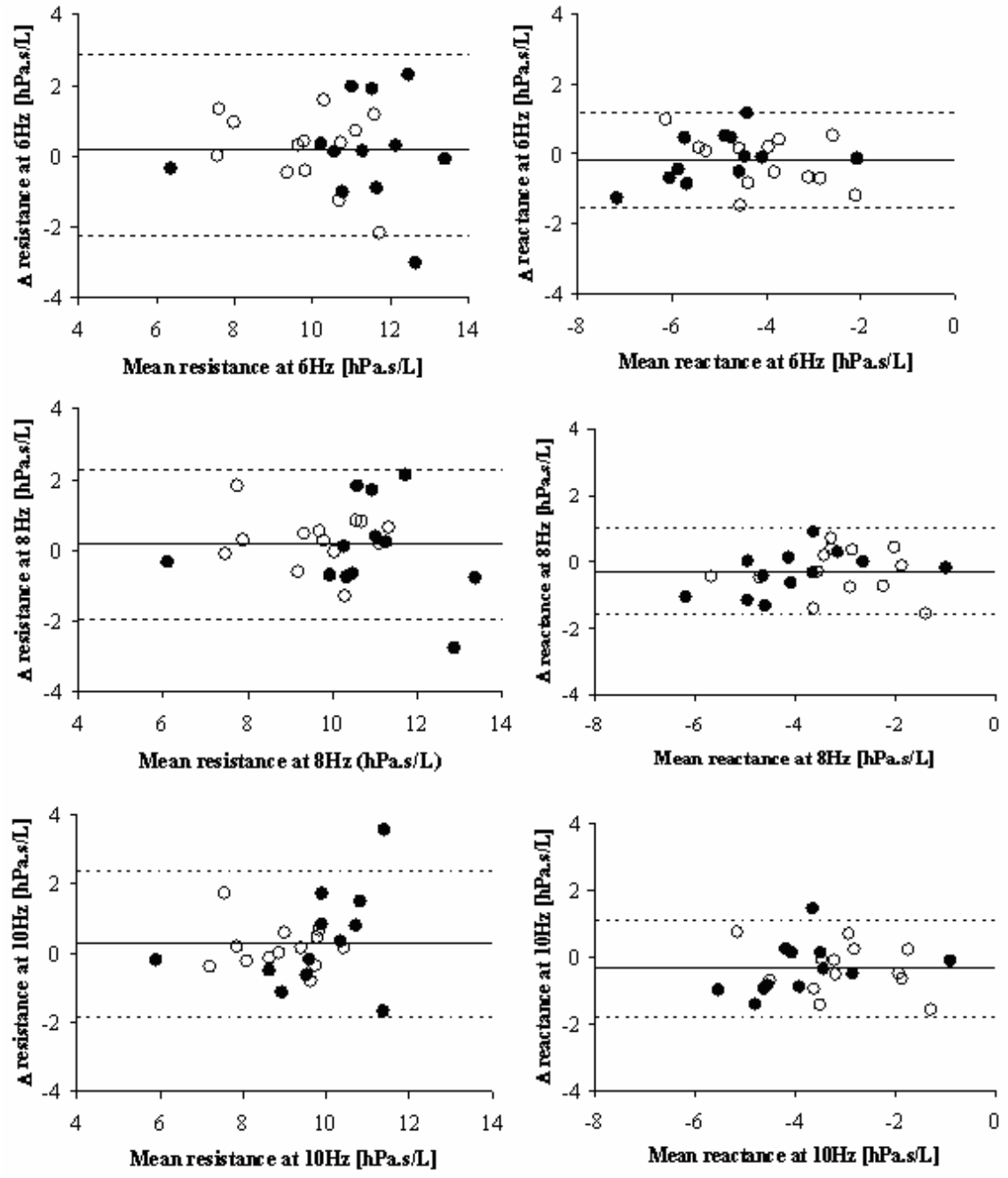

Figure 3.1: Bland Altman plots for resistance (Rrs) and reactance (Xrs) showing mean difference (___) with limits of agreement (---) for two sets of forced oscillation measurements made 15 minutes apart in children with cystic fibrosis. Measurements made in the absence (○) or presence (●) of respiratory symptoms are shown.

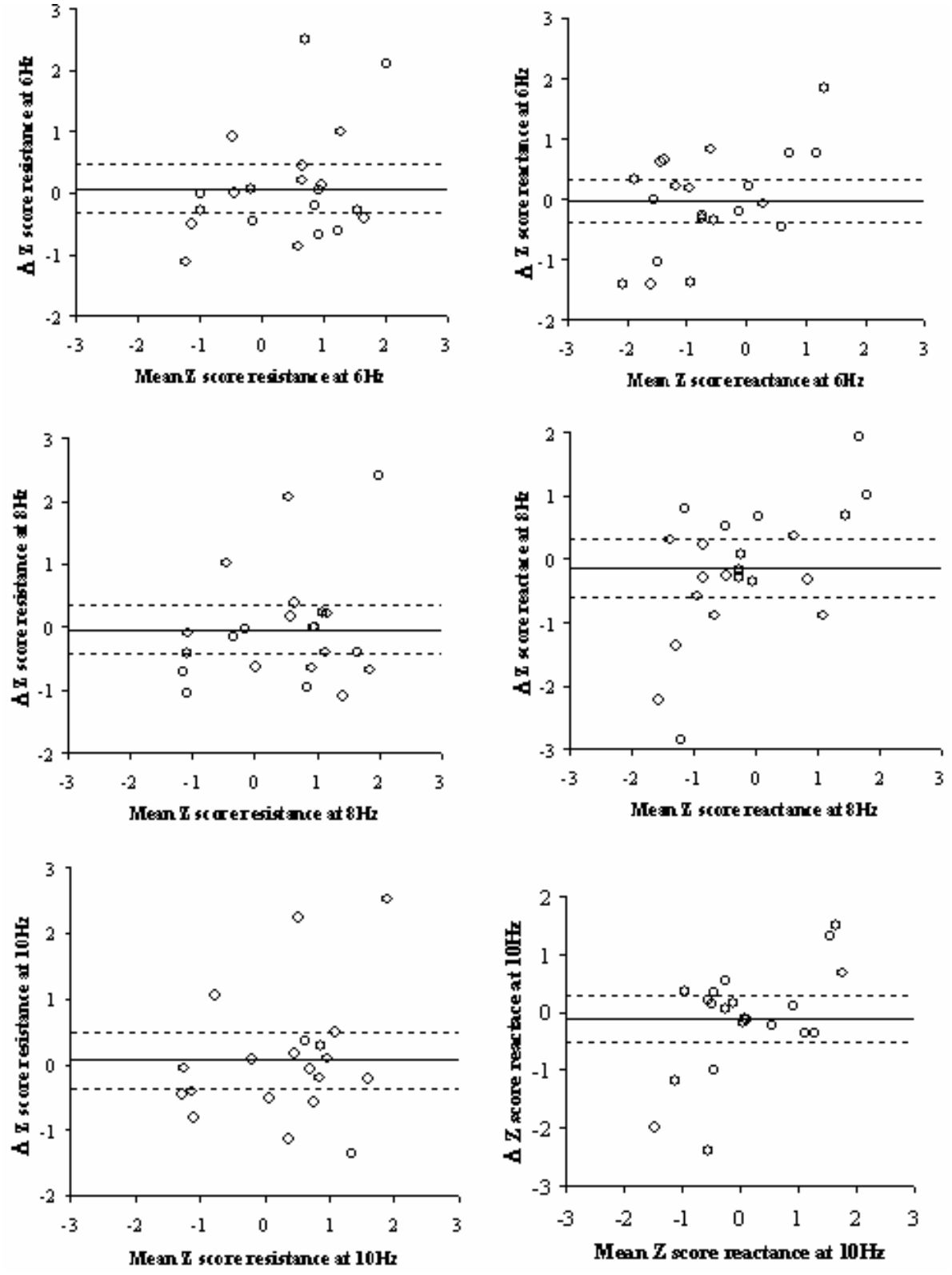

Figure 3.2: Bland Altman plots for resistance (Rrs) and reactance (Xrs) showing mean difference (___) with limits of agreement (---) for two sets of forced oscillation measurements made 3 months apart in children with cystic fibrosis.

Table 3.3: Mean Z score difference and 95% confidence limits of agreement of the difference between two asymptomatic clinic visits.

FOT variable*	Asymptomatic to asymptomatic visit		
	Mean difference	95% confidence limits of agreement	
		Upper	Lower
Rrs6	0.08	0.47	.127
Rrs8	-0.04	0.34	.046
Rrs10	0.07	0.50	.008
Xrs6	-0.01	-.146	.297
Xrs8	-0.14	-.255	.209
Xrs10	-0.11	-.119	.214

*Rrs=resistance (Hz), Xrs=reactance (Hz).

3.2.2 Respiratory Function

Z scores were calculated for 57 children (of 59 children) with a height >92cm. Technically acceptable measurements were obtained, on average, after 2 to 3 visits. As a group, children with CF had significantly increased Rrs compared to the healthy reference population with Z scores for Rrs6 ($p<0.0001$), Rrs8 ($p<0.0001$) and Rrs10 ($p<0.0001$) significantly different from zero (table 3.4). Even when children were classified asymptomatic (n=26) at the time of testing, Z score Rrs6 ($p=0.049$), Rrs8 ($p=0.007$) and Rrs10 ($p=0.01$) were significantly increased from zero. When compared to the healthy population the Z scores for Xrs in children with CF as a group were significantly different from zero at Xrs6 ($p<0.0001$), Xrs8 ($p=0.003$) and Xrs10 ($p=0.004$) (Table 3.4). These differences were primarily due to the children who were symptomatic at the time of testing.

Children who had symptoms at the time of testing had increased Rrs and decreased Xrs, at all frequencies, compared to the healthy reference group (Table 3.4). Children classified as currently symptomatic had increased Rrs6 (p=0.02), but not Rrs8 (p=0.13) or Rrs10 (p=0.21), and decreased Xrs6 (p=0.02), Xrs8 (p=0.05) and Xrs10 (p=0.01) compared to children asymptomatic at the time of testing. The power of the performed test where alpha=0.05 for Rrs8 and Rrs10 was 0.323 and 0.241, respectively. There were no differences between children who were asymptomatic or symptomatic at the time of testing for age, height, weight or gender (Table 3.1).

Table 3.4: Mean (SD) Z score resistance and reactance at 6, 8 and 10Hz for children with cystic fibrosis compared with healthy children.

	Whole population	Compared to healthy (p value)	Asymptomatic	Symptomatic
n	57	-	26	31
Rrs6	0.66 (1.02)	<0.0001	0.34 (0.84)*	0.96 (1.09)**
Rrs8	0.76 (1.03)	<0.0001	0.54 (0.92)*	0.95 (1.09)**
Rrs10	0.70 (1.07)	<0.0001	0.50 (0.95)*	0.86 (1.14)**
Xrs6	-0.76 (1.16)	<0.0001	-0.36 (0.98)	-1.10 (1.21)**
Xrs8	-0.60 (1.44)	0.003	-0.19 (1.27)	-0.94 (1.50)**
Xrs10	-0.34 (1.26)	0.004	0.11 (0.97)	-0.72 (1.36)**

Rrs=resistance; Xrs=reactance; Values are mean (SD); *denotes p value <0.05 compared to healthy; **denotes p value <0.01 compared to healthy.

Analysis of changes in lung function in children over the transition from an asymptomatic to a symptomatic episode, was conducted on individual children using a paired t-test. Respiratory function was assessed initially at an asymptomatic clinic visit and at a consecutive clinic visit 2.32±0.85 months later where the child presented with respiratory symptoms. Respiratory function from an asymptomatic episode to a symptomatic episode in 25 children, significantly worsened for Rrs6 (p=0.011), Rrs8 (p=0.036), Rrs10 (p=0.026), but not Xrs6 (p=0.074), Xrs8 (p=0.521) and Xrs10 (p=0.535) (Table 3.5). The power of the performed test where alpha=0.05 for Xrs(6-10Hz) was >0.800.

To determine the clinical relevance of statistical changes in respiratory function, these differences were compared against the repeatability of lung function over a 3 month period (see section 3.2.1). The upper 95% confidence interval limits for changes in respiratory function between two consecutive asymptomatic clinic visits were used a cut-offs for clinically relevant changes. As reported in Table 3.5, up to 60% of children had a mean change in lung function outside the confidence limits. Therefore, respiratory function significantly worsened in the presence of symptoms between clinic visits, and changes were outside the limits of variability over this time.

Table 3.5: 95% limits of agreement between two concurrent asymptomatic clinic visits, and mean difference in FOT from asymptomatic to symptomatic clinic visit.

FOT variable*	Asymptomatic to asymptomatic visit		Asymptomatic to symptomatic visit		
	95% limits of agreement		Mean difference	St dev of difference	% outside CI limits
Rrs6	-.207	.127	-.422[f]	.762	60
Rrs8	-.336	.046	-.363[f]	.816	64
Rrs10	-.457	.008	-.335[f]	.704	48
Xrs6	-.146	.297	.355	.948	56
Xrs8	-.255	.209	.153	1.172	56
Xrs10	-.119	.214	.143	1.134	36

*Rrs=resistance (Hz), Xrs=reactance (Hz); [f]Asymptomatic v symptomatic visit difference $p<0.05$

Longitudinal evaluation of respiratory function and associations with the presence of symptoms was conducted on 39 children (after 10 months of follow-up this reduced to 6 children). Follow-up time from initial asymptomatic clinic visit ranged from 3 weeks to 22 months. In the period of consecutive symptomatic visits that followed an initial asymptomatic visit (t=0), using GEE, there were significant increases in Z scores for Rrs (p value; Rrs6: p=0.033; Rrs8: p=0.044; and Rrs10: p=0.011) over the course of the follow-up, but not Xrs (Xrs6: p=0.121; Xrs8: p=0.105; and Xrs10: p=0.231) (Figure 3.3).

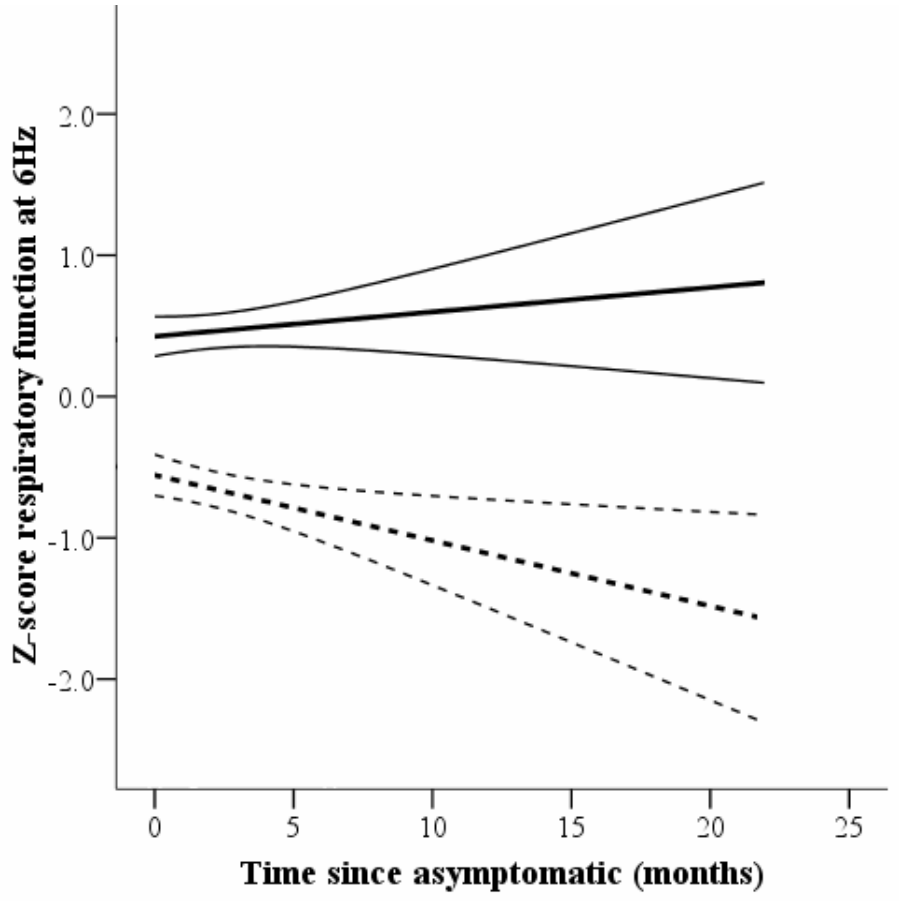

Figure 3.3: Mean change (95% CI) in Z score respiratory function at concurrent symptomatic visits for Rrs6 (—) and Xrs6 (---) from asymptomatic episode (T=0).

3.2.3 Respiratory Function and Age

Lung function was assessed at time of BAL in 47 children at 96 visits. At the time of annual review children were well enough to undergo general anesthesia and BAL. In this cohort, children had a total of 1 (n=15), 2 (n=16), 3 (n=11) or 4 (n=4) visits. At the time of lung function 40% (38/96) of the BAL samples were infected. Children who were classified as infected had the following micro-organisms isolated from BAL: *Pseudomonas aeruginosa* (n=12), *Staphylococcus aereus* (n=13), *Aspergillus species* (n=6), *Candida* (n=5), *Stenotrophomonas maltophilia* (n=5), *Haemophilus influzenae* (n=6), *Streptococcus pneumoniae* (n=2), *Parainfluenza* (n=1), *Staphylococcus epidermidis* (n=1), *Respiratory Syncytial Virus* (n=1), *Serratia species* (n=1), Group C *Streptococcus* (n=2) and/or *Achromobacter xylosoxidans* (n=1). Children classified as uninfected at their lung function visit had no micro-organism isolated (n=30), isolated

colonies (n=11) or mixed oral flora (n=26). Eight children who less than 92 cm tall were excluded from analysis. Z scores were available for 39 children at 87 visits at 8Hz and 10Hz, and on 83 visits at 6Hz.

There was a significant worsening of Rrs6 (Slope=0.20; 95%CI=0.03, 0.38; p=0.02) with increasing age (Figure 3.4). There were no associations with age and Rrs8 (Slope=0.09; 95% CI=-0.08, 0.26; p=0.31) and Rrs10 (Slope=0.07; 95% CI=-0.10, 0.24; p=0.43) (Figure 3.4). Adjusting for the presence of infection had no affect on results.

There was a significant decrease in Xrs6 with increasing age (Slope=-0.34; 95%CI=-0.49, -0.19; p<0.001). There were no associations with age and Xrs8 (Slope=-0.05; 95%CI=-0.19, 0.10; p=0.51) and Xrs10 (Slope=-0.06; 95%CI=-0.21, 0.10; p=0.43) (Figure 3.4). Adjusting for the presence of infection had no affect on results.

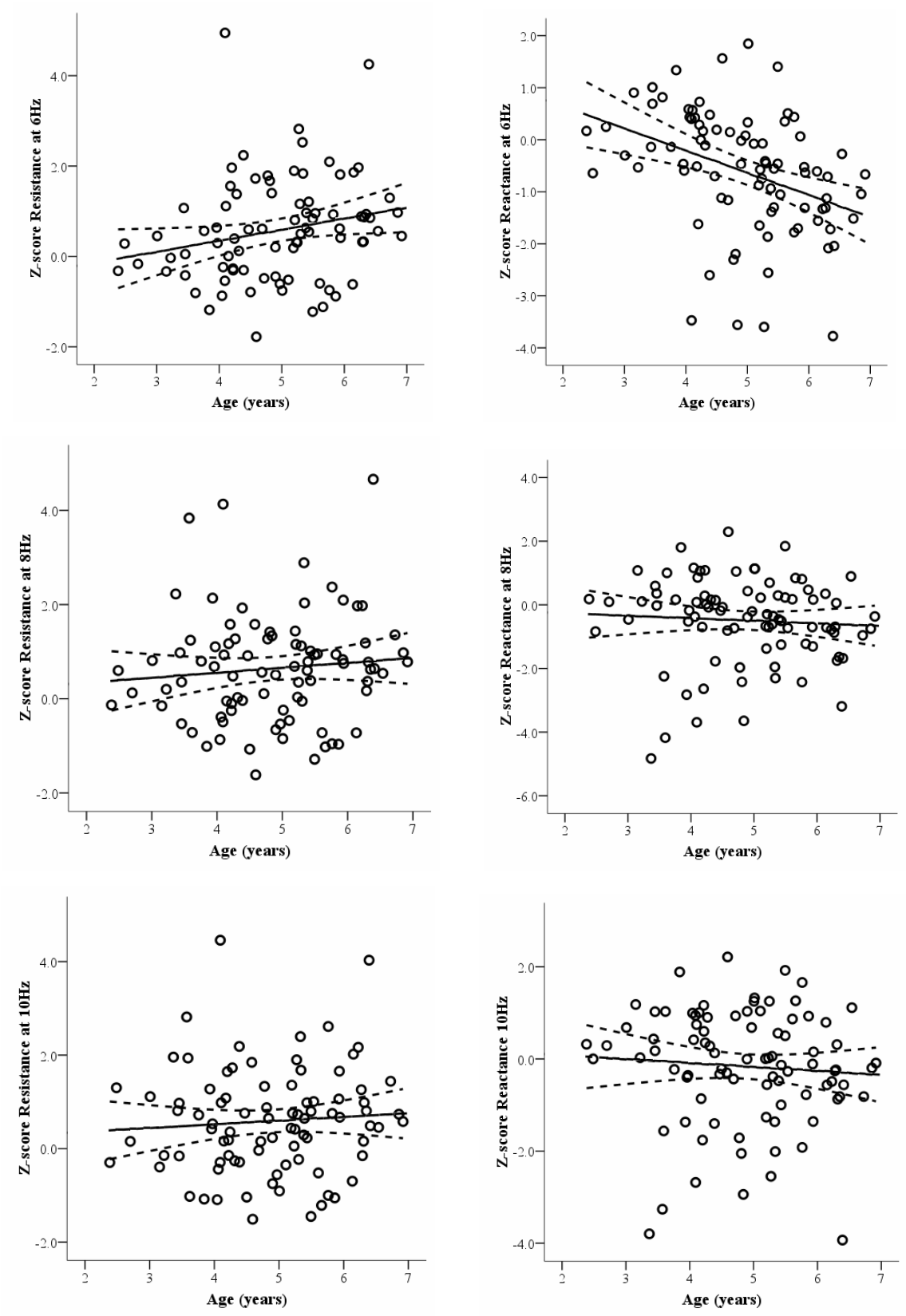

Figure 3.4: Regression (—) and 95% confidence intervals (----) of lung function versus age at the time of annual review in children with CF.

3.2.5 Bronchodilator Response

As shown in the table below, compared with the healthy reference population, children with CF had significantly different age, height, weight and Z score lung function ($p<0.05$). Approximately one third (24/78) of the healthy group were atopic and 17.9% (14/78) were exposed to tobacco smoke. Neither atopy nor passive smoke exposure influenced baseline lung function, absolute or relative BD responses measured by any of the FOT variables (Mann-Whitney test, $p > 0.05$ for all tests).

Table 3.6: Demographics and mean (SD) lung function of healthy reference population and children with CF.

	Healthy	**Cystic Fibrosis**	**p value**
n	78	48	-
Age (years)	4.9 (0.8)	4.4 (1.1)	**0.01**
Sex (M:F)	36:42	18:30	0.22
Height (cm)	110.1 (5.6)	103.0 (8.5)	**<0.001**
Weight (kg)	19.3 (2.8)	17.2 (3.5)	**0.001**
Symptomatic	**-**	56%	-
Genotype			
ΔF508 Homozygous	-	29	-
ΔF508 Heterozygous	-	18	-
Other	-	1	-
Z score respiratory function*			
Rrs6	0.01 (1.04)	0.53 (0.99)	**0.007**
Rrs8	0.08 (0.99)	0.57 (0.95)	**0.009**
Rrs10	-0.04 (1.00)	0.54 (1.00)	**0.002**
Xrs6	-0.02 (0.99)	-0.68 (1.01)	**0.001**
Xrs8	-0.06 (1.01)	-0.50 (1.35)	**0.043**
Xrs10	-0.09 (1.07)	-0.21 (1.19)	0.55

Values are mean (SD); *Rrs=resistance (Hz), Xrs=reactance (Hz); **Bold** indicates $p<0.05$.

The magnitude of the absolute BDR was related to baseline lung function, i.e. those with higher resistance had a larger BDR ($p<0.03$). The magnitude of the absolute BDR was not dependant on any other factors including age, sex, height and weight with the exception of Rrs6 and age in the healthy reference group ($p=0.017$). Absolute change in Z score was dependant only on baseline lung function ($p<0.028$) and not age, sex, height or weight, with the exception of age in the healthy group ($p=0.017$). Percent change of BDR was not dependant on age, sex, height, weight or baseline lung function with the exception of Rrs6 ($p=0.022$), Xrs8 ($p=0.037$) and Xrs10 ($p=0.037$) in the healthy reference group.

Adjusting for all covariates (age, height, weight and baseline lung function) using multivariate linear regression, there were no differences in magnitude of BDR in the healthy reference population and children with CF. This is noted with the exception of a statistically significant smaller BDR in children with CF for Rrs. Specifically this included absolute BDR at Rrs6 ($p=0.035$) and Rrs8 ($p=0.034$), percent BDR for Rrs6 ($p=0.034$), and Z score BDR at Rrs6 ($p=0.033$) and Rrs8 ($p=0.028$). As demonstrated in Table 3.7 the actual difference between the groups is small.

Table 3.7: Predicted mean (standard error) values of bronchodilator response in healthy children and children with CF.

FOT variable†	Absolute difference (hPa.s/L)		Percent difference (%)		Z score difference	
	Healthy	CF	Healthy	CF	Healthy	CF
n*	78	48	78	48	78	46
Rrs6	**-1.87 (0.14)**	**-1.35 (0.80)**	**-20.43 (01.55)**	**-15.03 (1.96)**	**-0.97 (0.07)**	**-0.69 (0.10)**
Rrs8	**-1.76 (0.11)**	**-1.33 (0.15)**	-20.09 (1.32)	-15.52 (1.74)	**-0.99 (0.06)**	**-0.74 (0.09)**
Rrs10	-1.57 (0.10)	-1.23 (0.13)	-19.17 (1.28)	-15.12 (1.68)	-0.99 (0.06)	-0.77 (0.09)
Xrs6	0.82 (0.11)	0.83 (0.14)	23.78 (2.95)	23.41 (3.75)	0.67 (0.08)	0.65 (0.11)
Xrs8	0.94 (0.08)	0.96 (0.11)	29.72 (2.94)	29.86 (3.89)	0.91 (0.08)	0.92 (0.11)
Xrs10	0.96 (0.08)	0.98 (0.11)	29.40 (2.83)	30.04 (3.70)	0.88 (0.08)	0.89 (0.10)

Data reported not adjusted for age, height or weight; †Rrs=resistance, Xrs=reactance; **Bold** type $p<0.05$; *Number of healthy children and children with CF at 6Hz=68 and 44, 8Hz=78 and 47. For Z score difference number of children with CF at 6Hz=43, 8Hz=45, 10Hz=46.

Using a paired t-test analysis there was a statistically significant improvement in lung function in children with CF following inhalation of BD for all FOT variables ($p<0.0001$). There were no differences in the BDR between children with CF who were asymptomatic or symptomatic at the time of lung function test and any FOT variables. The power of the performed test where alpha=0.05 was >0.60 for all lung function variables for relative change in lung function, although for Z score change in lung function this varied from 0.1 to 0.81.

There were no differences in BDR in children who were uninfected or infected at the time of lung function. The power of the performed test where alpha=0.05 was >0.60 for all lung function variables. At the time of analysis 27 children were symptomatic. Information on infections was obtained from the most recent BAL within the year prior to BDR assessment. No information was available on 4 children, 27 children were uninfected and 17 had a detectable pathogen. Of the children classified as 'uninfected', 7 had isolated colonies and 3 had mixed oral flora. Children with an infection had the presence of: *Staphylococcus aureus* (n=5), *Haemophilus influenzae* (n=3), *Pseudomonas aeruginosa* (n=2), *Stenotrophomonas maltophilia* (n=2), *Aspergillus species* (n=2), *Streptococcus pneumoniae* (n=2), *Candida* (n=1), *Pneumococcus* (n=1), *Group C Streptococcus* (n=1) and *Parainfluenzae* (n=1).

The lung function characteristics of the healthy group were used to determine the change in lung function that constitutes a significant BDR. From previously published data the limits of agreement for percent BD responses in the healthy group were set to be -42%, -37%, and -39% for Rrs6, Rrs8 and Rrs10, respectively (taken from the 5^{th} percentiles), and 61%, 67%, and 63% for Xrs6, Xrs8, and Xrs10, respectively (taken from the 95^{th} percentiles) (104). Using these criteria up to 8% of children with CF had a significant BD response (table 3.8).

The limits of agreement for change in Z scores in the healthy group are described in Table 3.8 (104). Using these cut-off's a change in respiratory function of greater than 1.88 of a Z score for Rrs6, and 1.73 of a Z score for Xrs6 were classified as a significant BD response (104). From these criteria, up to 14% of children with CF had a significant BD response.

Using Z score changes as a criteria yielded a higher number of children with CF classified as responding to BD's (up to 14%), while the criteria using a percent change identified no children as having a BDR for Rrs and only up to 8% of children having a significant BD for Xrs. Xrs parameters tended to yield more responders than the Rrs parameters, although there were no differences in the number of responders in children with CF compared with the healthy reference group (Fishers exact test, $p>0.05$).

Table 3.8: Number (and percent) of healthy children and children with CF with a significant bronchodilator response.

	FOT variable*	Cut-off for Significant change	Healthy	CF
Percent (%)	**Rrs6**	42%	3 (4.4%)	0 (0.0%)
	Rrs8	37%	3 (3.8%)	0 (0.0%)
	Rrs10	39%	3 (3.8%)	0 (0.0%)
	Xrs6	61%	3 (4.4%)	3 (6.8%)
	Xrs8	67%	4 (5.1%)	3 (6.3%)
	Xrs10	63%	3 (4.4%)	4 (8.3%)
Z score	**Rrs6**	-2.24	3 (4.4%)	1 (2.3%)
	Rrs8	-2.03	3 (3.8%)	3 (6.7%)
	Rrs10	-1.88	3 (3.8%)	5 (10.9%)
	Xrs6	1.73	3 (4.4%)	6 (13.9%)
	Xrs8	2.35	4 (5.1%)	5 (11.1%)
	Xrs10	2.37	4 (5.1%)	3 (6.5%)

*Rrs=Resistance (Hz), Xrs=Reactance (Hz)

3.3 Discussion

This chapter focused on the validation of the FOT in young children with CF in a clinical setting. The between-test repeatability in young children with CF was similar to healthy children. Children with CF had increased Rrs and decreased Xrs compared to a healthy reference population and lung function was worse in the presence of respiratory symptoms. Children with CF neither exhibited a BDR different to healthy children, nor was response influenced by symptoms or infection.

3.3.1 Repeatability and baseline respiratory function

The SD of changes between FOT measurements over a 15 minute period in young children with CF (Rrs: 1.07 – 1.25 hPa.s/L; Xrs: 0.67 – 0.74 hPa.s/L) were similar to that reported in studies in healthy children of similar age ranges (Rrs: 0.55 – 1.84 hPa.s/L; Xrs: 0.57 – 1.41 hPa.s/L) (48). The reported 15 minute between-test repeatability was not influenced by symptoms. The repeatability of respiratory function between two asymptomatic clinic visits was investigated to assess the "disease" effect (i.e. the potential for a true change in lung function due to disease-related pathophysiology). Mean Z score difference in Rrs6 (0.08) and Xrs6 (0.01) reported in this chapter over a 3 month period is similar to that reported for Rrs5 (0.03) and Xrs5 (0.09) in the Nielsen study over a 1 month period (26). The Z score CR in this thesis (Rrs=1.84, Xrs=2.06) was similar to that of Nielsen *et al.*, study (Rrs=1.87, Xrs=2.57) and the 14 day repeatability in healthy children recorded by our group (unpublished data) for Rrs6 (1.67) and Xrs (1.35) (26). These data suggests that in young children with CF, repeatability may not be a function of disease or clinical history.

As a group, children with CF had significantly worse lung function, with higher Rrs between 6 and 10Hz and lower Xrs between 6 and 10Hz compared to a healthy reference population. However, most children with CF had pulmonary function within the normal range, as defined as being within two standard deviations (±2 Z scores) of the mean of the healthy reference population. These results are comparable with other measurements of lung function in preschoolers with CF including the interrupter technique (26, 50, 76), MBW (71, 151) and IOS (26, 152). The fact that the majority of children with CF fall into the normal range is not surprising as, in general, children had

mild symptoms with less than 5% of children presenting to clinic with respiratory signs or respirataory tract infection and 54% of children having no detectable bacteria or isolated colonies at their most recent BAL. Some children did have abnormal lung function; 11% had Rrs6 and 12% had Xrs6 outside the healthy range. This increased to 19% for both Rrs6 and Xrs6 in children with CF classified as currently symptomatic. This suggests that measurements outside the normal range may be indicative of clinically relevant disease.

Deterioration of lung function is well described in older children and adults with CF using spirometry. The associated decline in FEV_1 percent predicted is reported between 2% and 4% per year (84, 153-157). These studies included patients over the age of six years and generally had a long follow-up period of greater than four years. In younger children, progressive changes in lung function are less well described. In a 3 year longitudinal study of 30 children with CF, Nielsen *et al*., reported significant deterioration in Rint of 0.3 of a Z score per year and for Rrs5 a change of 0.4 of a Z score per year, with sRaw and Xrs5 remaining stable (26). These changes were mainly observed in the older children with lung function in the younger children having increased variability, but remaining fairly stable. Only 16% of children were under 4 years at the start of the Nieslen *et al.* study and children up to 11 years old were included in the analysis (26). The authors did not report whether older children were more likely to have a change in lung function than preschoolers. In a study in infants, Ranganthan *et al*., demonstrated no changes in Z score over a period of 6 months using RVRTC in 34 children (mean age at first visit=28.4 weeks) (94). While there were no observed changes, the follow-up time of 6 months in this group was relatively small, and only two lung function measurements recorded per child.

This thesis reports the associations with age and lung function at the time of annual review in children aged 2 to 7 years. This was not a longitudinal analysis, although children had between 1 and 4 lung function tests included. Lung function significantly worsened with age by 0.2 of a Z score per year for Rrs6 ($p=0.02$) and by 0.3 of a Z score per year for Xrs6 ($p<0.001$). This progressive change was not observed at any other frequencies. Due to low coherence, data at 6Hz was only recorded at 83 visits, compared to 87 visits at 8Hz and 10Hz. It appears that data at 6Hz may be more

sensitive to progressive changes in lung function compared to 8Hz or 10Hz, although missing data at this frequency may affect the reliability of obtaining measurements.
This thesis reports that compared to healthy children, lung function is worse in young children with CF and progressively worsens with age. These changes in lung function occur early in life and have manifested before children are old enough to perform acceptable spirometry. The information FOT provides on changes in lung function during the early years of life will aid in the clinical assessment and tracking of children during the preschool years when early damage to lung may be occurring.

3.3.2 Respiratory function and symptoms

In CF the presence of respiratory symptoms are observed early in life and increase in frequency and intensity. Associations between respiratory symptoms and abnormal lung function become more apparent in older children with CF, possibly representing the progression of lung damage with age (85).

Tepper *et al*., reported the differences in lung function in infants with CF diagnosed within the first 13 months of life with or without the presence of respiratory symptoms to normal controls (91). Children were classified according to symptoms at diagnosis and included the groups; failure to thrive with no respiratory symptoms and failure to thrive with respiratory symptoms. Those without respiratory symptoms had lung function no different to the normal controls (91). Those with respiratory symptoms however had higher FRC, lower mixing index and lower V'maxFRC compared to the normal controls (91). Total respiratory system compliance was no different. This chapter reports similar findings in preschoolers where, compared to healthy controls, lung function (Rrs and Xrs) was no different in children with CF who were asymptomatic but was worse in those with respiratory symptoms.

In a later study in children with CF up to 28 months of age, Tepper *et al*. reported children with respiratory symptoms had worse V'maxFRC compared to those who were asymptomatic (92). Similarly, Godfrey in children less than 6 months old reported children with mild symptoms had airway obstruction while those with severe symptoms also had hyperinflation and low compliance (93). In children less than 3 years old Brennan *et al*. reported no differences in lung function using LFOT between symptom

groups (42). An inconsistency between the above studies is the classification of symptoms. In the Tepper and Godfrey studies respiratory symptoms were defined as cough, wheezing or 'marked respiratory symptoms'. In the case of the Brennan study children were classified generally as clinically well or unwell. The lack of clearly defined objective measurements of the presence or absence of symptoms may have an effect on the resulting differences in lung function. While one study may define children as generally unwell, another may define as asymptomatic.

In this chapter children were defined as symptomatic according to the respiratory symptom questionnaire described in chapter 2 of this thesis. Children were simply classed as asymptomatic or symptomatic and severity of symptoms was not gauged. Children were classified as symptomatic in the presence of cough, wheeze, crackles or respiratory tract infection. As a group, children with CF who were symptomatic had worse Rrs6 and Xrs (6-10Hz) than children who were asymptomatic. This extends into the preschool age group the reports in infants where the presence of symptoms was associated with worse lung function. We then extended this analysis further to investigate change in lung function between an asymptomatic and a symptomatic clinic visit.

Based on the results of the repeatability between two asymptomatic visits (as discussed above), the criteria used for defining a significant increase in lung function between visits was the upper 95% confidence limits. These limits were between 0.34 and 0.50 of a Z score for Rrs, and between 0.12 and 0.26 of a Z score for Xrs. We reported more than half of children with CF had significantly increased Rrs and decreased Xrs (except at 10Hz) from an asymptomatic clinic visit to a symptomatic clinic visit. However, this number is probably an overestimation due to the low standard deviation of the difference between two asymptomatic measurements.

Studies in young children and infants with CF by Brennan *et al.,* and Ranganathan *et al.,* both reported no associations with lung function in the presence of or a history of respiratory symptoms. In a longitudinal study Tepper *et al*., reported after a one year follow-up that V'maxFRC in infants with respiratory symptoms reported at diagnosis was worse than children with no respiratory symptoms at diagnosis (92). In preschool children, lung function using the interrupter technique was worse in those with a history

of respiratory symptoms (76). Although it should be noted that the group of children with a history of CF-related symtpoms was considerably smaller (n=8), than children without symptoms (n=31) which may affect statistical outcome.

In the present study we reported the association between the duration of respiratory symptoms and decline in lung function. The longitudinal data confirms what was observed between consecutive clinic visits where Rrs worsens in the presence of symptoms while deterioration of Xrs is not as pronounced. The greater variability of Xrs may have masked small changes. Over the course of the follow-up of up to 1½ years lung function continues to decline, demonstrating the detrimental effect of persistent symptoms over time. As follow-up time increases and the number of children with persistent symptoms decrease, the confidence intervals of the data increase. These data are the first of their kind that represents significant longitudinal changes in children with symptoms and suggests the detrimental effect on respiratory function over an extended period of time in children with persistent symptoms.

Previous studies have reported a higher incidence of symptoms in children with an infection, and the presence of symptoms (among other causes) may be reflective of lower airway infections. The data presented in this chapter link the presence of respiratory symptoms with worsening lung function. This indicates worsening lung function may be a reflection of lung damage and highlights the possibility of the FOT as a tool for use in the clinical assessment of children with CF in this age group.

3.3.3 Bronchodilator response

Children with CF exhibited a statistically significant improvement in lung function following inhaled BD, although the response was no greater than that observed in to healthy children. The cut-offs used for a significant bronchodilator response was based on the 95th percent confidence interval of the 15 minute repeatability in healthy children previously published by our group (104). As the 15 minute repeatability in children with CF was similar to healthy children the same cut-offs for a significant BDR were used. These were -37% for Rrs8 and 67% for Xrs8. The BD criteria used in this chapter are similar to those previously reported in the literature for Rrs5 by Hellinckx *et al.*, (49) at -41% and Malmberg *et al.*, (74) at -37%, although larger than reported by Nielsen and Bisgaard (24) at -28% and Hellinckx *et al.* (87) at -12%. However, the latter Hellinckx

study may have underestimated BDR as the cut-off criteria used was the CV of a healthy population. Nielsen and Bisgaard also reported cut-off for Xrs5 at 45%, and Hellinckx at 25%, again lower than the limits used in this thesis (24).

Using cut-off values at 8Hz for the FOT may offer the best balance between acceptable measurements based on coherence and the number of responders for BDR assessment. As described later, a change in lung function at 6Hz appears to report the most number of responders followed by 8Hz then 10Hz. Data at 6Hz was unavailable in 11% (112/126) of children due to low coherence, compared to 1% (125/126) of children at 8Hz. However, respiratory function measurements were obtained during routine clinic visits and attempts to increase coherence at 6Hz was not attempted. Therefore, reporting of data at 8Hz is the best compromise in obtaining measurements and the number of responders.

Using the reported percent change cut-offs, a maximum of four children with CF had what constituted a significant response. The number of children with CF with a significant BDR increased to up to 14% when cut-offs were defined as a change in Z score. However, changes in Z score do not adjust for baseline Z score, which is greater in children with CF. Therefore the increased number of children with CF having a significant BDR is likely to be an effect of this.

Based on the 95% confidence intervals of a BDR for the healthy population, a significant BD response was similar for 6, 8 and 10Hz for Rrs (37-42%) and Xrs (61-67%). A percent change from baseline using absolute values is recommended for determining a significant BDR as baseline lung function is taken into account. A difference in absolute values or Z score does not account for this. Also, due to height restrictions of the reference population, Z score data may not be able to be calculated for the entire CF population.

As a group children with CF did not have a greater BDR response compared to healthy children, nor was response affected by clinical status. The few studies have reported BDR in children with CF report similar findings. In a longitudinal study Nieslen *et al*., reported no difference in BDR between healthy children and children with CF using sRaw (26). While children with CF had a statistically greater BDR compared to healthy

children with Xrs5 using IOS, this was dismissed as a type I statistical error, and was probably due to two outliers (26). In a separate study using the FOT in older children (mean age=12.2±3.1 years) Hellinckx *et al*., described 13 of 20 children with CF had a greater than normal BDR with Rrs6 (87). However, the cut-offs Hellinckx *et al*. used was a >12% change, the within-subject variability of the test in children with CF. Using these cut offs the authors may have overestimated the number of responders (87).

No relationships between symptoms and magnitude of BDR have been reported in preschoolers (76) and infants (102). Although, in the preschool study the children were classified as having a history of respiratory symptoms rather than current symptoms, and in the infant study half of the CF group had a history of wheeze, which may have adversely affected results (76). In adults (7-49 years) Hordvick reported the magnitude of a BDR was greater during periods of exacerbation compared to routine clinic visits, although response during this time was not influenced by severity of disease (p=0.449) (103). The lack of BDR in children with CF reported in this chapter not surprising, as cough and not bronchospasm is the main symptom in CF. However, as associations with BDR and periods of exacerbations have been observed in older children, a significant change in BDR may be clinically significant (103).

Children with CF had a reduced BDR compared to healthy children for Rrs. This difference is most likely a statistical difference and not a physiological difference caused by small numbers in the sample population. The constitution of the CF airway may also have an effect on the response to a BD. Areas of inflammation may lead to increased stiffness of the CF lung, as demonstrated by significantly worse Xrs compared to healthy children. This increased lung stiffness may limit the dilation of the airways, especially in the periphery, which is embedded in the parenchyma.

This chapter describes the results of lung function measurements using the FOT in a clinic population of preschool children with CF. We demonstrated that lung function in this group is worse compared to a healthy reference group and that children with CF with current symptoms had worse lung function compared to children with CF who were asymptomatic.We also report the association betewen duration of symptoms and decline in lung function in individual children using a longitudinal analysis. Although the presence of symptoms has been associated with infections, children can never-the-less be asymptomatic but still have a lower airway infection (42). Therefore, whilst this

chapter has reported associations between symptoms and lung function, an analysis of changes in respiratory function and acquisition of infection will add value to the use of the FOT in clinical assessment. This study supports the current view in the literature that children with CF do not exhibit a greater BDR than healthy children, and contributes the finding that the presence of symptoms or presence of infection does not affect the magnitude of BDR in children with CF.

4

Markers of lung disease and relationships with respiratory function using forced oscillations

4. Marker of lung disease and relationships with respiratory function using forced oscillations

This section focuses on the relationships between FOT variables and markers of lung disease in CF. Associations between lung function and infection, especially with *P. aeruginosa*, are identified. The presence of inflammation and structural abnormalities are also discussed, although no associations with lung function were reported.

4.1 Methods

4.1.1 Collection of data

Measurement protocols for the FOT and procedures for BAL and HRCT have been previously described in the General Methods Section. Children were classified as symptomatic based on the Respiratory Symptom Questionnaire for Children with Cystic Fibrosis discussed in Chapter Two of this thesis.

Lung function using FOT was collected the morning of, or the day prior to, HRCT and BAL. Lung function was also collected 3 monthly during clinic in the year prior to HRCT. Worst FOT was reported as the lowest recorded FOT measurement in the year preceding HRCT. Data collected from the BAL visit was excluded if the visit was a three month follow-up following *P. aerugionsa* eradication program.

Methods for detection of inflammatory markers have been described in the General Methods section. Neutrophil elastase activity was classified as detectable if NE was >0.20 μg/ml (limit of detection 0.20 μg/ml). Cytology was reported in 41 children, and inflammatory markers in 39 children.

Children were classed as infected if BAL fluid contained $\geq 10^5$ cfu/ml, or as uninfected if BAL fluid contained $<10^4$ cfu/ml or mixed oral flora. Children classified as 'never infected' had no bacteria isolated from any previous BAL. Children classified with a 'past infection' had a pathogen isolated from at least one previous BAL. Data from children admitted to hospital during the time of follow-up were excluded from analysis.

Structural abnormalities, namely presence of bronchiectasis, bronchial wall thickening and air trapping from HRCT were described. Other structural abnormalities that are commonly scored for in older children and adults, such consolidation, cysts, bullae etc were not recorded as these abnormalities are rarely seen in infants and young children. All HRCT data were presented as binary variables as there is yet no validated scoring system in young children and infants.

4.1.2 Statistical analysis

BAL

Independent t-tests were used to determine differences between groups for age, height and weight. Fishers exact test was used for symptoms (presence/absence) and sex (male/female). A Bartlett's test was used to determine differences in lung function between children with CF and the reference population (150). Independent t-tests were used to determine the relationship with presence of infection and inflammation, and presence of infection and lung function. The effect of an infection with *P. aeruginosa* on lung function was analysed using a regression model with covariates current and past infections included. Trend analyses between infection groups (never infected/previous infection/current infection) were conducted using ANOVA with Sidak posthoc test. Regression analysis was used to examine associations between FOT variables and inflammatory markers and cell counts. Inflammatory markers, TCC/ml, neutrophils/ml, IL-1β and IL-8 were log10 transformed to correct for skewed distributions. The influence of the presence of NE on lung function was analysed using a regression model with the presence of infection included as a covariate.

HRCT

Independent t-tests were used to determine differences between groups for age, height and weight. Fishers exact test was used for binary presence of symptoms and sex. Correlations with FOT and HRCT were conducted using a regression analysis.

4.2 Results

Data was collected on 43 individual children at their first BAL visit with associated FOT data and are described in Table 4.1.

Table 4.1: Mean ± standard deviation of anthropometrics of children at the time first bronchoalveolar lavage.

	First visit	Uninfected	Infected
Number of children	43[†]	23[¶]	20[‡]
Demographics			
Age (years)	4.28 ± 1.03	4.08 ± 0.81	4.51 ± 1.22
Height (cm)	101.8 ± 6.85	100.3 ± 5.94	103.5 ± 7.57
Weight (kg)	16.5 ± 2.80	16.1 ± 2.20	16.9 ± 3.37
Sex (M:F)	20:23	9:14	11:9
Genotype			
ΔF508 Homozygous	24	15	9
ΔF508 Heterozygous	17	7	10
Other	2	1	1
Microbiology			
No pathogen ($<10^1$ cfu/ml)	11	11	0
Isolated colonies ($10^1 – 10^4$ cfu/ml)[*f*]	2	2	0
Mixed oral flora	10	10	0
Pathogen ($\geq10^5$ cfu/ml)			
Pseudomonas aeruginosa	8	0	8
Staphylococcus aureus	6	0	6
Aspergillus species	3	0	3
Haemophilus influenzae	2	0	2
Other*	8	0	8
Symptomatic	14 (33%)	9 (39%)	5 (25%)
Inflammatory markers			
TCC/ml fluid retrieved $x10^6$	0.87 ± 2.51	0.42 ± 0.39	1.39 ± 3.64
Neutrophils/ml fluid retrieved $x10^4$	19.4 ± 23.0	11.1 ± 16.0	29.0 ± 26.5
IL-8 ng/ml	2.54 ± 5.39	1.15 ± 2.38	4.23 ± 7.36
IL-1β pg/ml	78.9 ± 177.4	23.3 ± 19.3	146.9 ± 250.7
Neutrophil elastase μg/ml	2.29 ± 6.52	0.59 ± 1.44	4.27 ± 9.22

[†]n=41 TCC/ml & IL-1β, n=40 neutrophil/ml & IL-8, n=39 NE; [¶]n=22 TCC/ml & IL-8, n=21 neutrophil/ml & NE [‡]n=19 TCC/ml & neutrophil/ml, n=18 IL-8, IL-1β & NE; [*f*]Aspergillus species; ****Stenotrophomonas maltophilia*** (n=2), Candida (2), ***Streptococcus pneumoniae*** (2), Parainfluenzae (1), ***Staphylococcus epidermidis*** (1).

4.2.1 Inflammation

There were no differences in age (p=0.170), height (p=0.140), weight (p=0.373) or sex (p=0.232) between children who did or did not have a detectable infection at the time of BAL. There were no differences between infection groups for the presence of symptoms (p=0.256), or treatment with antibiotics at the time of BAL. A total of 11 children were not receiving any antibiotic treatment (uninfected v^s infected, n=6 v^s n=5) while the number of children who were receiving treatment was similar for Augmentin (14 v^s 11), Tobramycin (4 v^s 3), Cephalex (2 v^s 1) and other medication including Timentin, Septrim and Resprim (1 v^s 3).

The following inflammatory markers were increased in presence of infection: log TCC/ml fluid retrieved $x10^6$ (p=0.038), log neutrophil/ml fluid retrieved $x10^4$ (p=0.009) and log IL-1β (p=0.001). There was a trend for increased levels of log IL-8 in the presence of infection (p=0.072). There was an association with the presence of free NE and the presence of an infection (p=0.020). There was a tendency for increased levels of free NE in the presence of an infection (p=0.059).

Lung function decreased in the presence of increasing levels of the proinflammatory cytokine IL-1β [Rrs6 (R=0.37, p=0.02); Rrs8 (R=0.38, p=0.02); Rrs10 (R=0.30, p=0.06); Xrs6 (R=-0.49, p=0.002); Xrs8 (R=-0.41, p=0.009); Xrs10 (R=-0.47, p=0.002)] (Figure 4.1). Levels of IL-1β were below the lower limit of detection in 17 of the 41 children assessed. After adding the presence of an infection as a covariate to the model these associations were no longer significant for Rrs (Rrs6, p=0.26; Rrs8, p=0.19; Rrs10, p=0.33). The associations with Xrs remained after adjusting for the presence of an infection (Xrs6, p=0.03; Xrs8, p=0.05; Xrs10, p=0.02).

There was a tendency for increased levels of IL-8 to be associated with increasing Rrs [Rrs6 (R=0.31, p=0.06), Rrs8 (R=0.30, p=0.06), Rrs10 (R=0.27, p=0.09)] and decreasing Xrs [Xrs6 (R=-0.40, p=0.15), Xrs8 (R=-0.21, p=0.19), Xrs10 (R=-0.28, p=0.09)] (Figure 4.2). After adjusting for the presence of infection as a covariate in the model no associations were reported for Rrs (Rrs6, p=0.23; Rrs8, p=0.20; Rrs10, p=0.21) or Xrs (Xrs6, p=0.06; Xrs8, p=0.39; Xrs10; p=0.23).

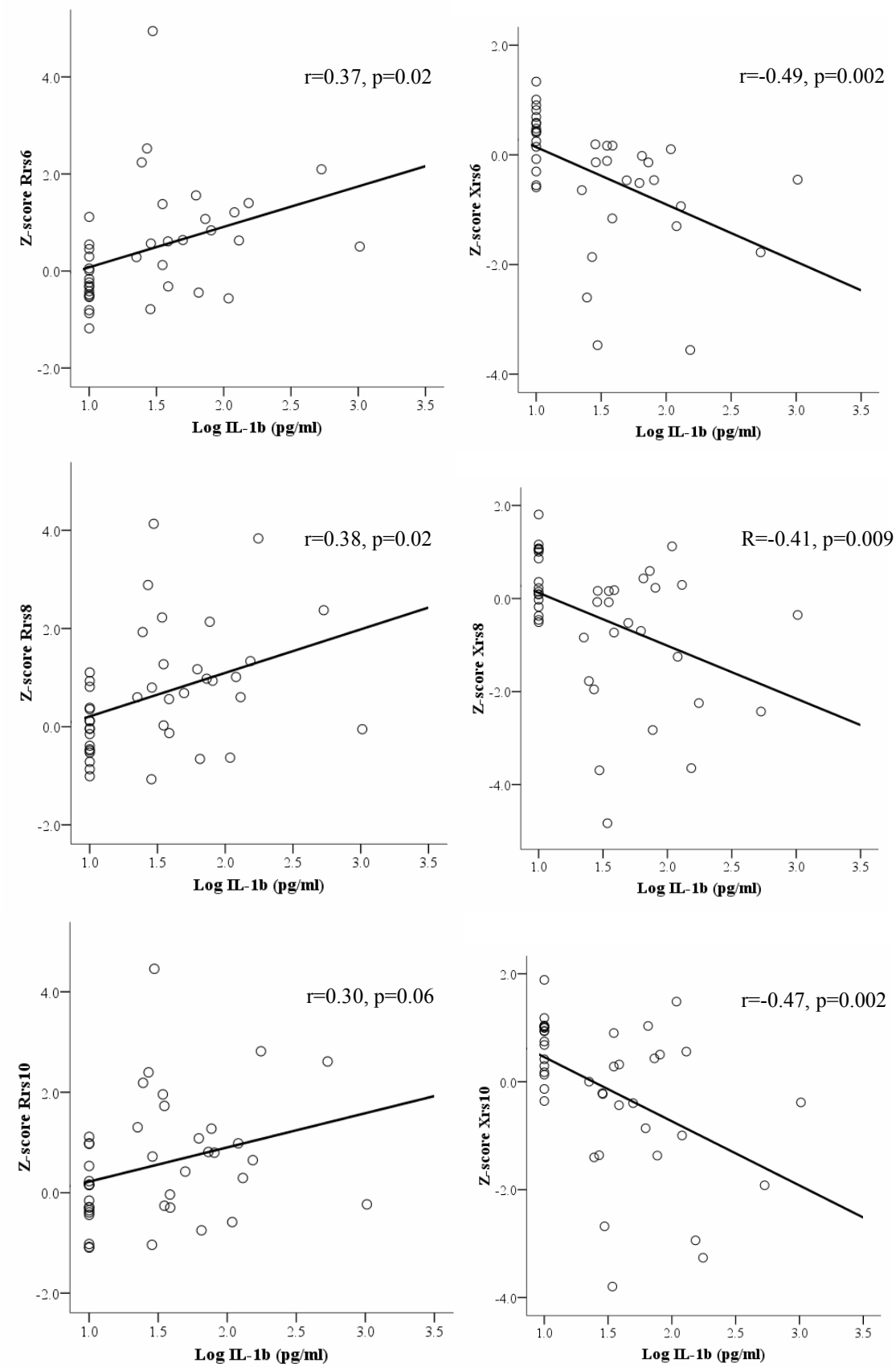

Figure 4.1: Associations between log IL-1β (pg/ml) and resistance and reactance.

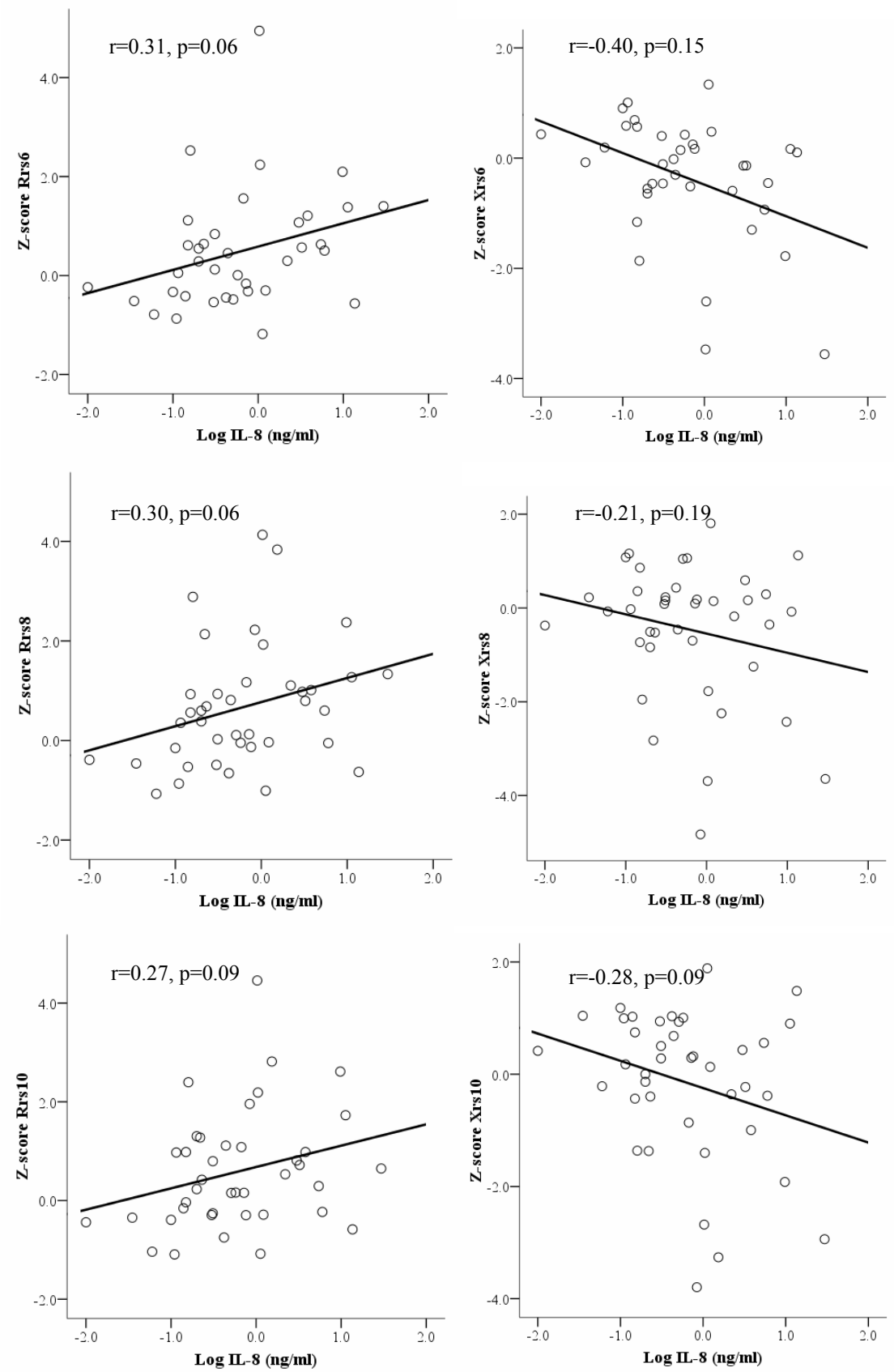

Figure 4.2: Associations between log IL-8 (ng/ml) and resistance and reactance.

No relationships were identified between any FOT variable and TCC/ml fluid retrieved or neutrophils/ml fluid retrieved from BAL (Table 4.2).

Table 4.2: Statistical p-values for associations between inflammation and FOT variables

FOT variable*	Log TCC/ml[†]	Log neutrophils/ml[f]
Rrs6	0.47	0.34
Rrs8	0.33	0.24
Rrs10	0.31	0.52
Xrs6	0.34	0.13
Xrs8	0.27	0.58
Xrs10	0.46	0.22

*Rrs=Resistance (Hz), Xrs=Reactance (Hz); [†]total cell count per ml of bronchoalveolar lavage fluid retrieved; [f]neutrophils per ml of bronchoalveolar lavage fluid retrieved.

Sixteen children had detectable levels of NE, with levels ranging between 0.36 to 29.75 μg/ml, of whom 4 (25%) were symptomatic and 11 (69%) had detectable infection on BAL. Twenty three children had undetectable levels of NE, of whom 7 (30%) were symptomatic and 7 (30%) were infected. The association between the presence and absence of NE in BAL and lung function was analysed using a regression model. Children with free NE detected had an increased mean Z score for Rrs between 0.55 and 0.70, which reduced to 0.3 of a Z score after adjusting for the presence of an infection. The presence of free NE was associated with a mean Z score decrease for Xrs between -0.66 and -0.85, which reduced to a change of between -0.39 and -0.64 after addition of infection as a covariate to the model. No relationships reached statistical significance (Table 4.3).

Table 4.3: Mean Z score difference in lung function between children with and without detectable levels of free neutrophil elastase.

FOT variable*	Without adjusting for infection		After adjusting for infection	
	Mean Z score difference (95% CI)	p value	Mean Z score difference (95% CI)	p value
Rrs6	0.70 (-0.09, 1.49)	0.08	0.33 (-0.51, 1.17)	0.43
Rrs8	0.63 (-0.16, 1.42)	0.12	0.33 (-0.50, 1.15)	0.43
Rrs10	0.55 (-0.23, 1.33)	0.16	0.34 (-0.49, 1.17)	0.41
Xrs6	-0.66 (-1.43, 0.96)	0.09	-0.39 (-1.21, 0.44)	0.35
Xrs8	-0.85 (-1.79, 0.10)	0.08	-0.64 (-1.67, 0.37)	0.21
Xrs10	-0.85 (-1.70, 0.00)	0.05	-0.63 (-1.54, 0.27)	0.17

*Rrs=resistance, Xrs=reactance.

4.2.2 Infection

As a group, children with CF had increased Rrs (6-10Hz) and decreased Xrs8 compared to the healthy reference population (Table 4.4). When subdivided according to infection status on BAL culture, lung function in uninfected children did not differ from the healthy population, whereas lung function was significantly reduced from the healthy population in those with a current infection (Table 4.4). Children who were classified as infected had significantly increased Rrs (6-10Hz) and decreased Xrs (6-10Hz) compared to children who were uninfected (Table 4.4). These differences persisted after adjusting for the child's age and for the presence of respiratory symptoms at the time of the BAL.

Table 4.4 Respiratory function in children with CF who were uninfected or infected at the time of BAL.

	Children with CF	Uninfected	Infected	p value
Number of children	43‡	23†	20§	
FOT variables (Z score)#				
Rrs6	0.46 ± 1.16¶	0.13 ± 0.80	1.04 ± 1.32¶	**0.01**
Rrs8	0.66 ± 1.21¶	0.20 ± 0.90	1.19 ± 1.31¶	**0.01**
Rrs10	0.58 ± 1.19¶	0.21 ± 0.99	1.01 ± 1.27¶	**0.03**
Xrs6	-0.33 ± 1.16	0.03 ± 0.78	-0.81 ± 1.29¶	**0.03**
Xrs8	-0.51 ± 1.53¶	-0.06 ± 1.28	-1.02 ± 1.67¶	**0.04**
Xrs10	-0.16 ± 1.32	0.27 ± 1.12	-0.65 ± 1.38¶	**0.02**

‡n=39 at 6Hz; †n=22 at 6Hz; §n=17 at 6Hz; #Rrs=resistance, Xrs=reactance; ¶$p<0.05$ compared to healthy reference population; **bold** indicates $p<0.05$ for comparison between infection groups.

Infection with *P. aeruginosa* was associated with worse lung function. Eight children had a confirmed *P. aeruginosa* infection with 1 child co-infected with *Staphylococcus aureus* and another with *Aspergillus*. For 2 children this was the first time *P. aeruginosa* had been isolated. The effect of a *P. aeruginosa* infection was analysed using a regression model incorporating current infection (other than *P. aeruginosa*) and previous infection with any pathogen. Children infected with *P. aeruginosa* had a significantly increased mean Z score (95% CI) for Rrs [Rrs6: 1.47 (0.51, 2.42), p=0.004; Rrs8: 1.03 (0.05, 2.02), p=0.04; Rrs10: 1.01 (0.01, 2.02), p=0.05]. Infection with *P. aeruginosa* was associated with a mean Z score decrease in Xrs [(Xrs6: -1.17 (-2.15, -0.19), p=0.02; Xrs8: -1.18 (-2.52, 0.16), p=0.08; Xrs10: -0.95, (-2.09, 0.18), p=0.10] which did not reach statistical significance. Data at 8Hz is shown in Figure 4.3.

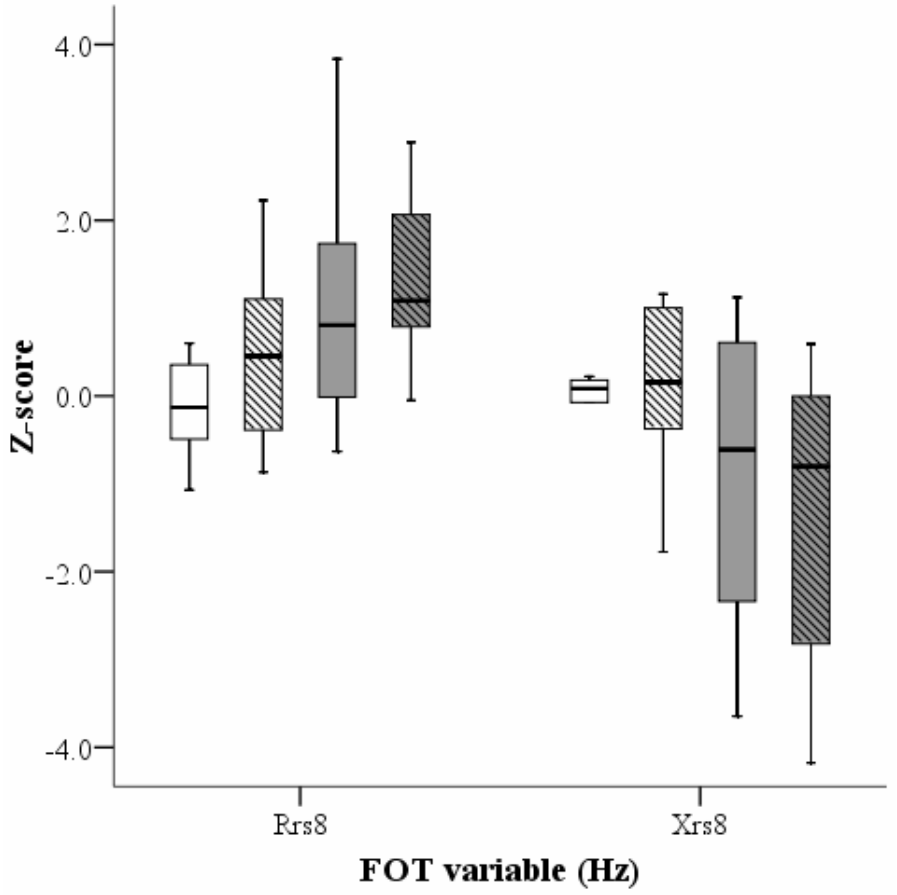

Figure 4.3: Z score resistance and reactance at 8Hz in children who were never infected (○) (n=9), those with a past infection (▨) (n=14), those with an infection other than *P. aeruginosa* (●) (n=12) and those with a confirmed *P. aeruginosa* infection (▩) (n=8) at BAL.

Examining infection history in more detail, children were divided into three categories based on infection history for those who had never had a detectable pathogen at BAL (n=9), those who were currently uninfected but had a previous infection (n=14), and those with a current infection (n=20). There were no differences among the three groups for age, height, weight, sex or symptom status (Table 4.5). There was a significant trend for increasing Rrs (6-10Hz) and decreasing Xrs at 6Hz between children who never had a detectable infection and those with a past or current infection (Table 4.5). From posthoc analysis, lung function in those with a past infection was not significantly different from the never infected group (Table 4.5)

Table 4.5: Demographics and mean lung function ± SD of children with no past infection ever, previous infection, and current infection.

	No detectable infection ever	Past detectable infection	Current detectable infection	p value
N	9	14[†]	20[§]	
Demographics				
Age (years)	3.96 ± 1.05	4.15 ± 0.63	4.51 ± 1.22	0.36
Height (cm)	99.1 ± 5.60	101.1 ± 0.22	103.5 ± 7.57	0.27
Weight (kg)	15.8 ± 2.37	16.4 ± 2.13	16.9 ± 3.37	0.60
Sex (M:F)	4:5	4:10	11:9	0.26
Symptoms	2 (22%)	7 (50%)	5 (25%)	0.24
Genotype				
ΔF508 Homozygous	5	10	9	
ΔF508 Heterozygous	4	3	10	0.45
Other	0	1	1	
FOT variable[#]				
Rrs6	-0.26 ± 0.55	0.20 ± 0.90	1.04 ± 1.32	**0.018**
Rrs8	-0.20 ± 0.60	0.46 ± 0.98	1.19 ± 1.31	**0.008**
Rrs10	-0.09 ± 0.81	0.41 ± 1.08	1.01 ± 1.28	**0.039**
Xrs6	0.19 ± 0.66	-0.08 ± 0.87	-0.81 ± 1.30	0.095
Xrs8	0.11 ± 0.73	-0.17 ± 1.55	-1.02 ± 1.67	**0.046**
Xrs10	0.48 ± 0.69	0.13 ± 1.33	-0.65 ± 1.38	**0.027**

[†]n=13 at 6Hz; [§]n=17 at 6Hz; [#]Z score lung function, Rrs=resistance, Xrs=reactance; **bold** indicates $p<0.05$

4.2.3 High Resolution Computed Tomography

Characteristics of patients with HRCT and FOT are described in Table 4.6. Direct associations between HRCT abnormalities and FOT were investigated in 34 children who had FOT assessed on the morning of or day prior to HRCT. Worst lung function in the year prior to HRCT and HRCT finding was assessed in 21 children. Bronchial wall thickening was the most prevalent HRCT finding, followed by bronchiectasis (Table 4.6).

Table 4.6: Characteristics of patients with FOT/HRCT data

	Correlated FOT/HRCT	**Worst FOT in year prior to HRCT**
Number of children	34	21
Demographics		
Age (years)	5.00 ± 1.10	5.24 ± 0.79
Height (cm)	105.8 ± 7.82	107.9 ± 6.64
Weight (kg)	17.9 ± 3.40	18.4 ± 2.85
Sex (M:F)	18:16	9:12
Genotype		
ΔF508 Homozygous	16	13
ΔF508 Heterozygous	16	7
Other	2	1
Microbiology		
No pathogen ($<10^1$ cfu/ml)	9	4
Isolated colonies ($10^1 – 10^4$ cfu/ml)	3	2
Mixed oral flora	11	9
Pathogen ($\geq 10^5$ cfu/ml)		
Pseudomonas aeruginosa	6	4
Staphylococcus aureus	4	2
Aspergillus spp	1	1
Haemophilus influenzae	1	0
Other*	5	2
Symptomatic	12 (35%)	6 (29%)
HRCT findings		
Bronchiectasis	19 (56%)	12 (57%)
Bronchial Wall Thickening	20 (59%)	13 (62%)
Air Trapping	15 (44%)	7 (33%)

**Stenotrophomonas maltophilia* (2), *Candida* (3)

There were no associations between the presence of structural abnormalities and worse lung function (Table 4.7). In cases where there is an approach to statistical significance it probably represents a statistical artefact rather than any real change. The time between worst lung function measurement and HRCT scan was 4.90 ± 4.77 months.

Table 4.7: Statistical p-values for associations between presence of structural abnormalities in the lung and respiratory function.

FOT variable*	Presence of structural abnormality identified by HRCT		
	Bronchiectasis	Bronchial wall thickening	Air trapping
Mean Rrs6	0.04	0.41	0.56
Mean Rrs8	0.09	0.26	0.84
Mean Rrs10	0.07	0.53	0.74
Mean Xrs6	0.16	0.17	0.87
Mean Xrs8	0.25	0.25	0.45
Mean Xrs10	0.19	0.32	0.54
Worst Rrs6	0.27	0.09	0.52
Worst Rrs8	0.43	0.08	0.65
Worst Rrs10	0.16	0.07	0.90
Worst Xrs6	0.76	0.44	0.29
Worst Xrs8	0.35	0.49	0.26
Worst Xrs10	0.41	0.46	0.28

*Rrs=resistance (Hz), Xrs=reactance (Hz), mean=mean respiratory function of the group, worst=worse respiratory function in the year prior to HRCT.

4.3 Discussion

This chapter focused specifically on the relationship between lung function using FOT and inflammation, infection and structural abnormalities. The results from this chapter reported associations between lung function and the presence of infection, in particular with *P. aeruginosa*. The relationship was stronger with measurements of Rrs than Xrs and a linear trend was seen between groups of children who had never been infected, those with past infection and those with current infection. Once infection was considered, there were no relationships between pulmonary inflammation and lung function. No associations between lung function and structural damage were observed, although presence of structural damage was mild and localised. The observed relationships between respiratory function and the presence of an infection indicate the potential of the FOT for use in the clinical assessment of children with CF when alternative invasive measures such as BAL are not able to be performed.

4.3.1 Lung function and inflammation

Reports of the effect of inflammation on lung function have been limited to studies in infants (< 3 years) (42, 90, 111, 116) or in older children and adults (> 6years) (158-161). The studies in older children and adults have all reported associations with spirometry and inflammation, while studies in younger children report varying results. A major difference between studies in young children and older children is the issue of sampling of lung fluid. Older children are able to expectorate sputum for analysis, while in young children fluid from the lung is obtained by other methods. BAL is the gold standard method for detection of lower airway infections in young children although OPC have also been used for the detection of upper airway respiratory infections.

Bronchscopy and BAL are performed under general anaesthesia in young children. Samples of BAL fluid are taken from nominated areas of the lung, representing a small section of the overall lung. However inflammation in the lung can be heterogeneous (162) and while inflammation may not be identified in the lobe that is being sample by BAL, there may be inflammation at another site in the lung. For example, in this thesis the right middle lobe was the area lavaged, although the presence of structural abnormalities varied between the lobes (right upper=42%, left upper=34%, right middle=27%, left middle=29%, right lower=30%, left lower=33%). Therefore we may not have identified inflammation or infection in some children due to limitations in

sampling. This may have masked any difference in inflammatory markers, or reduced the differences in seen in infected versus non-infected children. When comparing measurements of lung function and inflammation/infection, it is important to recognise the fact that lung function using FOT is representative of the whole lung, while BAL is representative of a section of the lung. Also, inflammation may persist for a period of time after an infection has been cleared (116) and so lung damage caused by inflammation may be observed on lung function despite no infection being detected.

Differences between studies in procedures for analysis of lavage fluid may have an effect on inflammatory marker levels. For example, all studies instilled up to 3 aliquots of 1ml/kg of saline into the lungs, however aliquots used for analysis of inflammatory markers were either pooled (90, 111), the first aliquot used (116), or aliquots 2 and 3 pooled (42). The present study used the standard protocol of pooling aliquots 2 and 3 for analysis of inflammation, and aliquot 1 for identification of pathogens. The difference between aliquots used for analysis may affect the levels of inflammatory markers detected. It has previously been shown that using aliquots other than the first, or pooling aliquots with the first, levels of inflammation may be underestimated (115).

Varying results on associations between lung function and burden of inflammation have been reported in young children. Brennan *et al*., in a study of children up to 5 years old with CF reported relationships with inflammation and lung function using LFOT (42). As discussed in the literature review, FOT at low frequencies needs to be performed during apnoeic periods and is impractical for routine clinical use. However, at low frequencies the LFOT is representative of the peripheral lung and data reported in the Brennan study suggests changes in LFOT is associated with early lung disease (42). The Brennan study complements an earlier study by Dakin *et al*. that reports relationships with specific compliance and air trapping, both measurements of the airway periphery, and inflammation (90). Dakin used a combination of nitrogen washouts and single breath occlusion passive deflation flow-volume technique, both conducted under anaesthesia (90). No other studies have reported associations with inflammation and lung function using RVRTC, nitrogen washout or helium dilutions (111, 116).

Two studies in children within the 2 to 7 year age range have reported no associations with neutrophils or IL-8 and FRC in 3 year olds (111) and IL-8 and spirometry in 7 year

olds(129). However IL-8 was measured by exhaled breath condensates in the Bodini study that used spirometry (129), and in the Rosenfeld study that used FRC, there was period of up to 28 days prior to or following BAL that lung function measurements were made (111). This chapter also reported no associations between lung function and cell burden (log total cell count or log neutrophils) or inflammatory markers (IL-1β, IL-8, NE) after adjusting for the presence of an infection. This indicates that the presence of infection may have a greater impact on lung function than inflammation.

This chapter reports that inflammation is not associated with lung function once infection is taken into account. In this thesis, a small range of inflammatory markers were used, but those included were associated with neutrophilic inflammation and thus should be relevant to alterations in lung structure and function. In some children inflammation was below the lower limits of the assay sensitivity, in particular for IL-Iβ. The onset of inflammation maybe delayed from the start of an infection, or may persist for some time after the infection has been eradicated. Inflammation is associated with lung injury, and the consequent structural damage may have an effect on lung function. To better understand these relationships a longitudinal investigation into the progressive changes in lung function prior to and following a confirmed infection with information on inflammation and structural damage is needed.

Previous studies have demonstrated relationships with lung function measurements specific to the peripheral airways and the presence of inflammation in young children with CF. However, these studies are mainly in infants and use measurements of lung function that require anaesthesia or sedation and therefore cannot be performed frequently, unlike FOT that can be used when children are awake and alert. This thesis did not report any relationships between levels of inflammation and lung function. In fact the presence of infection had a greater impact on lung function than inflammation

4.3.2 Lung function and infection

There is no consistency in the literature regarding associations between lung function and pulmonary infection in young children with CF. In a study of 2 to 7 year old children, Nielsen *et al*. (26) failed to detect any significant relationships between lung function and *P. aeruginosa* infection using IOS (Rrs5 and Xrs5), the interrupter technique (Rint), plethysmography (sRaw) and spirometry (FEV_1 and FVC). Similarly,

Vilozni *et al*. and Aurora *et al*. reported no associations between $FEV_{0.5}$ or FEF_{25-75} and *P. aeruginosa* infection (71, 89). In a study in infants (<3 years), Nixon *et al.* reported a trend for worse $FEV_{0.5}$ (p=0.06) in children with an infection confirmed by BAL (116).

Aurora *et al,* (71) reported the effect of a *P. aeruginosa* infection on lung clearance index (LCI) using MBW and sRaw using body plethysmography in 2 to 6 year olds with CF. They reported a higher LCI in children with a *P. aeruginosa* infection, although sRaw was not different (71). A study by Beydon *et al*. (76) reported no effect of *P. aeruginosa* on Rint or helium dilution (measuring functional residual capacity: FRC). Rosenfeld *et al*. (111) also reported no difference in percent predicted FRC using helium or nitrogen dilution in 3 year olds with an unspecified infection of $>10^5$ cfu/ml or with a *P. aeruginosa* infection. Rosenfeld *et al.* reported significantly lower FRCs in 2 year olds with an infection, and in 1 year olds with a *P. aeruginosa* infection (111).

In the present study, lung function was measured when children were clinically stable and well enough to undergo BAL under general anaesthesia. Thus the associations reported with current infection are likely to represent a 'best-case' scenario. Infection was detected in BAL from 46% of children, with the most common organisms isolated being *P. aeruginosa* (8/20) and *S. aureus* (6/20). Infection by BAL culture was associated with worse lung function. In particular worse Rrs, but not Xrs (with the exception of Xrs6) was seen in children with a confirmed *P. aeruginosa* infection, extending into the preschool age the reports in infancy (71, 111, 116). Relationships with *P. aeruginosa* infection and Xrs8 and Xrs10 were not statistically significant (p=0.08 and p=0.10 respectively) and this is most likely an effect of small sample size (n=8) and increased variability in Xrs measurements.

The present study differs in experimental design from many of the above studies that showed no effect of *P. aeruginosa* infection on lung function. In the present study lung function was performed on the morning of or day prior to BAL, ensuring contemporaneous assessment of infective status and lung function. Other studies correlated lung function with the presence of infection that may have occurred up to 6 months prior to lung function, during which time infection may have been cleared or commenced (26, 71, 76, 111). The aim of the present study was to determine cross-sectional relationships with lung function at the time of a confirmed infection and not

the long term effect of *P. aeruginosa* infection. Longitudinal studies are required to show whether changes in lung function are permanent, or whether lung function improves with successful eradication of infections.

Secondly, infection was confirmed using BAL exclusively whereas previous studies have used oropharyngeal cultures (OPC), sputum or a combination of techniques (26, 71, 76, 89). OPC has a low positive predictive value to detect lower respiratory infections and has been shown to be less sensitive than BAL or sputum (126, 128). Young children find it difficult to expectorate sputum therefore the use of OPC rather than BAL may underestimate the presence of lower respiratory airway infections in the younger age group.

We compared lung function between groups of children who had no current infection, those currently infected with organisms other than *P. aeruginosa* and those currently infected with *P. aeruginosa*. The significant linear trend for lower lung function across these groups supports the clinical consensus that pulmonary infection with *P. aeruginosa* has more serious consequences than infections with other organisms. The results of this study also caution against grouping children currently infected with organisms other than *P. aeruginosa* with children not currently infected. This may be one explanation why other studies have failed to show an adverse effect of *P. aeruginosa* infection on lung function.

The present study reported the effect previous infections on lung function in preschool aged children. No other study has reported this effect on lung function. The present study reported a significant trend with increasing (worse) Rrs and decreasing (worse) Xrs and the presence of infection. This demonstrates that following infection the effect on lung function may be long-term. The numbers of children with no detectable infection ever were lower (n=9) and children were younger (3.7 years) although the difference was not significant. However, we have shown in the previous chapter than lung function does not worsen with age, and any differences with age were more likely to occur in the older age group. Difficulties arise in the analysis of these data as, following infection, treatments vary due to bacteria isolated, the presence of symptoms or an exacerbation and the ‘recovery’ period of the lungs to an infection. Longitudinal studies in the months following an infection are needed to clearly address this issue.

4.3.3 Lung function and structural abnormalities

Manifestations of lung disease through airway wall thickening and loss of respiratory epithelium begin in the peripheral rather than central airways with damage observed in children as young as 5 months (135). In this cohort of children with a mean age of 5 years, over 50% had evidence of bronchiectasis and bronchial wall thickening as identified by an experienced paediatric respiratory radiologist. However, these structural abnormalities were considered mild as they were generally limited to one zone of the left or right lung (upper, mid or lower zone) with less than 50% of the area affected.

While weak relationships between spirometry and HRCT scores have been observed, HRCT is reported to be a more sensitive measure of lung disease than spirometry (4, 136, 163). This is attributed to the presence of structural abnormalities in children with normal lung function (133, 138, 139) and changes in HRCT over a period of 2 years while lung function using spirometry did not change (138).

Measures of airway resistance by body plethysmography (Raw) (138) and FVC (138, 139) were not associated with lung structure with the exception of FVC in some studies (133-135). Both Raw and FRC are related to lung volumes and are not sensitive to the peripheral lung. Most studies included patients up to 18 years old, some with severe lung disease. Therefore it is difficult to compare these studies to this thesis which is reporting on lung function in children under 7 years old with mild lung disease.

Relationships between HRCT and measures of the peripheral airways including FEF_{25-75} using spirometry, LCI, and forced expiratory flows (RVRTC) have been reported (134, 137, 139, 141). This suggests that lung function measurements that represent the peripheral lung may be sensitive to structural damage. In these studies, patients aged up to 20 years were included in the analysis although Gustafsson's study included 16 children under the age of 10 years (134, 139, 141). However, the authors did not report whether these children had milder lung damage compared to the older age group (141). In the Gustafsson study, the observed trend for worse lung function was only seen in those with worse structural damage identified by worse HRCT score (141). Martinez et al. reported a relationship with forced expiratory flows using RVRTC and the ratio between airway and lumen size in children under 5 years (137). These relationships were typically weak ($r^2<0.4$) with the exception of wall to lumen area and $FEV_{0.5}$

(r^2=0.66) (137). The Martinez study was conducted on 11 children and the distribution of both lung function and lung size was small within the group, with 3 outliers possibly affecting results. However, this study demonstrates the presence of abnormalities, particularly in the lower lobes in children with CF.

In the present study, children were classified as either having the presence or absence of specific structural abnormalities in the lungs and severity was not gauged. We found no associations between FOT variables and HRCT outcomes. The lack of relationship is not unexpected for a number of reasons. Firstly, as reported by de Jong *et al*., progressive structural damage was not reflected by spirometry with most patients lung function falling in normal limits despite structural abnormalities (4). In the present study, most children's respiratory function was within normal limits, and the structural damage was mild as described above. It is not known the extent of structural damage required before changes in lung function are observed.

Secondly, at the time of HRCT children were under general anaesthesia, and lung volume was raised to an inflation pressure of 20cmH_2O while lung function was recorded when children were awake during tidal breathing. The increased lung volumes at HRCT are required to identify structural damage to the very periphery of the lung. FOT is not performed at this lung volume and it is not known exactly how much of the very peripheral airways contribute to lung function using FOT, or how lung function using this method may be affected by gas trapping.

Finally, the HRCT procedure used in this study was a 3 slice scan. This 3-slice method may not give an accurate representation of total lung structure and may influence HRCT results depending on the location of structural damage. It is important to recognise that HRCT and lung function measure different properties of the lung. Also, as recognised by de Jong *et al.*, lung function tests can fluctuate for reasons other than structural damage, for example infection (4). For this reason, in the present study the presence of infection was included in the analysis as a confounder.

Relationships between structural abnormalities and lung function are not clear. Reports suggest measurements of lung function that are representative of the peripheral airways may be more sensitive than measures of lung volume to changes in structural

abnormalities (134, 139, 141). The present study reports on preschool children with mild structural lung damage, and therefore the lack of relationships between lung function and structure may be a factor of this. In this preliminary analysis, the presence or absence of structural abnormalities was reported. To extend this investigation, observation of relationships between severity and extent of structural abnormalities in the lung and respiratory function would be useful to help understand at what level of lung damage changes in lung function are seen.

This chapter investigated the association with lung function and factors known to be associated with damage to the lung in CF. A predominant characteristic of CF is the presence of inflammation and infections in the airways leading to lung damage. Previous studies have identified that this process begins early in life with inflammation detectable in some infants even when asymptomatic (42, 117). This chapter reports the relationship between infection, especially with *P. aeruginosa*, and lung function. Consistent with the literature, no associations between lung function and HRCT were identified with most children having normal lung function despite the presence of structural abnormalities. These results have clinical implications in the ability to detect changes in lung function and possibly relate these changes to clinically relevant lung disease. This will aid in the regular clinical assessment of young children, where information on lung disease is irregular and invasive, and spirometry is difficult to perform.

5

Summarising discussion

5. Summarising Discussion

The FOT has been shown to be a useful tool to measure lung function in the preschool age group. However, there is relatively little known about interpretation of clinical significance of lung function using the FOT in young children with CF. The main aims of the studies presented in this thesis were to validate the FOT in preschool children with CF and investigate the relationships between lung function and gold-standard measures of lung damage. The purpose was also to evaluate the use of the FOT in the clinical assessment of children with CF in the preschool age group.

Initially this involved validation of the use of the FOT in a clinical setting in young children with CF. Feasibility was not assessed as this was not a naïve population and children were encouraged to practise at home before attempting the FOT for the first time. This thesis is the first study to report the 15 minute repeatability of the FOT in children with CF, which was similar to that in healthy children. From this the criterion a significant change in BDR was described. Children with CF did not exhibit a greater BDR than healthy children, nor was response affected by the presence of symptoms or infection.

This thesis reported that children with CF had worse lung function compared to healthy children. Lung function was worse in the presence of symptoms and was further reduced with persistent symptoms. This demonstrated that lung function using the FOT provides information on the respiratory system and reports differences in lung physiology in children with CF. The presence of symptoms can be, amongst other things, associated with lung disease. The worsening of lung function in children with CF who were symptomatic demonstrates the FOT is reflective of physiological changes in the lung.

This thesis reported lung function was worse in the presence of an infection, particularly with *P. aeruginosa*. Worse respiratory function was reported between groups of children who had never been infected, those with a past infection, and those with a current infection. These results demonstrate the FOT is reflective of lung infection, and that pulmonary infections may have a long term impact on lung function. The FOT therefore has the potential to provide information during routine clinical visits, between

annual BAL reviews, and for the clinical assessment and management of children with CF.

Brennan *et al.*, reported in a study in young children with CF associations between lung function using LFOT and the total number of cells and neutrophils in BAL fluid, and levels of IL-8 and LTB_4 (42). In this thesis, no relationships between inflammatory markers and lung function using FOT were reported. Although children in the Brennan *et al*. study were younger, the percent of children with an infection in the present study was lower (50% compared to 42%). Levels of inflammation in children with an infection in the present study were also lower compared to the Brennan *et al.* study, although levels of inflammation in children without an infection were similar. Dakin *et al.* also reported associations between levels of inflammation and lung function in children with CF, although again levels of inflammation were higher than in the present study.

The lack of relationship between inflammation and lung function in the present is perhaps a factor of low levels of inflammation in these children, and perhaps the FOT is not sensitive to detect these influences on lung function. The mechanisms involved in initiating inflammation in the lung of children with CF are controversial and the presence of infection is a major factor. Longitudinal studies performed from birth in children identified by new born screening would be required to adequately address this question. Tracking levels of inflammation and lung function over time, during periods of high levels of inflammation, will give us an understanding of the short and long term impact of inflammation on lung function.

As reported in previous studies, lung function was not related to structural damage identified by HRCT. HRCT has been reported as more a sensitive measure of lung damage than lung function (4, 136, 163). However, it is unclear at what severity and extent of structural damage changes in respiratory function are induced. Investigations are needed into the long-term effect of structural changes in infancy and their affect on lung function in later life are needed to investigate this.

A tool to objectively classify young children as symptomatic was needed, although until now no such measure existed (42). In this thesis a respiratory symptom questionnaire

for young children with CF was developed and validated. This enabled children to be uniformly classified as asymptomatic or symptomatic, and matching lung function data described those with symptoms as having worse lung function. The respiratory symptom questionnaire for children with CF (RSQCCF) provides a measure of symptom classification in this population of children and has the potential, if combined with other objective measures such as lung function and past infection status, to be used as a tool to classify the current clinical status of young children with CF.

5.1 Methodology of the FOT

Cut-offs for determining clinically significant changes in lung function using the FOT in children are required. This gives us information on what level of change in lung function may indicate the presence or onset of lung disease, as well as providing criteria for significant improvement of worsening of lung function during clinical trials. Criteria for determining these changes are based on the repeatability of the test. Reports on changes in lung function outside the repeatability of the test describe 'real changes' in lung function. This thesis reported the repeatability of two FOT measurements taken 15 minutes or 3 months apart. The upper 95% confidence limits for the repeated measurements are reported in Table 5.1.

Table 5.1: Upper 95% confidence limits for repeatability of Z score measurements

FOT variables*	15 minute repeatability	3 month repeatability
Rrs6	0.37	0.47
Rrs8	0.36	0.34
Rrs10	0.45	0.50
Xrs6	-0.37	-0.37
Xrs8	-0.54	-0.60
Xrs10	-0.60	-0.51

*Rrs=resistance, Xrs=reactance;

Based on these 95% confidence limits, a change in lung function of about 0.6 of a Z score is outside the limits of repeatability. However, if we look at the mean difference in lung function based on clinical criteria (Table 5.2) a change in lung function of 0.6 of a Z score seems an underestimation to observe clinical changes.

Table 5.2: Mean Z score difference in lung function between different clinical groups studied cross-sectionally.

FOT variables*	Cystic fibrosis vs Healthy		Asymptomatic vs symptomatic		Infected vs uninfected	
	Mean	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
Rrs6	-0.67^{f}	-1.03	-0.61^{f}	-1.13	-1.02^{f}	-1.71
Rrs8	-0.68^{f}	-1.02	-0.41	-0.95	-0.99^{f}	-1.67
Rrs10	-0.73^{f}	-1.09	-0.36	-0.93	-0.80^{f}	-1.50
Xrs6	0.74^{f}	1.12	0.74^{f}	1.33	0.84^{f}	1.51
Xrs8	0.53^{f}	0.95	0.74^{f}	1.49	0.96^{f}	1.87
Xrs10	0.25	0.65	0.83^{f}	1.47	0.92^{f}	1.69

*Rrs=resistance, Xrs=reactance; ^{f}denotes significant associations.

From these results a change in lung function greater than the upper confidence limit of 0.6 of Z score would be more appropriate. Table 5.3 gives the positive predictive value and the negative predictive values of a Z score of 0.8, 1.0 and 1.2 in the population of children with and without a current infection. From these results a change of 1.0 of a Z score gives the best positive and negative predictive value. However, to verify whether this criterion is correct longitudinal studies will be need to be conducted. This would involve collection of repeated measurements of lung function collected over a period of time, combined with information on infection and inflammation with BAL, and lung structure with HRCT. This prospective approach will also provide information on the specificity and sensitivity of this lung function test and the criteria used for clinically significant changes in lung function.

Table 5.3: Positive and negative predictive values of Z score lung function in children without an infection at the time of BAL

	0.8 of a Z score		1.0 of a Z score		1.2 of a Z score	
	PPV (%)	**NPV (%)**	**PPV (%)**	**NPV (%)**	**PPV (%)**	**NPV (%)**
Rrs6	75	70	75	70	75	65
Rrs8	67	68	72	72	70	61
Rrs10	63	63	69	67	60	58
Xrs6	88	68	88	68	83	64
Xrs8	73	63	73	63	80	64
Xrs10	82	66	82	66	78	62

PPV=positive predictive value, NPV=negative predictive value; Rrs=resistance, Xrs=reactance;

Criteria for acceptable measurements using the FOT have been discussed previously (67). Even though the FOT requires minimal patient co-operation the way children perform the test will affect results. While this thesis reports no 'disease' effect on repeatability, if children are unwell they are less likely to co-operate. In this thesis I have reported FOT measurements at 6, 8 and 10 Hz. Data at 6Hz was the least reliable with low coherence in up to 9% of measurements. Reliability of data increased with increasing frequency (Table 5.4). However, from Table 5.3 the most suitable frequency to determine differences in lung function was 6Hz, followed by 8Hz and 10Hz. Therefore, reporting of data at 8Hz may offer the best balance between reliability and sensitivity. An alternative would be to examine the frequency-dependence of resistance, using all valid data points between 4 and 24 Hz. While this may appear to be attractive, stringent acceptability criteria wauled need to be developed for young children.

Table 5.4: Measurements (%) missing due to low coherence at given frequencies in cross-sectional studies.

Study	6Hz	8Hz	10Hz
Baseline and symptoms	0	0	0
Bronchodilator response	8	2	0
Infection and inflammation	9	0	0
High resolution computed tomography	0	0	0

In young children objective measures of lung disease currently involve the use of techniques, such as BAL and HRCT, which cannot frequently be performed due to need of anaesthesia. These techniques provide us with information on infection and lung structure for the clinical assessment of lung disease in children. The data presented in this thesis demonstrates the association between infection and FOT measurements in young children with CF and indicates the potential use of the FOT in providing information that can be used routinely as part of children's clinical assessment or as an outcome measure for clinical trials. Information on lung function in this preschool group will aid in the assessment of children with CF presenting to clinic, however it does not provide an alternative to, nor is it a surrogate for, measures of infection and inflammation (BAL), or lung structure (HRCT).

5.2 Future applications of the FOT in CF

This thesis has reported the use of the FOT in the clinical assessment of children with CF based on relationships with markers of lung disease. The clinical assessment of children with CF will not be limited to use of the FOT, and investigations into using the FOT in combination with other tests to determine what provides the best information about children's health are essential.

In a recent manuscript, Davis *et al*. discussed endpoints for clinical trials in children younger than 6 years old with CF (164). In this review the potential use of the FOT for endpoints in clinical trials was discussed due to guidelines and reference data available and the non-invasive nature of the test (164). This thesis reports on the repeatability and the clinical relevance of FOT measurements in children with CF, factors that are essential for defining endpoints for clinical trials. The use of the FOT as an outcome measure for clinical trials in young children with CF will aid in the assessment of treatments and interventions in this group. This will lead to an improvement in health outcomes early in life.

Lung function measurements are also used to define pulmonary exacerbations. Pulmonary exacerbations in CF are usually characterised by a period of worsening respiratory symptoms and are often accompanied by systemic symptoms. In children able to perform spirometry a decline in FEV_1, as well as other physical findings and patient history, are used to define an exacerbation (165). In children unable to perform

spirometry there are currently no standardised definitions for exacerbations, and questionnaires that include information on lung function are not relevant. This thesis has described a reduction in lung function using FOT in symptomatic children and therefore the potential to be used, in combination with information about symptoms in the definition of a pulmonary exacerbation in young children. Collectively this may provide more information about underlying disease severity.

Longitudinal observations of lung function from early in life could provide useful information about the effects of infection on the developing lungs. For example such measurements could provide information regarding the effects of early infection with *P. aeruginosa*, antibiotic prophylaxis and early interventions on lung function and development.

Investigation of lung function in children from the preschool years using FOT to late childhood using spirometry would provide information if FOT during these early years could serve as a predictor of lung function using spirometry in later childhood. Previous studies have examined the relationship between FOT and spirometry to identify commonality between the two tests (50, 83, 87, 166). However, it is difficult to directly compare these two measures of lung function as tests are conducted differently and measure different aspects of lung mechanics. Investigations on lung function in infancy using LFOT and relationships with FOT in the preschool years will provide information on lung mechanics using forced oscillations longitudinally from infancy through to childhood. These data will provide information on the progression of lung function in early childhood, and may provide an outcome measure in determining optimal interventions and treatments over a period of time.

5.3 Diseases other than CF

The population investigated in the studies presented in this thesis included children between the ages of 2 and 7 years with CF. The relationships reported in this thesis between lung function and markers of lung damage were specific to this group, although the FOT has the potential to be used in the clinical assessment of children with other respiratory illnesses. Other studies have investigated use of the FOT in young children with respiratory illnesses including asthma (13, 19, 21, 25, 43, 49-52) and neonatal lung disease (30, 32, 46, 47). Further investigations into the use of the FOT in respiratory

diseases that affect the peripheral lung, as well as the clinical relevance of these, will validate the use of the FOT to provide clinically relevant information and assist in the detection and treatment of early lung disease.

The FOT has the potential to extend beyond the preschool age group to other groups that find it difficult to perform forced expiratory manoeuvres. These include the elderly, mentally and physically disabled and those requiring oxygen. Few studies have investigated the use of the FOT in elderly (167-169) although further research is required for feasibility, reproducibility and clinical relevance of measurements. The FOT also has the potential to provide information on lung function in diseases that affect the peripheral lung in adults such as chronic obstructive pulmonary disease, bronchiolitis obliterans or occupational lung disease, where spirometry may be insensitive to early stages of lung disease. In these cases the FOT may provide information on lung damage that is occurring early in the course of the disease. Reference data is required for adult populations as well as reproducibility measurements to determine clinically relevant changes in lung function.

5.4 Concluding remarks

The FOT is a standardised measurement of lung function with appropriate reference values available for use in preschool children. This thesis has reported the repeatability of respiratory function data using the FOT which is similar between healthy children and children with CF and is not affected by disease status. The results of this thesis show associations with lung function using the FOT and symptoms and infection, and suggest that regular measurements of lung function using the FOT may provide useful clinical information on lung disease in young children. We described a change of 1.0 of a Z score may be interpreted as a clinically relevant change in respiratory function and these changes may be reflective of lung disease.

6

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6. References

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7

Appendices

7. Appendices

7.1 Respiratory Symptom Questionnaire for Children with Cystic Fibrosis.

1a. Has your child had a cough in the ***last month***?
□No *(go to qu. 2)*□Yes *(answer below)*

1b. **Was the cough produced?**
□With physio/exercise/treatment **only** *(go to qu. 2)*
□Spontaneously *(answer below)*
□Both *(answer below)*

1c. **How often was it produced?**
□Rarely (monthly)
□Occasionally (weekly)
□Frequently (daily)
□Constant (many times per day)

2a. Does your child have a ***current*** cough?
□No *(go to qu. 3)*□Yes *(answer below)*

2b. **Is it different to last month?**
□No *(go to qu. 3)*
□Yes *How is it different?*_____

3a. Did your child produce sputum in the ***last month***?
□No *(go to qu. 4)*□Yes *(answer below)*

3b. **Was sputum produced?**
□With physio/treatment/exercise only *(go to qu. 4)*
□Spontaneously *(answer below)*
□Both *(answer below)*

3c. **Was the sputum production?**
□Small amount (less than a teaspoon), light
□Increased (more than a teaspoon), dark, thick
□Other (plugs, etc) _____

4a. Does your child ***currently*** produce sputum?
□No *(go to qu. 5)*□Yes *(answer below)*

4b. **Is it different to last month?**
□No *(go to qu. 5)*
□Yes *How is it different?*_____

5. Did your child have a cold in the ***last month***? □No □Yes

6. Does your child ***currently*** have a cold? □No □Yes

7a. Has your child been admitted into PMH in the last 3 months?
□No *(go to qu. 8)* □Yes *(go to qu. 7b)*

7b. Was it for respiratory reasons?
□No *(go to qu. 8)* □Yes *How long did your child stay?*____

8. What medications did your child take in the ***last month*** and what medications are they ***currently*** taking? *(tick all that apply)*

Taking now	Taken in last month	**Antibiotics**	Taking now	Taken in last month	**Asthma**	Taking now	Taken in last month	**Enzyme**	Taking now	Taken in last month	**Steroid/ Other**
☐	☐	Augmentin Duo	☐	☐	Flixotide	☐	☐	Pancrease	☐	☐	Prednisolone
☐	☐	Azithromycin	☐	☐	Seretide	☐	☐	Creon 5 000	☐	☐	Pusle Methyl Prednisolone
☐	☐	Clarithromycin	☐	☐	Serevent	☐	☐	Creon 10 000	☐	☐	Pulmozyme (Dornase alfa)
☐	☐	Keflex	☐	☐	Singuilair	☐	☐	Creon 25 000			
☐	☐	Resprim	☐	☐	Ventolin	☐	☐	Cotazym	☐	☐	Other_____ _____
☐	☐	Tobramycin	☐	☐	_____	☐	☐	_____			
☐	☐	_____									

9. Is your child exercising the □Same □More □Less than normal?

10. Is there anything else you would like to tell us about your childs respiratory health that has occurred or worried you since your last visit?_____

_____

_____

……..

Clinician Section

Is wheeze detectable? □None □Isolated □Multilobe □Diffuse with poor airflow

Are crackles detectable? □None □Isolated □Multilobe □Diffuse with poor airflow

Is there any current respiratory tract infection? □No □Lower □Upper

Has a change in medication been prescribed? □No □Yes *Why?*_____

_____

Do you have any additional comments about clinical status?_____

_____

_____